Carotenoid coloration is related to fat digestion efficiency in a wild bird

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SHORT COMMUNICATION

Carotenoid coloration is related to fat digestion efficiency in a wild bird

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Abstract Some of the most spectacular visual signals found in the animal kingdom are based on dietarily derived carotenoid pigments (which cannot be produced de novo), with a general assumption that carotenoids are limited resources for wild organisms, causing trade-offs in allocation of carotenoids to different physiological functions and ornamentation. This resource trade-off view has been recently questioned, since the efficiency of carotenoid processing may relax the trade-off between allocation toward condition or ornamentation. This hypothesis has so far received little exploratory support, since studies of digestive efficiency of wild animals are limited due to methodological difficulties. Recently, a method for quantifying the percentage of fat in fecal samples to measure digestive efficiency has been developed in birds. Here, we use this method to test if the intensity of the carotenoid-based coloration predicts digestive efficiency in a wild bird, the house finch (*Haemorhous mexicanus*). The redness of carotenoid feather coloration (hue) positively predicted digestion efficiency, with redder birds being more efficient at absorbing fats from seeds. We show for the first time in a wild species that digestive efficiency predicts ornamental coloration. Though not conclusive due to the correlative nature of our study, these results strongly suggest that fat extraction might be a crucial but overlooked process behind many ornamental traits.

Keywords Sexual selection · Steatocrit · Ornamentation · Metabolism · Coccidia

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Introduction

Carotenoid-based ornaments account for some of the most spectacular visual signals of animals (Svensson and Wong, 2011). Studies have successfully linked carotenoid coloration to oxidative stress resistance, immunocompetence, parasite resistance, stress response, and foraging efficiency (Hill and Farmer 2005; Perez-Rodriguez et al. 2010; Simons et al. 2012) with a general assumption of these molecules being limited resources for wild organisms, causing trade-offs in allocation of carotenoids to different physiological functions. However, this resource trade-off view of the associations between condition and ornamentation is not supported by many studies in which resources are provided ad libitum, but ornamentation still varies among individuals (Hill 2014). It has been suggested that processing of dietary carotenoids (Toews et al. 2017), efficiency of cellular respiration (Hill 2011), or digestive efficiency (Galvan et al. 2016) might be more important than dietary carotenoid availability in linking coloration to individual condition.

Studies of digestive efficiency in wild animals are limited, partly due to methodological difficulties related to non-invasive assessment. Therefore, the theory that physiological mechanisms related to carotenoid signaling depend on the efficiency of digestive machinery has never been tested. Not all consumed food is available for metabolism due to

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inefficiencies in the digestive process, and consistent differences exist in energetic processing between individuals (Nespolo and Franco 2007). Efficiency of nutrient absorption might be especially important in small birds that have significantly shorter small intestines and less small intestine surface area than similarly sized mammals (Caviedes-Vidal et al. 2007). Accordingly, digestion efficiency is a trait of individual quality that may be advertised with sexual signal traits in birds.

Recently, a method for quantifying the percentage of fat in fecal samples and thus measuring digestive efficiency (based on the acid steatocrit method) has been developed in birds (Tran et al. 1994; Meitern et al. 2016). Here, we use this method to test for the first time if digestive efficiency predicts the intensity of the carotenoid-based coloration in a wild animal, the house finch (*Haemorhous mexicanus*). As carotenoids are fat-soluble molecules (di Mascio et al. 1991), measurement of fat digestion efficiency is relevant in the context of carotenoid assimilation. We predict that males with redder (i.e., female preferred) coloration have higher digestion efficiency (lower steatocrit) than birds with drab, yellow plumage. In addition, we measure levels of circulating carotenoids and triglycerides to test if the possible association between digestion efficiency and coloration is mediated directly through individual differences in carotenoid and fat absorption. We also assess intestinal parasite (coccidia) levels to see if digestion efficiency is related to the intensity of infection, as coccidian parasites can reduce intestinal fat absorption (Major and Ruff 1978), and this way may negatively impact condition and carotenoid coloration.

