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Carotenoids in bird testes: Links to body carotenoid supplies, plumage coloration, body mass and testes mass in house finches (*Carpodacus mexicanus*)

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ABSTRACT

Carotenoid pigments can be allocated to different parts of the body to serve specific functions. In contrast to other body tissues, studies of carotenoid resources in the testes of animals are relatively scarce. We used high-performance liquid chromatography to determine the types and concentrations of carotenoids in the testes of house finches (*Carpodacus mexicanus*). Additionally, we examined the relationships between testes carotenoid concentrations and carotenoid pools in other body tissues, as well as body mass, testes mass and plumage coloration. We detected low concentrations of several carotenoids – lutein (the predominant carotenoid), zeaxanthin, anhydrolutein, ß-cryptoxanthin, ß-carotene and an unknown carotene – in the testes of wild house finches. We also found that testes lutein levels were significantly and positively associated with circulating lutein levels, while the concentration of zeaxanthin in testes was positively associated with zeaxanthin levels in liver, though in this instance the relationship was much weaker and only marginally significant. Furthermore, lutein levels in testes were significantly negatively associated with testes mass. Finally, plumage coloration was not associated with either the concentration of carotenoids in the testes or relative testes mass. These results suggest that testes carotenoids are reflective of the pool of circulating carotenoids in house finches, and that plumage coloration is unlikely to signal either the carotenoid content of testes tissue or a male's capacity for sperm production.

1. Introduction

Recent integrative studies of carotenoids in animals have deepened our understanding of the morphological, behavioral, immunological and fitness roles of these pigments. An emergent physiological theme in this research is that carotenoid allocation to different parts of the body (e.g. integument, liver, retina) permits different functions (McGraw and Toomey, 2010). For example, adipose and liver tissue may be used as carotenoid storage sites (Negro et al., 2001) to be accessed at a later time when additional carotenoid resources are required (e.g. molt, migration), while retina tissue is suggested to accumulate carotenoids for photoprotection and spectral tuning (Thomson et al., 2002a, 2002b; Vorobyev, 2003). Carotenoids have also been identified in the ovaries (Goodwin, 1950; Miki et al., 1982; Czeczuga-Semeniuk and Wolczynski, 2005) and eggs (Blount et al., 2000; Dierenfeld et al., 2002; Biard et al., 2009) of several animal taxa, where yolk carotenoids act as antioxidants and immunostimulants to aid in offspring development (Saino et al., 2003; Tanvez et al., 2009). Similarly, a handful of studies have reported on the occurrence and distribution of carotenoid pigments in testes tissue (e.g. Goodwin, 1950; Tsushima et al., 1989; Stahl et al., 1992). However these studies encompass a limited range of taxa. Moreover, studies of the carotenoid resources in the testes of birds, a model system for studies of carotenoid pigments, are entirely lacking.

The majority of studies examining the carotenoid content of testes tissue have focused on molluscs (e.g. limpets) and echinoderms (e.g. sea urchins), where research primarily aims to understand the importance of carotenoids for commercial aquaculture (e.g. Plank et al., 2002). Echininone, cryptoxanthin, zeaxanthin and ß-carotene have all been identified from the testes of limpets (Mollusca: Gastropoda; Goodwin, 1950; Goodwin and Taha, 1950). Similarly, echinenone and ß-carotene have been isolated from the testes tissue of sea urchins (Echinodermata: Echinoidea), along with lutein, zeaxanthin, astaxanthin, canthaxanthin and α-carotene (Lamare and Hoffman, 2004; Tsushima, 2007). In humans, several carotenoids have been isolated from testicular tissue, including lycopene, ß-carotene, α-carotene and cryptoxanthin (Stahl et al., 1992). Interestingly, the carotenoid profile of human testicular tissue is similar to the profile obtained from plasma, but exceeds levels found in both liver and adipose tissue (Stahl et al., 1992; Clinton, 1998), suggesting that these pigments are strategically allocated to testicular tissue for specific functions.

