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PIGMENTS AND FEATHER STRUCTURE OF THE REDPOLLS, *CARDUELIS FLAMMEA* AND *C. HORNEMANNI*

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ABSTRACT.—Spectral profiles of intact carotenoid-bearing feathers and of pigments isolated from polls and breasts of Common and Hoary redpolls (*Carduelis flammea* and *C. hornemanni*) were compared to help clarify the taxonomic relationship of the two taxa. Pigments were identified by thin-layer chromatography and by partitioning experiments. Two carotenoids were present in all samples, apparently echinenone and lutein. Color differences between taxa result from differences in absolute concentrations and changes in the relative concentration of the two pigments. Orange feathers have relatively high concentrations of lutein. Pigments are restricted to feather rami. Those most intensely colored (from polls) have flattened rami and lack barbules. New breast feathers are tipped with an unpigmented band. During late winter the band becomes worn exposing the colored rami. The most intensely pigmented breast feathers (usually those of Common Redpolls) lose their barbules as spring and summer progress.

The specific distinction of the Common Redpoll (*Carduelis flammea*) and the Hoary Redpoll (*C. hornemanni*) has been questioned by Williamson (1961), Harris et al. (1965), and Troy (1980). These taxa differ in the color of their carotenoid-bearing feathers, particularly those of the breast, as well as in other characteristics. Common Redpolls have red breasts and Hoary Redpolls have pink breasts (Coues 1861, Grinnell 1900, Newton 1972).

Color variations may result from either unique pigments or changes in pigment concentration. Feather structure may modify the reflective properties of pigments and, hence, the observed color (Dyck 1966, Brush 1969). In this study, we analyzed the pigments and structure of the carotenoid-bearing breast and poll feathers of redpolls. Our primary objective was to determine the proximate reasons for observed color differences between the two redpolls and, consequently, to clarify the taxonomic relationship between them.

METHODS

Feathers used in this study came from redpolls collected in Alaska. Troy (1980) demonstrated continuous variation of plumage and morphological variables between Common and Hoary redpolls; our feather samples for this study were taken from redpolls at the extremes of the Common–Hoary gradient.

Feathers were dry-mounted on slides and examined with a light microscope. Variations in the distribution of pigment, form of the barbs, and occurrence of barbules were noted. The color of intact patches of feathers was measured spectrophotometrically. Samples of breast or poll feathers were scanned from 400 to 625 nm, using a Perkin-Elmer 552 Recording Spectrophotometer equipped with an integrating sphere. A sample window of 6 × 8 mm was used. Preliminary scans over a wider range of wavelengths indicated that most absorption in the visible spectrum occurred within this range. For comparison, we also scanned white redpoll feathers (taken from the flank region). Samples were compared to a standard white surface (Perkin Elmer #103750) by the spectrophotometer during each scan. The integrating sphere detects the light energy reflected from the feather surface. Measurements can be read in either transmission or absorption mode; these bear a reciprocal relationship to each other. The instrument is so designed as to capture light reflected from the feather surface. In the absorbance mode, the spectral characters of the reflected light are determined; this is physically identical to the process of measuring the absorbance spectrum of a pigment in solution. The transmission mode measures the reciprocal of this spectrum and is recorded as percent transmission.

Feather carotenoid pigments were extracted in alkaline (KOH) ethanol, then transferred to hexane. These solutions were analyzed spectrophotometrically to measure absorbance in the same range of wavelengths as was used for intact feathers. Comparing the spectral profiles of the intact feathers with those of the isolated pigments indicated the effect of structure on the apparent color of the feather.

The soluble pigments were analyzed further

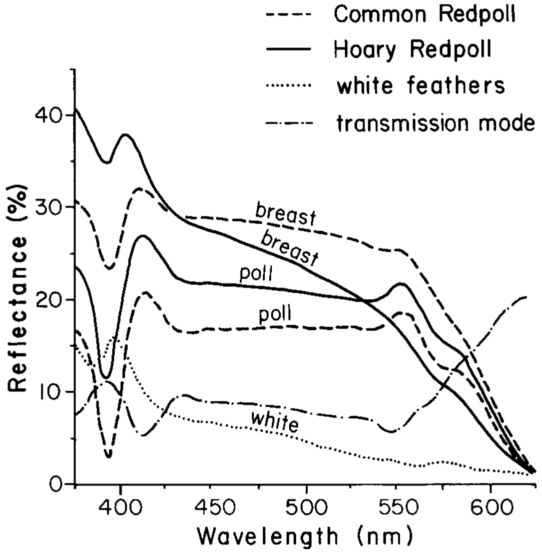

FIGURE 1. Spectral profiles of pigments in vivo of poll and breast feathers from Common and Hoary redpolls. A sample of white flank feathers is included for comparison. The reflected light was read in absorbance mode to make the curves compatible with those in Figure 2. A curve in transmission mode is included for comparison.