Methods

Thirty-six male house finches were caught with basket traps in January 2017 from the Phoenix metropolitan area, AZ, USA, between 8 a.m. and 1 p.m. Birds were brought into aviary and fed ad libitum with sunflower seeds and water. Sample collection (blood from alar vein, 20 μL per bird, fecal samples, photographs for color) started after 5 p.m. on the same day.

We measured the concentration of plasma carotenoids by high-performance liquid chromatography (HPLC) methods (McGraw et al. 2002). The severity of endoparasitism by coccidian protozoans (*Isospora* spp.) was estimated following previously established methods (McGraw and Hill 2000; Giraudeau et al. 2014; Brawner et al. 2000). Fresh feces were preserved in potassium dichromate solution. After fecal float and microscope slide preparation, we scored oocyst load from 0 to 5 based on a logarithmic scale. Plumage coloration of captured male finches was measured according to Lendvai et al. (2013). We took photos of the breast feathers in a room with standard lighting. In Photoshop (vers. 20170309.r.207), we highlighted each plumage area using the "magic wand" tool and extracted the RBG values. Steatocrit was measured according to Meitern et al. (2016). Bird droppings in deionized water and 5 M perchloric acid homogenate were collected into the capillary tube, centrifuged at 13,000 rpm, and photographed. Length of the upper fat layer and solid bottom layer was quantified using ImageJ. Steatocrit was expressed as a percentage of fat in the non-aqueous matter. Triglycerides were measured with Cayman Triglyceride Colorimetric Assay Kit (item 10010303).

All statistical tests were performed in the R computing environment (vers. 3.3.2) with $\alpha = 0.05$ and were two-tailed. To test whether and how various traits (plumage hue, total carotenoids, triglycerides, coccidia endoparasitism) predict variation in steatocrit levels, we used ANCOVA with site as a factor to control for inter-site differences. Details about sample collection, sample analysis, and data analysis are available as Electronic Supplementary Materials.

Data availability Data are available from the Dryad Digital Repository (Madonia et al. 2017).

Results

Hue positively predicted steatocrit levels (hue $\beta = 0.032 \pm 0.016$; $F_{1,31} = 4.21$; $p = 0.048$; Fig. 1). We found no links between steatocrit and plasma carotenoids, triglycerides, or coccidian infection levels (all $p > 0.05$; Table 1), although we found a significant site*steatocrit interaction predicting carotenoid levels. However, follow-up regression analyses revealed that neither site had a slope significantly different from zero (see Electronic Supplementary Materials).

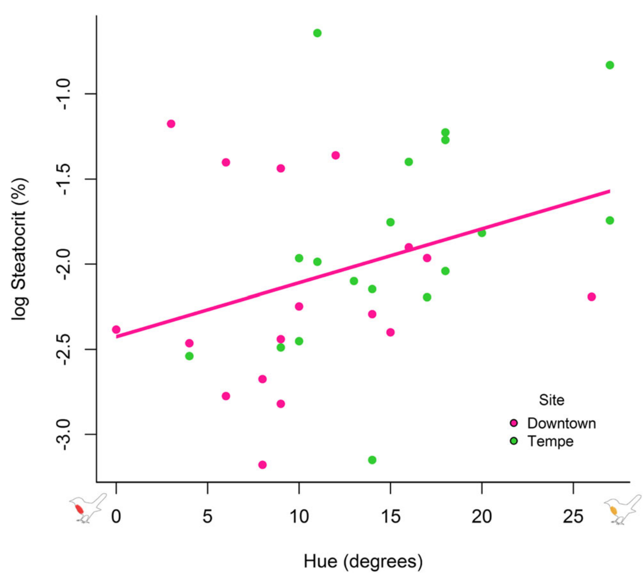

Fig. 1 Sexual ornamentation predicts digestive efficiency in male house finches. Lower hue values correspond to male redness. Higher steatocrit values indicate a higher proportion of fat in fecal matter