Carotenoids are responsible for the red, orange and yellow coloration observed in a range of taxa (e.g. fish, invertebrates, birds; Wedekind et al., 1998; Vershinin, 1999; McGraw, 2006a). Carotenoids

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also have important physiological properties that make them valuable for self-maintenance functions (i.e. immunocompetence, antioxidant defense; Møller et al., 2000; Blount et al., 2003; McGraw and Ardia, 2003; but see Costantini and Møller, 2008). Importantly, in many species, carotenoid-based colors are considered honest signals of individual health and quality (e.g. Hill, 1991; Grether et al., 1999; Pike et al., 2010). For example, in male zebra finches (*Taeniopygia guttata*), bill coloration is positively correlated with the concentration of carotenoids in circulation, which is in turn positively associated with the immune responsiveness of individuals (McGraw and Ardia, 2003). Under the assumption that carotenoids are present in the testes, we hypothesized that plumage hue (i.e. redness) might also be associated with the levels of carotenoids in testes tissue. Moreover, the phenotype-linked fertility hypothesis suggests that phenotype may indicate a male's functional fertility (Sheldon, 1994). In birds, larger testes produce more sperm (de Reviers and Williams, 1984; Møller, 1988). Thus, relative testes size reflects a male's capacity for sperm production and, ultimately, his ability to avoid sperm depletion and gain fertilization success. We therefore hypothesized that male plumage hue might also reflect relative testes mass in male house finches.

We used high-performance liquid chromatography (HPLC) to identify carotenoids in the testes of wild male house finches (*Carpodacus mexicanus*) and examined the relationships between testes carotenoids and the carotenoid content of plasma and liver tissue, as well as other morphological traits like body mass, testes mass and plumage coloration. We chose to study *C. mexicanus* because it is a model species for studies of sexual selection, plumage coloration and carotenoid ecology (e.g. Hill, 1990, 2002; McGraw et al., 2006a). Male house finches display carotenoid-based plumage coloration ranging from pale yellow to intense red, and males with redder (more carotenoid-enriched) plumage are preferred as mates by females (Hill, 1990), have fewer parasites (Thompson et al., 1997), breed earlier in the season and produce more offspring in a year (Hill et al., 1999; McGraw et al., 2001). Our first objective was to examine the relationships between levels of different carotenoids across tissue types to identify whether or not carotenoid supplies in testes were more similar to body carotenoid stores (i.e. liver) or the current, mobilized pool (i.e. plasma) of pigments. Additionally, we tested whether or not carotenoid concentrations declined with increasing testes mass, since previous work in avian eggs (e.g. quail, *Coturnix japonica*, McGraw, 2006b) showed a decrease in carotenoid concentration with increasing yolk size. We also examined the relationship between carotenoid-based plumage coloration and the carotenoid content of testes tissue. Next, we tested whether levels of plasma and liver carotenoids predict plumage hue in male house finches during the breeding season, when individuals are not undergoing feather molt, because previous work demonstrates that circulating carotenoid levels (i.e. ß-cryptoxanthin) predicts plumage redness in molting male house finches (McGraw et al., 2006a). Finally, as a test of the phenotype-linked fertility hypothesis, we examined the relationship between plumage coloration and relative testes mass.

Positive associations between different carotenoids within tissue types have been observed in both molting male house finches (McGraw et al., 2006a) and zebra finches (McGraw and Toomey, 2010). Therefore, we predicted that males exhibiting high concentrations of carotenoids in one endogenous tissue would also tend to have more carotenoids in other tissues (e.g. males circulating higher lutein levels in plasma would also have higher lutein levels in liver and testes). Following from this prediction, and because plasma carotenoid levels are associated with integument coloration in a range of avian species (McGraw, 2006a; McGraw et al., 2006a), we also predicted that males with redder plumage would have higher levels of carotenoids in plasma, liver and testes. Finally, we did not formulate specific predictions concerning the direction of the relationship between testes mass and plumage coloration because both positive and negative associations are predicted from theory: the phenotype-linked fertility hypothesis predicts a positive relationship between these traits (Sheldon, 1994), whereas sperm competition theory predicts a trade-off between sexual signals and ejaculate investment (Parker, 1998). Furthermore, empirical studies examining the relationship between plumage coloration and testes size have reported mixed results (e.g. Merilä and Sheldon, 1999; Dale, 2000).