using thin-layer chromatography (TLC) to determine if a sample contained more than one pigment and to compare samples from different birds or from breasts and polls of a single bird. Several solvent systems were tested for their ability to resolve redpoll pigments. A mixture of benzene and acetone (98:2) produced the clearest resolution and gave the most consistent results.

Pigments were identified by three methods: (1) partitioning coefficients in hexane-methanol (Petracek and Zechmeister 1956), (2) absorbance maxima as compared with published values (Foppen 1971), and (3) direct comparisons by co-chromatography with carotenoids of known identity. Reference canthaxanthin and other carotenoids came both from commercial preparations and from isolates prepared at the University of Connecticut.

RESULTS

Samples of actual breast and poll feathers yielded similar reflectance spectra (Fig. 1). Patterns identical to these were obtained from four sets of feather samples. Each set contained four samples: a poll and a breast feather, both from a Common and a Hoary redpoll. Hoary Redpoll breasts differed from Common Redpolls in having less absorbance at high wavelengths and in having a peak near 405 nm instead of near 415 nm. Overall, the Hoary Redpoll breast profile was intermediate between those of Common breasts or Hoary polls and white feathers.

An additional series of poll feathers representing a broad range of colors (two shades of orange, three shades of red, and mosaics) had absorbance profiles similar to the breasts and polls shown in Figure 1. They differed mainly in the intensity of the peaks, indicating differences in concentration rather than the presence of different pigments.

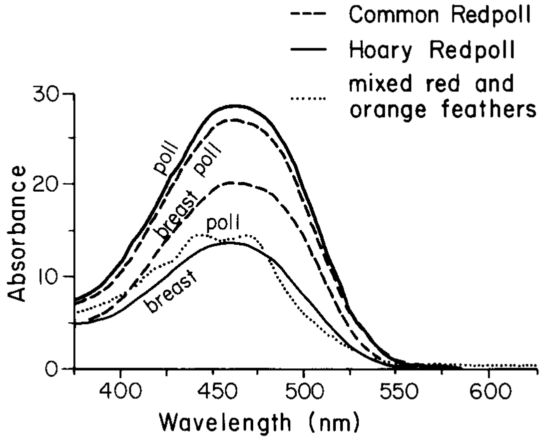

FIGURE 2. Absorbance spectra of soluble pigments in hexane from poll and breast feathers from Common and Hoary redpolls. The spectrum of a sample extracted from a mosaic (red and orange feathers) is also shown.

The spectra of the carotenoids isolated from redpoll feathers had distinct peaks compared to those of intact feathers (Fig. 2). All breasts and most polls had similar profiles, differing only in relative concentration. Several polls (one illustrated) had spectra with two peaks; all of these contained either orange or mosaics of red and orange feathers. The in vitro spectra differed from those obtained in vivo, demonstrating that properties of the feather other than the presence of carotenoids significantly modified color.

In most of the 16 samples examined using TLC, two pigments were separated. The slower-moving pigment generally appeared much less intense and may have been present in all samples. The slow pigment was most intense in poll samples with orange feathers. The chemical and spectral results are consistent with echinenone being the more concentrated, faster pigment in TLC. The partitioning coefficient of 92:8 in hexane-methanol compared favorably to the expected value of 93:7 (Petracek and Zechmeister 1956). Similarly, pure echinenone in hexane has an absorbance maximum at 459 nm, a value close to the rather broad maxima of our samples (Fig. 2).

