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PROXIMATE BASIS OF VARIATION IN CAROTENOID PIGMENTATION IN MALE HOUSE FINCHES

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ABSTRACT.—In the wild, male House Finches (*Carpodacus mexicanus*) vary in plumage color from pale yellow to bright red. I investigated the proximate basis of this variation in plumage brightness, as well as the basis for variation in the extent of ventral carotenoid pigmentation. Regardless of their age or coloration in the wild, captive males converged on a similar appearance after completing prebasic molt on a standardized diet, with significantly less variance in coloration than is found among wild males. Captive males that were fed a diet deficient in carotenoid pigments grew pale yellow feathers; males fed a diet supplemented with β-carotene grew pale orange feathers; and males fed a diet supplemented with canthaxanthin grew bright red feathers. Stored carotenoids did not appear to be an important source of feather pigments. Red males captured from the wild just prior to fall molt and fed a carotenoid-deficient diet did not grow more colorful feathers than males that had been held in captivity on a carotenoid-deficient diet for six to nine months prior to fall molt. In a wild House Finch population in southeastern Michigan, the mean plumage coloration of yearling males was significantly drabber than the mean coloration of older males, although both groups displayed approximately the same range of coloration. Wild males tended to become brighter between their first and second springs, but thereafter, males tended to display a similar plumage coloration between years. The extent of ventral carotenoid pigmentation (color-patch size) also was partly dependent on carotenoid intake. Captive males whose diet was supplemented with canthaxanthin produced significantly larger patches after captive molt than before captive molt, and canthaxanthin-supplemented males also expressed significantly larger patches than males in the carotenoid-deficient or β-carotene-supplemented groups. Among wild males, there was a significant positive correlation between patch size and plumage brightness. *Received 17 September 1990, accepted 24 July 1991.*

THE PHYSIOLOGY of plumage coloration that results from carotenoid pigmentation has been a topic of research for more than a century (Bogdanov 1856, 1858 cited *in* Voitkevich 1966; Volker 1938). It has long been known that birds cannot synthesize carotenoid pigments and must obtain them from food (Goodwin 1950, Brush 1978). With few exceptions, however, studies of bird coloration have been aimed at elucidating the biochemical and genetic basis of general color patterns among species (Fox and Hopkins 1966, Brush and Johnson 1976, Troy and Brush 1983, Buckley 1987). The basis for variation among individuals in a population has received little attention, despite a growing need in studies of the function of coloration for an understanding of the proximate basis of individual variation (Kodric-Brown 1985, 1989, Hill 1990, 1991).

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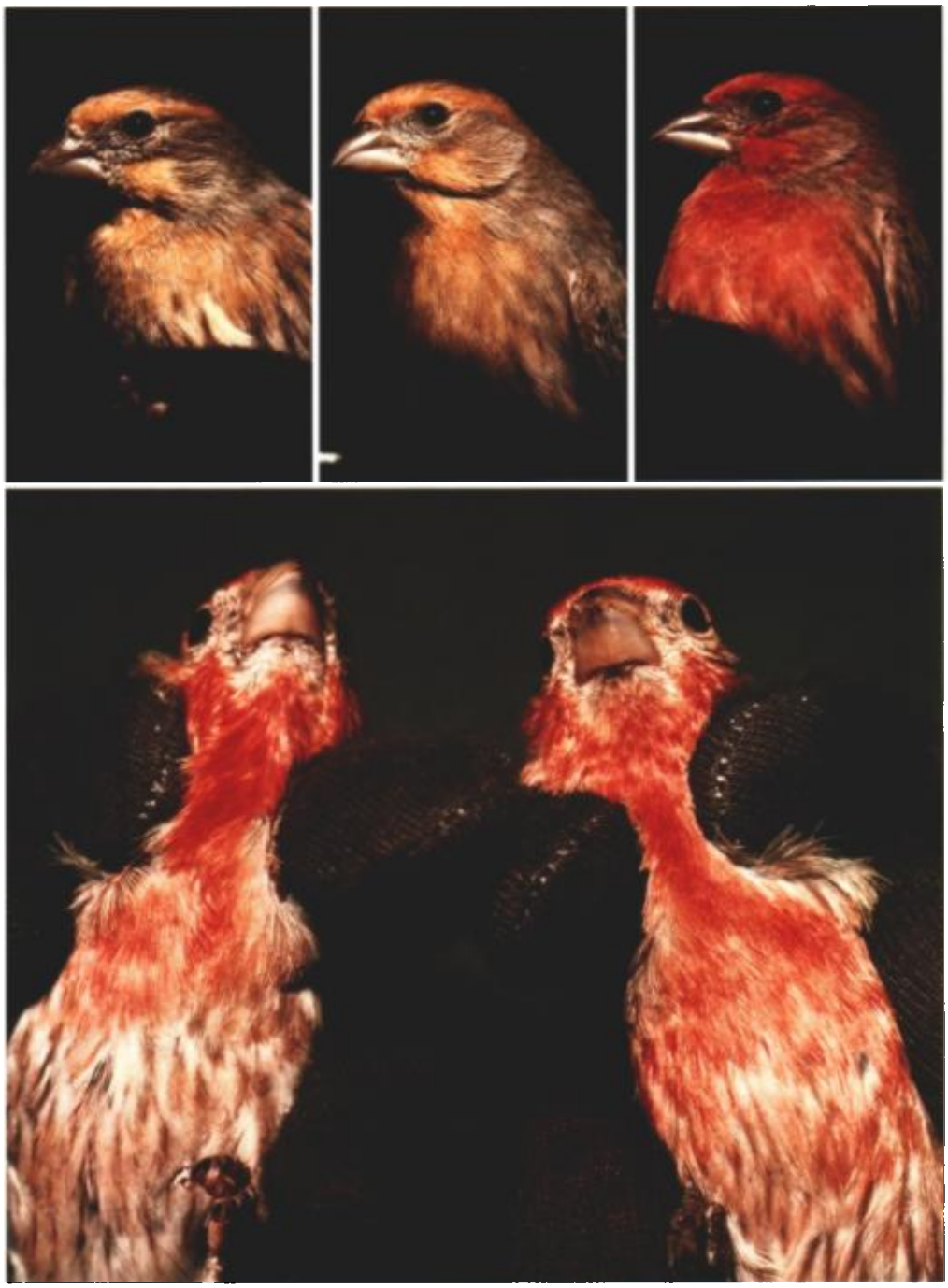

FRONTISPIECE. Male House Finches (*Carpodacus mexicanus*) that underwent captive molt on: (Upper Left) carotenoid-deficient diet; (Upper Middle) β-carotene-supplemented diet; (Upper Right) canthaxanthin-supplemented diet. (Lower) Two male House Finches captured in Ann Arbor, Michigan in May 1990 showing natural variation in extent of ventral carotenoid pigmentation. Males had similar plumage coloration, but extent of ventral carotenoid pigmentation (patch size) is substantially greater on male to the right.

