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# Seasonal, sexual, and quality related variation in retinal carotenoid accumulation in the house finch (*Carpodacus mexicanus*)

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## Summary

**1.** Studies of visual ecology generally focus on the tuning of the eye to the spectral environment. However, the environment may also shape vision if the availability of nutrients or other extrinsic stressors impact eye structure or function.

**2.** Carotenoids are diet-derived pigments that accumulate in the retinas of birds, where they provide photoprotection and tune colour vision. In domesticated species, carotenoid accumulation in retina is dependent on dietary intake, but little is known about the variability in or control of these pigments in the eyes of wild animals.

**3.** Carotenoids are also deposited in the integument of many animals, where they generate colourful sexually selected displays that communicate information about individual health and nutrition. We hypothesize that retinal carotenoid accumulation is subject to the same health and nutritional constraints as the use of carotenoids in colour signals.

**4.** As a first test of this hypothesis, we examined retinal carotenoid accumulation in relation to season, sex, body condition, circulating plasma carotenoid concentrations, and plumage colouration in a free ranging population of house finches (*Carpodacus mexicanus*) – a model species for studies of carotenoid ecology.

**5.** Retinal carotenoid accumulation varied considerably among individuals and differed significantly among seasons, with the highest levels observed in late fall and winter. Body condition and plasma zeaxanthin levels were significantly positively correlated with retinal carotenoid accumulation, but retinal carotenoid concentrations did not differ between the sexes. Plumage redness covaried positively with retinal carotenoid concentration as well, though this relationship was no longer significant when accounting for seasonal variation.

**6.** Our results, although correlational, do suggest that retinal carotenoid accumulation is a variable trait that may be influenced by environmental and physiological conditions, raising the intriguing possibility that plumage colouration and colour vision could be linked through a common biochemical mechanism.

**Key-words:** colour vision, HPLC, visual ecology

## Introduction

Studies of the ecology and evolution of animal signals have surged in recent decades, and we now have detailed understandings of the function, efficacy, and information content for many forms of visual, acoustic, chemical, and electric communication (Bradbury & Vehrencamp 1998; Espmark *et al.* 2000; Maynard *et al.* 2003). Comparatively less attention has been paid to the evolutionary ecology of signal reception. Sensory systems are traditionally considered a property of the species that is optimized to detect specific stimuli (e.g. prey, predators, mates) under particular environmental conditions (Ryan 1990; Endler 1992; Endler & Basolo 1998) and are often modelled as a constant component of the signalling interaction (e.g. Endler & Mielke 2005; Delhey & Peters 2008). Because sensory systems are complex and finely tuned, there is great potential for individual variation arising from the costs of developing and maintaining sensory organs and neural networks. Growing evidence indicates that the developmental environment can influence the structure and function of sensory systems (Cronin *et al.* 2001; Kröger *et al.* 2003;

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Fuller *et al*. 2005; Shand *et al*. 2008). However, much less is known about how environmental conditions, including resource availability and other environmental stressors, affect mature sensory systems.

Vision is the dominant sensory system for many bird species and is a model for the study of sensory ecology and evolution (Bennett & Thery 2007). Birds have six classes of retinal photoreceptors: four classes of cone photoreceptors producing tetrachromatic colour vision, as well as achromatic sensitive double cones, and rods (Hart 2001). Within the cone photoreceptors and the principle member of the double cones, there are carotenoid-pigmented oil droplets that absorb incoming light before it reaches the visual pigment (Goldsmith *et al.* 1984; Bowmaker *et al*. 1997). Here, carotenoids protect the photoreceptor from harmful ultraviolet (UV) radiation and narrow the spectral sensitivity of the photoreceptor, enhancing colour discrimination and colour constancy in variable lighting environments (Vorobyev *et al*. 1998; Vorobyev 2003). Birds cannot synthesize carotenoids but must acquire them from their diet (Goodwin 1984), and complete exclusion of carotenoids from the diet of domesticated quail (*Coturnix japonica*) produces individuals with colourless oil droplets (Meyer 1971; Duecker & Schulze 1977; Wallman 1979; Bowmaker *et al*. 1993). These carotenoid-free individuals, although still capable of colour vision, have altered colour preferences (Duecker & Schulze 1977; Bowmaker *et al*. 1993) and spectral sensitivities (Wallman 1979). Based on these observations from domesticated species, it is possible that environmental availability of carotenoids may affect retinal accumulation and colour vision in wild birds. However, nothing is known about how retinal carotenoid accumulation varies in any wild animal species.