2. Materials and methods

2.1. General field methods and sample collection

In May 2010 (4–11), we captured 30 adult male house finches using basket traps at baited feeding sites on the campus of Arizona State University in Tempe, AZ, USA. Thus males were sampled during the peak of the breeding season, when testes are at their maximum size (Hamner, 1968), allowing us to avoid seasonal effects on testes mass, body mass and plumage coloration. For all birds, we measured body mass using an electronic balance (to the nearest 0.01 g) and tarsus length using digital calipers (to the nearest 0.01 mm). Additionally, for each bird, we collected a small blood sample (<80 μL) from the brachial vein and collected the plasma (via centrifugation) for later carotenoid analysis (see below). For each bird, we measured the hue (i.e. true color, measured in integer units around a 360° color wheel with red set at 0°) of crown, breast and rump plumage using a hand-held Colortron II reflectance spectrophotometer. Each plumage patch was measured twice and, from the six total measures, we calculated an overall plumage hue for each bird (McGraw and Hill, 2000). We chose to focus on hue because recent studies demonstrate that variation in plumage hue is detectable (Toomey and McGraw, 2012), related to feather carotenoid content (Butler et al., 2011) and relevant to mate choice (e.g. Hill, 1994a; Toomey and McGraw, 2012) in house finches. Birds were then euthanized via rapid decapitation, testes were immediately dissected and wet mass for both left and right testis was obtained using an electronic balance (to the nearest 0.001 mg) and testes were stored in cryotubes at −80 °C for later carotenoid analysis (see below). We also collected a section of the liver (right lobe) from each bird, and all tissue samples (plasma, liver, testes) were immediately frozen at −80 ° C for later carotenoid analysis. For all birds, combined testes mass (hereafter referred to as simply testes mass) was measured as the sum of the wet mass of the left and right testis. All morphological measurements were taken by one of us to minimize sampling error. All procedures concerning animal care and treatment were in accordance with the regulations of the Animal Care and Use Committee of Arizona State University (protocol # 09-1054R).

2.2. Carotenoid analysis

Carotenoids were analyzed for all tissue samples (30 testes, 30 plasma, 30 liver) within 6 months of sample collection; all samples were subject to similar storage periods. Following McGraw et al. (2002), we extracted carotenoids from 20 μL of plasma by adding 200 μL of ethanol followed by 200 μL of methyl *tert*-butyl ether (MTBE). After centrifugation, the supernatant was collected and evaporated to dryness under nitrogen. For liver and testes, we weighed tissue samples to the nearest 0.001 mg (testes: 0.006–0.28 g; liver: 0.01–0.013 g) and transferred the tissue to a mortar. Due to their small mass, we were not able to analyze the left and right testis individually; thus all testes carotenoid data were obtained from samples combining both the left and right testis. We then added 3–4 mL of 1:1 hexane/MTBE to the mortar, ground the tissue with a pestle and collected the solvent. The process was repeated three or more times, and each time the solvent was collected and placed into a glass culture tube. The samples were then centrifuged, and the supernatant was recovered and evaporated to dryness. Finally, for all samples, the residue was reconstituted in methanol:acetonitrile:

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dichloromethane (42:42:16, v/v/v) for HPLC analyses (sensu McGraw et al., 2006a).

Carotenoid types were identified by comparing retention times and light-absorbance maxima to authentic standards, and the concentration of each carotenoid type was determined using external standard curves. Detectable amounts of lutein were found in the testes tissue of the majority of males (97%), while zeaxanthin, ß-carotene and an unknown carotene (carotene B) were found in lower concentrations with less regularity. Finally, an additional two pigments – anhydrolutein and ß-cryptoxanthin – were also identified in a small number of samples ($n=2$ and 1, respectively), but at levels near the detection limit; consequently, these carotenoids were excluded from statistical analyses.