One of the few species for which the biochemical basis of carotenoid coloration and individual color variation has been studied in detail is the House Finch (*Carpodacus mexicanus*), a sexually dichromatic passerine in which males display carotenoid pigmentation that varies continuously within populations from pale yellow to bright red (Michener and Michener 1931, Gill and Lanyon 1965, Hill 1990). All male House Finches show the same basic pattern of pigmentation, with carotenoid pigment concentrated in three "patches" of feathers on the crown-eyestripe, throat-breast, and rump. The extent of the throat-breast patch (hereafter called the ventral patch) varies substantially both within and between populations.

House Finches of all ages undergo one annual (prebasic) molt in the late summer and fall (Stangel 1985) at which time males acquire their plumage coloration for the following year. Prior to their first prebasic molt, male House Finches show no carotenoid pigmentation. Throughout this paper, I will refer to males that have not undergone a first prebasic molt as juveniles, males in a first definitive plumage acquired through a first prebasic molt as yearlings, and males in subsequent plumages as ASY (after second year).

Brush and Power (1976) analyzed the plumage of male House Finches with different plumage coloration and found that yellow plumage contained β-carotene, orange plumage contained β-carotene plus isocryptoxanthin, and red plumage contained β-carotene and isocryptoxanthin plus echinenone. To evaluate the extent to which color variation among male House Finches reflects variation in intake of carotenoid pigments, Brush and Power (1976) conducted a series of controlled feeding experiments. They fed one group of wild-caught captive males a nutritious but carotenoid-deficient diet of seeds and water, and a second group the same diet but with canthaxanthin added to their water. Canthaxanthin is a red carotenoid that House Finches assimilate and use to pigment their plumage as they would naturally use echinenone. Brush and Power (1976) allowed the birds in both groups to complete the annual fall molt and then examined the coloration of their plumage. Despite variation in plumage coloration among the males prior to treatment, all males in the canthaxanthin-supplemented group grew uniformly bright-red plumage, while males in the carotenoid-deficient group grew uniformly pale-yellow plumage. The diets of the males were then reversed, and several months later a pigmented region of the throat of each bird was plucked to induce molt. Once again, males on the canthaxanthin-rich diet grew red feathers and those on a carotenoid-deficient diet grew drab-yellow feathers.

These experiments demonstrated that the appearance of a male House Finch is dependent on the carotenoid content of its diet and that, under different dietary conditions, individual males have the capacity to display opposite extremes of character expression. These were pioneering studies, and they remain the best analyses of the basis of individual variation in carotenoid pigmentation in birds. However, these studies left several fundamental questions unresolved. Brush and Power (1976) reported that birds fed canthaxanthin were "uniformly bright red," but they did not quantify the plumage coloration of males. Thus, they did not adequately address the extent to which color variation persists within a population when carotenoid intake is standardized. Quantification of the extent of plumage variation among males before and after feeding experiments is essential for an understanding of the extent to which individual variation in pigmentation is a function of carotenoid access rather than variation in the use of ingested pigments. In addition, Brush and Power (1976) presented evidence that House Finches can use stored carotenoids, but their data did not indicate the extent to which male House Finches can rely on stored carotenoids in pigmenting their plumage. They also did not address the effect of age on a male's ability to display carotenoid pigments. And finally, the experiments conducted by Brush and Power (1976) did not address the role of diet in determining the size of colored patches of feathers.

I conducted a series of controlled feeding experiments and concurrent field observations of a wild population of House Finches aimed at clarifying and extending the results of Brush and Power (1976). I used a standardized scoring system to quantify male plumage coloration and patch size. In the feeding experiments, I used known-age male House Finches, and I scored individuals before and after they underwent molt on a diet in which their intake of various carotenoid pigments was controlled. My goal

was to gain a better understanding of the proximate basis of individual variation in plumage coloration in male House Finches.

Methods

General methods.—I conducted the field portion of this research in 1988–1990 on the main campus of the University of Michigan, Ann Arbor. House Finches were trapped at feeding stations, scored for plumage coloration and ventral patch size, banded, and released. About 80% of the resident males were banded in 1988, and about 90% in 1989 and 1990. For age comparisons, I used only males whose age was certain. Yearling males were banded either in the nest or in juvenile plumage in the previous year, and ASY males were present in adult plumage for at least a second breeding season.

I captured House Finches for captive flocks away from the study population at feeding stations in southeastern Michigan and southwestern Ohio. Most birds were aged at the time of capture by examining their skulls for extent of pneumatization (July through November; Klimkiewicz 1980). Birds were housed in unisex flocks in large flight cages (2.5 × 2.5 × 4 m) on the roof of the Museum of Zoology on the University of Michigan campus. All birds were provided water treated with "Vita-sol" multivitamins (8 In 1 Products Inc., Hauppauge, New York) and were fed a basic diet of "oil" sunflower seeds and commercial finch seed. In 1988, birds were provided with "Frank's Wee Bird Seed" (Frank's Nursery and Crafts, Detroit, Michigan), which contained canary seed (13% by weight), rape seed (13%), flax (13%), white millet (50%), red millet (10%), and thistle (1%). In 1989, I switched to "Kaytee Wild Finch Food" (Kaytee Products Inc., Chilton, Wisconsin), which contained canary seed (33%), niger seed (20%), rape seed (12%), finch millet (10%), white millet (10%), red millet (5%), flax (5%), and calcium granules (5%). This latter source of finch seed likely provided a better source of β-carotene than the seed used in 1988. The Kaytee product was more yellow in coloration and, as described below, males achieved a brighter plumage than on the product from Frank's Nursery and Crafts. Seed was provided *ad libitum* by placing it in hanging feeders. This basic diet was augmented according to the specific experiment.

