CAROTENOID PIGMENTS IN MALE HOUSE FINCH PLUMAGE IN RELATION TO AGE, SUBSPECIES, AND ORNAMENTAL COLORATION

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Abstract.—Like males of many bird species, male House Finches (*Carpodacus mexicanus*) have patches of feathers with ornamental coloration that are due to carotenoid pigments. Within populations, male House Finches vary in expression of ornamental coloration from pale yellow to bright red, which previous research suggested was the result of variation in types and amounts of carotenoid pigments deposited in feathers. Here we used improved analytical techniques to describe types and amounts of carotenoid pigments present in that plumage. We then used those data to make comparisons of carotenoid composition of feathers of male House Finches at three levels: among individual males with different plumage hue and saturation, between age groups of males from the same population, and between males from two subspecies that differ in extent of ventral carotenoid pigmentation (patch size): large-patched *C. m. frontalis* from coastal California and small-patched *C. m. griscomi* from Guerrero, Mexico. In all age groups and populations, the ornamental plumage coloration of male House Finches resulted from the same 13 carotenoid pigments, with 3-hydroxy echinenone and lutein being the most abundant carotenoid pigments. The composition of carotenoids in feathers suggested that House Finches are capable of metabolic transformation of dietary forms of carotenoids. The hue of male plumage depended on component carotenoids, their relative concentrations, and total concentration of all carotenoids. Most 4-keto (red) carotenoids were positively correlated with plumage redness, and most yellow carotenoid pigments were negatively associated with plumage redness, although the strength of the relationship for specific carotenoid pigments varied among age groups and subspecies. Using age and subspecies as factors and concentration of each component carotenoid as dependent variables in a MANOVA, we found a distinctive pigment profile for each age group within each subspecies. Among *frontalis* males, hatch-year birds did not differ from adults in mean plumage hue, but they had a significantly lower proportion of red pigments in their plumage, and significantly lower levels of the red piments adonirubin and astaxanthin, but significantly higher levels of the yellow pigment zeaxanthin, than adult males. Among *griscomi* males, hatch-year birds differed from adults in plumage hue but not significantly in pigment composition, though in general their feathers had lower concentrations of red pigments and higher concentrations of yellow pigments than adult males. Both adult and hatch-year *frontalis* males differed from *griscomi* males in having significantly higher levels of most yellow carotenoid pigments and significantly lower levels of most red carotenoid pigments. Variation in pigment profiles of subspecies and age classes may reflect differences among the groups in carotenoid metabolism, in dietary access to carotenoids, or in exposure to environmental factors, such as parasites, that may affect pigmentation. *Received 18 January 1999, accepted 11 June 2001.*

Carotenoid pigments are responsible for the bright red, orange, and yellow coloration of

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plumage. Birds obtain those carotenoids exclusively through their diet. No animal has been shown unequivocally to be capable of *in vivo* synthesis of carotenoids (Goodwin 1984, 1986; Schiedt 1990). In birds, dietary carotenoids may either be deposited directly into feathers or chemically changed from ingested forms prior to pigment deposition, typically by addition or elimination of oxygen groups to one

or both end rings of the molecule (Davies 1985, Goodwin 1986, Tyzckowski and Hamilton 1986a, b; Brush 1990, Schiedt 1990).

The House Finch (*Carpodacus mexicanus*) is a sexually dichromatic passerine bird species in which males display bright, carotenoid-based patches of color on their crowns, throats, breasts, and rumps, and male House Finches vary in expression of that ornamental coloration from a bright red to a dull yellow (Michener and Michener 1931, Hill 1990, 1993a). The carotenoid pigments responsible for colorful plumage in the House Finch and the pigmentary basis for variation among males in expression of that coloration were first studied by Brush and Power (1976). They attributed plumage color variation to differences in constituent carotenoids in feathers. Red birds had the most complex assemblage of pigments, consisting of β-carotene, a group of unidentified mixed xanthophylls, orange isocryptoxanthin, and red echinenone; orange birds had the same subset of carotenoids without echinenone; and yellow birds lacked both echinenone and isocryptoxanthin. Recent analyses of several congeneric finch species of the Palearctic Carduelinae done by Stradi et al. (1995a, b; 1996, 1997), using new analytical techniques, revealed a more complex pattern that differed substantially from that described by Brush and Power (1976).

The proximate basis of variation in carotenoid-based plumage coloration in House Finches is of interest beyond improved understanding of the physiological control of avian pigmentation. Plumage redness in House Finches has been shown to be a primary criterion used by females in choosing mates (Hill 1990, 1991, 1994a). In addition, plumage brightness in male House Finches is correlated with overwinter survival (Hill 1991), nutritional condition during molt (Hill and Montgomerie 1994), parasite load (Thompson et al. 1997, Brawner et al. 2000), and provisioning of females during incubation (Hill 1991). It has been proposed that male plumage brightness is an honest signal of male condition, because carotenoids may be scarce resources in the environment and carotenoid-based color displays may be costly to produce (Hill 1994b, 1996a, 2002). A thorough understanding of the signal content of carotenoid-based ornamental displays can only be achieved, however, through an understanding of the proximate control of variation among

males in expression of these displays (Hill 1992, 1996a, 2002).

Feeding experiments conducted with captive House Finches have demonstrated that variation among males in plumage hue and saturation is dependent upon carotenoid access during molt (Brush and Power 1976, Hill 1992, 1993a). When males are held in flight cages and fed a standardized diet, variation in ornamental plumage coloration is minimized (Hill 1992, 1993a). Moreover, after molting in captivity under conditions of standardized carotenoid access, males from populations that are typically drab in coloration grow ornamental plumage coloration that is indistinguishable from that grown by males from populations that are typically bright in coloration (Hill 1993a). However, the degree to which access to dietary carotenoids affects expression of carotenoid-based plumage coloration in the wild remains controversial (Hill 1994c, 2002; Hudon 1994a, Thompson et al. 1997, Inouye 1999). Birds are capable of endogenous modification of ingested carotenoids prior to deposition into target tissues (Fox et al. 1969, Davies 1985, Schiedt et al. 1985, Tyzckowski and Hamilton 1986b, c, d; Brush 1990, Hencken 1992). Therefore, mechanisms involved in the digestion, absorption, transport, modification, and deposition of dietary carotenoids may contribute to plumage color variation (Hill 1999, 2002). Furthermore, viral, bacterial, and coccidial infections may have a significant effect on expression of ornamental plumage coloration by male House Finches (Thompson et al. 1997, Nolan et al. 1998, Hill and Brawner 1998, Brawner et al. 2000).

There is substantial variation in expression of ornamental plumage coloration in House Finches not just among males within populations, but also among populations and subspecies (Moore 1939, Hill 1993a). There are approximately 15 subspecies of House Finches in North America (Moore 1939, Hill 1996b), each of which has had a unique evolutionary history for thousands of years (Moore 1939). Each subspecies is characterized by specific plumage traits, some of which involve carotenoid coloration (Moore 1939, Hill 1996b). Two subspecies are studied in this paper: *C. m. frontalis*, originally native to coastal California but now introduced to the Hawaiian Islands and the eastern United States and Canada, and *C. m.*

griscomi, found in a relatively small region of southern Mexico. Male House Finches from the *frontalis* population have much more extensive ventral carotenoid pigmentation (larger patch size) than those from the *griscomi* population (Moore 1939, Hill 1993a), but some adult male *griscomi* have more intense red coloration than any male *frontalis* (Hill 1993a).