Of the carotenoids commonly found in birds (Brush 1982), the only one with a reflectance maximum in the range of the low wavelength peak of the orange-polled birds (Fig. 2) is lutein (maximum absorbance at 445 nm). We did not do any partitioning experiments on this pigment because we could not isolate it in adequate amounts from the more concentrated echinenone; certain observations, however, suggest that it was lutein. During the initial extractions some pigment always remained hypophasic. Strongly hypophasic carotenoids typically have two hydroxyl (OH) groups. Of avian pigments only lutein, zeaxanthin and isozeaxanthin are dihydroxic. The latter two have absorbance maxima higher than lutein (450 and 451 nm, respectively; Foppen 1971) and, hence, are unlikely to be the slow pigment. In addition, the initial partitioning resulted in a coefficient lower than would be expected for echinenone; i.e., more pigment was transferred to methanol than expected. This result would be expected if the second pigment contained hydroxyl groups. Canthaxanthin and isozeaxanthin are the only pigments other than echinenone reported in cardueline finches (Brush 1982). Co-chromatography revealed that the slow pigment in redpolls had a lower mobility than canthaxanthin. The absorptive maximum of canthaxanthin occurs at 467 nm (Foppen 1971), even higher than echinenone; hence, it could not be the slow pigment. Therefore, we tentatively identified the pigments in redpolls as echinenone and lutein, the fast and slow pigments respectively.

Microscopic examination of feathers revealed morphological differences between Common and Hoary redpolls. These differences were related primarily to patterns of wear, which affect the conspicuousness of the colored feathers because carotenoid deposits were restricted to the rami, or axes, of feather barbs. We distinguished three stages of wear of breast feathers. When fresh, and through most of the winter, redpoll breast feathers were tipped with an unpigmented band. On specimens collected in early spring, this band was usually abraded and more of the pigmented rami were exposed. As the breeding season progressed barbules were lost, exposing the rami and producing more intense color. Except for the loss of barbules, pigmented breast feathers did not appear to be modified structurally. Most Hoary Redpolls did not reach this third stage of wear. Since Hoary Redpolls are less intensely pigmented than Common Redpolls, a relationship between the amount of pigment and the loss of barbules is suggested. The most intensely pigmented Hoary Redpoll appeared pink (as opposed to red) and had only a limited loss of barbules. Poll feathers were broad, flat, and without barbules in all specimens examined.

DISCUSSION

The observed differences in coloration were due primarily to pigment concentration, exaggerated by structural modification of the feathers. Breast feather barbules of Hoary Redpolls were more resistant to wear than those of Common Redpolls. Thus a combination of differences in pigment concentration, pigment distribution, and feather structure produced the observed color differences. Pigment composition was not found to be the cause of observed color differences between the redpoll taxa.

Several structural features affected feather color, the first being the nature and the amount of pigment in the feather. The absolute amount of pigment present produced a range of colors in individual feathers from yellow to "burgundy" red. A second factor was the distribution of pigments. As is the case in many birds, carotenoid pigments in redpolls were restricted to feather rami. The pigmented rami of poll feathers were broad and flattened and lacked barbules, presumably enhancing the intensity of the display of the poll (Brush and Seifried 1968).

Since we used extreme examples of Common and Hoary redpolls, the differences noted are likely the ends of a gradient of concentrations and relative proportions of the two pigments. In nature, the extent and intensity of pigments are much more varied and intermediately colored birds occur. Thus, these data support the conclusions of Williamson (1961), Harris et al. (1965), and Troy (1980) that the recognition of two species of redpolls is unwarranted. We agree broadly with Short (1976), who considered color, especially that due to carotenoid pigments, to be a poor taxonomic character. The reasons for this include convergence on specific end products, universality of certain biochemical pathways, and general lack of species-specific end products (Brush 1982). Nevertheless, at lower taxonomic levels carotenoid differences may be related to taxonomic or adaptive differences among populations (Brush 1970, Johnson and Brush 1972, Brush and Johnson 1976). Overall color provides only weak evidence for any taxonomic decision, but color differences may be adaptive in themselves or may be correlated with other, more reliable taxonomic differences.

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LITERATURE CITED

BRUSH, A. H. 1969. On the nature of "contingin." Condor 71:431–433.

BRUSH, A. H. 1970. Pigments in hybrid, variant and melanic tanagers (birds). Comp. Biochem. Physiol. 36:785–793.

BRUSH, A. H. 1982. Carotenoids in wild and captive birds, p. 539–562. *In* J. C. Bauernfeind [ed.], Carotenoids as colorants and vitamin A precursors. Academic Press, New York.

BRUSH, A. H., AND H. SEIFRIED. 1968. Pigmentation and feather structure in genetic variants of the Gouldian Finch, *Poephila gouldiae*. Auk 85:416–430.

BRUSH, A. H., AND N. K. JOHNSON. 1976. Evolution of color differences between Nashville and Virginia's warblers. Condor 78:412–414.