I scored the overall coloration of males by quantifying coloration of seven pigmented plumage regions: four areas on the underside (see Fig. 1 for the size and orientation of these ventral regions), plus the crown, eyestripe and rump. I recorded the plumage coloration of each region as a three-number code that recorded the hue, intensity (chroma), and tone (value) of the coloration by comparison to color chips in the *Methuen Handbook of Colour* (Kornerup and Wanscher 1983). Hue scores ranged from colorless (1) through yellow (ca. 2–4) and orange (ca. 5–8) to red (ca. 9–11). Intensity ranged from 1 to 8 and tone from 1 to 6. I then added the 21 plumage scores that resulted from this analysis to derive a single index value, which was used as an estimate of the overall plumage brightness of a male's coloration (for additional details, see Hill 1990).

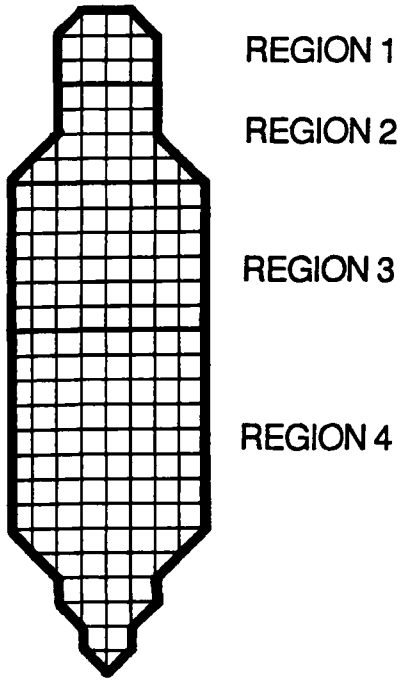

Fig. 1. Template used to score ventral plumage coloration and extent of ventral carotenoid pigmentation (patch size) in male House Finches. Plumage coloration scored for each of four regions, and patch size measured by counting number of squares containing plumage with carotenoid pigmentation. Grid squares were 3 mm^2. Region 1 is anterior to other regions.

In addition to scoring the coloration of males, I also recorded the extent of ventral carotenoid pigmentation using a gridded transparency overlay (Fig. 1). I positioned the transparency over the ventral side of a male and adjusted the transparency so that the top of region 1 was positioned at the base of the lower mandible of the bird. I then counted the number of squares that covered feathers pigmented with carotenoids. Size variation among the birds that I handled was insignificant and, thus, the standard template fit all males adequately. I scored the plumage coloration and measured the patch size of all males at the time of capture and again after the completion of prebasic molt in captivity.

Both plumage-coloration and patch-size measurements were repeatable. I independently rescored the

plumage coloration and ventral patch size of 40 males and calculated the repeatability (R) of these measures using the interclass correlation coefficient (plumage score, $R = 0.98$, $F_{39,40} = 181.44$, $P < 0.0001$; patch size, $R = 0.88$, $F_{39,40} = 11.22$, $P < 0.0001$; Lessells and Boag 1987).

Experiment 1.—In June 1988, I partitioned a group of 56 males into two groups, which I housed in separate flight cages. I fed the males in one group (5 yearling and 15 older males) the basic diet plus *ad libitum* chopped apples. I fed males in the other group (20 yearling and 16 older males) the basic diet plus *ad libitum* chopped carrots or sweet potatoes. Apples contain only a trace of carotenoids (Gebhardt et al. 1982) and were used as a control for the effects of fresh vegetables on the carrot-supplemented group. Carrots and sweet potatoes contain large quantities of β-carotene, but have few xanthophylls (i.e. red carotenoids; Gebhardt et al. 1982, Bureau and Bushway 1986), so these were used as a source for β-carotene. Birds in both treatment groups consumed large quantities of apples or carrots and sweet potatoes, and they molted healthy-looking plumage in the late summer and fall in synchrony with wild House Finches. I scored the plumage coloration of males in this experiment in mid-October 1988 after all birds had completed their prebasic molt.

Experiment 2.—In June 1989 I partitioned a group of 48 males into three treatment groups, which I housed in separate flight cages. I fed males in one group (3 yearling and 22 adult males) the basic diet plus chopped apples coated with canthaxanthin (Roxathin Red 10 WS, Hoffmann-LaRoche; ca. 0.01 g/gram of apples) and water treated with the same (ca. 0.001 g/milliliter of water). Males in a second group (1 yearling and 10 older males) received the basic diet plus chopped apples coated with 10% water-dispersible β-carotene beadlets (Hoffmann-LaRoche, ca. 0.01 g/gram of apples) and water treated with the same (ca. 0.001 g/milliliter of water). Males in the third group (12 adult males) received untreated water and apples.

RESULTS

Plumage color in relation to diet.—In experiment 1, males on the carrot-supplemented diet molted into plumage that was significantly brighter than the plumage of males on the carotenoid-deficient diet ($t = 9.34$, df = 54, $P < 0.0001$, one-tailed t-test; Table 1). However, after captive molt, males from both treatment groups were much less colorful than typical wild males in southeastern Michigan (carotenoid deficient, $t = 16.89$, df = 566, $P < 0.0001$; carrot supplemented, $t = 15.24$, df = 582, $P < 0.0001$; one-tailed t-test; Table 1). For both groups, the mean post-treatment plumage score was significantly lower than the mean pre-treatment plumage score (carotenoid deficient, $t = 15.67$, df = 28, $P < 0.0001$; carrot supplemented, $t = 3.65$, df = 30, $P < 0.001$; one-tailed paired t-test; Table 1).

Males on the carotenoid-deficient diet in experiment 2 grew pale-yellow plumage (Frontispiece) that was substantially less colorful than that of typical wild males ($t = 11.23$, df = 558, $P < 0.0001$; one-tailed t-test; Table 1), but significantly brighter than that of males fed a carotenoid-deficient diet in 1988 ($t = 2.60$, df = 30, $P = 0.014$; two-tailed t-test; Table 1). The difference in plumage coloration between males from the two carotenoid-deficient treatment groups was likely a result of the different seed mixtures provided to the males in the two groups. A richer (and yellower) seed mix that probably provided more β-carotene was used in experiment 2, and males grew brighter plumage. The mean post-treatment plumage score of males in the carotenoid-deficient group in experiment 2 was significantly lower than their mean pre-treatment score ($t = 10.94$, df = 11, $P < 0.0001$; one-tailed paired t-test; Table 1).