In contrast to the plasticity of expression of the color (hue, brightness, and saturation) of carotenoid-based plumage coloration, differences between *frontalis* and *griscomi* males in expression of size of ventral patches of ornamental coloration reflect fixed genetic differences between populations. When they are fed a low-carotenoid diet, both *frontalis* and *griscomi* males grow drab yellow plumage; when they are fed a red-carotenoid-supplemented diet, they grow bright red feathers (Hill 1993a). Regardless of diet treatment and plumage color, *griscomi* males always display a small patch of ornamental color, and *frontalis* males always display a relatively large patch of color (Hill 1993a). Moreover, hybrid males produced by crossing a *griscomi* female with a *frontalis* male showed a patch size intermediate to the parent types (Hill 1993a). Thus, there are some fixed genetic differences between subspecies at least in distribution of carotenoid pigments in the plumage. Whether there are also differences among subspecies in types and amounts of carotenoid pigments that color feathers has not previously been investigated.

In this study, we identified and quantified the carotenoids responsible for male House Finch plumage coloration in the subspecies *frontalis* and *griscomi.* Our objectives were, first, to elucidate the pigmentary basis for extreme variation in expression of ornamental plumage coloration among males observed within age classes and subspecies. To do that, we investigated how types and amounts of carotenoid pigments in feathers affected both hue (redness) and saturation of plumage coloration among individual males. Alhough we studied males in only two subspecies, a wide range of hues, saturations and patch sizes of ornamental plumage color has been recorded in *frontalis* and *griscomi* (Hill 1993a). Second, within both subspecies, we compared carotenoid pigments of hatch-year versus older males to analyze extent to which feather pigmenation was affected by age. Finally, to investigate the proximate ba-

sis for differences between subspecies in carotenoid display, we compared their patch sizes and mean hues, total carotenoid abundances, and carotenoid composition of their feathers.

METHODS

Sample collection.—We collected 62 hatch-year (≤1 year) and 69 adult male House Finches during 2–13 August 1992 from two locations ~12 km apart in San Jose County, California (Coyote Creek Riparian Station in Alviso and a private residence in the city of San Jose) and 59 hatch-year and 32 adult males during 9–16 September 1992 from various sites within 20 km of Chilpancingo, Guerrero, Mexico. Individuals were captured with mist nets or feeder traps. All birds were aged postmortem on the basis of degree of ossification of the skull (Pyle et al. 1987). Hatch-year birds had incompletely ossified skulls and had hatched in the same calendar year in which they were collected. Adults had completely ossified skulls and were one or more years old. The sex of each bird was confirmed by gonadal examination.

The plumage color of each bird was quantified by visual comparison of feathers to color plates in the *Methuen Handbook of Colour* (Kornerup and Wanscher 1983; see Hill 1990, 1992, 1998 for a detailed description of the color scoring methods). Birds used in this study were undergoing molt when they were collected. Most hatch-year males had only a relatively small patch of newly emerged breast feathers for color scoring. For those birds with limited ornamental plumage, we scored only the hue of their breast plumage. For most adult males and a few hatch-year males, molt had progressed far enough to allow us to estimate a complete plumage color score, including hue, saturation, and tone (=chroma or brightness) following Hill (1992). Thus, we analyzed the relationship between plumage hue and plumage pigments for all males, but we analyzed the relationship between saturation and plumage pigments for only 23 adult *frontalis* males and only 17 adult *griscomi* males. Tone, the third of the tristimulus color descriptors (Hill 1998), varied little among males in our visual assessment and was not used in comparisons. After color scoring, individuals were euthanized, and each bird's pelt was collected. Pelts were then covered with sodium chloride and wrapped in aluminum foil to protect feathers from prolonged exposure to light, which can result in photo-oxidation of the pigments.

Determination of absolute and relative carotenoid concentrations.—Feathers having yellow, orange, or red color (indicative of carotenoid pigmentation) were plucked from the pelt, washed with a commercial detergent solution (0.1% w/v), and air dried. Colored barbules were then cut from the feathers and weighed. A 5 mg aliquot of colored barbules was

washed in hexane, then finely ground, using an ultrasonic homogenizer, in methanol to solubilize the pigments. The resulting fluid extract was filtered, evaporated under nitrogen, and stored in the dark at −20°C.

Carotenoid pigments were isolated via high performance liquid chromatography (HPLC) using two sequential reverse-phase C18 columns (250 × 4 mm I.D.). The mobile phase was 70:30 acetonitrile:methanol administered at a flow rate of 0.5 ml min⁻¹. Eluents were scanned at wavelengths between 230 and 600 nm with a diode array detector. Peak areas were integrated at 450 nm. Data were recorded as three-dimensional chromatograms using HP Chem Software (Hewlett-Packard, Palo Alto, California).

Quantitative determination of carotenoids was completed using visible-light spectrophotometry. Once a component carotenoid was isolated via HPLC, it was evaporated to dryness and redissolved in methanol, then the visible spectrum of the pigment was recorded. The carotenoid concentration in feathers (micrograms carotenoids per grams feather) was calculated according to the formula ($A_{max} \times$ volume of extract [ml] $\times 10^4$) / ($E \times$ feather mass [g]), where A_{max} is the absorbance recorded at the maximum wavelength (λ_{max}) of the pigment sample, and E is the extinction coefficient at 1% per centimeter of the relevant carotenoid in the solvent (methanol). Values for E for most carotenoids are published in Davies (1976) but an E of 2200 was used when the precise value was unknown (e.g. for 3-hydroxy-echinenone). Areas under the peaks recorded at the time of HPLC analysis were determined and used to calculate both absolute carotenoid concentrations and relative abundances of individual carotenoids in each sample.

All biochemical extractions and analyses of carotenoids were performed by R.D.S. and coworkers at the University of Milan. A detailed description of the methods is given in Stradi et al. (1995a). All standards, except 4-oxo-rubixanthin, were generously donated by Hoffman-LaRoche (Basel, Switzerland).