Table 1 Model ANCOVA outputs for the variables studied

Response variable	Predictor	Sum of squares	F	p
log Steatocrit	Hue	1.42	$4.21_{1,31}$	0.048
	Site	0.23	$0.68_{1,31}$	0.41
	Hue*site	0.64	$1.89_{1,31}$	0.18
log Triglycerides	log Steatocrit	0.001	$0.0047_{1,29}$	0.94
	Site	1.889	$8.8_{1,29}$	0.005
	log Steatocrit*site	0.002	$0.0076_{1,29}$	0.93
Carotenoids	log Steatocrit	35.1	$0.23_{1,32}$	0.62
	Site	116.1	$1.59_{1,32}$	0.21
	log Steatocrit*site	3.9	$4.26_{1,32}$	0.047
log Steatocrit	Coccidia	0.35	$0.96_{1,32}$	0.33
	Site	0.99	$2.67_{1,32}$	0.11
	Coccidia*site	0.06	$0.17_{1,32}$	0.67

Discussion

Here, we show that carotenoid-based plumage coloration may be a predictor of steatocrit levels in house finches. Carotenoid pigments cannot be synthesized de novo and must be acquired through diet (McGraw 2006). Thus, individuals better able to extract, assimilate, and transport carotenoids gain an advantage in terms of the amount of pigments that can be allocated to the coloration of their ornaments. Alternatively, according to the shared pathway hypothesis (Hill 2011), aspects of carotenoid utilization (i.e., ketolation of carotenoids) as well as digestion efficiency can be tied to the efficiency of respiratory reactions, resulting in a correlation between coloration and steatocrit.

At the proximate level, carotenoids extracted from the diet are bound to lipoproteins that deliver lipids to peripheral tissues in the body (McGraw and Parker 2005). Individuals with higher levels of fat might be better able to take up carotenoids from diet and circulate more carotenoids through blood. McGraw and Parker (2005) found that (a) blood cholesterol levels were highly positively correlated with both plasma-carotenoid concentration and beak pigmentation and that (b) an experimental increase/decrease of cholesterol levels was associated with an elevation/reduction of blood carotenoids and beak color in zebra finches (*Taeniopygia guttata*). In line with this study, our result suggests that the ability to extract fat from the food could be an essential component of the process leading to plumage coloration. However, we did not find a significant relationship between circulating levels of carotenoids and steatocrit scores in our study of wintering birds, a finding which lends more support toward the shared pathway hypothesis (Hill 2011).

We did not find a correlation between plasma triglyceride levels and steatocrit. In Meitern et al. (2016), this association was found in some, but not all experimental time points, suggesting that it might depend on time of day. Meitern et al. (2016) showed that experimental infection with coccidian parasites influences steatocrit levels. Although coccidian parasites are common in house finches and infection negatively influences plumage coloration (Brawner et al. 2000), we did not find a relationship between steatocrit and coccidian levels in the current study. Another component that can influence the intestine health and the digestive efficiency is the composition of the gut microbiome, a field of study that is now emerging in ecological studies (Kohl 2012). To the best of our knowledge, no studies have ever assessed how the gut microbiome composition influences carotenoid assimilation in any taxa. The abundant human literature on this topic shows that microbiome composition is a key player of nutrient absorption and especially of fat extraction (i.e., Krajmalnik-Brown et al. 2012) and it is thus possible that the gut microbiome composition of some individuals might give them an advantage to assimilate fat and carotenoids.

Another question is how sexual selection might link ornamental color to digestive efficiency. House finches have biparental care; the male feeds both female and offspring. Males with efficient digestion may be able to spare more food for offspring; indeed, females prefer to mate with red males, who make more feeding visits (Hill 1991). Therefore, females may receive fitness benefits by choosing a male with efficient digestion. In sum, digestive efficiency may underlie both a male's ability to feed offspring but also to survive and maintain long-term pair bonds, ultimately leading to enhanced fitness.

To conclude, we have shown for the first time in a wild species that digestive efficiency predicts ornamental coloration. Though not conclusive due to the correlative nature of our study, these results suggest that fat extraction might be a crucial but overlooked process behind many ornamental traits.

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Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This research was approved by ASU Institutional Animal Care and Use Committee by animal protocol number 15-1401RRFC43.

Conflict of interest The authors declare that they have no competing interests.

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