2.3. Statistical analysis

We examined four main associations: (1) relationships between levels of different carotenoid concentrations across tissue types, (2) relationships between testes carotenoid concentrations and testes mass, (3) relationships between male plumage coloration and tissue carotenoid levels, and (4) relationship between plumage coloration and relative testes mass. More specifically, we first examined the relationship between testes carotenoids and carotenoids in other tissues (i.e. plasma, liver) using linear models. Separate models were run for lutein, zeaxanthin and 'carotenes' (because carotene B was a very minor pigment in testes, we lumped it together with ß-carotene for analyses). We also included relative testes mass in these models by including both (ln-transformed) body mass and testes mass as covariates in our analyses; a more robust statistical approach than the use of residuals from a regression between the two variables (Darlington and Smulders, 2001; García-Berthou, 2001). We then examined the relationships between levels of different plasma and liver carotenoids (lutein, zeaxanthin, ß-cryptoxanthin and ß-carotene) using Spearman rank correlations. Next, we examined the relationship between male plumage coloration and testes carotenoid concentrations (lutein, zeaxanthin and ß-carotene) using linear models. To account for the contributions of minor carotenoids, we also used a Spearman rank correlation test to examine the relationship between plumage hue and total carotenoid concentration of the testes. Similarly, to determine if body tissue carotenoids predicted male coloration, we used linear models to examine the relationship between plumage hue and total carotenoid concentrations of both plasma and liver. Finally, we used a linear model to examine the relationship between male coloration and relative testes mass. As before, we estimated relative testes mass by including both (ln-transformed) body mass and testes mass as covariates in our models.

In all but the final model, interaction terms were not significant and including all possible interaction terms did not significantly improve model fits, so they were omitted. For our final model (relationship between plumage hue and relative testes mass), we retained the interaction between body mass and testes mass, as this model was a significantly better fit of the data. Model assumptions were checked by visual examination of plotted residuals and normality testing of residuals. All statistics were performed using the R (2.12.0) software package (R Development Core Team, 2010), and all residuals were normally distributed.

3. Results

3.1. Carotenoid profiles: testes, plasma and liver

Four main carotenoid pigments were detected in the testes of wild male house finches (Table 1): lutein, zeaxanthin and ß-carotene, as well as a carotene that we could not identify (which we label carotene B). Lutein was the most prevalent pigment in the testes. Zeaxanthin and ß-carotene were recovered relatively less frequently, while carotene B was only recovered from a single individual. Excluding carotenoids only recovered from a single individual, the most concentrated pigment was ß-carotene, followed by lutein and then zeaxanthin (Table 1).

The carotenoid profiles of both plasma and liver tissue were similar to that of the testes. Both tissue types contained lutein, zeaxanthin, ß-cryptoxanthin and ß-carotene. In addition, plasma samples contained anhydrolutein and carotene B (though each of these carotenoid types occurred at low frequencies). Carotene B was also present in liver, as was a second unknown carotene (which we label carotene A; Table 1).

3.2. Relationships between levels of different carotenoids across tissue types

We found that testes lutein concentration was significantly positively associated with plasma lutein concentration and significantly negatively related to testes mass (Table 2, Fig. 1). In contrast, neither liver lutein concentration nor male body mass explained significant variation in testes lutein concentration (Table 2). Testes zeaxanthin concentration was not significantly related to testes mass, body mass or plasma zeaxanthin concentrations, but there was a marginally significant, positive relationship between the concentrations of zeaxanthin in liver and testes (Table 2). The concentration of carotenes in testes was not significantly related to circulating carotene levels, liver carotene levels, testes mass or body mass (Table 2). Finally, levels of lutein and zeaxanthin in male plasma and liver were not significantly intercorrelated, whereas levels of ß-cryptoxanthin and ß-carotene were significantly positively correlated between plasma and liver (Table 3).

3.3. Relationship between plumage coloration and carotenoid content of body tissues

In contrast to previous studies of molting male house finches, we found that neither plasma nor liver carotenoid concentrations significantly predicted plumage hue of males during the breeding season (Table 4). Furthermore, we found that male plumage hue was not significantly predicted by the concentration of any carotenoid type in the testes (Table 4, Fig. 2A–C). Similarly, male plumage coloration was not

Table 1
Carotenoid types and concentrations in the testes, plasma and liver of house finches (*Carpodacus mexicanus*).