Statistical analyses.—According to Hill (1998), correlation between hue scores derived by visual comparison to the plates in Kornerup and Wanscher (1983) and hues measured with a spectrometer are significantly positive ($n = 55$, $r = 0.96$, $P = 0.0001$) and linear, so hue scores were considered continuous variables (rather than ranks) in all analyses. That permitted use of parametric statistical analyses when relevant assumptions could be met. For analyses involving the survey of many repetitions of the same statistical test across several variables, we used the sequential Bonferroni correction to assess statistical significance of P-values (tablewide $\alpha = 0.05$; Rice 1989).
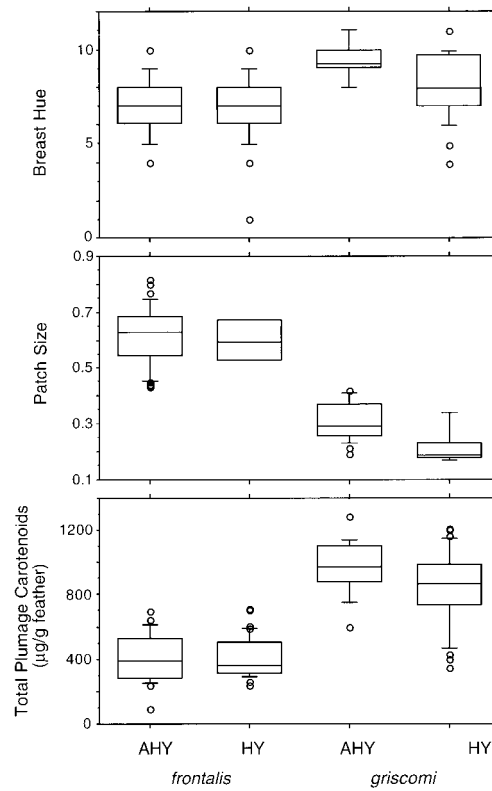

FIG. 1.   Comparison of adult male House Finches from the subspecies *frontalis* (California) and *griscomi* (Mexico). (A) Breast hue (scored by comparison with color chips, see text) where yellow is 4–5, orange is 6–8 and red is 9–11. (B) Patch size (proportion of ventral surface with carotenoid pigmentation). (C) Total carotenoid present in plumage (see text for methods). Plotted are the medians, 10th, 25th, 75th percentiles, 90th percentiles, and outlying data points.

RESULTS

Patch size, plumage hue, and age effects.—As was found in a previous study comparing *griscomi* and *frontalis* populations (Hill 1993a), males from those two subspecies differed in both mean proportion of ventral surface covered by carotenoid pigmentation (patch size) and mean plumage color (hue; Fig. 1). Adult males from the *griscomi* subspecies had significantly smaller patches of color (Mann-Whitney U-test, $z = 6.73$, $P = 0.0001$, $n = 69, 39$; Fig. 1) but significantly redder breast hues ($z = 7.81$, $P = 0.0001$, $n = 65, 30$; Fig. 1) than adult males from the *frontalis* subspecies. Similarly, hatch-year males from the *griscomi* subspecies had significantly

TABLE 1. Common and structural names of carotenoid pigments isolated from the feathers of House Finch of two subspecies, *Carpodacus mexicanus frontalis* and *C. m. griscomi*. Main absorption maxima in nanometers in methanol are indicated for each carotenoid.

Common name	Structure	Absorption maxima (nm)		
RED PIGMENTS				
Astaxanthin	3,3'-dihydroxy-β, β-carotene-4, 4'-dione			474
Adonirubin	3-hydroxy-β, β-carotene-4, 4'-dione			474
Canthaxanthin	β, β-carotene-4, 4'-dione			473
4-Oxo-rubixanthin	3-hydroxy-β, ψ-caroten-4-one	438	470	490
3-Hydroxy-echinenone	3-hydroxy-β, β-caroten-4-one			465
Echinenone	β β-caroten-4-one			461
YELLOW PIGMENTS				
Canary xanthophylls	ε,ε-caroten-3, 3'-dione	416	440	470
	3'-hydroxy-ε, ε-caroten-3-one	416	440	470
3'-Dehydro-lutein	3-hydroxy-ß, ε-caroten-3'-one	424	445	474
Lutein	β, ε-carotene-3, 3'-diol	421	445	473
Zeaxanthin	β, β-carotene-3, 3'-diol	422	450	481
β-Cryptoxanthin	β, β-caroten-3-ol	428	449	473
β-Carotene	β, β-carotene	427	449	475

redder breast plumage than hatch-year *frontalis* males ($z = 4.29$, $P = 0.0001$, $n = 65, 59$; Fig. 1). Few hatch-year males had grown sufficient new plumage when they were scored to allow an estimation of patch size; yet for the small sample available, hatch-year males of the *griscomi* subspecies had significantly smaller patches than *frontalis* males ($z = 2.24$, $P = 0.03$, $n = 3, 5$; Fig. 1).

Unlike *frontalis* males, *griscomi* males exhibit delayed plumage maturation, in which hatch-year males grow only small patches of ornamental plumage (Hill 1996b). Not surprisingly, therefore, adult males had significantly redder breast patches than hatch-year males in *griscomi* ($z = 3.51$, $P = 0.0004$, $n = 32, 59$; Fig. 1), as well as significantly larger mean patch sizes ($z = 2.62$, $P = 0.009$, $n = 5, 35$; Fig. 1). Among *frontalis* males, there were no significant differences in mean breast hue or mean patch sizes between age classes (hue: $z = 0.09$, $P = 0.93$, $n = 62, 69$; patch size: $z = 0.19$, $P = 0.85$, $n = 3, 69$; Fig. 1).

Component carotenoids and individual variation in plumage hue.—In both subspecies, the same 13 carotenoids were extracted from feathers: astaxanthin, adonirubin (=phoenicoxanthin), canthaxanthin, 4-oxo-rubixanthin, 3-hydroxy-echinenone, echinenone, ε,ε-caroten-3,3'-dione, 3'-hydroxy-ε,ε-caroten-3-one, 3'-dehydrolutein, lutein, zeaxanthin, β-cryptoxanthin, and β-carotene (Table 1). All 13 component carotenoids were identified by their retention times during HPLC and spectral characteristics at λ_{max} that

agreed with standards. A typical three-dimensional chromatogram of a red male selected from the San Jose, California population of *frontalis* is shown in Figure 2 and illustrates characteristic retention times and spectra of plumage carotenoids. In both subspecies, the most abundant carotenoids were lutein, appearing at a retention time of 12.0 min, and 3-hydroxy-echinenone, with a retention time of 21.5 min.

For the purpose of later analyses, we classified these carotenoids into (1) red pigments (4-keto-carotenoids) and (2) yellow pigments (Table 1). We make that distinction and use the terms ''yellow'' and ''red'' pigments because it aids in understanding the combined contribution of different classes of pigments to the plumage coloration of House Finches, even though some yellow carotenoids can produce a range of hues from yellow to orange and some red carotenoids can appear orange depending on their concentration (see Stradi 1998). The component carotenoids we classed as red appeared as red bands and those classed as yellow appeared as yellow bands on thin-layer chromatography plates (C. Y. Inouye pers. obs.). See also Stradi (1998) for characteristic hues of carotenoid pigments.

