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Interspecific Variation in Plasma Hue in Relation to Carotenoid Plumage Pigmentation

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Much of the bright yellow, orange, and red coloration of plumage results from carotenoid pigmentation (Brush 1978, Goodwin 1984). Carotenoids are unique among plumage pigments in that they cannot be synthesized by birds de novo; they must be ingested (Goodwin 1984, Brush 1978, 1990). An understanding of carotenoid pigmentation, therefore, requires an understanding of how birds get carotenoids from food into developing feathers. Unfortunately, the processes of carotenoid uptake, transport, and metabolism remain poorly understood (for reviews, see Brush 1978, 1990), with carotenoid transport perhaps the least studied aspect of carotenoid pigmentation (Brush 1990).

To get from food in the gut to growing feathers, carotenoids must be transported in the blood. Trams (1969) was among the first to determine the carotenoid content of blood in relation to plumage coloration. He compared the blood of bright-red-plumaged Scarlet Ibises (*Eudocimus ruber*) with the blood of closely related but white-plumaged White Ibises (*E. albus*). As predicted, the blood of Scarlet Ibises contained much greater concentrations of red carotenoid pigments, as well as greater concentrations of a high-density lipoprotein that Trams (1969) speculated was the carrier protein for the carotenoids. Trams' study was conducted with birds that were not molting and, thus, at a time when carotenoid pigments in the blood would not be of use to the birds as feather pigments (although both species have red bills and/or legs that may require a more-or-less constant supply of carotenoid pigments to retain their color). More recently, Hill et al. (1994) found that the plasma of molting House Finches (*Carpodacus mexicanus*) was frequently colored orange or red, which suggests presence of carotenoid pigments. Moreover, Hill et al. (1994) found a positive correlation between the redness of the plasma of molting male House Finches and the redness of growing feathers, as well as a significant difference between the plasma color of females, which have little carotenoid pigmentation, and males, which have large carotenoid ornaments. Together, these studies demonstrated that gross interspecific and intersexual differences in the carotenoid-based plumage coloration are reflected in the levels of carotenoid pigments in the blood and, thus, that at least some control of carotenoid pigmentation comes before uptake by and deposition in the growing feather follicle (Hill et al. 1994).

I extend previous studies by comparing the plasma color of 14 bird species of birds (12 in Passeriformes, 1 in Piciformes, 1 in Columbiformes). I assumed that the ability to transport carotenoid pigments in the blood is a special adaptation associated with display of plumage or other integumentary structures pigmented with carotenoids. Consequently, I predicted that during molt bird species with plumage pigmented with carotenoids would have significantly redder plasma (on a yellow-orange-red continuum, reflecting higher concentrations of carotenoids or more xanthophylls being transported in the blood) than bird species lacking carotenoid displays.

Methods.—I captured birds in mist nets and traps in a residential area of Auburn, Alabama (32°30'N, 85°20'E). Most species of birds in temperate North America undergo an extensive prebasic molt in the late summer and early fall (Pyle et al. 1987). Accordingly, birds in this comparison were sampled between mid-July and mid-September, and I included only birds undergoing molt of body plumage. Each bird was banded and classed by age and sex where possible. Age was determined only as HY (hatching year; i.e. in calendar year in which birds hatched), AHY (after hatching year; i.e. having hatched in a previous calendar year), or U (unknown). From each bird, I collected approximately 50 μl of blood in a microhematocrit tube after nicking the brachial vein. Blood in the microhematocrit tubes was immediately spun for 2 min at 10,000 rpm in a IEC Clinical Centrifuge, and the hue of the plasma portion of the blood was scored by comparison to the *Methuen Handbook of Colour* (Kornerup and Wansher 1983; for more on plasma scoring method, see Hill et al. 1994). Because carotenoids reflect light in a portion of the electromagnetic spectrum visible to humans, the presence of carotenoids as well as general classes of carotenoids (e.g. yellow carotenes or red xanthophylls) can be detected with the human visual system (Brush and Power 1976). A critical assumption of my study is that the redness of plasma is a function of the type and quantities of carotenoids contained (for further justification of this assumption, see Brush and Power 1976, Hill et al. 1994).

Before blood sampling was conducted, species were classed as having carotenoid-based plumage coloration or lacking carotenoid-based plumage coloration. House Finches, Northern Cardinals (*Cardinalis cardinalis*), and Northern Flickers (*Colaptes auratus*) are

known to have carotenoid-based plumage coloration (Test 1969, Brush and Power 1976, Hudon 1991). Two species of wood-warblers (family Parulidae) with yellow plumage coloration are known to have carotenoid-based plumage coloration (Brush and Johnson 1976), but the wood-warbler species sampled in this study, Common Yellowthroat (*Geothlypis trichas*), has not been tested. The pigment composition of the other species is not known, but it is well established that melanin pigmentation produces earth tones while carotenoid pigments produce bright yellow, orange, and red coloration (Ralph 1969, Brush 1978). Thus, presence or absence of bright red/orange/yellow plumage coloration was used to classify birds as having or lacking carotenoid-based plumage coloration.

