Behavioral Ecology Vol. 14 No. 6: 909–916
DOI: 10.1093/beheco/arg085

Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration

Kristen J. Navara and Geoffrey E. Hill
Department of Biological Sciences, 331 Funchess Hall, Auburn University, Auburn, AL 36849-5414, USA

Carotenoid pigments can directly enhance the immune responses of vertebrates, and they are used by many animals to create ornamental color displays. It has been hypothesized that these two functions of carotenoid pigments are linked: animals must trade off use of carotenoid pigments for immune function versus ornamental display. We tested two key predictions of this hypothesis with captive American goldfinches, *Carduelis tristis*, a species with extensive carotenoid-based plumage coloration. First, we tested whether the immune systems of male goldfinches are carotenoid limited during molt by supplying treatment groups with low, approximately normal, or high dietary access to lutein and zeaxanthin. Dietary treatment had a significant effect on plumage and bill color but not on immunocompetence. We compared the cell-mediated and humoral immune responses and the course of disease after infection for males in the different treatments. We observed no significant effect of the carotenoid content of diet on immune response or disease resistance. Second, we tested whether there was a positive relationship between immune function and expression of ornamental coloration by comparing both the pre- and posttreatment plumage coloration of males to their immune responses. We failed to find the predicted trade-off between ornament display and immune function. These findings do not support the hypothesis that songbirds with extensive carotenoid-based plumage displays trade off the use of carotenoids for ornamentation versus immune function. *Key words:* American goldfinch, *Carduelis tristis*, PHA, plumage, sexual selection, SRBC. *[Behav Ecol 14:909–916(2003)]*

Many of the brilliant red, orange, and yellow color displays of vertebrates are the result of carotenoid pigments deposited in the integument. Unlike other biological pigments, carotenoids cannot be synthesized de novo by animals; they have to be obtained from the diet (Goodwin, 1984; Völker, 1938). Thus, carotenoid-based color displays reflect the amount and types of carotenoids that individuals ingest (Grether et al., 1999; Hill, 1992, Hill et al., 2002), which could, in turn, signal the foraging and competitive ability of individuals (Hill, 2002). Ingestion of quantities of appropriate carotenoid pigments, however, does not guarantee maximum ornamentation. Independent of carotenoid access, animals in good nutritional condition produce brighter color displays than do those in poor nutritional condition (Frischknecht, 1993; Hill, 2000; Hill and Montgomerie, 1994). A variety of parasites also depress expression of carotenoid-based color displays (Brawner et al., 2000; Hill, 2000; Houde and Torio, 1992; McGraw and Hill, 2000; Milinski and Bakker, 1990; Thompson et al., 1997). Expression of carotenoid-based coloration, therefore, is dependent on several distinct agents of condition.

Carotenoid-based integumentary coloration has been found to be an important criterion in female choice for a number of species of birds and fish (Hill, 1999), and a primary benefit of choosing brightly colored males is likely information about the foraging ability, nutritional condition, and parasite loads of prospective mates (Hill, 2002; Houde, 1997). An alternative explanation for carotenoid-based color displays is that they evolved because of the immuno-enhancing properties of carotenoids (Lozano, 1994; Møller et al., 2000). Carotenoids directly bolster the immune systems of vertebrates by stimulating effector T-cell function, enhancing macrophage and cytotoxic T-cell capacities, as well as stimulating T- and B-lymphocyte proliferation (Bendich, 1989; Chew, 1993; Jyonouchi et al., 1994). Furthermore, carotenoids act as free radical scavengers that "mop up" the free radicals produced during daily metabolic and immunological functions (Burton, 1989; Machlin and Bendich, 1987). If animals with carotenoid-based color displays have immune systems that are carotenoid limited, then potential immunological benefits would be forfeited when carotenoids are used for the production of ornamental displays instead of immune enhancement. Individuals in better condition would have less need to use carotenoids for immune function and could, therefore, deposit more carotenoid pigments into the integument. In this way, females could assess male quality based on the size or color of the ornamental display.

This carotenoid-immunocompetence trade-off hypothesis has not been tested directly in a species with extensive carotenoid display (for review, see Hill, 1999; Møller et al., 2000). Previous studies have found that parasitic infection has a suppressive effect on expression of carotenoid-based ornamentation (Brawner et al., 2000; Hill, 2000; Houde and Torio, 1992; McGraw and Hill, 2000; Milinski and Bakker, 1990; Thompson et al., 1997; Zuk et al., 1998) and, likewise, that degree of carotenoid ornamentation can act as a signal of lower parasite loads (Bletner et al., 1966; Ruff et al., 1974). A positive relationship has been found between intensity of carotenoid-based coloration and various measures of immune function (Dufva and Allander, 1995; Figuerola et al., 1999; Merilä et al. 1999; but see Birkhead et al. 1998; Bortolotti et al. 1996; Seutin, 1994; Skarstein et al. 2001; Weatherhead, 1990; Weatherhead et al. 1993; for review, see Møller et al., 1998; Shykoff and Widmer, 1996), and carotenoids have been found to have immuno-enhancing effects in mammals (Bendich,

Address correspondence to K.J. Navara. E-mail: navarkr@auburn.edu.

Received 3 August 2002; revised 6 February 2003; accepted 4 March 2003.

