

Carotenoid access and intraspecific variation in plumage pigmentation in male American Goldfinches (*Carduelis tristis*) and Northern Cardinals (*Cardinalis cardinalis*)

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Summary

1. Behavioural ecologists have shown much interest recently in the factors that promote variation in carotenoid-based colour displays. Because vertebrates cannot synthesize the pigments needed to attain this form of bright coloration, most research has focused on the effect of dietary access to pigments on carotenoid ornamentation.
2. However, few studies have considered experimentally how the availability of carotenoid pigments in the diet influences variability in the expression of carotenoid-based coloration.
3. Dietary access to carotenoids was manipulated in captive groups of male American Goldfinches (*Carduelis tristis* L.) and Northern Cardinals (*Cardinalis cardinalis* L.) to assess the role of pigment acquisition in shaping intraspecific variation in carotenoid-based plumage pigmentation.
4. Substantial variation was detected in the plumage displays of captive male goldfinches and cardinals within a diet treatment, such that birds with unlimited access to the same diet type displayed carotenoid-based plumage variation that did not differ significantly from that typical of wild males from each species. Additionally, the natural plumage brightness that male goldfinches displayed at capture predicted the degree to which they grew colourful plumage under controlled conditions in our experiments.
5. These results suggest that carotenoid-based plumage variation in male goldfinches and cardinals is less sensitive to pigment availability in the diet than colour variation observed in other studied taxa. Differences in ornamental coloration among males from these two passerine species may instead reflect their ability to utilize ingested pigments physiologically.

Key-words: Carotenoid pigments, House Finch, ornamental coloration, plumage variation, sexual selection

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Introduction

Some of the most striking features of organisms are the brilliant colours that are displayed by male animals. Colour ornaments range from ultraviolet to red, and tremendous variation exists in the degree of expression of bright coloration both within and among species. To date, evolutionary biologists have focused primarily on understanding variation in coloration among males within a species and how the brightness of individuals relates to aspects of mate quality and sexual selection (reviewed in Andersson 1994; Savalli 1995).

Carotenoid-based colour displays have been of particular interest as animal signals (reviewed in Olson &

Owens 1998; Hill 1999). Carotenoid pigments are responsible for most of the bright red, orange and yellow colours seen in animals (Fox 1976). In a variety of species in which males display carotenoid-based ornamental coloration, females prefer to mate with the most intensely pigmented males (e.g. Kodric-Brown 1985; Burley & Coopersmith 1987; Hill 1990; Burley, Price & Zann 1992; Johnson, Dalton & Burley 1993; Sundberg 1995). However, the proximate mechanisms underlying variation in carotenoid-based signal expression remain contentious and unresolved. Understanding the environmental, physiological and genetic factors that control carotenoid display is a critical step toward identifying the benefits that females receive from mating with brightly coloured males.

Early work with zoo animals demonstrated that captive animals deprived of natural diets were far less

colourful than those in the wild (Delacour 1928; Wackernagel 1959). We have subsequently learned that carotenoid pigments cannot be synthesized *de novo* by vertebrates, and that individuals must ingest pigments in the diet before they can be deposited in their integument (Völker 1938; Goodwin 1984). These observations led to the hypothesis that the degree of expression of carotenoid-based coloration primarily reflects dietary access to pigments (Brush 1978, 1981; Endler 1983; Hill 1990). Support for this idea has come from conditions in the wild in which changes in male coloration are associated with spatial or temporal variation in environmental carotenoid availability (Slagsvold & Lifjeld 1985; Ryan, Moloney & Hudon 1994; Hudon *et al.* 1996; Witmer 1996; Linville & Breitwisch 1997; Grether, Hudon & Millie 1999) as well as from captive experimental studies in which carotenoid content in the diet is manipulated (Fox & McBeth 1970; Fox, McBeth & Mackinney 1970; Brush & Power 1976; Kodric-Brown 1989; Hill 1992, 1993a).