For both hatch-year and adult males from both subspecies, the concentration of specific red carotenoid pigments tended to be positively correlated with plumage redness, whereas concentration of specific yellow carotenoid pigments tended to be negatively correlated with plumage redness (Table 2). Although few of
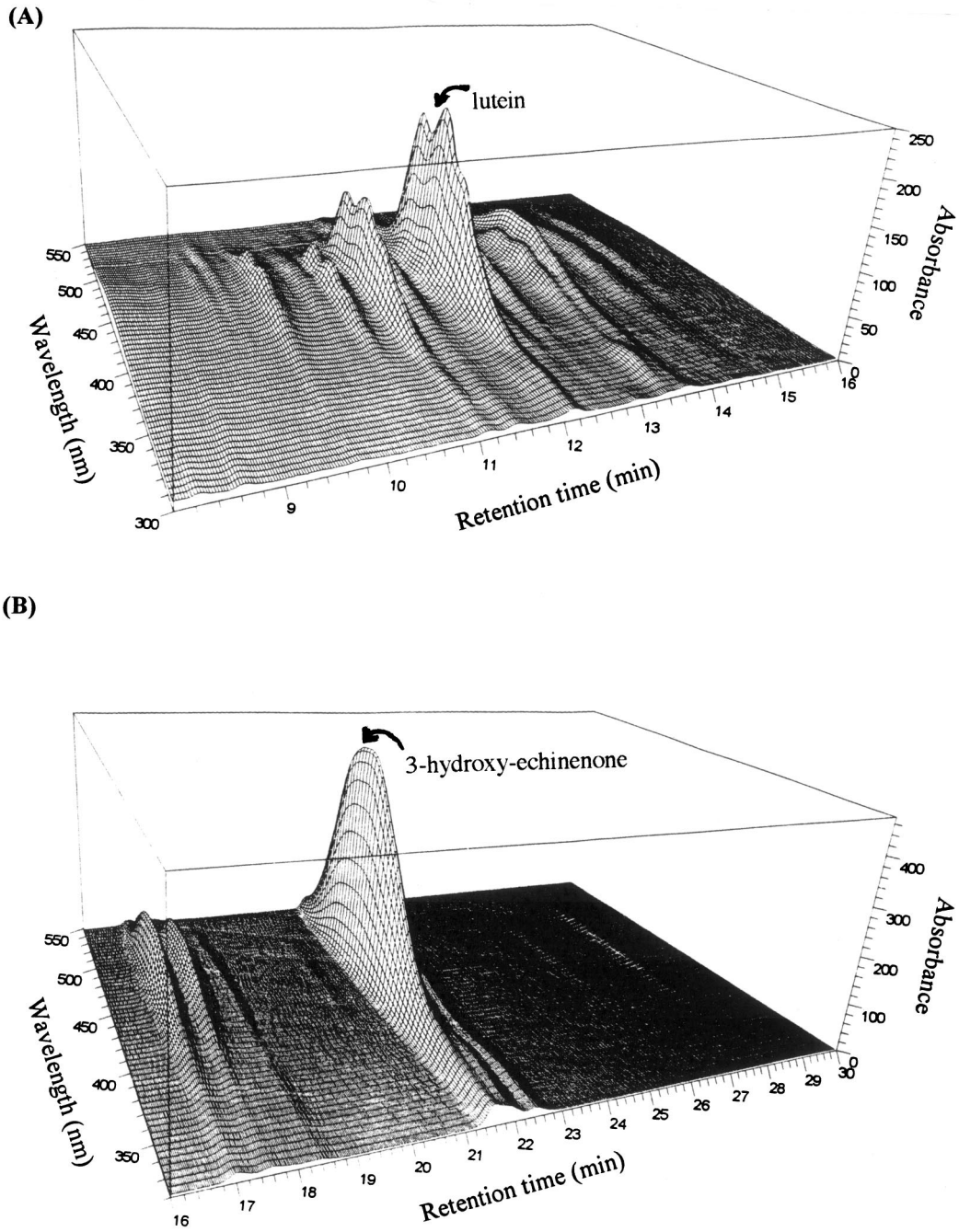
(A)

(B)

Fig. 2.   Three-dimensional chromatograms exhibiting the typical carotenoid patterns of a red male House Finch from San Jose, California. Retention time during HPLC is plotted along the *x*-axis and is separated horizontally into (A) 0–16 min and (B) 16–30 min. Absorbance (unitless) is plotted along the *z*-axis, and its range differs for (A) 0–250 and (B) 0–500. Wavelength (nanometers) is shown along the *y*-axis. The two most prominent peaks are indicated: the yellow carotenoid, lutein, with a retention time of 12 min, and the red carotenoid, 3-hydroxy-echinenone, with a retention time of 21.5 min.

TABLE 2. Pearson correlation coefficients between the concentration of specific plumage carotenoids and the hue of feathers from which they were extracted for hatch-year (HY) and adult (AHY) males from two House Finch subspecies.

Carotenoid	C. m. frontalis		C. m. griscomi	
	HY (n = 39)	AHY (n = 19)	HY (n = 28)	AHY (n = 15)
Red Carotenoids				
Astaxanthin	0.51[*]	0.08	0.14	−0.01
Adonirubin	0.43	0.15	0.44	−0.15
Canthaxanthin	0.19	0.64	0.46	−0.04
4-Oxo-rubixanthin	0.26	0.51	0.51	−0.32
3-Hydroxy-echinenone	0.57[a]	0.18	0.53	−0.20
Echinenone	0.18	0.25	0.22	0.23
Yellow Carotenoids				
ϵ, ϵ-caroten-3, 3'-dione	0.05	−0.03	−0.19	0.51
3'-hydroxy-ϵ, ϵ-caroten-3-one	−0.08	−0.45	−0.21	0.45
3'-Dehydro-lutein	−0.08	−0.46	−0.23	0.08
Lutein	−0.10	−0.46	−0.33	0.09
Zeaxanthin	0.09	0.002	−0.41	0.22
β-Cryptoxanthin	0.11	0.11	0.06	−0.09
β-Carotene	−0.05	−0.39	−0.22	−0.13

[a] $P < 0.05$ after Bonferroni correction.

those correlations were significant after Bonferroni correction, overall trend is highly significant with 19 of 24 correlations between hue and red pigments positive and 17 of 28 with yellow pigments negative (Fisher's exact test, P = 0.005). Note, however, that relative contribution of various component carotenoids varied among age groups and subspecies. For instance, 3-hydroxy-echinenone was the best predictor of male breast patch hue in hatch-year *frontalis* males but was a relatively poor predictor of breast hue for adult *frontalis* males, though both correlations were positive (Table 2). Likewise, 3-hydroxy-echinenone was the best predictor of male breast patch hue in hatch-year *griscomi* males, but was a poor predictor of breast hue for adult *griscomi* males, and in the opposite direction (Table 2). Proportion of red pigments in the plumage was a good predictor of breast patch hue for both hatch-year and adult *frontalis* (r = 0.60, P = 0.0001, n = 39, and r = 0.48, P = 0.04, n = 19, respectively) and hatch-year *griscomi* (r = 0.63, P = 0.0004, n = 28) males (Fig. 3). Thus, in general, redder males had a higher proportion of 4-keto-carotenoids, but the relationship did not hold for adult *griscomi* males (r = −0.19, P = 0.49, n = 15). Overall, relationship between hue and proportion of red pigments was significant and positive (ANCOVA, F = 24.7, df = 4 and 96, P < 0.0001) with no significant differences
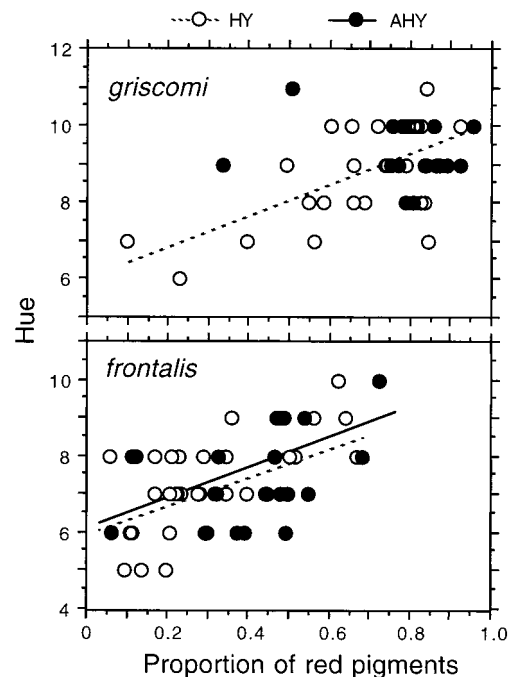