Males provided with a β-carotene-supplemented diet in experiment 2 molted pale orange plumage (Frontispiece) that was significantly brighter than that of males on the carotenoid-deficient diets in experiment 1 ($t = 7.28$, df = 29, $P < 0.0001$; one-tailed t-test; Table 1) or experiment 2 ($t = 3.39$, df = 21, $P < 0.0001$, one-tailed t-test; Table 1), but less colorful than the average wild male in southeastern Michigan ($t = 8.28$, df = 557, $P = 0.0001$; one-tailed t-test; Table 1). The appearance of these males was very similar to that of males fed carrots in 1988, and there was no significant difference in the mean plumage scores of the two groups ($t = 0.38$, df = 45, $P = 0.70$; two-tailed t-test; Table 1). β-carotene-supplemented males in experiment 2 also had a mean post-treatment plumage score that was significantly lower than their mean pre-treatment plumage score ($t = 5.49$, df = 9, $P < 0.0001$; one-tailed t-test; Table 1).

All males fed a diet supplemented with canthaxanthin in experiment 2 attained bright reddish plumage (Frontispiece) that was significantly more colorful than that of males from either of the other treatment groups in experiment 2 ($P < 0.05$ for both comparisons; Scheffé pairwise F-test; Table 1). The intensity and tone of the plumage of these canthaxanthin-supplemented males were like the brightest males

TABLE 1. Plumage brightness scores of male House Finches before and after captive molt on specified diets.

Diet group[a]	Age[b]	*n*	Pre-treatment $\bar{x} \pm$ SD	Post-treatment $\bar{x} \pm$ SD	t[c]	P[c]	F_S[d]	P[d]
Wild unmanipulated	U	548	145.0 ± 12.0	—	—	—	—	—
			Experiment 1					
Carotenoid deficient	Y + A_t	20	—	99.4 ± 6.2	—	—	—	—
	Y	5	—	105.0 ± 5.1	—	—	—	—
	A_t	15	144.8 ± 10.3	97.5 ± 5.5	15.7	0.0001	3.5	0.02
	A_1	5	149.4 ± 4.4	96.6 ± 6.0	15.8	0.0001	1.8	0.25
Carrot supplemented	Y + A_t	36	—	114.2 ± 5.4	—	—	—	—
	Y	20	—	113.9 ± 5.3	—	—	—	—
	A_t	16	132.1 ± 18.3	114.6 ± 5.7	3.7	0.001	10.3	0.001
	A_1	2	130.0 ± 7.1	110.0 ± 7.1	—	—	—	—
			Experiment 2					
Carotenoid deficient	A_t	12	149.3 ± 7.5	105.8 ± 7.7	11.7	0.0001	1.1	0.25
	A_1	2	158.0 ± 5.6	92.6 ± 1.4	—	—	—	—
β-carotene supplemented	Y + A_t	11	—	114.9 ± 4.5	—	—	—	—
	Y	1	—	112	—	—	—	—
	A_t	10	148.7 ± 9.1	114.4 ± 4.2	6.5	0.0001	4.7	0.05
	A_1	2	160.0 ± 2.8	110.0 ± 2.8	—	—	—	—
Canthaxanthin supplemented	Y + A_t	25	—	146.2 ± 3.5	—	—	—	—
	Y	3	—	146.5 ± 0.07	—	—	—	—
	A_t	22	138.8 ± 17.3	146.0 ± 2.1	1.9	0.03	24.7	0.001
	A_1	3	143.3 ± 21.1	147.7 ± 2.1	—	—	—	—

[a] Wild males captured in Ann Arbor, Michigan between 1 February and 1 July 1988–1990. Carotenoid-deficient diet (untreated water and plain apples), carrot-supplemented diet (untreated water and chopped carrots), β-carotene-supplemented diet (water and apples treated with 10% β-carotene beadlets), and canthaxanthin-supplemented diet (water and apples treated with 10% canthaxanthin beadlets).

[b] U = age unknown; Y = yearling; A_t = two years old or older; A_1 = two years old or older, and captured just prior to fall molt.

[c] Paired two-tailed *t*-test comparing pre- and post-treatment means.

[d] *F*-test comparing pre- and post-treatment variances.

found in the wild, but the hue of their plumage was slightly more orange than the brightest red males from southeastern Michigan. Consequently, no canthaxanthin-supplemented males achieved plumage scores quite as high as the most colorful wild males, and the mean plumage scores of canthaxanthin-supplemented males and wild males were very similar (t = 0.52, df = 571, P = 0.60; two-tailed t-test; Table 1). The mean post-treatment plumage score of males in the canthaxanthin-supplemented group in experiment 2 was significantly greater than the mean pre-treatment score (t = 1.88, df = 21, P = 0.04; one-tailed paired t-test; Table 1).

Patch size in relation to diet.—Dietary intake of carotenoids affected not only the coloration of male plumage, but also the extent of ventral pigmentation. There were no differences in mean patch size among treatment groups before captive molt (F = 1.47, df = 4 and 70, P = 0.22; ANOVA; Table 2), and captive molt on a carotenoid-deficient diet in experiments 1 and 2, or a β-carotene-supplemented diet in experiment 2 did not significantly affect patch size (carotenoid-deficient experiment 1, t = 0.32, df = 14, P = 0.75; carotenoid-deficient experiment 2, t = 1.02, df = 11, P = 0.33; β-carotene supplemented, t = 0.85, df = 9, P = 0.43; paired two-tailed t-test; Table 2). However, males in the carrot-supplemented group in experiment 1 and the canthaxanthin-supplemented group in experiment 2 had significantly larger ventral patches after captive molt than before (carrot supplemented, t = 2.37, df = 15, P = 0.03; canthaxanthin supplemented, t = 9.66, df = 21, P < 0.0001; paired two-tailed t-test; Table 3). In addition, canthaxanthin-supplemented males had significantly larger patches than either β-carotene-supplemented males in experiment 1 (t = 9.36, df = 43, P < 0.0001) or experiment 2 (t = 8.13, df = 34, P < 0.0001; two-tailed t-test; Table 2), or carotenoid-deficient males in experiment 1 (t = 10.24, df = 59, P < 0.0001) or experiment 2 (t = 7.15, df = 35, P < 0.0001; two-tailed t-test; Table 2). There were no differences in patch size between β-carotene-supplemented and carotenoid-deficient groups in either experiment 1 or 2 (experiment 1, t = 1.78,

TABLE 2. Proportion of ventral plumage of male House Finches with carotenoid pigmentation before and after captive molt on specified diets.