In contrast, a considerable amount is known about the accumulation of carotenoids in the sexually selected ornaments of wild animals like birds. Carotenoid-based avian colours have emerged as popular subjects for investigating the costs and content of animal signals (Blount & McGraw 2008). Elaborate colouration is linked to dietary carotenoid intake in wild birds and fishes (Grether *et al.* 1999; Hill *et al.* 2002). Carotenoid pigments also serve other roles, including antioxidant defence and immune-system enhancement (Vershinin 1999; McGraw 2006), and in many species carotenoid colouration is an honest indicator of individual health and quality (reviewed in Hill 2006). Because plumage and retinal carotenoids share a common dietary source, their accumulation may be subject to the same environmental and physiological constraints. In effect, carotenoid accumulation in the eye may be condition-dependent allowing only the best individuals to obtain key visual benefits of retinal carotenoid deposition.

In our first study on this topic, we sought to correlate the accumulation of retinal carotenoids in individuals of a free-ranging animal species with several traditional, environmentally variable metrics, such as season, body condition, and carotenoid-based plumage colouration. We did this in house finches (*Carpodacus mexicanus*) – a model avian species for studying the costs and benefits of carotenoids and colouration in animals (Hill 2002). The red, orange, and yellow carotenoid-based plumage colour of male house finches is a classic example of sexually selected carotenoid-based colouration, and much is known about carotenoid physiology and signalling behaviour of this species (reviewed in Hill 2002). Males with redder (more carotenoid enriched) plumage have fewer parasites (Thompson *et al*. 1997), grow their feathers faster (Hill & Montgomerie 1994), and have higher concentrations of carotenoids in their diet (Hill *et al.* 2002), plasma, and liver (McGraw *et al.* 2006) during feather growth. The condition dependence of plumage colouration has been confirmed with experimental manipulations of dietary carotenoids (Hill 1992), caloric intake (Hill 2000), and parasite load (Hill & Brawner 1998; Brawner *et al.* 2000; Hill *et al.* 2004). Females prefer to mate with males with the reddest plumage (Hill 1990; Hill 1991; Hill *et al.* 1999; Oh & Badyaev 2006), such that they acquire mates that are in the best nutritional and health condition.

We measured retinal and plasma carotenoid levels and body condition of male and female adult house finches, as well as male plumage colouration, throughout the year. We predicted that retinal carotenoids levels would correlate positively with body condition and male plumage colouration. We also hypothesized that retinal carotenoid accumulation would be a function of plasma carotenoids being delivered to the eye, as is the case for other tissues (e.g. liver) in house finches (McGraw *et al.* 2006). Therefore, we predicted that accumulation of carotenoids in house finch retinas would vary seasonally, like plasma carotenoid levels do in house finches (K.J. McGraw & L.L. Washington unpublished data) and other songbirds (Isaksson *et al.* 2007; Deviche *et al.* 2008), and peak when plasma carotenoids are at their annual high. Sex differences in plasma carotenoids are minor in this species, occurring only for a few carotenoids during the molt period (McGraw *et al.* 2006); based on this limited information, we did not anticipate dramatic sex differences in retinal carotenoid accumulation.

## Methods

### SAMPLE COLLECTION

We captured wild house finches on the Arizona State University campus in Tempe, Arizona, USA in basket traps at baited feeding stations between the hours of 08.00 and 13.00 h (*sensu* McGraw *et al.* 2006). We captured male (M) and female (F) adult finches during five different time periods in 2006–2007: 2–10 March 2006 ($n = 15$M, 15F), 2–10 June 2006 (19M, 19F), 17 July–14 August 2006 (18M, 15F), 24–28 November 2006 (15M, 15F), and 3–4 February 2007 (15M, 15F). In the March, June, and July–August sampling periods, adult birds were readily identified by plumage characteristics (Hill 1993) and hatch year birds were excluded from the sample. A subset of the adult birds were banded in previous years with United Sates Fish and Wildlife Service bands and could be assigned to one of two age classes: 1 year old (19M, 11F), or 2 or more years old (4M, 4F). In the July–August period, we also sampled six hatch year (HY) birds to assess age related variation in retinal accumulation; however, these individuals were excluded from all other analyses. From all individuals, we collected a plasma sample (~80 μL) from the wing

**Table 1.** The retention times ($R_t$), absorbance maxima ($\lambda_{max}$), putative identity, and mean ± S.E. concentration of carotenoids in the house finch retina ($n = 161$)

