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COMMENTS & REPLIES

Identifying anatomical sites of carotenoid metabolism in birds

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Carotenoid metabolism has long interested plant and animal biochemists (Goodwin 1986; Lu and Li 2008). Identifying tissue sites and enzymes responsible for carotenoid transformations (e.g., β-carotene to vitamin A) has been challenging. Colorful birds have recently become a model for studying carotenoid nutrition and metabolism, in the context of sexual selection and honest signaling (McGraw 2006). In a recent paper published in Naturwissenschaften, del Val et al. (2009) described carotenoid profiles in tissues of male common crossbills (*Loxia curvirostra*), with the aim of localizing metabolic site(s) for a ketocarotenoid pigment—3-hydroxy-echinenone (3HE)—present in red feathers. They found 3HE in blood and liver, unlike previous studies of colorful songbirds where metabolized integumentary carotenoids were found only at peripheral tissues (e.g., beak, legs; McGraw 2004). Thus, the authors concluded that the liver was the site of ketocarotenoid synthesis in crossbills. Here, I outline the limitations of such descriptive pigment analyses, when carotenoid types are present in various tissues, and discuss fruitful directions for future lines of research in this area.

Because carotenoids are dietary in origin in animals but can be modified in body tissues enzymatically, careful food and physiological studies allow for accurate identification of pigment origins and transformations. Traditional rigorous methods for identifying carotenoid metabolic sites, processes, and products in animals include direct enzyme identification (Wyss 2004), isotope labeling of precursors (Burri and Clifford 2004), and by inference from ex vivo chemical reactions (Khachik et al. 1998) or where carotenoid types exist in no other tissue type (McGraw 2004). del Val et al. (2009) undertook none of these types of investigation. The first step in such research is to rule out a dietary source to the pigment, but the authors did not study food carotenoids in crossbills; they sampled only liver, skin, and feathers from accidentally field-killed animals and drew blood from molting birds. While red carotenoids are not currently thought to be common in diets of herbivorous land birds (e.g., rubixanthin in rose hips, rhodoxanthin in *Taxus* berries), this is a key assumption to biochemically validate for any species, given the paucity of information on avian food carotenoids.

del Val et al. (2009) based their assertion that 3HE was metabolized in the liver of crossbills on the observation that 3HE was the most proportionally concentrated (and most variably occurring) carotenoid in liver. However, data on other types of body carotenoids were not presented in this paper, the molt status of dead birds was not given, and with small sample sizes (n=7) no statistics were performed. Thus, we have no context for understanding which and how certain carotenoid pigments occurred in different crossbill tissues. Follicles actually contained proportionally more 3HE than liver (61% vs. 35% of total carotenoids, respectively), and while the authors argue that this is expected (given that "maturing follicles would just accumulate this red derivative until the pigment would be incorporated finally into growing feathers during moult"; p. xxx), this makes the non-null assumption that follicles preferentially retain 3HE over other circulating carotenoids.

Even if we assume that 3HE is not dietary in origin, alternate tissues with carotenoid metabolism capabilities were not studied. The oxygenase enzyme that cleaves β-carotene

This is a comment to del Val E, Senar JC, Garrido-Fernández J, Jarén M, Borràs A, Cabrera J, Negro JJ (2009) The liver but not the skin is the site for conversion of a red carotenoid in a passerine bird. Naturwissenschaften doi:10.1007/s00114-009-0533-x.

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into retinal in chickens is expressed in duodenum, kidney, lungs, and liver (Wyss 2004). Upstream duodenal formation of 3HE would be especially key to rule out as a synthesis site here, given high levels of the metabolite anhydrolutein in duodenum and liver of zebra finches (*Taeniopygia guttata*) and other estrildids (McGraw et al. 2002). It is also plausible that liver accumulates high concentrations of carotenoids that have been delivered from non-digestive tissues, given its dual blood supply (e.g., from lung/heart via the hepatic artery).

What the study by del Val et al. (2009) clearly provides is evidence that crossbill liver and blood can concentrate 3HE. It certainly leaves open the possibility that liver *may be a site* for ketocarotenoid metabolism, but does not identify the site or any sites. Previous studies have emphasized a peripheral feather or beak-tissue origin to red, metabolized integumentary carotenoids, because internal tissues sampled and the bloodstream lacked such carotenoids (McGraw 2004). Clearly this straight-forward, tissue-specific distribution of carotenoids is not the case in all birds, and especially in such instances, it is due time, however difficult it may be, to undertake the definitive studies of carotenoid metabolism in colorful birds. Collaborations with enzymologists and molecular geneticists will yield information about enzyme localization and expression in tissues, even in wild birds and including season- and sex-specificity. Carotenoid labeling studies, using stable isotopes or radiotracers, have a foundation in the domesticated bird literature (Bhosale et al. 2007) and, when diet can be controlled and body tissues sampled, will provide an even larger window into carotenoid metabolic processes in birds.

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