One of the best studied of all animals displaying carotenoid-based ornamental coloration is the House Finch (*Carpodacus mexicanus*). Remarkably, it is the only avian species for which the effect of carotenoid access on intraspecific variation in carotenoid pigmentation has been studied experimentally. Male House Finches vary in their expression of carotenoid-based coloration from bright red to orange to pale yellow (Hill 1993b). Hill (1992, 1993a) investigated proximate control of pigment displays in House Finches by feeding groups of captive males standardized amounts of carotenoid pigments to determine the degree to which they could express plumage that was as bright and as variable as wild birds. He found that captive males that were fed the same diet displayed significantly less variation in carotenoid-based plumage coloration than wild males (Hill 1992, 1993a). Moreover, he found that birds that had naturally red plumage before completing moult in captivity failed to grow brighter plumage than males who displayed naturally drab plumage before the experiment. These results provide the foundation upon which much of our understanding of the proximate bases for variation in carotenoid-based signals has been built, and support the hypothesis that differences in pigment acquisition play an important role in generating variability in carotenoid-based ornamental coloration.

Because the effect of pigment access in the diet on the expression of carotenoid-based coloration has been tested experimentally only in the House Finch, a species that displays highly variable breeding plumage compared with other birds, it seems that more research on the mechanisms underlying variation in carotenoid ornaments is warranted. In this study, we designed feeding experiments similar to those for House Finches to assess how variation in dietary access to carotenoid pigments affects plumage brightness and plumage variation in two other North American passerines exhibiting carotenoid-based ornamental color-

ation – the American Goldfinch (*Carduelis tristis* [Linnaeus]) and the Northern Cardinal (*Cardinalis cardinalis* [Linnaeus]).

American Goldfinches, like House Finches, are sexually dichromatic cardueline finches from the family Fringillidae. Males exhibit carotenoid-based breeding plumage pigmentation on the throat, breast, back, and rump that varies in colour from bright yellow to drab yellow, while females are mostly olive and have only a small patch of yellow throat feathers (Middleton 1993). Also similar to House Finches, female goldfinches prefer to mate with the most colourful males (Johnson, Dalton & Burley 1993), and male coloration is an honest indicator of the degree of parasitic infection during moult (Olson 1996; McGraw & Hill 2000). The Northern Cardinal is a sexually dichromatic passerine species from the family Emberizidae. Male cardinals exhibit carotenoid-based plumage coloration throughout the year that varies from bright red to drab red, whereas red carotenoid pigmentation in females is restricted to the bill, crest, tail and underwings (Halkin & Linville 1999). Although it is not yet clear whether females choose mates based on variation in male plumage brightness (Wolfenbarger 1999a), redder males begin breeding earlier in the season, defend a higher-quality territory and fledge more offspring in a season than do less red males (Wolfenbarger 1999b). Brighter males also provide a greater proportion of a pair's feeding efforts to nestlings (Linville, Breitwisch & Schilling 1998). By observing the effect of a carotenoid shortage in the wild on the plumage brightness of cardinals, Linville & Breitwisch (1997) found that pigment availability in the diet can influence carotenoid-based colour displays in this species, but, as with American Goldfinches, the effect of dietary access to pigment on intraspecific variation in carotenoid-based plumage has yet to be tested.

Following the methods of Hill (1992, 1993a), groups of male goldfinches and cardinals were housed in captivity during moult and all males in each group were provided with a diet that contained the same type and amount of carotenoid pigments. The coloration of natural plumage that males displayed at capture as well as the subsequent plumage grown under our experimental conditions were scored. Plumage colour variation among captive males fed the same diet during moult was compared with that found in wild males of each species. The relationship between the plumage brightness of individual males prior to our feeding experiments and that displayed after completing a captive moult under controlled conditions was also examined. We predicted that, if dietary access to carotenoids has a strong impact on plumage variation in the wild, we would see little variation in plumage brightness among males fed the same diet type in captivity. Similarly, the natural plumage colours that males grew in the wild and brought into captivity with them should not predict the degree to which they express bright coloration after our feeding experiments.