| Peak No. | $R_t$ (min) | $\lambda_{max}$ (nm) | | | Carotenoid | Concentration (μg/retina) |
|---|---|---|---|---|---|---|
| 1 | 3·12 | (356·4) | 373·0 | 393·5 | galloxanthin (A)[1] | 0·042 ± 0·0007 |
| 2 | 3·32 | | 380·2 | 394·0 | galloxanthin (B)[1] | 0·267 ± 0·007 |
| 3 | 3·55 | | 381·3 | 397·1 | galloxanthin (C)[1] | 0·146 ± 0·004 |
| 4 | 6·13 | | 336·1 | 457·6 | astaxanthin (A)[2] | 0·014 ± 0·002 |
| 5 | 6·68 | | 375·4 | 468·5 | astaxanthin (B)[2] | 0·019 ± 0·0004 |
| 6 | 7·10 | | 368·2 | 469·7 | astaxanthin (C)[2] | 0·042 ± 0·001 |
| 7 | 7·94 | | | 478·2 | astaxanthin (D)[2] | 0·309 ± 0·008 |
| 8 | 8·24 | | | 477·0 | astaxanthin (E)[2] | 0·031 ± 0·002 |
| 9 | 10·0 | | | 474·6 | astaxanthin (F)[2] | 0·062 ± 0·002 |
| 10 | 8·17 | (423·0) | 446·7 | 475·8 | 3′-epilutein[3] | 0·086 ± 0·002 |
| 11 | 8·87 | (421·3) | 446·7 | 474·6 | lutein[2] | 0·047 ± 0·001 |
| 12 | 11·05 | | 453·9 | 479·4 | zeaxanthin[2] | 0·107 ± 0·002 |
| 13 | 14·40 | (422·5) | 447·9 | 477·0 | unknown | 0·041 ± 0·0006 |
| 14 | 18·21 | (420·0) | 443·0 | 472·1 | ε-carotene[4] | 0·017 ± 0·0001 |

The $\lambda_{max}$ values in parentheses denote the location of shoulders in the absorbance spectra and letters following the carotenoid name denote different isomers.
[1]Identified based on the descriptions of Goldsmith *et al.* (1984) and Toyoda *et al.* (2002) and quantified based on the calibration curve for lutein. [2]Identified and quantified by comparison to purified standards. [3]Identified based on the descriptions of Khachik *et al.* (2002). [4]Identified based on the descriptions of Goldsmith *et al.* (1984) and quantified based on calibration curve for β-carotene.

vein, morphological measurements (body mass and tarsus length), and scored the hue of the crown, breast, and rump plumage of males with a visible-light Colortron™ reflectance spectrophotometer (Light Source Inc. San Rafael, California; see McGraw & Hill 2000 for description and justification). In our analyses, we used the mean hue value from the three colourful plumage patches, and calculated body condition as the residual of the linear regression of body mass on tarsus length (Duckworth *et al.* 2001). We euthanized the birds within 1 h of capture and collected the retinas from both eyes. Retinas and plasma samples were stored at –80 °C for up to 6 months prior to carotenoid analysis. We collected birds under United States Fish and Wildlife Service permit #MB088806-0, and Arizona State Game and Fish scientific collecting permit SP797514. All procedures were approved by the Institutional Animal Care and Use Committee at Arizona State University (protocol #06-874R).

## CAROTENOID ANALYSIS

We measured carotenoid accumulation in the left retina of each individual with high performance liquid chromatography following previously described methods (Toomey & McGraw 2007). We chose to analyze a single retina because preliminary analyses indicated that accumulation in the left and right retina was significantly positively correlated ($r^2 = 0{\cdot}55$, $F_{1,30} = 34{\cdot}68$, $P < 0{\cdot}0001$, unpublished data). Briefly, we ground each retina for 3 min at 30 Hz in a ball mill (MM200, Retsch GmbH & Co. KG, Haan, Germany) in 2 mL of 1 : 1 hexane: *tert*-butyl methyl ether (MTBE). The ground retinas and solvent were centrifuged for 5 min at 3000 r.p.m., at which point the solvent fraction was transferred to a fresh tube, evaporated to dryness under a stream of nitrogen, resuspendend in 1 : 1 (vol/vol) MTBE, split into two equal samples, and dried again. We split the samples in half to maximize our recovery of retinal carotenoids by performing two different saponification procedures: one half of the sample was resuspended in 1 mL of a 0·02 M solution of NaOH in methanol (a weak base treatment for analysis of ketocarotenoids) and the other was resuspended in 1 mL of a 0·2 M solution of NaOH in methanol (a strong base treatment for analysis of xanthophylls; Toomey & McGraw 2007). The samples were then capped under nitrogen gas and incubated in the dark at room temperature for 4 h. After this time, we extracted the carotenoids from the saponification solution with 3 mL hexane : MTBE, dried the samples under nitrogen, and resuspended them in 200 μL of HPLC mobile phase consisting of 44 : 44 : 12 (vol/vol/vol) methanol : acetonitrile : dichloromethane. We injected 50 μL of each sample into a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, Massachusetts) fitted with a Waters YMC Carotenoid 5·0-μm column (4·6 mm × 250 mm). Retinal carotenoids were identified by comparing their retention times and absorbance spectra to those of external standards of purified zeaxanthin (DSM Inc., Heerlen, Netherlands), astaxanthin (Sigma, St. Louis, MO), lutein, and β-carotene (CaroteNature, Lupsingen, Switzerland). When external standards were not available, we identified carotenoids based upon published absorbance values and quantified them with external standard curves of available reference carotenoids (see Table 1 for details). We measured plasma carotenoid levels following the methods of McGraw *et al.* (2006).

