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PROBABLE DIETARY BASIS OF A COLOR VARIANT OF THE CEDAR WAXWING

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Abstract.—Pigments in the tail band of a recently discovered orange form were isolated and compared to those of normal Cedar Waxwings (*Bombycilla cedrorum*). The main pigment in the yellow feathers was identified as a canary-xanthophyll. The orange variants had, in addition, significant amounts (up to *c.* 40%) of the red carotenoid rhodoxanthin. Rectrix color differed widely among variant individuals, as a result of differences in the total amounts of carotenoids and the duller melanins. Red waxy appendages of the Cedar Waxwing and the tail band of the red-banded Japanese Waxwing (*B. japonica*) contained astaxanthin and two unidentified red acidogenic carotenoids.

Canary-xanthophylls and acidogenic carotenoids are modified metabolically from dietary precursors. Rhodoxanthin, by contrast, is obtained directly from the diet and, presumably, deposited unmodified in the variant tail feathers. We believe that many of the orange individuals, most of whom are juveniles, ingested rhodoxanthin as nestlings. The relatively recent occurrence of the orange color implies a new food source or a change in food choice by the adults.

VARIANTE EN COLORACIÓN EN *BOMBYCILLA CEDRORUM*: PROBABLE EFECTO DE LA DIETA

Resumen.—Se aislaron los pigmentos que componen el anaranjado recientemente descubierto en la banda del rabo de individuos del picotero *Bombycilla cedrorum,* y se compararon con los pigmentos en la coloración normal de la especie. El pigmento principal amarillo, resultó ser xantófilo-canario. La variante anaranjada contenía además, cantidades considerables (hasta un 40%) de rodoxantina (carotenoide rojo). El color de las rectrices varió considerablemente entre los individuos, debido a diferencias en la cantidad de carotenoides y melanina. El rojo ceroso en el picotero norteamericano y la banda en el rabo en el picotero japonés (*B. japonica*) contienen astaxantina y dos carotenoides acidogénicos que no han podido ser identificados. Tanto el xantófilo-canario como los carotenoides acidogénicos son elementos de la dieta modificados metabolicamente. Por el contrario, la rodoxantina es obtenida directamente de la dieta y probablemente depositada sin modificarse en las plumas del rabo. Los autores creen que muchos de los individuos anaranjados, la mayoría de estos juveniles, ingirieron rodoxantina cuando eran polluelos. El nuevo hallazgo de anaranjado en el rabo de estas aves implica una novel fuente de alimentos o cambios recientes en la dieta de los adultos.

Individual Cedar Waxwings (*Bombycilla cedrorum*) in eastern North America may have a tail band that is orange instead of the usual yellow. This color is not found in museum specimens older than about 30 years and recently has increased dramatically in frequency. Furthermore, it is most common in juvenile birds (Parkes and Wood, pers. comm.). K. C. Parkes and D. S. Wood have documented the geographical distribution of the new form, and its sudden appearance in time.

Seasonal and sexual dichromatism involving carotenoid pigments are well known in birds. The chemical changes that produce such differences

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have been reported in a few species (Brush 1967). Color morphs are less common, but their chemical basis in several species is well known (Brush and Seifried 1968, Johnson and Brush 1972, Völker 1964). It is possible to correlate such chemical differences in feather pigments with metabolic processes and, by extension, to speculate on their genetic basis (reviewed in Brush 1981). In these and other cases (see Buckley 1987), morphs are of long term occurrence and appear to be stable in the population. The sudden appearance of a color variant in the Cedar Waxwing is therefore intriguing. Accordingly, we undertook an analysis of the pigments in the rectrices of orange-banded and yellow-banded Cedar Waxwings, and the red-banded Japanese Waxwing (*B. japonica*). We expected that characterization of the pigment differences between the orange- and yellow-banded individuals would elucidate the cause of dimorphism in this previously monomorphic species.

METHODS

Tail feathers from individual Cedar Waxwings of both normal and variant colors (Table 1; collected from June 1968 (CM 143398) to August 1984 (CM 162781), in Westmoreland and Allegheny counties, Pennsylvania) and from a Japanese Waxwing (ex-AMNH 665434, no data) were supplied by the Carnegie Museum of Natural History. Additional samples, including a rectrix bearing a waxy appendage like those found on secondary remiges of adult Cedar Waxwings (UCONN 9152; Westport, Connecticut), came from the University of Connecticut Museum of Natural History. We weighed each set of feather tips. Tail band colors were assessed using color swatches (Smithe 1975), and the carotenoid pigments extracted in warm, acidified (HCl) pyridine (Völker 1936). This procedure preserved the structure of the feather, and did not solubilize the duller melanin pigments to any major extent. It also permitted the extraction of carotenoids from small amounts of starting material, as was necessary here.