Tissue	Lutein	Anhydrolutein	Zeaxanthin	ß-Cryptoxanthin	ß-Carotene	Carotene A	Carotene B	Total carotenoids
Testes	0.06 ± 0.007 (0.97)	0* (0)	0.03 ± 0.007 (0.87)	0* (0)	0.11 ± 0.02 (0.47)	–	0.11 (0.03)	0.14 ± 0.03 (0.97)
Plasma	7.13 ± 0.60 (1.0)	0.007 ± 0.007 (0.03)	1.80 ± 0.18 (1.0)	0.35 ± 0.08 (0.64)	0.58 ± 0.11 (0.57)	0 (0)	0.34 ± 0.20 (0.13)	10.20 ± 0.83 (1.0)
Liver	6.72 ± 4.25 (1.0)	0 (0)	2.79 ± 2.10 (0.9)	0.17 ± 0.11 (0.17)	4.04 ± 1.17 (0.87)	1.75 ± 0.47 (0.67)	1.16 ± 1.16 (0.03)	16.63 ± 8.94 (1.0)

Samples were analyzed using high-performance liquid chromatography (HPLC). Values (mean ± SE) for plasma are in μg mL^{-1}; values for liver and testes are in μg g^{-1}. Values in parentheses indicate proportion of birds with measurable levels of each carotenoid type. Dash indicates no carotenoids present in tissue.
*Anhydrolutein and ß-cryptoxanthin were identified in a small number of samples ($n=2$ and 1, respectively) at levels near the detection limit.

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Table 2
Relationships between carotenoid concentrations in testes tissue and concentrations in both plasma and liver tissue, as well as body mass and testes mass of male house finches.

Testes carotenoid	Predictor	ß	*t*	*P*
Lutein	**Plasma lutein**	**0.10**	**2.77**	**0.01**
	Liver lutein	0.01	1.87	0.08
	Body mass	5.76	1.75	0.09
	Testes mass	**−6.81**	**−3.17**	**0.004**
Zeaxanthin	Plasma zeaxanthin	0.17	1.29	0.21
	Liver zeaxanthin	**0.001**	**2.08**	**0.048**
	Body mass	0.19	1.02	0.32
	Testes mass	−0.18	−1.45	0.16
Carotenes	Plasma carotenes	−0.005	−0.23	0.82
	Liver carotenes	0.003	1.34	0.19
	Body mass	−0.13	−0.28	0.78
	Testes mass	−0.08	−0.26	0.80

Variables in bold are significant at $\alpha = 0.05$.

Table 3
Correlations among carotenoid concentrations within plasma (μg mL^{-1}) and liver (μg g^{-1}) samples of wild male house finches ($n = 30$) collected during the peak of the breeding season.

	Liver lutein	Liver zeaxanthin	Liver ß-cryptoxanthin	Liver ß-carotene
Plasma lutein	$r_s = 0.10$	$r_s = 0.09$	$r_s = -0.07$	$r_s = 0.10$
	$P = 0.61$	$P = 0.63$	$P = 0.70$	$P = 0.62$
Plasma zeaxanthin	$r_s = 0.16$	$r_s = 0.22$	$r_s = 0.05$	$r_s = 0.10$
	$P = 0.41$	$P = 0.24$	$P = 0.80$	$P = 0.59$
Plasma ß-cryptoxanthin	$r_s = 0.03$	$r_s = 0.05$	$\mathbf{r_s = 0.47}$	$r_s = 0.23$
	$P = 0.89$	$P = 0.82$	$\mathbf{P = 0.009}$	$P = 0.22$
Plasma ß-carotene	$r_s = 0.32$	$\mathbf{r_s = 0.38}$	$\mathbf{r_s = 0.50}$	$\mathbf{r_s = 0.58}$
	$P = 0.08$	$\mathbf{P = 0.04}$	$\mathbf{P = 0.005}$	$\mathbf{P = 0.0007}$

We followed recommendations in the recent literature and rejected the use of Bonferroni corrections to adjust α levels for multiple tests (Moran, 2003; Nakagawa, 2004). Therefore, results were considered statistically significant at $\alpha = 0.05$ (shown in bold).

significantly related to the total carotenoid concentration of the testes ($r_s = 0.08$, $P = 0.69$, $n = 30$, Fig. 2D).

3.4. Male plumage coloration and testes mass

Plumage hue was not significantly related to relative testes mass of male house finches. More specifically, in a model including both testes mass and body mass, plumage hue was not significantly associated with either body mass ($t = -1.19$, $P = 0.22$, n = 30) or testes mass ($t = 1.18$, $P = 0.25$, $n = 30$; Fig. 3), nor the interaction between these two variables ($t = -1.20$, $P = 0.24$, $n = 30$).