## STATISTICAL ANALYSES

Consistent with previous studies (Khachik *et al*. 2002; Bhosale *et al*. 2007), we report retinal carotenoid concentrations as μg of carotenoid per whole retina. For our analyses, we summed the concentrations of all isomers of a given carotenoid type (Table 1) and assessed the intercorrelations among retinal carotenoid types with a series of Pearson's product-moment correlations. The concentrations of all retinal carotenoid types were significantly intercorrelated (Table 2), so we summed them and used total retinal carotenoid concentration for subsequent analyses.

We used analysis of covariance (ANCOVA) to explore variation in total retinal carotenoid accumulation in relation to sampling period, sex, body condition, and the plasma concentrations of lutein and zeaxanthin. In our study, lutein and zeaxanthin made up > 91% of total circulating carotenoids and were the only plasma carotenoid types consistently observed in every individual (β–cryptoxanthin

**Table 2.** Pearson product-moment correlation coefficients for the relationships among carotenoid concentrations in the retinas of wild house finches ($n = 161$)

| | Astaxanthin | Galloxanthin | Lutein | Zeaxanthin | Unknown | ε-carotene |
|---|---|---|---|---|---|---|
| Astaxanthin | – | 0·27 | 0·39 | 0·41 | 0·37 | 0·19 |
| Galloxanthin | | – | 0·68 | 0·70 | 0·49 | 0·40 |
| Lutein | | | – | 0·77 | 0·51 | 0·30 |
| Zeaxanthin | | | | – | 0·86 | 0·39 |
| Unknown | | | | | – | 0·40 |
| ε-carotene | | | | | | – |

Isomers of each carotenoid type were pooled for analysis. The concentrations of all carotenoid types were significantly intercorrelated ($P \leq 0{\cdot}016$).

and β–carotene were also present in the plasma of 66 and 117 of 161 individuals, respectively, on average making up < 5% of total carotenoid concentration). There were no significant interaction terms in any of our models ($P > 0{\cdot}05$), so we retained only the main effects in final models. We used Tukey–Kramer *post hoc* comparisons to compare retinal carotenoid levels among the five sampling periods. We also repeated this analysis using a multivariate approach (MANCOVA) with the six retinal carotenoid types as dependent variables (see Appendix S1 in Supporting Information); however these results were similar to our analyses with total retinal carotenoid concentration and we chose to present only the univariate analyses.

To explore the influence of age on retinal carotenoid accumulation, we used a one-way ANOVA to compare levels among age classes of the subsample of known age birds and repeated the above ANCOVA analysis within the largest known age-class in our sample (1 year). Because plumage colouration was only measured for male finches, we used a separate ANCOVA model to analyze the relationship between mean plumage hue, sampling period, plasma concentrations of lutein and zeaxanthin, and retinal carotenoid accumulation. Because sampling period explained much of the variation in retinal carotenoid accumulation (see results), we also investigated seasonal variation in body condition, plumage hue, and plasma zeaxanthin with ANOVA. Our data met the assumptions of parametric statistics (normality and equal variance), an alpha level of $P = 0{\cdot}05$ was used throughout, and statistical analyses were carried out with SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

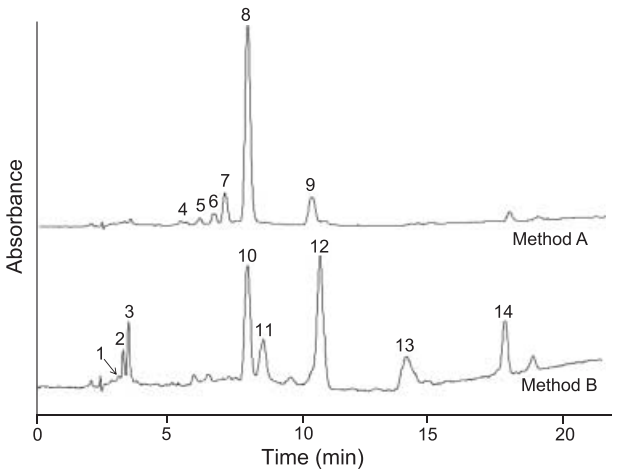


**Fig. 1.** Representative HPLC chromatograms (at 448 nm) of major carotenoids present in the retinas of house finches, recovered using method A (weak base) and method B (strong base) saponification. Numbers above peaks correspond to those listed in Table 1. Absorbance units are not relevant for this qualitative depiction and are omitted.