Results.—I first looked for biases in the age/sex classes of individuals sampled for the various species. I was particularly concerned with the representation of males and females and HY and AHY birds between species with and without carotenoid-based plumage pigmentation. For eight species, I was either unable to class most birds by age or sex, or too few individuals were captured. The five species for which I obtained age/sex ratios are: House Finch, 10 AHY/M, 5 AHY/F, 31 HY/M, 15 HY/F, 38 HY/U; Northern Cardinal, 2 AHY/M, 1 AHY/F, 7 HY/M, 9 HY/F, 5 HY/U; Rufous-sided Towhee (*Pipilo erythrophthalmus*), 1 AHY/F, 2 HY/U; Common Grackle (*Quiscalus quiscula*), 2 AHY/M, 1 HY/M; and Mourning Dove (*Zenaida macroura*), 3 AHY/M, 2 HY/U. I pooled data between the two species with carotenoid-based plumage coloration (House Finch and Northern Cardinal) and among species with no apparent carotenoid display (Rufous-sided Towhee, Common Grackle, and Morning Dove). There was a significant difference in the proportions of age/sex classes between species with and without carotenoid pigmentation ($X^2 = 8.28$, df $= 3$, $P = 0.04$; G-test for goodness of fit). The primary difference between the two groups was the over representation of HY birds in species with carotenoid plumage coloration.

Northern Cardinals and House Finches, each with extensive, bright-red carotenoid plumage displays, had the highest mean plasma hue scores (Fig. 1). Northern (yellow-shafted) Flickers have large patches of yellow carotenoid pigmentation on their underwings and very small patches of red carotenoid pigmentation on their heads. The single "yellow-shafted" flicker that was sampled had yellow-orange plasma with a higher hue score than any of the species lacking carotenoid-based plumage coloration (Fig. 1). The single White-eyed Vireo (*Vireo griseus*), a species with a small amount of yellow carotenoid-based plumage coloration, and the single Common Yellowthroat, a species with extensive yellow coloration, had plasma scores at the high end of the range recorded for species lacking carotenoid-based plumage coloration (Fig. 1). Most individuals of the other nine bird species, which lack carotenoid-based plumage coloration, had pale yellow plasma that scored 2 (presumably the color of plasma lacking carotenoid pigments; see Hill et al. 1994).

I used two approaches to compare statistically the plasma scores of species. First, for the six species represented by five or more individuals, I used an ANOVA to test for differences. I found a significant difference in the plasma hue among groups ($F = 10.60$, df $= 5$ and 144, $P < 0.001$). Using a Student-Newman-Keuls multiple-comparisons test (Abacus Concepts 1992) to determine which groups differed significantly, I found that the mean plasma scores of both Northern Cardinals and House Finches were significantly higher than the mean plasma scores of the other four species (all of which lacked carotenoid plumage pigmentation ($P < 0.05$ for all comparisons), but that there were no significant differences between cardinals and finches, or among the four species lacking carotenoid plumage coloration. Second, I pooled species into two groups—with and without carotenoid-based plumage coloration—and used a Student's t-test to compare the mean plasma color of the groups. Species with carotenoid-based plumage coloration had significantly higher mean plasma scores (with carotenoid plumage, $\bar{x} = 4.64 \pm$ SD of 1.76; without carotenoid plumage, $\bar{x} = 2.16 \pm 0.44$; df $=$ 166, $t = 8.60$, $P = 0.001$).

Discussion.—This is the first interspecific comparison of the relative quantities and/or types of carotenoids (as indicated by plasma color) present in the blood of birds during molt. I found that species with carotenoid-based plumage coloration had significantly redder plasma than species that lack carotenoid pigmentation. Moreover, although I had only a small sample of species with yellow carotenoid pigmentation, they had less-red plasma than species with red pigmentation.

My study focused on the differences among species in plumage pigmentation. Species sampled were chosen for their accessibility, not because they necessarily represented the most appropriate species to test. Variables other than plumage pigmentation that differed among species and that could potentially could have contributed to the observed differences in plasma color include diet (granivory, insectivory, frugivory, etc.), parasite loads, and evolutionary history. Although alternative hypotheses cannot be ruled out without more data, the observation that, as predicted, species with carotenoid-based plumage coloration had redder plasma than species lacking carotenoid pigmentation suggests that integumentary coloration and plasma coloration are functionally linked.