Materials and methods

AMERICAN GOLDFINCHES

Between 15 and 24 January 1999, 58 male goldfinches were captured in basket traps at established feeding stations in Lee County, Alabama. These males were divided randomly into four groups that ranged in size from 13 to 15 birds, and the groups were housed in separate outdoor flight cages (3.7 m long \times 1.5 m wide \times 2.4 m high). At capture, birds were aged as either first year or after first year based on plumage characteristics (Middleton 1974). Also at this time, the carotenoid-based plumage coloration of males was quantified using a Colortron™ reflectance spectrophotometer (Light Source, Inc., San Rafael, CA, USA), a device that objectively measures the hue, saturation and brightness of colour patches (Hill 1998; see below for more methodological details). Goldfinches replace their colourful breeding plumage in the autumn with a prebasic moult that leaves them with yellow carotenoid-based plumage only at their throats (Middleton 1993). Two saturation measurements were taken (see below for justification) from the throat of each bird with the Colortron™ and these scores were averaged to obtain our measure of winter plumage colour (mean percentage saturation).

For the duration of time spent in captivity, male goldfinches were fed an *ad libitum* diet of thistle, sunflower hearts and water. To keep males healthy, their water was treated with 6.6 drops l^{-1} of Premium Multi-Drops™ high-potency multivitamins (Eight in One® Pet Products, Inc., Hauppauge, NY) and 0.26 g l^{-1} of sulfadimethoxine (Sigma®, St. Louis, MO). Sulfadimethoxine is a drug used to treat coccidiosis (Brawner, Hill & Sundermann 2000), and treatment in this study fully suppressed coccidial infections (results presented in McGraw & Hill 2000). The experiment began on 1 March, approximately 2 weeks before males began their prealternate moult. Two of the captive groups of goldfinches ($n = 28$ males) received a carotenoid-supplemented diet while they were growing their colourful feathers in the spring. The other two groups of males ($n = 30$ birds) continued receiving the standard diet of seed and water for the duration of the study. Additional pigments to the carotenoid-supplemented groups were provided by dissolving beadlets of canthaxanthin (Roxanthin Red 10 WS, Roche Vitamins Inc., Parsippany, NJ; approx. 0.001 g ml^{-1} of water) in the birds' water. Although goldfinches do not obtain this pigment in their natural diet to colour their feathers (K. McGraw, G. Hill & R. Stradi, unpublished data), this red pigment is readily absorbed and deposited in plumage by other cardueline finches, including House Finches (Inouye *et al.* 2001), other red fringillids (Stradi 1998) and even by other yellow *Carduelis* species when it is available to them in the diet (R. Stradi, personal communication). Hill (1992, 1993a) used this same concentration of canthaxanthin

in his House Finch experiments, in addition to using an unsupplemented seed diet similar to that used in this study, to show that all House Finches fed the same diet converge on a similar plumage appearance. Thus, canthaxanthin was used in our study to maintain consistency across feeding experiments for different species and because it was readily available commercially.

Males completed their prealternate moult by 1 June, and at this time carotenoid-based breeding plumage coloration was scored with the Colortron™. As with winter coloration, mean saturation was used as our index of plumage brightness because this unit of measurement is most variable among wild goldfinches and is a significant mate choice criterion in this species (Johnson, Dalton & Burley 1993). This time, three measurements were taken from each of three pigmented regions on the body (nape, throat and breast) and these nine scores were averaged to compute mean plumage saturation. The same was done for a group of 12 wild male goldfinches in breeding plumage that were captured in Ithaca, NY, between 29 August and 5 September 1999. In a double-blind test, using a subsample of goldfinches from our study that were euthanized and rescored on 8 February 2001, we found that quantifying plumage saturation with the Colortron™ was highly repeatable ($r = 0.99$, $F_{18,19} = 396.3$, $P < 0.0001$) when depressing the unit firmly against the feathers of the birds.