## Results

### RETINAL CAROTENOID TYPES

We detected six major carotenoid types in the house finch retina, with a varying number of isomers (Fig. 1, Table 1). The house finch retina was dominated by astaxanthin (five isomers, 39% of total) and galloxanthin (three isomers, 37%), with lesser amounts of lutein (two isomers), zeaxanthin, ε-carotene, and one unidentified carotenoid (Table 1). Galloxanthin and ε-carotene are apparently unique to the retina of birds and have not been observed in the diet or other tissues of house finches (McGraw *et al.* 2006).

### INTERINDIVIDUAL VARIABILITY IN RETINAL CAROTENOID CONCENTRATION

There was considerable variation in total retinal carotenoid accumulation among individuals in our sample, ranging from 0·569 to 1·975 μg/retina, with a mean of 1·165 μg/retina and coefficient of variation (CV) of 24·5. This level of variation is consistent with sexually selected traits (Cuervo & Møller 1999) and much greater than presumably naturally selected traits measured in this study, such as body mass (CV = 7·4) and tarsus length (CV = 2·9), but less than the variation in total plasma carotenoid concentration (CV = 73·9) or total plumage carotenoid concentration (CV = 65·1; data used from McGraw *et al.* 2006).

### PREDICTORS OF RETINAL CAROTENOID ACCUMULATION

Retinal carotenoid accumulation differed significantly among sampling periods, with the highest levels in November 2006 and February 2007 (Table 3, Fig. 2a) – a period that includes prebreeding mate choice (Hill 1993). Although our study was limited to 1 year, we collected seven additional birds (5M, 2F) in May 2007 for another study, and these birds had relatively low retinal carotenoid levels (mean 0·936 ± 0·072 μg/retina), which is consistent with a cyclic seasonal pattern of retinal carotenoid accumulation (Supplementary Fig. S2a). Seasonal

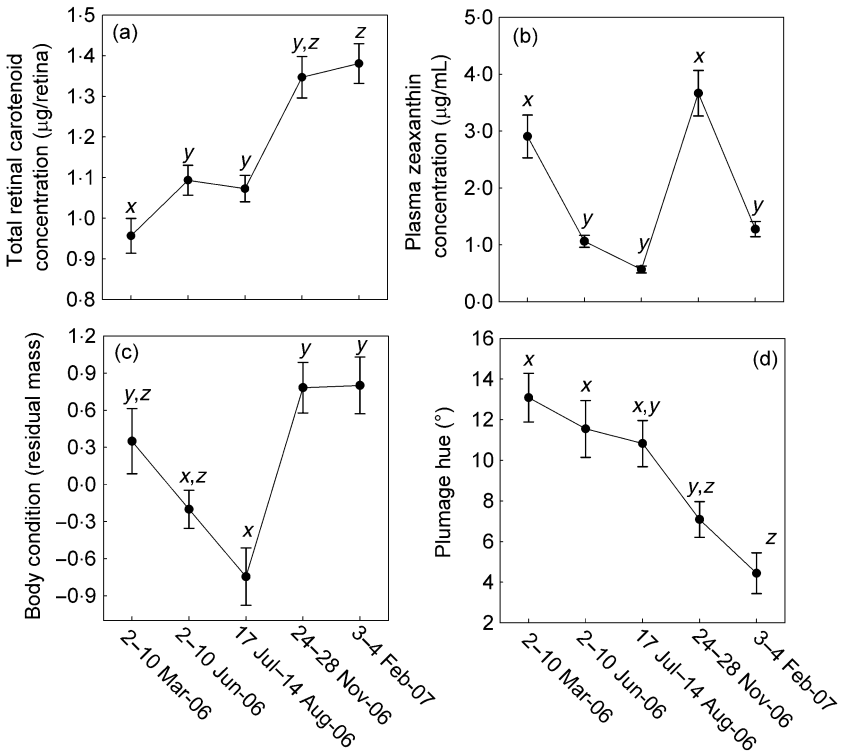


**Fig. 2.** Comparisons among sampling periods for: (a) mean ± SE retinal carotenoid concentrations, (b) mean ± SE plasma carotenoid concentrations, (c) mean ± SE mass/tarsus length residuals (a measure of body condition), and (d) mean ± SE male plumage hue (lower values indicate redder plumage). Within each panel, points that do not share a letter in common are significantly different (Tukey–Kramer *post hoc* tests, $P < 0{\cdot}05$).