COUES, E. 1861. A monograph of the genus *Aegiothus* with description of new species. Proc. Acad. Nat. Sci. Phila. 13:373–390.

DYCK, J. 1966. Determination of plumage colours, feather pigments and structures by means of reflection spectrophotometry. Dansk Ornithol. Foren. Tidsskr. 60:49–76.

FOPPEN, F. H. 1971. Tables for the identification of carotenoid pigments. Chromatogr. Rev. 14:133–298.

GRINNELL, J. 1900. Birds of the Kotzebue Sound region, Alaska. Pacific Coast Avifauna No. 1.

JOHNSON, N. K., AND A. H. BRUSH. 1972. Analysis of polymorphism in the Sooty-capped Bush Tanager. Syst. Zool. 21:245–262.

HARRIS, M. P., F. I. NORMAN, AND R. H. S. MCCOLL. 1965. A mixed population of redpolls in northern Norway. Brit. Birds 58:288–294.

NEWTON, I. 1972. Finches. Collins, Glasgow.

PETRACEK, F. J., AND L. ZECHMEISTER. 1956. Determination of partition coefficients of carotenoids as a tool in pigment analysis. Anal. Chem. 28:1484–1486.

SHORT, L. L. 1976. The contribution of external morphology to avian classification. Proc. XVI Int. Ornithol. Congr. (1974) 16:185–195.

TROY, D. M. 1980. A phenetic and karyotypic investigation of the systematics of the redpolls *Carduelis f. flammea* and *C. hornemanni exilipes*. M.Sc. thesis, Univ. of Alaska, Fairbanks.

WILLIAMSON, K. 1961. The taxonomy of redpolls. Brit. Birds 54:238–241.

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RECENT PUBLICATIONS

The Importance of Wildlife to Canadians.—F. L. Filion, S. W. James, J.-L. Ducharme, W. Pepper, R. Reid, P. Boxall, and D. Teillet. 1983. Canadian Wildlife Service, Environment Canada. 40 p. Paper cover. Source: Minister of Supply and Services, Ottawa, Canada. Catalogue No. CW66-62/1983E. This report presents the highlights of a comprehensive survey conducted in 1982, under the sponsorship of the Federal-Provincial Wildlife Conference. Questioning approximately 100,000 Canadians from all regions and walks of life, the study gauged the importance of wildlife to them. The results concerning participation in wildlife-related activities, expenditures on these activities, and attitudes toward wildlife are summarized here with text and many graphs. They quantify Canadians' high involvement and level of spending for wildlife-related activities, as well as their positive feelings toward wildlife and conservation. Although the data have not yet been completely analyzed, the report concludes by indicating some of their potential implications for wildlife management. These findings offer a lesson to wildlife agencies elsewhere about the value of consulting sociologists and economists.

How to Write and Publish a Scientific Paper, Second Edition.—Robert A. Day. 1983. ISI Press, Philadelphia. 181 p. $17.95 hardcover, $11.95 softcover. Source: ISI Press, 3501 Market St., University City Science Center, Philadelphia, PA 19104. Here is a revised version of an already outstanding manual on the presentation of science (noticed in Condor 82:75). Following the general plan of the original, it gives practical instructions on all aspects of scientific writing and publishing. Most of the chapters have been expanded and new ones have been added on the electronic manuscript, writing book reviews, and the oral presentation of papers. While the book can be consulted as a reference, it deserves to be read straight through, for its parts are interrelated and the writing itself is exemplary. Touches of humor make points and enliven the reading. This book is probably the best of its kind and deserves to be studied by any researcher, however experienced, who intends to publish an article. It can also be used by technical writers, editors, and teachers of scientific writing. Graduate students—if you can afford only one book this year, let this be it.

Eco-ornithological Glossary.—Rudolf Berndt and Wolfgang Winkel. 1983. Duncker & Humblot, Berlin. 79 p. Paper cover. DM 30. Source: Duncker & Humblot, P.O. Box 41-03-29, 1000 Berlin 41, Germany. This booklet, in German and English, gives definitions and translations for approximately 400 terms used in avian ecology. The entries range in length from a single word to nearly half a page, and the explanations are enhanced with many examples. The terms have been well translated into English by Rosemary Jellis, herself an ornithologist, and they can be looked up in either language. While this reference can be used merely for its definitions, it will be most valuable as a key for unlocking some of the foreign literature. American ornithologists now have less excuse than before for not keeping up with ideas and findings published in German. Index, list of selected references.