We transferred the carotenoids to hexanes in a separatory funnel. The pigments in the epiphase were washed with distilled water, concentrated under nitrogen, and stored over anhydrous sodium sulfate in the dark. Total absorbance and individual visible spectra were recorded on a Perkin-Elmer model 552 spectrophotometer. We determined the total carotenoid content of individual tail bands from the absorbance at 438 nm (λ_{max}) in hexane, with an extinction coefficient, $E^{1\%}_{1\mathrm{cm}}$, of 2500 (Britton 1985). Chemical reactions performed on whole extracts included reduction of carbonyl groups with sodium borohydride in methanol (Andrewes et al. 1974), acetylation of hydroxyl groups (acetic anhydride in pyridine, Andrewes et al. 1974) and treatment in alkaline (5% KOH) methanol (Partali et al. 1987). We separated pigments on silica gel IB and aluminum oxide IB thin-layer chromatographic (TLC) plates (Baker-flex, J. T. Baker Chem. Co., Phillipsburg, NJ) in a hexane: acetone (3:1) solvent system. We used a Waters HPLC instrument equipped with a Lambda Max model 481 LC variable wavelength detector to determine the relative

TABLE 1. Carotenoid content and composition of tail bands from normal and variant Cedar Waxwings. Band colors were assessed using Smithe (1975). The relative concentration of the dione pigment was calculated for the yellow pigments only.

Specimen	Band color	Carotenoid content (mg/g feather)	Percent rhodoxanthin (%)	Percent 3,3′-dione (% of yellow pigments)
Normal birds				
CM 153188	Spectrum Yellow (#55)	0.26	5.8	94
CM 152531	Spectrum Yellow (#55)	0.43	1.0	96
Variant birds				
CM 143398	Buff (#24)	0.12	22	62
CM 151128	Burnt Orange (#116)	0.37	44	82
CM 162781	Burnt Orange (#116)	0.38	24	92
CM 162773	Pratt's Rufous (#140)	0.62	36	59

concentration of identified pigments in each sample. The area under the curve of the individual pigments at 450 nm, a measure of concentration, was integrated by an attached model 740 data module. We used a Zorbax ODS (Du Pont, Wilmington, DE) reverse phase column to separate pigments. Elution with methanol (mobile phase) was performed at 0.5 ml/min. The capacity factor, k', a measure of mobility on HPLC, was calculated with the formula $k' = (t_R - t_0)/t_0$, where t_R was the pigment retention time and t_0 the column dead-time.

RESULTS

The brightly colored band of the rectrices in all Cedar Waxwings examined contained carotenoid pigments. A yellow pigment occurred consistently in large amounts in both normal and variant birds. We identified this carotenoid as ϵ,ϵ-carotene-3,3′-dione (Hudon and Brush, in prep.), a probable canary-xanthophyll (Brockmann and Völker 1934). This identification was based on chemical analysis, which revealed two carbonyl and no hydroxyl groups, and a sensitivity to alkaline conditions. Additional support came from the visible spectrum, with λ_{max} = 415, *438*, 468 nm (Matsuno et al. 1986). The capacity factor on HPLC (k') was 3.2. This pigment also predominated in the yellow belly feathers. A minor, yellow pigment of the tail band found in many individuals corresponded to 3′-hydroxy-ϵ,ϵ-caroten-3-one, another probable canary-xanthophyll (k' = 3.0) (Hudon and Brush, in prep.). Finally, an unidentified yellow pigment (k' = 2.45) was also detected in some individuals (see below).