One potential confounding variable is the age and sex of the individuals used to represent each species. In the sexually dichromatic House Finch, males have redder plasma than females and adult males have redder plasma than juvenile males (Hill et al. 1994). Male Northern Cardinals also have redder plasma than females (males, $\bar{x} = 5.1 \pm 1.2$, $n = 9$; females, $\bar{x}$

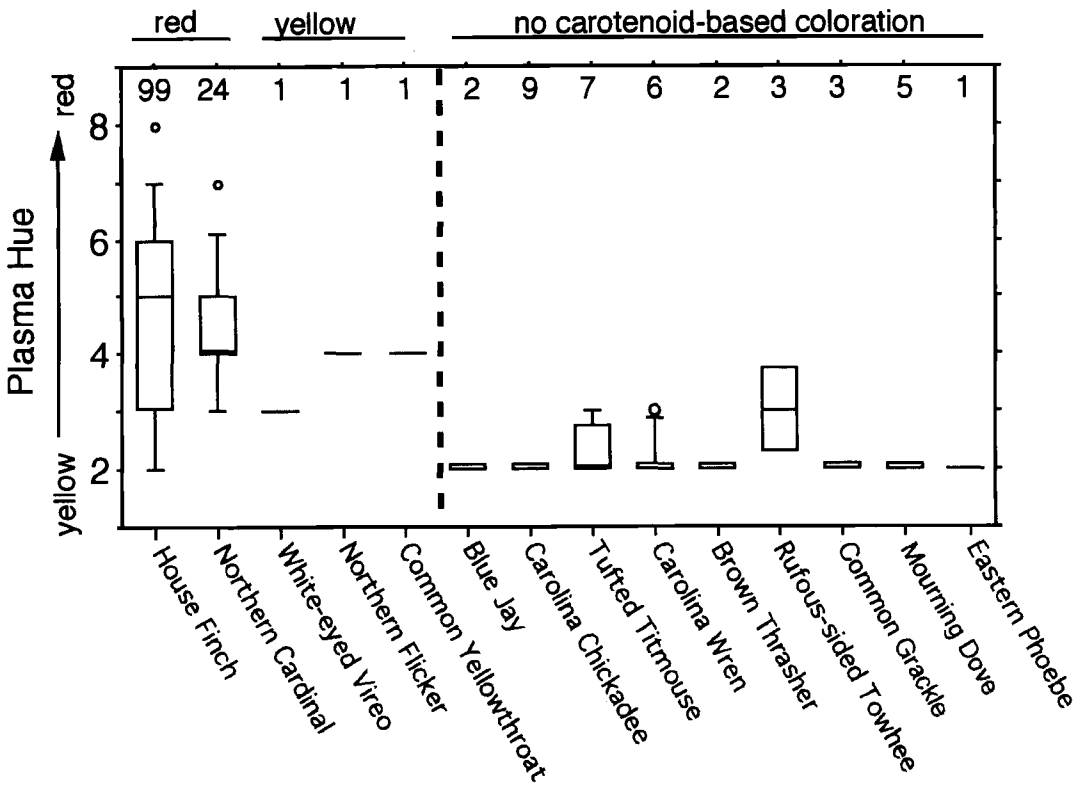

Fig. 1. Box plots of plasma hue scores for 13 species of birds sampled during molt in Alabama. Horizontal bars in box plots indicate the 10th, 25th, 50th, 75th, and 90th percentiles, and points give data for individuals outside this range. Numbers above box plots are sample sizes. Scientific names of species not covered in text: Eastern Phoebe (*Sayornis phoebe*), Carolina Chickadee (*Parus carolinensis*), Tufted Titmouse (*P. bicolor*), Carolina Wren (*Thryothorus ludovicianus*), and Brown Thrasher (*Toxostoma rufum*).

= 3.9 ± 0.9, $n = 10$; df = 17, $t = 2.58$, $P = 0.01$). The results of my study would be less convincing if individuals used to represent species lacking carotenoid-based display were composed of a higher proportion of females or juveniles than the individuals used to represent species with carotenoid-based displays. Analysis of age/sex ratios for the two groups of species indicates that, if anything, adult males were overrepresented in species lacking carotenoid plumage pigmentation. Thus, it seems very unlikely that biases in the age or sex classes sampled could have generated the patterns that I found. Moreover, because female Northern Cardinals and House Finches were, by necessity, included in comparisons, my study underestimates differences in plasma coloration between species with and without carotenoid plumage coloration. The differences between species that I present in this paper are really differences between individuals from species lacking carotenoid plumage coloration and males of species with carotenoid plumage pigmentation. That the differences remained significant, even when female House Finches and Northern Cardinals were included in the analysis, makes the observed pattern all the more convincing.