Diet group[a]	Age[b]	n	Pre-treatment $\bar{x} \pm$ SD	Post-treatment $\bar{x} \pm$ SD	t[c]	P[c]	F_S[d]	P[d]
Wild unmanipulated	U	548	0.66 ± 0.11	—	—	—	—	—
			Experiment 1					
Carotenoid deficient	Y + A	20	—	0.63 ± 0.09	—	—	—	—
	Y	5	—	0.66 ± 0.09	—	—	—	—
	A	15	0.61 ± 0.08	0.62 ± 0.10	0.3	0.75	1.4	0.25
Carrot supplemented	Y + A	36	—	0.67 ± 0.07	—	—	—	—
	Y	20	—	0.65 ± 0.06	—	—	—	—
	A	16	0.56 ± 0.19	0.69 ± 0.08	2.4	0.02	6.3	0.005
			Experiment 2					
Carotenoid deficient	A	12	0.67 ± 0.11	0.70 ± 0.05	1.0	0.33	5.9	0.01
β-carotene supplemented	Y + A	11	—	0.69 ± 0.02	—	—	—	—
	Y	1	—	0.66	—	—	—	—
	A	10	0.65 ± 0.16	0.69 ± 0.02	0.8	0.43	52.2	0.001
Canthaxanthin supplemented	Y + A	25	—	0.84 ± 0.06	—	—	—	—
	Y	3	—	0.84 ± 0.03	—	—	—	—
	A	22	0.60 ± 0.10	0.84 ± 0.06	9.7	0.0001	2.6	0.05

[a] See footnote *a* of Table 1.
[b] U = age unknown; Y = yearling; A = two years old or older.
[c] Paired two-tailed *t*-test comparing pre- and post-treatment means.
[d] *F*-test comparing pre- and post-treatment variances.

df = 54, $P = 0.08$; experiment 2, $t = 0.80$, df = 21, $P = 0.43$; two-tailed *t*-test; Table 2). Among wild males I found a significant positive correlation between patch size and plumage coloration ($r^2 = 0.32$, $n = 548$, $P = 0.0001$; Fig. 2).

Pre- and post-treatment color and patch-size variation.—Little variation in plumage coloration persisted among males within the treatment groups. Only ASY males were used in this comparison, because yearling males have streaky brown plumage with no carotenoid pigmentation prior to their first prebasic molt. In experiment 1, the variance in plumage coloration scores among experimental males prior to their captive molt was an order of magnitude greater than the variance in plumage scores after captive molt in the carrot-supplemented group ($n = 16$, $F_S = 10.3$, $P < 0.001$; Table 1; variance test following Sokal and Rohlf 1981:354–356) and three times greater in the carotenoid-deficient group ($n = 15$, $F_S = 3.5$, $P < 0.02$; Table 1). In experiment 2, variance in pre-treatment plumage scores was significantly greater than in post-treatment scores in both the β-carotene- and canthaxanthin-supplemented groups (β-carotene supplemented, $n = 9$, $F_S = 4.69$, $P < 0.025$; canthaxanthin supplemented, $n = 22$, $F_S = 11.07$, $P < 0.001$; Table 1). There was no difference in variance in pre- and post-manipulation plumage scores for the carotenoid-deficient group in experiment 2 ($n = 12$, $F_S = 1.05$, $P > 0.50$; Table 1). The variance in post-treatment plumage scores among males in this group was similar to the variance observed among males in other treatment groups. The lack of difference in variance among pre- and post-treatment males is due to a particularly low pre-treatment variance (Table 1). The variance in post-treatment plumage scores among males in the carotenoid-deficient group was still significantly less than that observed among wild males (carotenoid deficient, $n = 12$, and wild, $n = 548$; $F_S = 2.43$, $P < 0.05$).

Variance among males in patch size also tended to decrease when carotenoid intake was standardized. I found no difference in the variance of pre- and post-treatment patch sizes among males in the carotenoid-deficient group in experiment 1 ($n = 15$, $F_S = 1.37$, $P > 0.25$; Table 2), but for all other groups the variance in pre-treatment patch sizes was significantly greater than the variance in post-treatment patch sizes (carrot supplemented, $n = 16$, $F_S = 6.27$, $P < 0.005$; carotenoid-deficient experiment 2, $n = 12$, $F_S = 5.91$, $P < 0.01$; β-carotene supplemented, $n = 10$, $F_S = 52.23$, $P < 0.001$; canthaxanthin

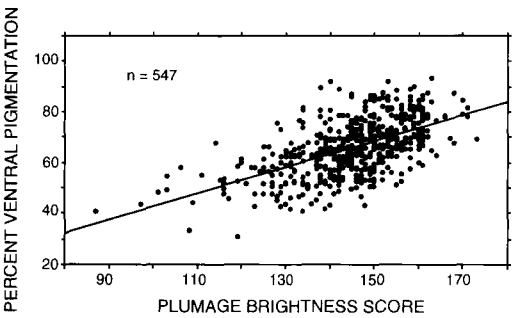

Fig. 2. Extent of ventral pigmentation as function of plumage brightness.

supplemented, $n = 22$, $F_S = 2.60$, $P < 0.05$; Table 2). Despite this drop in patch-size variance among males on a standardized diet, patch size remained substantially more variable than did coloration in the three treatment groups with 20 or more birds (carotenoid-deficient experiment 1, $n = 20$, $F_S = 2.30$, $P < 0.05$; carrot supplemented, $n = 36$, $F_S = 2.21$, $P < 0.025$; canthaxanthin supplemented, $n = 25$, $F_S = 3.00$, $P < 0.01$; F-test comparing coefficients of variance).