4. Discussion

Investigations of carotenoid accumulation in animal tissues have provided valuable insight into the allocation, specificity and functional role of these organic pigments. Analyses of internal tissues of animals may be particularly informative for understanding localized and specialized roles of carotenoids. In this study, we show for the first time that carotenoids are present in the testes of a bird species. Rowe and McGraw (2008) found previously that the semen of some birds (fairy-wrens) contained carotenoids, and our results suggest that the testes may be the direct, local point of origin for these pigments.

We detected a suite of testes carotenoids that are commonly found in avian diets (McGraw, 2006a), including lutein, zeaxanthin, ß-carotene and ß-cryptoxanthin. These pigments are also found in plasma and liver (McGraw et al., 2006a) as well as retina (Toomey and McGraw, 2009) of house finches. Anhydrolutein was also detected in small amounts and is presumed to be a metabolic derivative, putatively synthesized in the gut or liver of some birds (McGraw et al., 2002). Concentrations of these compounds in testes, however, were quite low: total carotenoid concentration in testis tissue represented just 2.5% of plasma concentrations and 1.5% of liver concentrations. Compared to humans, where carotenoid levels in testes exceed those in liver (Stahl et al., 1992; Clinton, 1998), testes carotenoids appear to contribute little to the overall carotenoid resources of finches. These levels are, however, consistent with the small amounts of carotenoids found in the ejaculates of birds (i.e. red-backed fairy-wren, *Malurus melanocephalus*), where carotenoid levels only represent 1-2% of plasma concentrations (Rowe and McGraw, 2008).

To gain an initial understanding of the ecological and physiological correlates of carotenoid levels in testis tissue, we compared testis carotenoid concentrations to concentrations in other body tissues. We found that testis lutein concentration was positively correlated with plasma lutein concentration. We also found that birds with more zeaxanthin in testes had more zeaxanthin in liver; in this case, however, the association was very weak and only marginally significant. Furthermore, zeaxanthin accounted for just 16% of total testes carotenoids, whereas lutein accounted for more than half of all carotenoids in testes. Thus, in general, it appears that testes carotenoids are more reflective of the availability of mobile, circulating pigments rather than long-term tissue stores. McGraw and Toomey (2010) suggested that breeding male zebra finches – also a short-lived, opportunistically breeding species – prioritize the maintenance of high circulating carotenoid levels in the plasma, as opposed to storage in tissues such as the liver, to keep pigments readily available for distribution to tissues for coloration or health purposes. Though our data are correlational, and thus we are limited in our ability to comment on physiological strategies of carotenoid accumulation, our results may suggest that wild male house finches also follow a strategy of

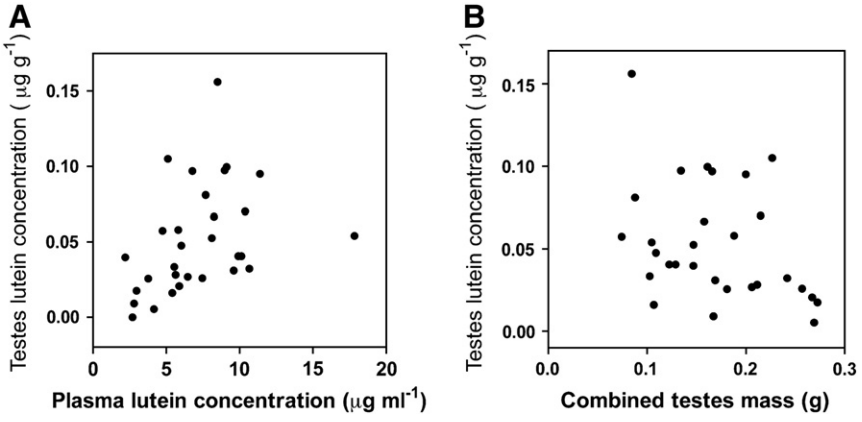

Fig. 1. Physiological predictors of testes lutein concentration in wild-caught, adult male house finches: (A) plasma lutein concentration, and (B) testis mass.

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Table 4
Relationship between plumage hue and carotenoid concentrations in plasma (μg mL^{-1}), liver (μg g^{-1}) and testes (in μg g^{-1}) in adult male house finches during the breeding season.