These observations have important implications. First, assuming that the coloration of plasma reflects the type and quantity of carotenoid pigments that it contains, the relationship between plasma color and plumage color among species supports the idea that carotenoid transport systems are shaped by the amounts of carotenoid pigments displayed in the integument (Trams 1969, Hill et al. 1994). It appears that species with the capacity to transport red or yellow carotenoid pigments in the blood are those that display red or yellow plumage, bill, or leg color. This agrees with the observation by Trams (1969) on nonmolting ibises that as the area of integument being pigmented by carotenoids increases the amount of carrier protein in the blood (and presumably other transport mechanisms) increases. Alternatively, all the species included in my study may have similar capacities to transport carotenoids, and the differences observed among species in plasma hue result from variation in the amount and type of carotenoids in-

gested (for a detailed discussion of effects of dietary carotenoids on display, see Hill 1992, 1994, Hudon 1994). The latter explanation seems less plausible, but feeding experiments are needed for a definitive test.

The second implication of this study is that, especially for species with red plumage, one can test whether a color display is carotenoid-based by examining plasma during molt. Species with plumage coloration that is carotenoid-based should have orange to red plasma. Species with plumage displays derived from melanins or other biochromes or from feather structures (and lacking carotenoid-based bill or leg color) should have pale yellow plasma. Of course, the best way to determine the basis for color display in a species is to conduct a careful biochemical analysis, but for field biologists examination of blood plasma can provide a quick and useful assay.

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Literature Cited

Abacus Concepts. 1992. Statview. Abacus Concepts, Inc., Berkeley, California.

Brush, A. H. 1978. Avian pigmentation. Pages 141–161 *in* Chemical zoology. Vol. 10, Aves (A. Brush, Ed.). Academic Press, New York.

Brush, A. H. 1990. Metabolism of carotenoid pigments in birds. FASEB J. 4:2969–2977.

Brush, A. H., and N. K. Johnson. 1976. The evolution of color differences between Nashville and Virginia's warblers. Condor 78:412–414.

Brush, A. H., and D. M. Power. 1976. House Finch pigmentation: Carotenoid metabolism and the effect of diet. Auk 93:725–739.

Goodwin, T. W. 1984. The biochemistry of carotenoids. Vol. 2, Animals, 2nd ed. Chapman and Hall, New York.

Hill, G. E. 1992. The proximate basis of variation in carotenoid pigmentation in male House Finches. Auk 109:1–12.

Hill, G. E. 1994. House Finches are what they eat: A reply to Hudon. Auk 111:221–224.

Hill, G. E., R. Montgomerie, C. Inouye, and J. Dale. 1994. Influence of dietary carotenoids on plasma and plumage color in the House Finch: Intra- and intersexual variation. Funct. Ecol. 8:343–350.

Hudon, J. 1991. Unusual carotenoid use by the Western Tanager (*Piranga ludoviciana*) and its evolutionary implications. Can. J. Zool. 69:2311–2320.

Hudon, J. 1994. Showiness, carotenoids, and captivity: A comment on Hill (1992). Auk 111:218–221.

Kornerup, A., and J. H. Wanscher. 1983. Methuen handbook of colour, 3rd ed. Methuen, London.

Pyle, P., S. N. G. Howell, R. P. Yunick, and D. F. DeSante. 1987. Identification guide to North American passerines. Slate Creek Press, Bolinas, California.

Ralph, C. L. 1969. Control of color in birds. Am. Zool. 9:521–530.

Test, F. H. 1969. Relation of wing and tail color of the woodpeckers *Colaptes auratus* and *C. cafer* to their food. Condor 71:206–211.

Trams, E. 1969. Carotenoid transport in the plasma of the Scarlet Ibis (*Eudocimus ruber*). Comp. Biochem. Physiol. 28:1177–1184.

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Seasonal Variation in Circulating Carotenoid Pigments in the House Finch

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Carotenoid pigments are responsible for much of the red, orange, and yellow coloration found in bird plumage (Brush 1978, 1990, Goodwin 1984). They are unique among known avian pigments in that they cannot be synthesized by birds and must be ingested (Goodwin 1950, Brush 1978, 1990). Thus, expression of carotenoid-based plumage coloration depends on a bird's ability to ingest, transport, and modify carotenoid pigments (Brush 1978, 1990). Some bird species are known to have sophisticated mechanisms for efficient absorption, transport, metabolism, and deposition of carotenoid pigments (studies summarized