**Table 3.** ANCOVA table depicting the effects of body condition, sampling period, sex, and circulating plasma carotenoid concentrations on retinal carotenoid accumulation

| Factor | d.f. | *F* | *P* |
|---|---|---|---|
| Body condition | 1,159 | 4·07 | **0·045** |
| Sampling period | 4,159 | 16·96 | **< 0·001** |
| Sex | 1,159 | 0·48 | 0·48 |
| Plasma lutein | 1,159 | 0·01 | 0·91 |
| Plasma zeaxanthin | 1,159 | 10·29 | **0·0016** |

variation could have resulted from differences in the ages of birds sampled in each period. However this is unlikely because there was no significant difference in retinal accumulation among known age birds (HY, 1 year, & ≥ 2 years, $F_{4,42} = 1{\cdot}87$, $P = 0{\cdot}17$) and if we limit our analysis to a single age-class (1 year olds), season remains a significant predictor of retinal accumulation ($F_{4,22} = 9{\cdot}66$, $P = 0{\cdot}0006$) and follows a qualitatively similar pattern to the whole sample (Supplementary Fig. S2b).

Body condition was a significant predictor of retinal carotenoid accumulation (Table 3), and body condition varied significantly among sampling periods with the highest levels in November 2006 and February 2007, when retinal carotenoid accumulation was also highest ($F_{4,160} = 9{\cdot}66$, $P < 0{\cdot}0001$, Fig. 2a,c). Across sampling periods, individuals in better condition had higher concentrations of retinal carotenoids (Fig. 3a). Retinal carotenoid accumulation also was significantly positively related to plasma zeaxanthin concentrations (Table 3, Fig. 3b). Plasma zeaxanthin concentrations differed

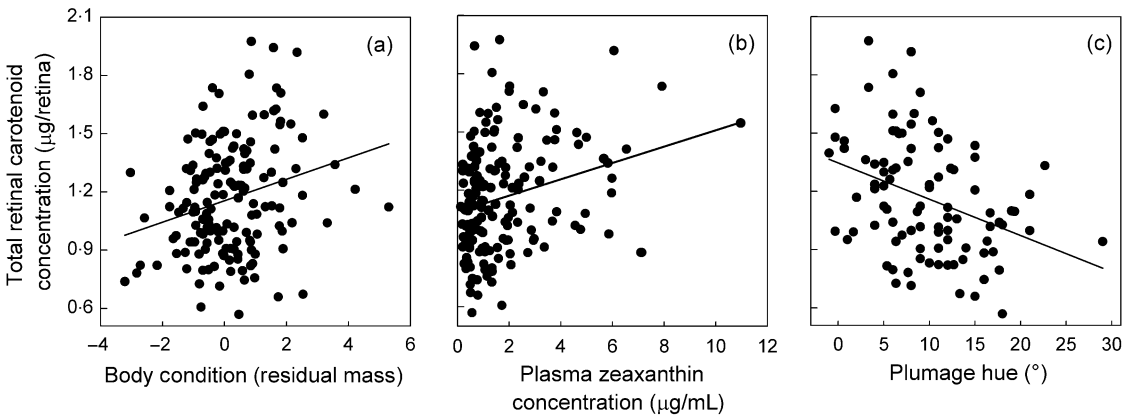


**Fig. 3.** Predictors of retinal carotenoid accumulation: (a) body condition, (b) circulating plasma zeaxanthin levels, (c) male plumage hue (lower hue values indicate redder plumage).

significantly across sampling periods ($F_{4,160} = 28{\cdot}61$, $P < 0{\cdot}0001$), but this pattern was not consistent with temporal differences in retinal accumulation (Fig. 2a,b).

Retinal carotenoid accumulation did not differ significantly between the sexes (Table 3), and male plumage hue was not a significant predictor when sampling period was taken into consideration ($F_{1,79} = 0{\cdot}19$, $P = 0{\cdot}67$). However, plumage hue differed significantly among sampling periods ($F_{4,79} = 9{\cdot}69$, $P < 0{\cdot}0001$) in a pattern that was consistent with retinal accumulation. Across sampling periods, there was a significant correlation between retinal carotenoid accumulation and male plumage hue, with redder males having higher levels of retinal carotenoids ($r_s = -0{\cdot}378$, $P = 0{\cdot}0005$, Fig. 3c). However, within any given season, mean plumage hue was not a significant predictor of retinal carotenoid accumulation ($P > 0{\cdot}19$).