FIG. 3. The relationship between breast hue and proportion of 4-keto (red) carotenoid pigments for hatch-year and adult male House Finches from the subspecies (A) *C. m. frontalis* and (B) *C. m. griscomi.*

between ages ($F = 0.03$, df = 1 and 96, $P = 0.86$) or subspecies ($F = 1.68$, df = 1 and 96, $P = 0.20$), and no significant variation in slopes ($F = 0.52$, df = 1 and 96, $P = 0.47$).

Component carotenoids and individual variation in plumage saturation.—By visual assessment, intensity (saturation) of breast patch coloration varied modestly among males with 21 of 23 *frontalis* and 12 of 17 *griscomi* males assigned a color saturation of 6 or 7. Because few males had lower or higher intensity scores, we classified males into two groups with high-saturation males having scores of seven or higher and low-saturation males with six or lower. We had saturation scores for too few hatch-year males in either subspecies to allow us to explore differences between age classes, so the following analyses are based on data for both age classes combined. In each case, the same trends were found within adult males alone.

Saturation of plumage coloration is thought to be a function of the concentration of pigments in plumage (Ryan et al. 1994), so we compared the mean pigment concentration of males in the two saturation categories. High-saturation *frontalis* males had a significantly higher concentration of red ($t = 2.29$, $P = 0.03$, $n = 10, 13$) but not yellow ($t = 0.64$, $P = 0.53$) or total ($t = 1.39$, $P = 0.18$) pigments than low-saturation males and a significantly higher proportion of pigments that were red ($t = 2.89$, $P = 0.008$). However, high-saturation *griscomi* males did not have a higher concentration of red pigments ($t = 1.09$, $P = 0.30$, $n = 10, 7$), but they did have a significantly lower concentration of yellow pigments ($t = 3.34$, $P = 0.005$) and a significantly higher proportion of red pigments in their plumage ($t = 2.36$, $P = 0.03$). As in *frontalis* males, high- and low-saturation *griscomi* males did not differ significantly in total pigments ($t = 0.06$, $P = 0.95$). Thus, color saturation in the breast patch of *frontalis,* but not *griscomi,* males was positively related to concentration of red pigments. The saturation of *griscomi* breast patch was negatively related to concentration of the yellow pigments. Despite the assumption that saturation reflects pigment concentration, there appears to be only a weak link between various measures of pigment concentration and plumage saturation.

Subspecies comparisons.—Although the same 13 carotenoid pigments were found in the plumage of both *frontalis* and *griscomi* males, the prevalence of various component caroten-
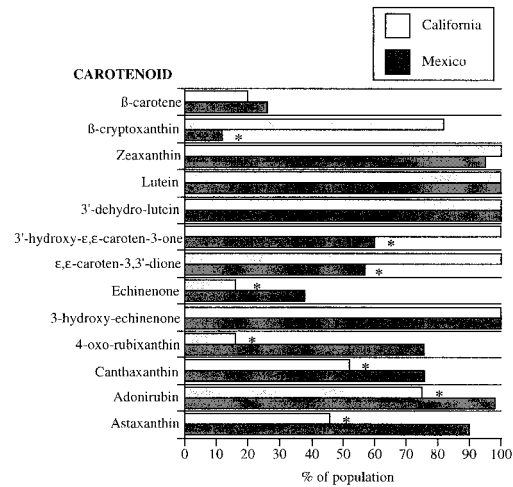

FIG. 4. Prevalence of carotenoids in plumages of male House Finches in the subspecies *griscomi* ($N = 43$) and *frontalis* ($N = 58$). Values shown are percentages of males displaying the respective carotenoid in their plumages. Data for adults and yearlings were pooled. An asterisk (*) indicates a significant difference between the subspecies ($P < 0.05$, Fisher's exact tests, see text).

oids differed between the subspecies. Lutein, 3'-dehydro-lutein, and 3-hydroxy-echinenone appeared in plumages of all males sampled in both populations (Fig. 4). Zeaxanthin, ϵ,ϵ-caroten-3,3'-dione, and 3'-hydroxy-ϵ,ϵ-caroten-3-one also occurred in all *frontalis* males (Fig. 4). Proportion of males with β-cryptoxanthin, 3'-hydroxy-ϵ,ϵ-caroten-3-one, ϵ,ϵ-caroten-3,3'-dione, 4-oxo-rubixanthin, adonirubin, and astaxanthin in their plumages differed significantly between the subspecies (Fisher's exact tests, all $P < 0.05$ after Bonferroni correction; Fig. 4). In contrast, there were no significant differences in proportion of adult and hatch-year males that had each of the carotenoids in their plumage in either subspecies (Fisher's exact tests, all $P > 0.05$ after Bonferroni correction).

Mean ($\pm$SE) total carotenoid concentration was more than twice as high in *griscomi* (885.4 $\pm$ 33.5 μg carotenoids/g feather) than in *frontalis* (405.5 $\pm$ 17.7) males, and this difference is highly significant (ANOVA, $F = 185.5$, df = 1 and 97, $P < 0.0001$; Fig. 1) with no significant age effect ($F = 2.96$, df = 1 and 97, $P = 0.09$). Using both subspecies and age as factors, we also found significant differences between *frontalis* and *griscomi* males (ANOVA, all $P < 0.05$ after Bonferroni correction, no interaction
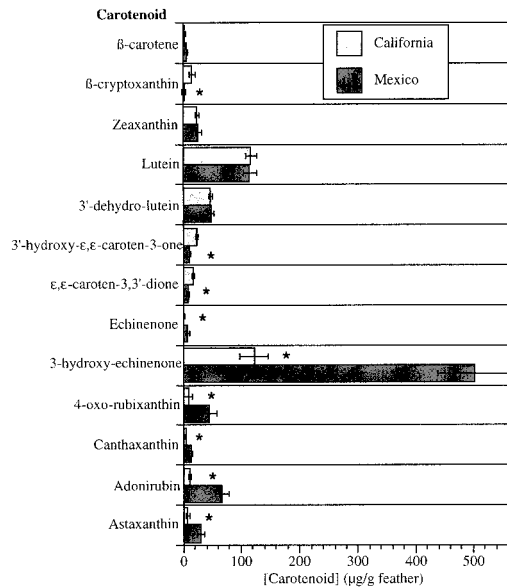
FIG. 5. Mean (±95% CL) concentrations (micrograms pigment per gram feather) of carotenoid pigments extracted from the feathers of male House Finches in the subspecies *griscomi* and *frontalis.* An asterisk (*) indicates a significant difference between the two populations (*P* < 0.05, *t*-tests, see text).