Between the two cages of male goldfinches within each diet treatment, there were no significant differences in either mean plumage saturation (carotenoid-unsupplemented: $t = 1.20$, $P = 0.47$; carotenoid-supplemented: $t = -0.74$, $P = 0.24$) or plumage variation (carotenoid-unsupplemented: $F = 1.66$, $P = 0.36$; carotenoid-supplemented: $F = 1.13$, $P = 0.83$). We also found no significant differences between the age classes in plumage saturation (carotenoid-unsupplemented: $t = 1.47$, $P = 0.15$; carotenoid-supplemented: $t = 0.57$, $P = 0.57$) or plumage variation (carotenoid-unsupplemented, $F = 1.20$, $P = 0.76$; carotenoid-supplemented, $F = 1.28$, $P = 0.67$). Thus, we pooled males from the two cages in each diet treatment for further statistical analyses (see below).

NORTHERN CARDINALS

Ten male cardinals (five first-year, five after-first-year) were captured in mistnets in Lee County, AL, USA, between 14 and 21 August 1997. All males were housed in a single cage identical to those described above for goldfinches. Captive cardinals were fed an *ad libitum* diet of white millet, red millet, sunflower seeds, and vitamin- and medically treated water. No carotenoid supplements were provided to these birds. All males completed their prebasic moult in captivity by 1 October, and at this time plumage coloration was scored with the Colortron™. Like goldfinches, male cardinals vary in pigment saturation (Linville, Breitwisch & Schilling 1998), so three areas of pigmentation on the

breast were measured with the spectrophotometer and these three saturation scores were averaged to determine mean breast saturation for each individual. For comparison, 16 wild males in breeding plumage were captured from Lee County, Alabama, between 3 October and 20 October 1997 and the colour of their breast plumage was scored in a similar fashion. Because there were too few captive birds for which it was possible to quantify previous carotenoid-based breeding coloration ($n = 5$ adults), comparisons of plumage expression in the wild with that in captivity for individual males are not presented.

STATISTICAL ANALYSES

Tests were performed for normality (Shapiro–Wilk W -test) and equal variances (F -tests for equality of variance) in all analyses, and unpaired t -tests were used to compare differences in plumage saturation among captive groups and wild-caught birds when variables were normally distributed and had equal variances ($P > 0.05$). When $P < 0.05$ for either test, non-parametric Mann–Whitney U -tests were used (Z reported). F -tests for equality of variance were used to compare variance differences among the groups and determine how changes in diet type affected carotenoid-based plumage variation. For goldfinches only, simple linear regression was used to determine the extent to which a male's premoult plumage colour score at the time of capture was predictive of the brightness of his plumage after growing new feathers in captivity. Separate regressions were performed for the two groups of goldfinches that were fed different captive diets.

Results

AMERICAN GOLDFINCHES

When held on a plain seed diet in captivity during the prealternate moult, males grew pale yellow plumage that was significantly less saturated than the breeding plumage of wild males ($t > 14$, $P < 0.0001$; Fig. 1). On a captive diet supplemented with canthaxanthin, males grew plumage that was significantly more saturated than that of carotenoid-unsupplemented males ($t > 14$, $P < 0.0001$; Fig. 1) but did not differ significantly from the nuptial plumage of wild goldfinches ($Z = 1.7$, $P = 0.1$; Fig. 1). Interestingly, the plumage variation of wild males was not significantly greater than either that of carotenoid-supplemented ($F = 2.6$, $P = 0.07$) or carotenoid-unsupplemented males ($F = 0.83$, $P = 0.72$). In fact, the plumage of canthaxanthin-fed males tended to be more variable than that of wild males, and was significantly more variable than that of carotenoid-unsupplemented captive males ($F = 3.1$, $P = 0.004$). Male goldfinches not only failed to converge in plumage expression in captivity, but the winter plumage coloration that they brought into captivity with them was significantly