## Discussion

Here we present the first ecological study of retinal carotenoid variation and correlates in a population of wild animals. Prior work on these natural pigments in animal eyes has been restricted to domesticated species (Khachik *et al*. 2002; Toyoda *et al*. 2002; Bhosale *et al*. 2007) or has been qualitative and inferential, by deducing light absorption properties of retinal oil droplets (which contain carotenoids) using microspectrophotometry (e.g. Goldsmith *et al.* 1984; Hart *et al.* 2006). Recent visual ecological studies have shown environmental sensitivity of eye morphology in vertebrates; for example, developmental lighting conditions affect the frequency of retinal cone types in several fish species (Kroger *et al.* 2003; Fuller *et al*. 2005; Shand *et al*. 2008) as well as the absorbance properties of cone oil droplets in chickens (*Gallus gallus domesticus*; Hart *et al.* 2006) and of visual filtering pigments in mantis shrimp (Cronin *et al.* 2001). However, an ecological approach has yet to be applied to carotenoid nutrients in the eye or to the plasticity of the mature visual system. Here, we found that retinal carotenoid accumulation varied among adult house finches from the same population, differed significantly among seasons, and correlated with body condition and colouration.

Qualitatively, the carotenoids we detected in house finch retinas were similar to those reported from HPLC studies of Japanese quail (*Coturnix coturnix japonica*; Bhosale *et al*. 2007; Toomey & McGraw 2007) and match the absorbance spectra of cone oil droplets reported in microspectrophotometry studies of a number of bird species (Goldsmith *et al.* 1984; Bowmaker *et al*. 1997). Astaxanthin and galloxanthin were dominant carotenoids in the house finch retina but not present in the diet (unpublished data) or circulation. These retinal carotenoids are likely produced through the metabolic conversion of specific dietary precursors (Davies 1985). Because of the conserved nature of absorbance profiles in retinal oil droplets interspecifically, and now similar carotenoid profiles in two unrelated species – one domesticated and one wild – it may be that the types of carotenoids in the retina are highly conserved and genetically controlled among birds (Bowmaker *et al*. 1997). In fact, the presence of carotenoid pigmented oil droplets appears to be phylogenetically conserved even beyond birds. Animals ranging from very primitive lungfish (Bailes *et al*. 2006) to fish, turtles, and lizards possess colourful cone oil droplets (Douglas & Marshall 1999) and it will now be exciting to determine retinal carotenoid types in these groups for comparison to birds.

Quantitatively, however, we discovered considerable inter-individual variation in retinal carotenoid concentration in wild house finches, along with several ecological and physiological predictors of this variation. The strongest predictor of retinal carotenoid levels was season, with highest levels seen in late fall (November) and winter (February). As several extrinsic and intrinsic factors change with season, it is difficult to assess the precise source of this variation, especially in a correlational study, but diet has traditionally been targeted as a key modulator of carotenoid accumulation in animals. Dietary carotenoid intake predicts plumage colouration in molting wild house finches (Hill *et al.* 2002), and based on diet and plasma analyses (Beal 1907; Hill 1995) carotenoid accumulation appears to peak during molt (September–October) in this species, a few months before retinal carotenoid accumulation reached an annual high in our study. In fact, in a pilot experiment with captive house finches, we found that it took two months of a dietary carotenoid manipulation to appreciably affect carotenoid levels in retina (K.J. McGraw, E.A. Tourville & M.B. Toomey, unpublished data), which suggests together with data from wild animals that high carotenoid supplies in the molting diet could influence retinal carotenoid levels a few months later. This long-term dietary mechanism would be in line with evidence from domesticated birds that multi-generational carotenoid deprivation produces individuals with carotenoid-free colourless oil droplets (Meyer 1971; Bowmaker *et al*. 1993) and that long-term dietary carotenoid supplementation increases retinal carotenoid levels (Toyoda *et al*. 2002; Thomson *et al*. 2002a; Thomson *et al*. 2002b).

Often correlated with dietary carotenoid intake is plasma carotenoid concentration (e.g. Negro *et al*. 2000), and we found that levels of one carotenoid type in plasma – zeaxanthin – significantly and positively predicted retinal carotenoid concentration in wild house finches. This was true at the individual level, controlling for season and other factors. Based on radio-labelling and diet experiments, zeaxanthin also has been touted as particularly important, compared with other carotenoids, for retinal carotenoid accumulation and maintenance in domesticated species like chickens and quail (Davies 1985; Schiedt *et al*. 1991; Toyoda *et al*. 2002; Bhosale *et al*. 2007). Zeaxanthin is deposited directly into cone oil droplets but is also the putative precursor for the formation of other retinal carotenoids, including astaxanthin and galloxanthin (Davies 1985; Schiedt *et al*. 1991; Bhosale *et al*. 2007). Thus, it is possible that both dietary and physiological/metabolic factors (Brush 1990) underlie retinal carotenoid accumulation. This could be analogous to the control of plumage colouration in molting house finches, which has a dietary component (Hill *et al*. 2002) but also involves

conversion of yellow dietary pigments into red forms (Inouye *et al*. 2001) and is affected by carotenoid-independent nutritional state (i.e. has an 'energetic cost'; Hill 2000). The fact that male plumage redness was positively correlated with retinal carotenoid accumulation in our study is consistent with the notion that they have similar control mechanisms.