To investigate whether "naturally" colorful males are more efficient in their use of carotenoid pigments than "naturally" drab males, I looked at the association of relative plumage brightness scores (difference from the treatment-group mean) of each male before and after captive molt. Regardless of the treatment group, I found no significant positive correlations between relative pre- and post-treatment scores (carrot-supplemented experiment 1, $r^2 = 0.11$, $n = 16$, $P = 0.11$; carotenoid-deficient experiment 1, $r^2 = 0.14$, $n = 15$, $P = 0.09$; β-carotene-supplemented experiment 2, $r^2 = 0.27$, $n = 10$, $P = 0.08$; canthaxanthin-supplemented experiment 2, $r^2 = 0.01$, $n = 22$, $P = 0.60$; Figs. 3 and 4). For the carotenoid-deficient treatment group in experiment 2, there was actually a significant negative correlation ($r^2 = 0.37$, $n = 12$, $P = 0.04$; Fig. 4). I also found no significant positive correlations between pre- and post-treatment patch size (carotenoid-deficient experiment 1, $r^2 = 0.01$, $n = 15$, $P = 0.76$; carrot-supplemented experiment 1, $r^2 = 0.01$, $n = 16$, $P = 0.79$; carotenoid-deficient experiment 2, $r^2 = 0.09$, $n = 12$, $P = 0.33$; β-carotene-supplemented experiment 2, $r^2 = 0.002$, $n = 10$, $P = 0.90$; canthaxanthin-supplemented experiment 2, $r^2 = 0.001$, $n = 22$, $P = 0.89$).

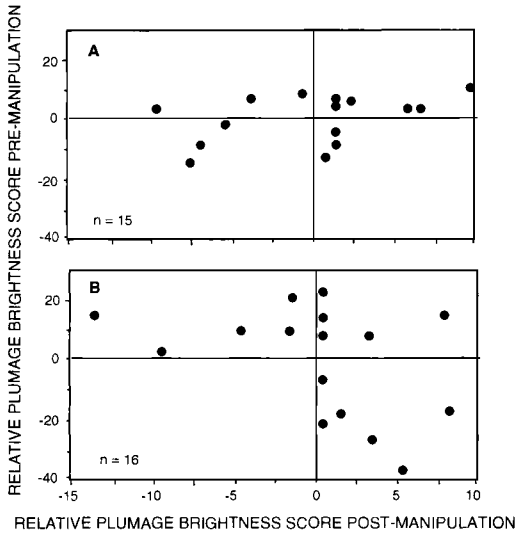

Fig. 3. Relationship of relative plumage coloration of male House Finches before and after captive molt in 1988 on: (A) carrot-supplemented (β-carotene-rich) diet; and (B) carotenoid-deficient diet. Values are differences from group mean for plumage brightness scores.

Age effects.—Captive feeding experiments indicated that yearling males have the same potential to be colorful or drab as do older males. In the carotenoid-deficient treatment group in experiment 1, yearling males actually had significantly higher plumage brightness scores than older males ($t = 2.70$, df = 18, $P = 0.015$, two-tailed t-test; Table 1), but the sample size was small. There were no significant differences in coloration between yearling and older males in the carrot-supplemented group in experiment 1 ($t = 0.40$, df = 34, $P = 0.70$; Table 1). Few yearling males were included in experiment 2, but as in experiment 1, they seemed to be able to acquire plumage that was as bright as that of older males. The two yearling males in the canthaxanthin-supplemented group had plumage scores very close to the group mean, as did the single yearling male in the β-carotene-supplemented group (Table 1). There were also no differences in patch size between yearling and ASY males in any treatment group (carotenoid-deficient experiment 1, $t = 0.74$, df = 18, $P = 0.47$; carrot supplemented, $t = 1.47$, df = 34, $P = 0.15$; canthaxanthin supplemented, $t = 0.05$, df = 23, $P = 0.96$; two-tailed t-test; Table 2).

In a wild southeastern Michigan population, I found that the mean plumage coloration

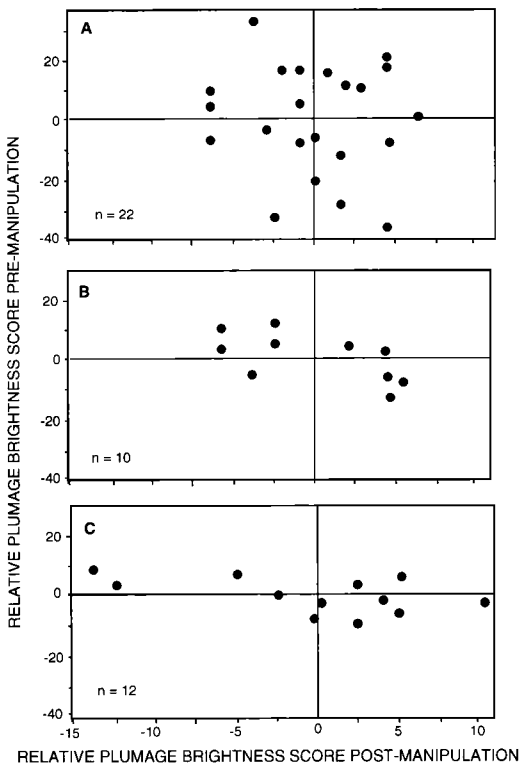

Fig. 4. Relationship of relative plumage coloration of male House Finches before and after captive molt in 1989 on: (A) canthaxanthin-supplemented diet; (B) β-carotene-supplemented diet; and (C) carotenoid-deficient diet. Values are differences from group mean for plumage brightness scores.