Plumage trait	Tissue	Carotenoid type	ß	t	P
Hue	Plasma	Lutein	−0.07	−0.11	0.92
		Zeaxanthin	1.32	0.56	0.58
		ß-Crypotoxanthin	−2.67	−0.82	0.42
		ß-Carotene	−0.71	−0.30	0.77
Hue	Liver	Lutein	1.86	1.34	0.19
		Zeaxanthin	−3.38	−1.27	0.22
		ß-Crypotoxanthin	1.12	0.21	0.84
		ß-Carotene	−0.78	−1.35	0.19
Hue	Testes	Lutein	17.76	0.35	0.73
		Zeaxanthin	18.50	0.34	0.74
		ß-Carotene	−7.92	−0.48	0.64

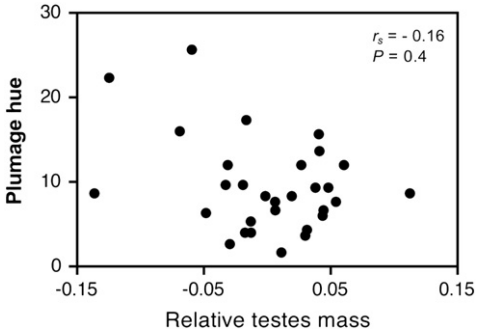

Fig. 3. Relationship between male plumage hue and relative testes mass (i.e. testes mass corrected for body mass). Statistics shown are from a post hoc Spearman rank correlation.

maximizing circulating carotenoid resources during the breeding season.

We also found that testes carotenoid concentration varied as a function of testes size: lutein concentrations in testis tissue decreased with increasing testes size. These results suggest that birds that develop larger testes might preferentially allocate carotenoids away from testes tissue, an accumulation pattern that is consistent with a strategy of prioritizing circulating carotenoid supplies. Future studies that experimentally manipulate dietary carotenoid intake or immune status may be better able to determine if the low concentrations of carotenoid pigments found in testes reflects such an allocation strategy or whether carotenoid deposition in testes is an entirely passive process.

The phenotype-linked fertility hypothesis proposes that sexual ornamentation and functional fertility are positively associated and that females benefit from choosing to mate with the most elaborately ornamented males either directly, via fertility assurance, or indirectly, via sons inheriting high-quality ejaculates (Sheldon, 1994; see also Trivers, 1972; Keller and Reeve, 1995; Pizzari et al., 2004). Because, in birds, larger testes produce more sperm (de Reviers and Williams, 1984; Møller, 1988), relative testes mass reflects a male's capacity for producing large numbers of sperm. An association between plumage coloration and testis size, with more ornamented males having larger testes relative to males with less elaborate coloration, has been reported for the black-headed grosbeak (*Pheucticus melanocephalus*, Hill, 1994b) and the greenfinch (*Carduelis chloris*, Merilä and Sheldon, 1999). Other studies, however, failed to find an association between these traits (e.g. red-billed quelea, *Quelea quelea*, Dale, 2000). Similarly, in this study, we found no support for a relationship between male coloration and relative testes mass.

Testis development is considered to be primarily under the control of gonadotrophic hormones (Froman, 1995), and testis tissue (i.e. leydig cells) is a major source of androgens (Lofts and Murton, 1973), which regulate variation in male reproductive behavior and morphology (Wingfield et al., 2001). Larger testes are also frequently associated with higher levels of circulating testosterone (e.g. Garamszegi et al., 2005; Denk and Kempenaers, 2006; Rowe et al., 2010). Furthermore, testosterone increases the bioavailability of carotenoids in plasma, resulting in the development of more elaborate carotenoid-dependent ornamental traits (see Blas et al., 2006; McGraw et al., 2006b), suggesting that carotenoid-based traits have the potential to indicate a male's investment in testis tissue. However, in house finches, elevated testosterone levels appear to delay molt and lead to drabber, less red plumage (Stoehr and Hill, 2001). Furthermore, testis development and the development of sexual coloration are temporally decoupled in