terms significant) in the mean concentrations of all carotenoids except β-carotene, zeaxanthin, lutein, and 3'-dehydro-lutein (Fig. 5). Levels of β-cryptoxanthin, 3'hydroxy-ε,ε-caroten-3-one, and ε,ε-caroten-3,3'-dione were significantly higher in *frontalis* males, whereas levels of echinenone, 4-oxo-rubixanthin, canthaxanthin, adonirubin, astaxanthin, and most notably 3-hydroxy-echinenone were significantly higher in *griscomi* males. In *frontalis,* adult males had significantly higher concentrations of the red pigment adonirubin than did hatch-year males, and significantly lower concentrations of the pigments astaxanthin, lutein, and zeaxanthin (ANOVA, all *P* < 0.05 after Bonferroni correction). That is an interesting result given that plumage of adult and hatch-year males from the *frontalis* population did not differ significantly in hue or saturation. Among *griscomi* males, only the concentration of adonirubin differed significantly between hatch-year and adult males.

The carotenoids present in the highest concentrations in male House Finch plumages were the red carotenoid 3-hydroxy-echinenone, and the yellow carotenoid lutein, together con-

stituting 69 ± 2.3% (mean ± 95% CI) of carotenoids by mass in the plumage of *frontalis* males and 58 ± 1.9% of carotenoids by mass in the plumage of *griscomi* males. The difference between subspecies, but not between age classes, was significant (ANOVA: subspecies, *F* = 53.2, df = 1 and 97, *P* < 0.0001; age, *F* = 1.30, df = 1 and 97, *P* = 0.26). The concentrations of those two carotenoids were significantly negatively related in *griscomi* (*r* = −0.58, *P* < 0.0001, *n* = 43), but not in *frontalis* (*r* = 0.07, *P* = 0.59, *n* = 58).

Four of the six most abundant carotenoids (≥4% of total carotenoids, by microgram per gram feather) found in the plumages of *frontalis* males were yellow (lutein, 3'-dehydro-lutein, zeaxanthin, and 3'-hydroxy-ε,ε-caroten-3-one, in decreasing abundance), whereas three of the five most abundant pigments in the plumages of *griscomi* males were red (3-hydroxy-echinenone, adonirubin, and 4-oxo-rubixanthin; Fig. 5).

To further analyze differences in total carotenoid composition between subspecies and age classes, we performed a multivariate analysis of variance (MANOVA) with all 13 carotenoid concentrations as dependent variables and both age and subspecies as factors. Only five of the carotenoid concentrations had normal distributions (Shapiro-Wilks' tests, *P* > 0.05) in each age class within subspecies sample and the others could not be normalized with transformations. MANOVA is robust to some departure from normality, so we ran that analysis using data for all 13 carotenoids. MANOVA using only the five carotenoids that had normal distributions resulted in exactly the same conclusions reported below.

The complete MANOVA (Pillai's trace, *F* = 4.3, df = 39 and 261, *P* < 0.0001) revealed significant age (*F* = 2.4, df = 13 and 85, *P* = 0.009) and subspecies (*F* = 21.1, df = 13 and 85, *P* < 0.0001) effects, and the interaction term was not significant (*F* = 1.4, df = 13 and 85, *P* = 0.18). We then performed separate MANOVA on each subspecies and age classes to further explore variation in plumage carotenoid composition. Those additional MANOVA revealed that adult and hatch-year males were significantly different in *frontalis* (*F* = 2.6, df = 13 and 29, *P* < 0.0001), but not *griscomi* (*F* = 1.4, df = 13 and 29, *P* = 0.21) and that *frontalis* and *griscomi* males differed significantly in the carotenoid
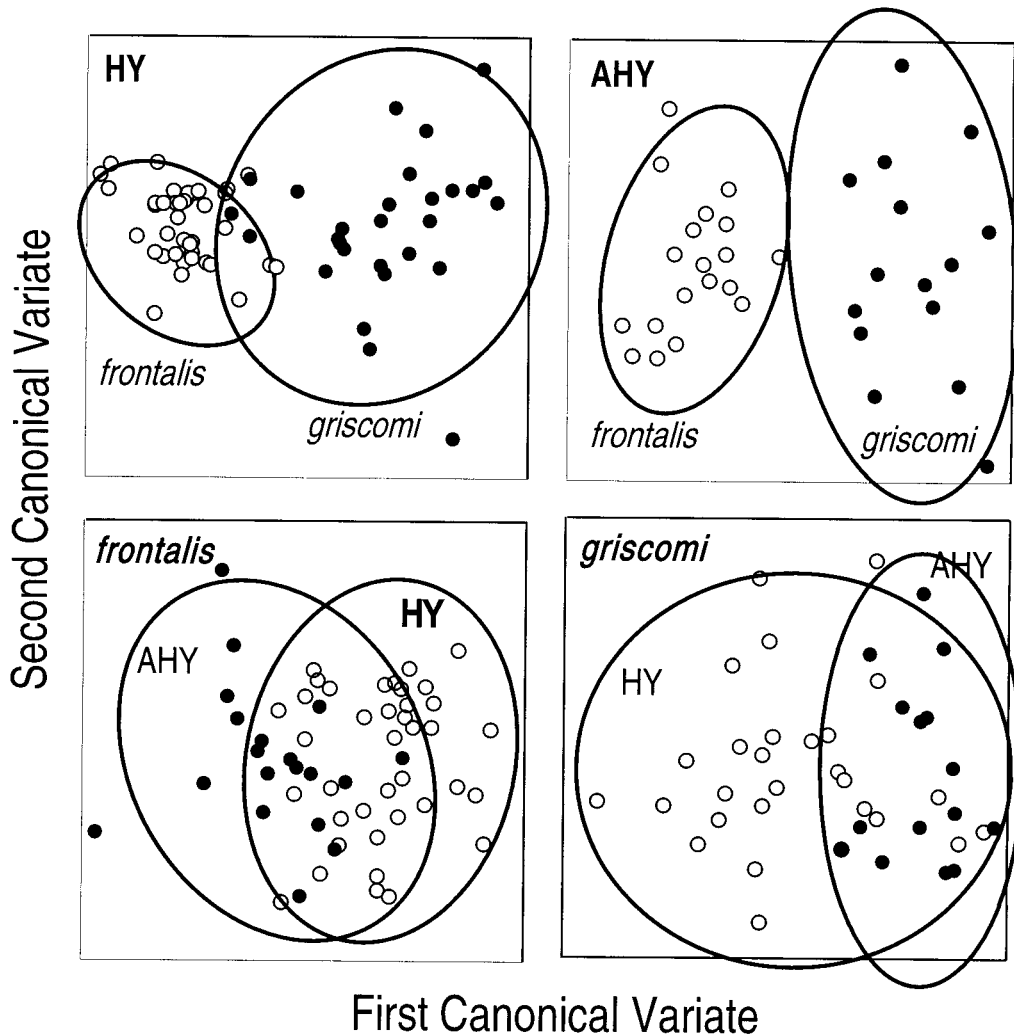
F<small>IG</small>. 6. Canonical variates plots showing variation between the subspecies *griscomi* and *frontalis* within each age class and variation between age classes HY (hatch-year) and AHY (adult) males within each subspecies. Canonical scores calculated from MANOVA using concentrations of all 13 component carotenoids; 95% confidence ellipses are shown. Axis units are not relevant in this study and have been removed for clarity.