Consistent with our hypothesis, retinal carotenoid accumulation was positively correlated with body condition and male plumage colouration. However, to establish if retinal carotenoid accumulation is 'condition-dependent' *per se*, the particular physiological processes associated with carotenoid uptake, transport, conversion, and use should now be examined in relation to the eye, and in the context of the many other allocations that have been previously studied (e.g. to colouration, egg yolk, antioxidant defence, immunomodulation, and internal tissue storage; McGraw 2006). The idea of carotenoid trade-offs has received increased attention recently in the literature on life-history evolution (Blount 2004; Clotfelter *et al*. 2007; Fitze *et al*. 2007; Peters 2007), but this work has yet to include the retina as an important allocation site for carotenoids. Health roles have stood at the centre of these studies (Lozano 1994), and we are now anxious to analyze how carotenoids may be diverted between health and retinal functions. However we do not suspect rapid health/retina trade-offs, if they exist, because avian retinal carotenoids appear to be stable to short-term changes in dietary carotenoid levels (Wang *et al*. 2007; authors' unpublished data).

Amidst all we attempted to account for in this correlational and ecological study of retinal carotenoids, it is also plausible that retinal carotenoids track more than internal supplies and needs, but also external demands like environmental light exposure. Carotenoids absorb harmful ultraviolet radiation and protect the retina from light-induced photodamage in quail (Thomson *et al*. 2002a; Thomson *et al*. 2002b). Hart *et al*. (2006) found that absorbance spectra of the retinal oil droplets of chickens reared in bright environments were shifted toward longer wavelengths compared with individuals from a dim environment. This spectral shift is likely the result of increase carotenoid accumulation; therefore, individuals may up-regulate retinal carotenoid accumulation to enhance photoprotection. In our study, however, we found that retinal carotenoid levels were highest in November and February, a period when solar radiation is relatively low (~400 mJ $m^{-2}$) compared to May and June (> 800 mJ $m^{-2}$, AZMET 2008, Phoenix Greenway station), when retinal carotenoid levels were low. The low levels of retinal carotenoid levels we see during the spring and summer months could instead be linked to (at least in part) photodegradation of retinal carotenoids (Mortensen & Skibsted 1999); however, the photostability of retinal carotenoids is not known. The variety of factors potentially affecting retinal accumulation provide ample opportunity for experimental manipulation and study, but what makes this variation in retinal carotenoid physiology particularly intriguing is its potential to affect colour vision and behaviour.

Functionally, retinal carotenoids accumulate in cone oil droplets and enhance colour vision by absorbing short-wavelength light and reducing the overlap of spectrally adjacent single cone photoreceptors (Vorobyev *et al*. 1998; Vorobyev 2003). Variation in carotenoid concentration should shift the cut-off wavelengths of the oil droplets, change the spectral overlap among photoreceptors, and alter an individual's colour vision. The absence of retinal carotenoids is known to alter the spectral sensitivity (Wallman 1979) and innate colour preferences of quail (Duecker & Schulze 1977; Bowmaker *et al*. 1993), though no empirical studies have been conducted within the natural range of variation in retinal carotenoid accumulation. We envision two key effects that retinal carotenoids could have on colour vision and sexual selection in house finches: (i) enhancing a male's ability to acquire colourful carotenoid-rich foods and thus develop colourful plumage, and (ii) facilitating female mate choice for colourful plumage in males. In our study, we uncovered two results consistent with these predictions. First, there was an overall correlation between retinal carotenoid levels and male plumage colouration, suggesting that better colour vision in a male may be associated with superior carotenoid intake and colouration. Second, retinal carotenoid accumulation was highest in the late fall and winter, coinciding with the pre-breeding period when house finches choose mates (Hill 1993). On this basis, one might also expect the sexes to accumulate different levels of carotenoids in retinas to facilitate these season-specific foraging or mating activities, but we found no evidence of sex differences in our study. Surely, to truly understand the functional importance of retinal carotenoid accumulation to avian colour vision and sexual selection, we need to merge microspectrophotometric assessments and visual models with the critical behavioural experiments and evidence. However, our initial correlational results do suggest that retinal carotenoid accumulation may be limited by environmental and physiological conditions, raising the intriguing possibility that plumage colouration and colour perception may be linked through a common biochemical mechanism.

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## Supporting Information

The following Supporting Information is available for this article:

**Appendix S1.** MANOVA analyses of accumulation of the six retinal carotenoid types in relation to body condition, sampling period, sex, and circulating lutein and zeaxanthin levels.

**Fig. S2.** Mean ± SE retinal carotenoid accumulation across sampling periods (a) for a sample including May 2007 that shows low levels consistent with data from 2006 and (b) a sample limited to 1-year-old birds.

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