of yearling male House Finches was significantly drabber than the mean coloration of older males (yearling: $\bar{x}$ = 146.0, SD = 8.6, n = 23; adult: $\bar{x}$ = 152.9, SD = 6.9, n = 74; t = 3.96, df = 95, P = 0.0001; two-tailed t-test; Fig. 5). By comparing the plumage scores of individual males between years, I was also able to investigate the degree to which individual appearance changes between years and the effect of age on annual plumage change. I recorded the plumage scores of 53 males in consecutive springs (when all males are in definitive plumage). In year 1 of the comparison, 17 of these males were at least two years old (known from banding data) and the remaining 36 were of unknown age. Because the latter group excluded known ASY males (both the 17 recaptured ASY males as well as a larger group of ASY males that returned but were not recaptured) and was composed entirely of previously unbanded males, it likely contained mostly yearling males. Males tended to get brighter with age (mean change = 6.7, t = 4.47, df = 52, P = 0.001, two-tailed paired t-test), but ASY males tended to increase by less than unknown-age males (ASY males, mean change = 4.2, t = 2.36, df = 16, P = 0.03; unknown-age males, mean change = 9.0, t = 3.9, df = 35, P = 0.0004; two-tailed paired t-test). Assuming that unknown-age males are mostly yearlings, this result suggests that males tend to increase more in plumage brightness between their first and second springs than between subsequent springs. In an evaluation of the association of year 1 and year 2 plumage scores of individual males, I found a significant positive correlation for ASY males (r^2 = 0.42, n = 17, P = 0.005; Fig. 6), but no significant correlation for unknown-age males (r^2 = 0.05, n = 36, P = 0.20; Fig. 6). Thus, it appears that, between their first and second years, males tend to increase substantially in plumage brightness and that brightness of first basic plumage is a poor predictor of the brightness of subsequent basic plumages. After their second prebasic molt, males tend to increase relatively little in plumage brightness.

Like plumage brightness, patch size increased significantly between years for unknown-age males (mean change = 0.11, t = 7.46, P = 0.0001; two-tailed paired t-test), but not for ASY males (mean change = 0.03, t = 1.37, df = 16, P = 0.18; two-tailed paired t-test). However, I found no significant differences in the mean patch size of wild yearling and ASY males captured in Michigan (t = 1.43, df = 95, P = 0.16; two-tailed t-test).

Carotenoid storage.—To test the extent to which House Finches can use stored carotenoids in pigmenting their plumage, I considered the pre- and post-treatment scores of 14 males captured in July, just prior to the start of their prebasic molt, which begins in late July or early August for House Finches in the eastern U.S. (Stangel 1985; Hill, unpubl. data). Eleven of these males were assigned to the carotenoid-deficient or β-carotene-supplemented treatment groups, and three to the canthaxanthin-supplemented group. These males had more than 11 months in the wild to store carotenoids for prebasic molt prior to being captured, but their plumage scores following captive molt were essentially the same as the scores of other males in their treatment groups that had been in captivity for six to nine months (Tables 1 and 2). Only the 1988 carot-

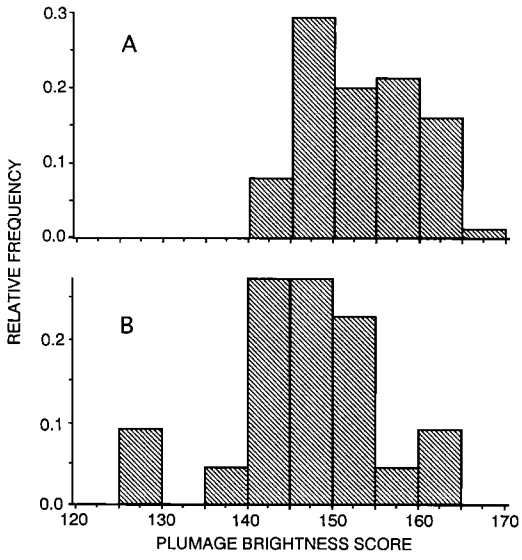

Fig. 5. Distribution of plumage brightness scores of known-age male House Finches captured in Ann Arbor, Michigan: (A) Two years old or older ($n = 74$); (B) yearling ($n = 23$).

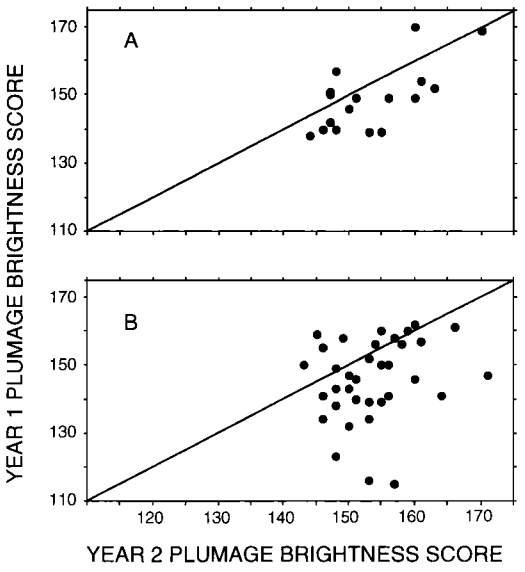

Fig. 6. Between-year change in individual plumage coloration. Individuals above diagonal decreased in plumage score; those below diagonal increased in plumage score. (A) Two years old or older in year 1; (B) age unknown in year 1.

enoid-deficient group and the pooled 1988 and 1989 β-carotene-supplemented groups included enough late-caught males for statistical comparison and, in both cases, there were no significant differences in the mean post-treatment plumage scores of early- and late-caught males (carotenoid deficient, $t = 0.30$, df = 18, $P = 0.77$; β-carotene supplemented, $t = 1.60$, df = 45, $P = 0.12$; two-tailed t-test; Table 1). None of the late-caught males fed a carotenoid-deficient or β-carotene-supplemented diet showed any hint of red or pink in their plumage. The failure of late-caught males to pigment their plumage with stored carotenoids did not appear to be an effect of handling or adjustment to captivity near the time of molt; the three late-caught males in the canthaxanthin treatment group grew red plumage like that of males that had been held in captivity on a carotenoid-deficient diet for six to nine months prior to captive molt ($t = 0.76$, df = 23, $P = 0.45$; two-tailed t-test; Table 1).