compositions of both adult ($F = 10.4$, df = 13 and 20, $P < 0.0001$) and hatch-year males ($F = 13.5$, df = 13 and 53, $P < 0.0001$).

Plots of the canonical variate scores from the MANOVA (Fig. 6) clearly show that subspecies are well separated within age classes with the discriminant function calculated from the carotenoid concentrations correctly predicting all adult males ($n = 19$ *frontalis* and 15 *griscomi*), all hatch-year *frontalis* ($n = 39$), and 89% of hatch-year *griscomi* ($n = 28$). The first canonical

variate comparing subspecies was significantly positively correlated with most red pigments (six in hatch-year males, four in adults) and significantly negatively correlated with some yellow pigments (three in hatch-year males, two in adults; all $P < 0.05$ after sequential Bonferroni correction). Similarly, the first canonical variate comparing age classes was significantly correlated with both red (two positive in *griscomi*, two negative in *frontalis*) and yellow pigments (one negative in *griscomi*, three positive

in *frontalis*). Thus, the first canonical variate (Fig. 6) nicely illustrates age classes and subspecies along a continuum from yellow to red pigments, though in reverse order when comparing age classes in *frontalis* males.

DISCUSSION

Pigmentary basis for individual variation in plumage coloration.—For male House Finches of the subspecies *C. m. frontalis* and *C. m. griscomi*, the hue of ornamental plumage depended primarily on proportion of red (4-keto-carotenoids) versus yellow pigments deposited in feathers. Relationship between proportion of red pigments and plumage redness was strong and significant for hatch-year and adult *frontalis* males and for hatch-year *griscomi* males, but there was no relationship between plumage redness and proportion of red pigments in adult *griscomi* males. It seems likely that proportion of red pigments was not correlated with plumage redness in adult *griscomi* males, because there was little detectable variation in hue. That is, all adult males were bright red with a hue of 9 or 10. Lack of variation in plumage hue among adult *griscomi* males would also explain weak relationships between hue and virtually all component carotenoids (Table 2), the weak negative relationships between plumage redness and concentration of several red pigments, and the weak positive relationships between plumage redness and concentration of several yellow pigments (Table 2).

Contributions of specific carotenoids to variation in plumage hue were different for the various subspecies and age classes of males. 3-Hydroxy-echinenone was the most abundant red pigment in the plumage of males from both subspecies and age classes, but its concentration was not always the best predictor of plumage redness. Likewise, lutein was the most abundant yellow pigment in the plumage of both subspecies and age classes that we sampled, but its contribution to plumage redness varied among groups. Total carotenoid concentration in feathers also had a weak but significant effect on plumage hue.

Our observations are similar to those reported for Common (*Carduelis flammea*) and Hoary (*C. hornemanni*) redpolls, in which hue differences among individuals were attributed to the concentrations of both lutein (a yellow carot-

enoid) and echinenone (a red carotenoid) as well as relative concentration of lutein—individuals with higher levels of lutein appeared more orange (Troy and Brush 1983). Similarly, the color polymorphism exhibited by the Sooty-capped Bush Tanager (*Chlorospingus pileatus*) is due to differing concentrations of lutein in the feathers (Johnson and Brush 1972). 3-Hydroxy-echinenone has also been found to be the primary carotenoid pigment responsible for red plumage color in several other *Carpodacus* finches, for example, *C. roseus* and *C. rubricilloides* (Stradi et al. 1995a, b; 1997), as well as the plumages of Pine Grosbeak (*Pinicola enucleator*; Stradi et al. 1996).

The same basic relationship between redness of integumentary display and proportion of red carotenoid pigments has also been observed in fish. In the stickleback (*Gasterosteus aculeatus*), red males had primarily the red carotenoid pigment astaxanthin in their skin whereas yellow males had primarily yellow pigments tunaxanthin and lutein in their skin (Wedekind et al. 1998).

The color saturation (intensity) of ornamental plumage appeared to depend primarily on concentration of carotenoids in feathers, but relationship between saturation and carotenoid concentration was different for *frontalis* and *griscomi* males. Among *frontalis* males, total concentration of red pigments was the best predictor of plumage color saturation, but among *griscomi* males, total concentration of yellow pigments was the best predictor of plumage intensity. Most *griscomi* males had abundant red pigments in their plumage, and variation in concentration of red pigments apparently had little influence on plumage color saturation. On the other hand, abundance of yellow pigments was variable, and that variation in yellow-pigment concentration appeared to determine plumage intensity in *griscomi* males. Conversely, most *frontalis* males had abundant yellow pigments but variable concentrations of red pigments. In that subspecies, the concentration of red pigments determined plumage color saturation. Overall, the relationship between color saturation and pigment concentration was not as strong as expected. However, color saturation was difficult to assess by the visual scoring methods used in this study. Hence, error in scoring saturation may have obscured patterns. Future studies that quantify plumage satura-

tion with a spectrophotometer may better re-
solve the issue of what pigment properties de-
termine plumage saturation.

Age effects.—We found significant differences
in plumage color and ventral patch size be-
tween adult and hatch-year males in *griscomi*
but not *frontalis.* The smaller patches of color
and drabber plumage of hatch-year *griscomi*
males is not surprising, because males from
that subspecies have been shown to have a dis-
tinctive and female-like first-year plumage
(Hill 1996b). In *frontalis* populations, hatch-
year males have generally been observed to be
less colorful than adults (Michener and Mich-
ener 1931, Gill and Lanyon 1965, Hill 1992,
1993b), but hatch-year and adult males exhibit
the same range of colors. What was surprising
was that, despite delayed plumage maturation,
adult and hatch-year *griscomi* did not differ sig-
nificantly in pigment composition of their
feathers. Clearly, many hatch-year *griscomi*
males had the same carotenoid composition in
their feathers as adult males (Fig. 6), but others
had more and higher concentrations of yellow
pigments. In contrast, despite their similarity
in appearance, adult and hatch-year *frontalis*
finches had significantly different pigment
compositions, suggesting that different carot-
enoid combinations may result in the same
plumage coloration.