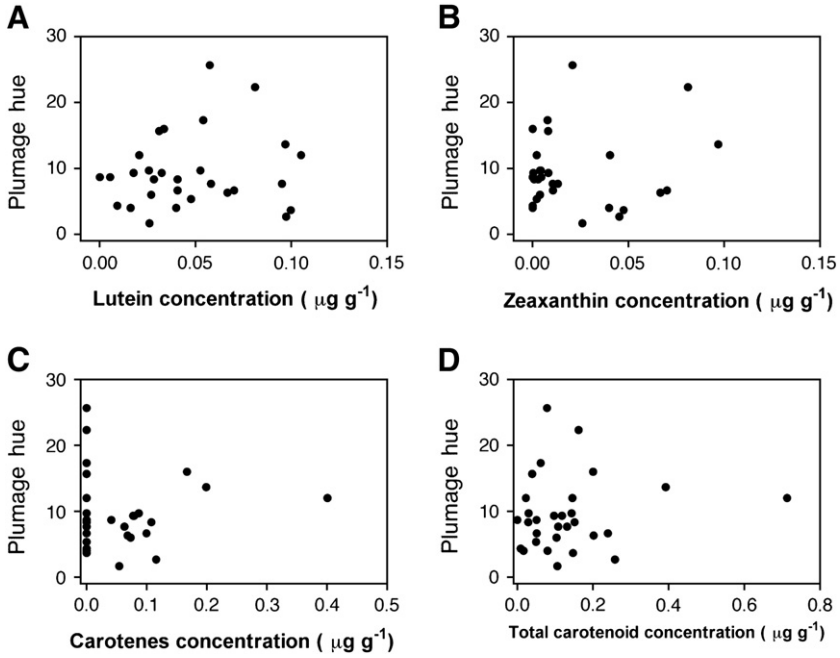

Fig. 2. Relationship between male plumage hue and the concentration of carotenoids in the testes of wild house finches: (A) lutein, (B) zeaxanthin, (C) carotenes (sum of ß-carotene and carotene B), and (D) total testes carotenoid concentration. Lower hue scores indicate redder plumage. There were no significant associations between plumage hue and carotenoid content of the testes (see text for statistical details).

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the house finch: in Arizona, males typically molt during September–October, a time when testis are regressed (Hamner, 1968). Consequently, an androgen-mediated relationship between testis development and sexual ornamentation is unlikely in house finches, and may explain why we did not observe a relationship between plumage hue and testes mass in the current study.

Importantly, our finding that plumage hue does not reflect male testes mass does not preclude the possibility that plumage may signal alternate aspects of male quality. For example, current hypotheses suggest that male phenotype might signal antioxidant availability and therefore a male's ability to combat oxidative stress (von Schantz et al., 1999; Blount et al., 2001). Carotenoids have been shown to serve as antioxidants (Burton and Ingold, 1984; Miki, 1991; Krinsky, 1998); however, the importance of carotenoids relative to other antioxidant molecules (e.g. vitamins) remains unclear (Hartley and Kennedy, 2004; Costantini and Møller, 2008). Nonetheless, recent studies indicate that carotenoid availability has a significant impact on male sperm competitiveness (Almbro et al., 2011), and that more elaborate carotenoid-based colors are associated with this higher competitive ability (Helfenstein et al., 2010; Pike et al., 2010). Moreover, dietary carotenoid therapy in humans has been linked to improvements in sperm quality and decreases in testicular oxidative stress-associated infertility (Gupta and Kumar, 2002; Ahmadi et al., 2006; Hekimoglu et al., 2009), suggesting that carotenoid accumulation in testes tissue may mediate testicular oxidative stress and thus play a role in maintaining male fertilizing capacity. In line with this notion is the possibility that carotenoid-based coloration may reflect the carotenoid resources of testes tissue. However, contrary to this idea, we found no evidence that plumage coloration indicates the carotenoid resources of the testes in male house finches.

Finally, although the goal of this study was to correlatively explore the physiological and morphological correlates of carotenoid levels in testis tissue, our findings offer some insight into the potential role of carotenoids in testes tissue. Specifically, the low concentrations of carotenoids found in the testes of male house finches suggest that carotenoids are unlikely to play a major antioxidant role in testes tissue, at least in this species. Nonetheless, this does not rule out the possibility that carotenoids may be of value in very small amounts or that testes carotenoids function synergistically with other non-carotenoid antioxidants to provide antioxidant defense for gametes. Thus, future empirical work investigating the role of carotenoids in testes tissue, as well as potential interactions between carotenoids and non-carotenoid antioxidants in the testes, has the potential to provide insight into the antioxidant protection mechanisms required for the production of functional sperm and broaden our understanding of the evolutionary and ecological importance of carotenoid pigments.

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