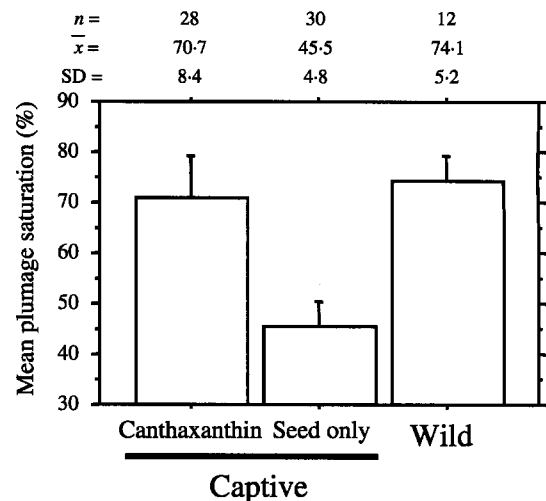


Fig. 1. The effect of dietary access to carotenoid pigments during moult on the expression of carotenoid-based plumage in captive and wild male American Goldfinches. Male plumage brightness was quantified with a hand-held Colotron™ reflectance spectrophotometer (Hill 1998). To supplement the diet of captive males with carotenoids, water-dispersible canthaxanthin beadlets were dissolved in water dishes; males kept on a carotenoid-deficient diet were fed the same foods but did not receive the carotenoid dose in their water. Bars represent means + 1 SD; group sample sizes, means and standard deviations are given at the top of the histogram.

predictive of the breeding plumage coloration that they attained in captivity, both for carotenoid-deficient males ($n = 30$, $r^2 = 0.17$, $P = 0.02$; Fig. 2a) and for males fed canthaxanthin ($n = 28$, $r^2 = 0.32$, $P = 0.002$; Fig. 2b).

NORTHERN CARDINALS

Among captive birds, no differences in plumage saturation ($Z = 0.1$, $P = 0.92$) or plumage variation ($F = 0.35$, $P = 0.33$) were found between the age classes. After pooling all captive birds, it was found that captive male cardinals fed a plain seed diet during moult grew significantly drabber carotenoid-based plumage than did wild males ($t = 10.4$, $P < 0.0001$; Fig. 3). Most importantly, however, plumage variation among these captive males did not differ from variation found in the plumage of wild males ($F = 0.74$, $P = 0.62$; Fig. 3).

Discussion

The information content of carotenoid-based integumentary displays has been the subject of widespread interest and debate among evolutionary biologists for more than a decade (Endler 1983; Kodric-Brown 1989; Hill 1990, 1992, 1994, 1999, 1992, 1994, 2000; Hill & Montgomerie 1994; Hudon 1994). The role of differential access to pigments in the diet in shaping intraspecific variation in ornamental pigmentation has received much attention because carotenoids are thought to be scarce in nature (Endler 1983; Hill 1990;

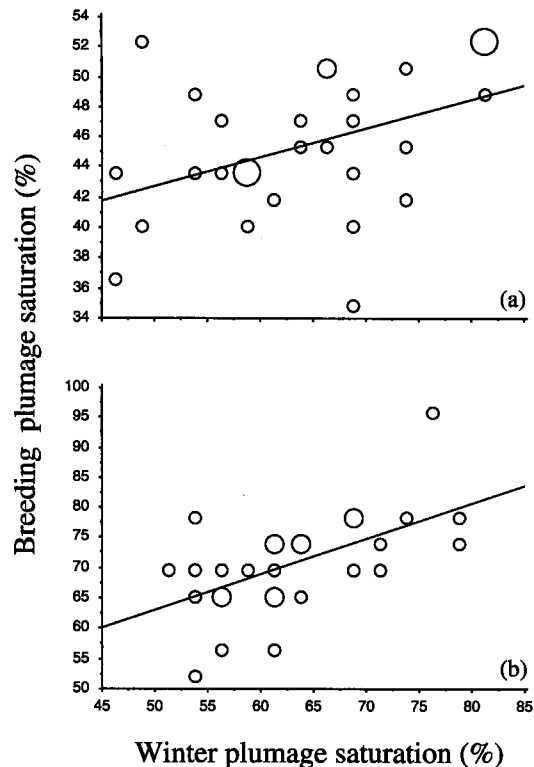


Fig. 2. Scatterplots of the relationship between winter and breeding plumage coloration for captive male American Goldfinches fed (a) carotenoid-unsupplemented and (b) carotenoid-supplemented diets. See legend in Fig. 1 for details of the carotenoid treatments. Point size is proportional to the number of overlapping observations.