Differences in the pigment composition of
the feathers of adult and hatch-year *frontalis*
males may reflect differences between those
age groups in physiological mechanisms in-
volved in feather pigmentation. Higher levels
of zeaxanthin and lutein and the lower levels of
adonirubin and astaxanthin in hatch-year com-
pared to adult *frontalis* males suggest that
hatch-year males may have a greater tendency
to deposit unmodified dietary carotenoids di-
rectly into the feathers. Thus, ability to convert
dietary precursors into 4-keto-carotenoids may
increase with age. Similar results have been
documented for female Red-winged Blackbirds
(*Agelaius phoeniceus*) in which epaulet color
changed from yellow in juveniles to orange in
adults (Miskimen 1980). Such changes could be
regulated by sex hormones (Stoehr and Hill
2001), such that ability to convert dietary ca-
rotenoids into the redder 4-keto-carotenoids is
enhanced by onset of sexual maturity. The age-
related differences in pigment composition
may also be due to differences between adult

and hatch-year birds in access to dietary carot-
enoids, levels of parasite infection, immuno-
competence, or general nutrition during molt.

*Implications for potential pathways of carotenoid
metabolism.*—Birds are known to be capable of
metabolically altering ingested carotenoids
(Fox et al. 1969, Schiedt et al. 1985, Tyczkowski
and Hamilton 1986a, b, c, d; Brush 1990,
Schiedt 1990). Much of the transformation and
modification of dietary carotenoids occurs by
the introduction of oxo- or hydroxy-groups
into the main β-ionone ring (Schiedt 1990), by
the alteration of end rings, for example, a β-
into an ε-ring (Davies 1985), or both. Stradi et
al. (1996) indicated that many cardueline finch-
es are capable of converting carotenoids by this
pathway, for example, converting dietary zea-
xanthin into astaxanthin that is deposited into
feathers.

Results from the present study suggest that
House Finches are also capable of the addition
of a keto group at the C-4 position, C-4′ posi-
tion, or both, thus explaining presence of red 4-
keto-carotenoids in the feathers. Keto groups at
the C-4(′) position functionally extend the
chain of conjugated double bonds in the carot-
enoid molecule, causing a bathochromic shift,
that is, shift of hue towards red (Hudon 1994b).
Thus, higher levels of 4-keto-carotenoids may
intensify redness of feathers, accounting for the
observation that redder House Finches had
higher proportions of 4-keto-carotenoids in
their plumages.

Many vertebrates have the ability to convert
β- into ε-end rings, which shortens the chain of
conjugated double bonds and alters carotenoid
color to bright yellow (Matsuno et al. 1985,
Miki et al. 1985). In birds, that may be done to
produce ''canary xanthophylls'' (Stradi et al.
1995a, b; 1997). House Finches may also be ca-
pable of those conversions, as demonstrated by
presence of the yellow carotenoids, ε,ε-caroten-
3,3′-dione and 3′-hydroxy-ε,ε-caroten-3-one, in
feathers. Those pigments may be produced
from dietary sources of lutein or zeaxanthin be-
cause House Finch diets probably do not con-
tain ε,ε-carotenoids (Inouye 1999). Many birds
have been shown to oxidize hydroxy- into keto-
groups at the C-3(′) position (Stradi et al. 1996),
and that mechanism may account for the oc-
currence of 3′-dehydro-lutein in House Finch
feathers, converted from dietary lutein.

Subspecies comparisons.—We found substantial differences in amounts and kinds of carotenoids in both hatch-year and adult males from the two subspecies. Those differences were large and consistent enough that we were able to use carotenoid concentrations to classify correctly to subspecies all adult males and 96% of hatch-year males on the basis of discriminant function analysis. In *frontalis* males, the yellow 3′-hydroxy-ϵ,ϵ-caroten-3-one and ϵ,ϵ-caroten-3,3′-dione were more likely to be found in plumage than the other component carotenoids. Those yellow carotenoids are most likely derived from dietary sources of lutein or zeaxanthin. On the other hand, the 4-keto-carotenoids, echinenone, 4-oxo-rubixanthin, canthaxanthin, adonirubin, and astaxanthin, in the plumage occurred in a greater percentage of the population of *griscomi* males. With the exception of astaxanthin, those carotenoids are probably not lutein- or zeaxanthin-derived. Those results indicate (a) that there may be differences between the two subspecies in levels of dietary lutein and zeaxanthin, and (b) that *griscomi* males demonstrate increased capacities for adding keto- functions at C-4(′), whereas *frontalis* males show increased capacities for converting β- into ϵ-end rings.

Carotenoid-based coloration as an honest signal.—In this paper, we describe the pigmentary basis for variation in color expression among male House Finches. The data, however, provide no direct information about what causes some males to have more red pigments in their feathers than other males. Diet may play a role in determining plumage coloration in males (Hill 1992). Male House Finches must either ingest red pigments (which are rare in the diet; Inouye 1999) or specific precursors to the red pigments that are ultimately deposited in the feathers. However, the role of dietary access to carotenoid pigments in determining variation in expression of plumage coloration among wild birds remains controversial (Hill 1994c, 1999; Hudon 1994a, Thompson et al. 1997, Inouye 1999). To convert precursors in their diet to red pigments in their feathers, male House Finches must establish and maintain complex physiological systems for carotenoid absorption, transport, and deposition (Allen 1987, Erdman et al. 1993, Parker 1996, Furr and Clark 1997, Hill 2002). Those systems require energy (Hill 1996a, 2002), although whether or not the energy required for carotenoid utilization is great enough to constrain expression of ornamental plumage coloration is unknown (Hill 1996a, Inouye 1999). There is evidence that nutritionally stressed House Finches produce less red plumage than birds that are not stressed even when they have access to the same carotenoid pigments (Hill 2000). Furthermore, parasites potentially play a large role in determining expression of carotenoids; coccidia may inhibit intestinal absorption of carotenoids and have been shown to decrease plumage redness upon molt in male House Finches (Hill and Brawner 1998, Brawner et al. 2000). Other diseases that affect the overall health of finches, including pox and *Mycoplasma galliceptum,* cause males to grow a less red plumage (Thompson et al. 1997, Nolan et al. 1998, Brawner et al. 2000). Carefully controlled studies will be required to determine the relative contribution of those various factors to the expression of ornamental plumage coloration in wild House Finches.

The differences in plumage coloration and plumage pigment composition that we observed between age classes, between subspecies, and among males within an age class and subspecies may be the result of any of the factors listed above acting alone or in combination. The different mix of carotenoid pigments in the plumages of hatch-year versus adult *frontalis* House Finches suggests that hatch-year males may utilize dietary carotenoid pigments differently than adult males, and such age-specific carotenoid utilization has potentially important implications for signal content of ornamental plumage coloration in this species (Hill 1990, 1994a, 2002). In addition, differences in the carotenoid composition of feathers of males from the two subspecies that we sampled suggest that those subspecies utilize different metabolic pathways in modifying dietary pigments. Whether those differences in pigment physiology evolved under sexual selection for brighter color display (Hill 1994c) and what the difference means for signal honesty in those two subspecies can perhaps be answered through a comparative study looking at pigment composition of other subspecies as well as the evolutionary relationships of those taxa.

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