In the orange-banded birds, and to a lesser extent in the yellow-banded individuals, we detected three closely migrating red pigments in addition to the major yellow pigment. This set of red pigments matched completely rhodoxanthin, isolated from Yew (*Taxus baccata*) berries, in the number of forms, their respective mobilities on TLC and HPLC (k' of from 4.7 to 5.3), color on TLC, and relative abundance. We believe this set of red pigments represents the three *cis-trans* isomers of rhodoxanthin (3,3′-diketo-retrodehydro-β-carotene) at the 6(6′)-exocyclic double bond (Kayser and Gemmrich 1984). A different set of red pigments were obtained from the red tail band of the Japanese Waxwing or the waxy appendages of Cedar Waxwings (Brush and Allen 1963). They included astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione), and two unidentified red carotenoids. All bound very tightly to the aluminum oxide TLC plates, a behavior observed consistently with 3-hydroxy, 4-keto-carotenoids. Their sensitivity to alkaline conditions in methanol lends further support to this identification (acidogenic carotenoids). Rhodoxanthin does not display such behavior. The waxy appendage on a Cedar Waxwing rectrix contained the same pigments found in the wing waxy appendages (astaxanthin) plus the pigments found in the tail band. In this individual the tail band was orange and contained rhodoxanthin.

We identified different color phenotypes of the tail band and evaluated different variables in typical representatives of each (Table 1). These included the total carotenoid content, the fractional contribution of rhodoxanthin, and the fraction of yellow carotenoids that was ϵ,ϵ-carotene-3,3′-dione. The two Spectrum Yellow individuals varied slightly in total carotenoid content and contained negligible rhodoxanthin (Table 1). Rhodoxanthin varied almost 5-fold between these two individuals.

The color of the tail band in the orange-banded birds varied in hue and saturation (or chroma). In order of increasing redness, there were bands that appeared Buff (#24), a poorly saturated color, Burnt Orange (#116) of high saturation, and Pratt's Rufous (#140) of moderate saturation, according to the Munsell notations (Smithe 1975). The tail bands of all the variants yielded considerable rhodoxanthin, up to 40% of the total carotenoid pigments in some individuals (Table 1). The two Burnt Orange individuals differed from the yellow-banded birds largely in respect to the deposition of increased amounts of rhodoxanthin. Orange tail bands varied greatly in their total carotenoid content (Table 1). The redness of the variant phenotype correlated broadly with pigment content. The Buff tail band contained about one-third as much pigment as the normal bands, whereas the Pratt's Rufous tail band contained almost twice as much. The Buff and Pratt's Rufous bands also contained relatively less ϵ,ϵ-carotene-3,3′-dione, while an unidentified pigment comprised most of the remainder of the yellow pigments (Table 1). These two variants, and also to a lesser extent the Burnt Orange-tailed birds, differed further from the yellow birds in the deposition of noticeable amounts of melanins, which produce black, grays, and browns, along with the carotenoids. In contrast, the yellow-banded birds had practically no residual melanins after extraction of the carotenoids in acidified pyridine.

DISCUSSION

Ultimately, in birds, all carotenoid pigments are derived from the diet (Brockmann and Völker 1934, Giersberg and Stadie 1933). Nevertheless, many carotenoids are the products of the activity of enzymes on dietary precursor carotenoids (Brush 1981). Prominent among the metabolites produced by birds are the canary-xanthophylls and 4-keto-carotenoids (Brockmann and Völker 1934, Fox et al. 1969, Völker 1962). Canary-xanthophylls are modified from lutein or lutein-like precursors (Brockmann and Völker 1934, Matsuno et al. 1986). The acidogenic pigments (3-hydroxy, 4-keto-carotenoids) present in the waxy tips of the Cedar Waxwing and the tail band of the Japanese Waxwing also result from enzymatic modification of dietary xanthophylls (Thommen 1971, Völker 1943). Xanthophylls (notably lutein) are widely distributed in plants, and constitute an important component of green leaves (Goodwin 1980). In contrast, apparently no animal produces rhodoxanthin enzymatically, as there are no reports of the endogenous production of rhodoxanthin in controlled feeding experiments.

Rhodoxanthin is found in some berries, notably in the plant genus *Taxus* (Goodwin 1980). It is also widespread in the leaves of gymnosperms (Czeczuga 1986), where it could be consumed by insects. Its known distribution in other plants is rather limited, and it is restricted to a few angiosperms (Kayser and Gemmrich 1984, Rahman and Egger 1973) and pteridophytes (Czeczuga 1985). Presumably, the carotenoid in the variant Cedar Waxwings originated from direct ingestion from berries or indirectly via insects (cp. Partali et al. 1987), and deposited unchanged in the feathers alongside the usual yellow pigment. Cedar Waxwings feed abundantly on berries year-round, but also ingest insects seasonally (Bent 1950). The young are fed on insects, but berries are added to the diet early in their lives (Bent 1950).