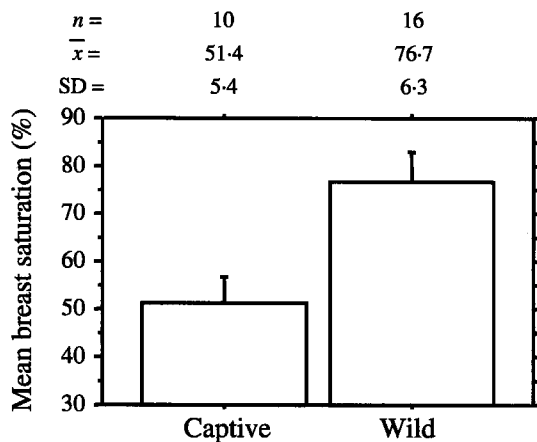


Fig. 3. The effect of dietary carotenoid availability on plumage brightness of captive and wild male Northern Cardinals. Captive males were fed an *ad libitum* diet of plain seed (sunflower and millet) and water throughout moult, whereas wild males were captured after they had completed moult in the autumn. Plumage brightness for all males was quantified using the Colortron™. See Fig. 1 for details of the histogram.

Grether, Hudon & Millie 1999), and thus only the superior foragers or competitors can acquire the pigments needed to display the brightest ornaments. Here we tested the effect of dietary access to carotenoid pigments during moult on the expression of bright plum-

age in two North American passerine species in which males display carotenoid-based plumage ornamentation. A large overall effect of pigment access on the extent to which males expressed bright plumage in both species was found. Compared with wild males, captive individuals that were fed basic seed diets during moult, which provided relatively few carotenoid pigments (McGraw *et al.* 2001), grew less colourful plumage. However, when the captive seed diets of male goldfinches was supplemented with canthaxanthin, the mean plumage saturation of captive males did not differ significantly from that of wild males. These data are consistent with those previously published for House Finches and support the idea that pigment access *per se*, and not the general effect of being held in captivity, underlies the drab carotenoid-based pigmentation that is common under captive conditions for many species (Hill 1994; Hudon 1994).

Although the plumage of male American Goldfinches and Northern Cardinals responded to the captive diet manipulations in the same general manner as House Finches – by growing relatively drab feathers when fed seeds and relatively bright feathers when fed a carotenoid-supplemented diet – there were distinct differences among these species in the variability of male plumage displays that provide valuable insight into the control of expression of carotenoid-based plumage coloration. In contrast to findings in the House Finch, where males display little plumage variation when fed a controlled diet in captivity, we observed as much variation in plumage expression among captive groups of male goldfinches and cardinals as was found in wild males from each species. The winter plumage colour that male goldfinches brought into captivity with them also strongly predicted the extent to which they expressed breeding plumage pigmentation after completing their prealternate moult in captivity. In other words, even when carotenoid access was standardized for captive males during moult, birds still grew variably coloured plumage, with naturally bright males growing relatively bright plumage and naturally drab males remaining relatively drab. These results suggest that carotenoid-based plumage variation in male goldfinches and cardinals is less sensitive to access to carotenoid pigments during moult than in House Finches, and that other factors contribute to within-sex variability in ornamental coloration in these two species.

The importance of access to pigments in determining the expression of carotenoid coloration among wild animals has been questioned by some (Hudon 1994; Bortolotti *et al.* 1996; Thompson *et al.* 1997; Negro *et al.* 1998), and it is now clear that other factors, including the general health and condition of individuals (Nicoletto 1991; Frischknecht 1993; Hill & Montgomerie 1994; Dufva & Allander 1995; Hill 2000) and levels of parasitism (Kodric-Brown 1989; Milinski & Bakker 1990; Houde & Torio 1992; Thompson *et al.* 1997; Harper 1999) can affect carotenoid pigmentation in birds and fish. In both House

