Hepatic conversion of red carotenoids in passerine birds

Esther del Val & Juan Carlos Senar & Juan Garrido-Fernández & Manuel Jarén & Antoni Borràs & Josep Cabrera & Juan José Negro

Identifying the metabolically active sites for derived carotenoids that colour avian feathers and determining whether or not different species may use different mechanisms of colour production are crucial for understanding the evolution of the ornament. However, the role of liver versus follicles, the two candidate sites suggested by previous authors, remains contentious (McGraw 2009; del Val et al. 2009).

The absence of certain carotenoids in the food of some red-coloured birds led to the proposal that the liver, as the most active metabolic site in any vertebrate, was the organ where those non-dietary carotenoids were metabolised (see del Val et al. 2009 and references therein). The fact that several studies failed to find traces of metabolically derived plumage carotenoids in liver and plasma (Stradi 1998;

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E. del Val (~) J. C. Senar A. Borràs J. Cabrera Behavioural & Evolutionary Ecology Associate Research Unit (CSIC), Natural History Museum, Passeig Picasso s/n, 08003 Barcelona, Spain e-mail: estherdelval@yahoo.es

J. Garrido-Fernández · M. Jarén Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero, 4, 41012 Seville, Spain

J. J. Negro Department of Evolutionary Ecology, Estación Biológica de Doñana (CSIC), C/ Americo Vespucio s/n, 41092 Seville, Spain Inouye 1999; McGraw 2004) led to the hypothesis that some colourful passerines might convert some red pigments directly in the skin. However, no conclusive studies confirming the occurrence of ketocarotenoid synthesis enzymes in feather follicles have so far been published, and there are no clear evidences to definitively confirm this proposal. In our recent paper in this journal (del Val et al. 2009), we demonstrated that the red derivative 3-hydroxyechinenone was indeed present in the liver and plasma of wild moulting crossbills. Although we have been criticised for using relatively few individuals for our liver analysis (McGraw 2009), ours is a qualitative evidence irrespective of sample size, and killing tens of birds would not had provided any additional support to the obvious.

It has been established that 3-hydroxy-echinenone has to be converted from other dietary carotenoids present in small amounts in all green plants, most probably β -cryptoxanthin (Stradi et al. 1996, see also McGraw 2004). Female crossbills display a yellow-greenish coloration based mainly on canary xanthophylls A and B (Stradi et al. 1996), and no trace of 3-hydroxy-echinenone has ever been found in their plumage. The fact that both sexes feed on the same diet (Cramp and Perrins 1994) strongly supports the idea that 3-hydroxy-echinenone is not of dietary origin and may be therefore converted from ingested yellow precursors. Furthermore, we also analysed by HPLC extracts of several potential food sources of common crossbills from our study area, including pine cones, buds, young needles, strobili, pollen, and various fruits. However, we found no trace of 3-hydroxy-echinenone in any of our samples (see Table 1).

The most parsimonious explanation for our findings is that 3-hydroxy-echinenone is directly converted from dietary yellow precursors all at once in the liver and that it is later transported via the bloodstream to those follicles specifically admitting the transformed pigment. The same

Table 1 Carotenoid content (mg/kg) of diffe	erent food sources consumed by moulting comm	on crossbills from our study area (mean±SE)
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	Lutein	Lycopene	β-Carotene	β -Cryptoxanthin	3-HE	Ν
Mountain pine (Pinus uncinata)						
Buds	80.41 ± 7.74	0	61.43 ± 6.06	0.42 ± 0.06	0	3
Young needles	13.07 ± 1.48	0	2.97 ± 0.25	1.14 ± 0.11	0	3
Male strobili	3.35 ± 0.57	0	0.43 ± 0.15	0.03 ± 0.01	0	9
Female strobili	4.15 ± 0.59	0	0.35 ± 0.19	0.05 ± 0.00	0	3
Green cones	8.37 ± 1.83	0	$3.55\!\pm\!0.78$	0.05 ± 0.01	0	12
Ripe cones	8.74 ± 1.40	0	$3.30 {\pm} 0.33$	0.06 ± 0.01	0	3
Scots pine (Pinus sylvestris)						
Female strobili	2.92 ± 0.34	0	0.28 ± 0.07	0.05 ± 0.00	0	3
Pollen	6.30 ± 0.32	0	1.24 ± 0.16	0.01 ± 0.00	0	3
Green cones	8.58 ± 0.83	0	2.15 ± 0.34	0.06 ± 0.00	0	3
European black pine (Pinus nigra)						
Green cones	13.47 ± 2.76	0	5.62 ± 1.43	0.08 ± 0.01	0	6
Fruits						
Unripe Common juniper (Juniperus communis)	6.00 ± 0.91	41.80 ± 1.91	$7.96 {\pm} 0.02$	13.64±0.26	0	2
Ripe Common juniper (Juniperus communis)	7.87 ± 1.16	0	3.77 ± 0.77	0	0	8
Currant (Ribes petraeum)	3.91 ± 0.25	0	1.95 ± 0.14	0	0	3
Unripe European mountainash (Sorbus aria)	4.68 ± 0.12	0	9.55 ± 2.76	0	0	2
Bloodtwig dogwood (Cornus sanguinea)	1.37	21.10	0	0	0	1
Invertebrates						
Lepidopteran larvae (Peribatodes sp.)	678.71	0	203.24	0	0	1

For details on the carotenoid extraction, identification, and quantification, see the Electronic supplementary material

physiological pathway that we suggest in our paper has been proposed in previous studies for explaining mechanisms of carotenoid coloration in red finches other than crossbills (e.g., Hill et al. 1994; Hill 2000). As McGraw (2004) acknowledged, it was suggested long ago (Brush 1990) that the liver was "the most logical site for metabolic processing" of derived plumage carotenoids.

Previous studies aimed at detecting 3-hydroxy-echinenone in bird liver and plasma but failing to do so may have differed from ours due to three reasons: (a) The model species did not convert the pigment in the liver nor in other internal organs, as suggested by McGraw (2009), because otherwise the converted pigment should circulate—and be detectable—in the bloodstream; (b) the timing for collecting the samples was inadequate but individuals may still convert the pigment internally at other times; and (c) the pigment was present in the analysed tissues but it was overlooked. The detection of the red derivative in the liver was difficult for us due to the presence of other non-carotenoid pigments at much higher concentrations, such as bilirubin and biliverdin, that masked the peak of 3-hydroxy-echinenone by setting the detection levels in the HPLC system in such a way that we had to zoom up significantly in the region where this red ketocarotenoid might had been present to actually detect it (see Results in del Val et al. 2009).

In any case, what needs an explanation is not the "logical" conversion of carotenoids in the liver, but the conversion in the feather follicles proposed by McGraw. Perhaps, as suggested by McGraw (2009), future studies using tracer/labelling methods can show the role of follicles in carotenoid synthesis, but in the meantime, the available data and the Occam's principle advice to support the hepatic conversion.

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