Birds that normally do not encounter rhodoxanthin in their diet, when fed the pigment, deposit it in carotenoid-containing feathers, irrespective of the feather's natural color (Völker 1955, 1957, 1958). Presumably, the processes involved in pigment deposition do not discriminate between rhodoxanthin and the normal carotenoid pigments (cp. with Kritzler 1943 for the use of capsanthin in feather pigmentation). Thus, no genetic changes need be invoked to explain the presence of orange-banded Cedar Waxwings. Indeed, diets that differ in rhodoxanthin level could lead to the observed color difference, with no requirement for an enzyme to produce the red pigment and of genetic information for the expression of the enzyme. We found rhodoxanthin in all birds examined, which suggests that the pigment is widely available. The orange-banded birds simply obtained more rhodoxanthin.

Additional evidence for dietary involvement comes from the unusual patterns of orange pigmentation exhibited by some variant individuals. For example, in one adult (USNM 566608, U.S. Natl. Mus.), the innermost rectrices (left r2, r3; right r1, r3, r4) were the normal yellow, but the outer rectrices (particularly left r5, r4 and right r5) were orange. In another bird, also an adult (UCONN 9152), the two innermost rectrices were yellow and the others orange. In this individual and a juvenile (UCONN 9750) some normally yellow anterior belly feathers were tinted red. Presumably, these patterns reflect changes in pigment levels and availability when the feathers were formed. It would be unusual for genetic changes to give rise to such a variety of individual patterns. As yet, there are no records of the band color in an adult Cedar Waxwing that was orange as a first year bird. Data of this type are likely to be scarce as banded juvenile waxwings are almost never recaptured in their second year (Parkes, pers. comm.).

According to the diet hypothesis, deposition of rhodoxanthin in the feathers should be contingent with its availability at the time of feather growth, and could be derived from the diet or internal storage sites. Thus, to be effective in juvenile birds, where the orange color prevails, the pigment would have to be available at the time of early growth and production of their rectrices. This probably happens in mid-July to August in eastern North America. The carotenoids could be obtained directly from the diet or indirectly from the egg yolk. The adults do not molt until later in the autumn (Dwight 1900) and presumably would not be affected by the presence of the pigment in the diet until that time. Possibly, the difference in frequency of incidence of the dimorphism between age classes can be attributed to the different time schedules of feather replacement between the age groups. Such a difference would be a function of seasonal changes in the environmental abundance of rhodoxanthin. A knowledge of the molt schedule of the yellow feather tracts in birds of different ages might be used to reconstruct the availability of the pigment in the environment. Alternatively, if the abundance of rhodoxanthin were relatively constant, a prevalence of the orange color in juvenile birds could arise from their high food intake as growing nestlings, and possible accumulation of rhodoxanthin.

In individuals with a Burnt Orange tail band, the incorporation of a dietary red pigment can account for the color change. In other variants the explanation must be more complex. The individual with a Buff tail band exhibited a highly reduced carotenoid deposition, in particular ϵ,ϵ-carotene-3,3′-dione, and increased melanin deposition, when compared with the yellow-banded birds. Similar phenotypes, characterized by low carotenoid and high melanin levels, have been produced experimentally in Eurasian Bullfinches (*Pyrrhula pyrrhula*) by limiting access to food (Schereschewsky 1929). Differences in the quantity or quality of food items might therefore have been responsible for the observed color differences. We might expect that nestlings, which are in a period of rapid growth, are more sensitive to dietary differences than adults. The quality of the diet is a function of the food items supplied by the parents. Presumably, in birds with yellow bands an adequate diet was available and the feathers resemble those in the adults.

Records from museum specimens indicate the appearance of orange feathers was relatively recent (Parkes and Wood, pers. comm.). They have existed in the birds from the northeast United States for less than 30 yr. If a dietary change is involved, the sudden appearance might reflect the appearance of a new food source, a change in the abundance of a native or established source, or a change in adult food choice. A study of the current geographical distribution and extent within localities of the phenomenon may resolve the cause of the dimorphism in the Cedar Waxwing. The possibility of finding variant nestlings at the nest would expedite the work of correlating the phenotypes with variables of the environment, and suggest possible solutions.

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