Discussion

The results of feeding experiments indicate that variation in plumage coloration among male House Finches is due to differential access to carotenoid pigments at the time of molt, and not to intrinsic differences among males in the ability to use or express carotenoid pigments. When the carotenoid intake of a group of captive males was standardized, regardless of whether carotenoid availability was high or low, all males converged on a similar appearance. The variance in plumage coloration that persisted among males on a standardized diet was, in all but one treatment group, significantly lower than the variance in the same group of males prior to treatment and, in all cases, significantly lower than the variance in plumage coloration found in wild males in southeastern Michigan. In addition, in all treatment groups a male's "natural" (pre-treatment) plumage coloration was a poor predictor of its relative coloration within a group after captive molt. If there were intrinsic differences among males in their ability to assimilate and express carotenoid pigments, then one would expect that males that are relatively bright in the wild would do better on a fixed intake of carotenoids than males that are relatively dull males in the wild. However, I found no correlation between the pre- and post-treatment plumage scores in any feeding experiments. One could argue that, in experiments in which males were provided with large quantities of β-carotene or canthaxanthin, any differences among males in the potential

to display coloration were overwhelmed by artificially high concentrations of carotenoid pigments. However, this argument cannot apply to experiments in which males were fed a carotenoid-deficient diet; in these treatment groups, there was no relationship between pre- and post-manipulation scores.

Ventral patch size was also affected by dietary intake of carotenoids. There was a strong positive correlation between patch size and coloration among wild males, and captive males that were fed a canthaxanthin-supplemented diet displayed a significantly larger mean patch size than wild males or males fed a carotenoid-deficient or β-carotene-supplemented diet. However, unlike expression of plumage coloration, expression of patch size did not appear to be completely diet-dependent. Standardizing carotenoid intake among groups of males significantly reduced variation in expression of patch size in all but one treatment group, but in the three treatment groups with a large sample size, there was significantly more post-treatment variance in patch size than in plumage brightness. Moreover, in the field I observed many individuals with similar coloration but quite different patch sizes (Frontispiece). Thus, it appears that there is little additive genetic variance for expression of plumage brightness (all males have the same potential to be colorful or dull), but that expression of patch size is in part due to genetic variance for expression of the trait (individuals differ in their potential for expression of patch size).

My observations also indicate that yearling male House Finches from Michigan do not differ from older males in their potential to display colorful plumage. In controlled feeding experiments, yearling males responded to various levels of dietary carotenoids in a manner that was similar to adult males. The only significant difference between yearling and older males in any treatment was that a small sample of yearling males that were fed carrots in 1988 displayed a mean plumage coloration that was significantly brighter than the plumage of older males that had been fed the same diet. As in previous studies on wild House Finches in New York (Gill and Lanyon 1965) and California (Michener and Michener 1931), my field observations in southeastern Michigan indicated that yearling males are less colorful on average than older males, although they display approximately the same range of plumage variation as older males. Given laboratory experiments indicating that yearling and ASY males have the same potential to display colorful plumage, the differences in plumage coloration between yearling and older males in the wild likely are a result of experienced older males gaining access to a greater quantity of carotenoids by outcompeting or foraging more efficiently than yearling males.

Recapture data suggest that male House Finches tend to increase substantially in plumage brightness between their first and second springs, but relatively little thereafter. Such age-specific individual change in plumage coloration would account for the differences in mean plumage scores of ASY and yearling males, and supports the hypothesis that differences in coloration between yearling and older males arise through differential access to carotenoids. Age-specific patterns of change in male coloration have been noted in many passerine species in which males display delayed plumage maturation (for a recent review, see Rohwer and Butcher 1988), but these examples involve the loss of a yearling-specific plumage, not a change in the brightness of definitive plumage as in House Finches. In two other passerine species in which the annual change in the coloration of individual males in definitive alternate plumage has been examined (Yellow Warbler, *Dendroica petechia*, Studd and Robertson 1985; Black-headed Grosbeak, *Pheucticus melanocephalus*, Hill 1987), adult males displayed a similar plumage coloration between years.

Another line of evidence is consistent with the hypothesis that differential access to carotenoid pigments is primarily responsible for variation among males in expression of plumage coloration. This involves the observation that male House Finches cannot use stored carotenoids as a significant source of feather pigments. It would seem much less likely that resource limitation plays a key role in determining individual expression of plumage coloration if male House Finches had an entire year between molts to accrue pigment. Brush and Power (1976) found traces of canthaxanthin in the plumage of captive male House Finches that had not ingested canthaxanthin for weeks prior to their molt. These investigators presented this observation as evidence that House Finches use stored carotenoids in pigmenting their plumage. However, I found bright-red male House Finches that were captured just prior to fall molt and

put on a carotenoid-deficient diet grew color-deficient plumage very similar to the plumage produced by males that had been fed a carotenoid-deficient diet for months prior to molt. Thus, although small quantities of stored carotenoids may be used in pigmenting feathers, which would account for the observation of Brush and Power (1976), stored carotenoids are not an important source of feather pigments in the House Finch.

Other studies of the importance of stored carotenoids in the pigmentation of feathers have yielded variable results. In a study of three species of African weaver finches, Kritzler (1943) found that, after three months on a carotenoid-deficient diet, *Euplectes afra* grew a normally pigmented yellow plumage, *Ploceus cucullatus* grew a slightly dulled yellow plumage, and *E. nigroventris* grew a pale-yellowish plumage (much drabber than typical plumage). In contrast, Ring-necked Pheasants (*Phasianus colchicus*) apparently do not store appreciable amounts of carotenoid pigments (Thommen 1971), and two months on a carotenoid-deficient diet was sufficient to cause flickers (*Colaptes auratus*) to grow virtually unpigmented feathers (Test 1969). When carrots were added to the diet of flickers, however, bright carotenoid pigments appeared in developing feathers in a few hours (Test 1969). Test's study indicates that birds can respond very rapidly to dietary carotenoids and that, for at least some species, a high daily intake of carotenoid pigments throughout the period of feather replacement may be essential for maximum plumage coloration.

The observation that expression of plumage coloration in male House Finches is a function of dietary intake of carotenoid pigments at the time of molt has important implications for hypotheses concerning the evolution of plumage coloration. Laboratory and field experiments have shown that male plumage coloration is an important criterion in female mate choice in the House Finch (Hill 1990, 1991) and that the plumage coloration of a male is a reliable indicator of its capacity to feed offspring (Hill 1991). Thus, male plumage coloration in the House Finch is precisely the sort of condition-dependent trait that is predicted by the honest-advertisement model of sexual selection (Zahavi 1975, Kodric-Brown and Brown 1984, Andersson 1986). However, the sources of carotenoids used by wild male House Finches in pigmenting their plumage and the factors that mediate access to carotenoid resources remain unknown.

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