1989; Bendich and Shapiro, 1986; Chew, 1993, 1996; Jyonouchi et al., 1994; Mortensen et al., 1997), fish (Christiansen et al., 1995), and chickens (Lawlor and O'Brien, 1995a,b; Tengerdy et al., 1990). These studies have been taken as evidence that carotenoid-based ornaments signal a direct trade-off between use of carotenoids for immune function and use of carotenoids for visual displays (Lozano, 1994; Møller et al., 2000b). However, none of these studies directly tests for such a color display/immune system trade-off. Although published studies suggest that carotenoid-based ornaments signal carotenoid access, nutritional condition, and parasite load (Hill, 1999, 2002), clearly a direct test is needed of the hypothesis that the immune systems of animals with extensive carotenoid displays are carotenoid-limited and, if this is the case, that there is a trade-off between the use of carotenoids for immune enhancement and use of carotenoids in color displays.

In the present study, we tested two key predictions of the carotenoid-immunocompetence trade-off hypothesis in the American goldfinch, *Carduelis tristis*, a sexually dichromatic songbird with extensive carotenoid-based yellow plumage coloration. The yellow plumage coloration of male goldfinches is a condition-dependent signal of male quality (McGraw and Hill, 2000) used by females in mate choice (Johnson et al., 1993). We held males in treatment groups that were fed either low, approximately normal, or high doses of the carotenoid pigments lutein and zeaxanthin, the primary pigments used by American goldfinches to produce yellow plumage pigments (McGraw et al., 2002). In May, during mid-molt, when there is active shuttling of carotenoid pigments into the integument, we subjected these goldfinches to a humoral immune challenge, a cell-mediated immune challenge, and to infection by a pathogen. We then assessed two key predictions of the carotenoid-immunocompetence trade-off hypothesis. First, we tested whether the immune systems of male goldfinches are carotenoid-limited during the molt period. Second, we looked for the predicted trade-offs between the use of carotenoid pigments for ornament display and use of carotenoid pigments for immune defense by assessing the relationship between pre- and postplumage coloration and immune function for males held in low-carotenoid environments.

METHODS

Experimental design

In February 2001, we captured American goldfinches in Lee County, Alabama, and determined the age and sex of the birds following the method of Pyle et al. (1987). American goldfinches are small sexually dimorphic passerines. Males exhibit a carotenoid-based bright yellow throat patch and a black melanin cap. Females are almost completely olive in color. We kept only yearling males to minimize preexposure to the pathogens used in this study. All birds were examined for signs of mycoplasmal conjunctivitis, as well as any other signs of avian disease, such as pox. Seventy-two healthy males were divided into groups of 24. These birds were then housed in three separate cages and quarantined for a period of 1 month. During this time, we carefully monitored the birds for signs of disease. Three of the birds displayed suspect behavior and were excluded from the study on day 2. Collection of the goldfinches took place when plumage dichromatism between males and females is subtle. As a result, eight of the 72 collected birds were later recognized as females and were excluded from the analysis.

Throughout the course of the study, we maintained the birds on ad lib sunflower and thistle seeds, which contain a low level of carotenoid pigments (McGraw et al., 2002). Water was supplemented with sulphadimethoxine (0.12 g/L water), to suppress Isosporan coccidiosis, an ubiquitous disease of goldfinches that will quickly kill aviary birds (McGraw and Hill, 2000). Beginning in March and continuing to the end of the experiment, we supplemented the bird's only source of water with one of three doses of dissolvable carotenoids: a low treatment (0.01 g/L water), a medium treatment (0.1 g/L water), or a high treatment (1.0 g/L water). Each treatment group contained 24 birds. The seeds that are commonly consumed by American goldfinches (e.g., thistle) contain lutein and zeaxanthin in a 70 : 30 ratio (McGraw et al., 2002), so we provided these carotenoids in this ratio to birds in the form of dissolvable starch-gel beadlets (supplied by Roche Vitamins Inc., Parsippany, New Jersey, USA). No data exist on the carotenoid contents of the diets of wild American goldfinches, so we estimated the concentration of carotenoids that would likely be consumed based on the quantities of carotenoids in the diets of house finches (Hill et al., 2002) and based on the responses of American goldfinches to previous feeding experiments (McGraw et al., 2002; Mcgraw and Hill, 2001). The difference in plumage and bill color grown by males on the three treatments (see results) suggests that we tested immune function over an appropriate range of carotenoid doses. After they had been maintained on these carotenoid treatments for 3 months (March–May), we presented all goldfinches with three immunological challenges.

Sheep red blood cell assay for humoral immune response

A challenge of the humoral immune response was performed in early May, when the birds were approximately midway through the molting process. To assess the humoral immune response, all goldfinches were inoculated intraperitoneally with 0.5 ml of a 5% sheep red blood cells (SRBCs) (Colorado Serum Company, Denver, Colorado, USA) in phosphate-buffered saline (PBS). Cells were washed twice in PBS and resuspended to the desired concentration. Antibodies produced in response to the SRBCs were quantified after 2 weeks by taking 50 µl of blood from the brachial vein and performing a standard hemagglutination assay (Hay and Hudson, 1989). Although all birds were subjected to this test, only 41 samples were used for the hemagglutination assay owing to technical difficulties in the laboratory. In short, 20 µl plasma was serially diluted in 20 µl PBS (1 : 2 . . . 1 : 1024) in 96-well v-bottom plates. Wells 11 and 12 served as negative and positive controls and did not contain any plasma. Instead, these wells were loaded with 20 µl PBS or 20 µl antisheep hemolysin (Colorado Serum Company, Denver, Colorado, USA), respectively. Next, 20 µl of a 2% SRBC suspension in PBS was added to each well. The plates were incubated at room temperature for 24 h. Finally, wells containing plasma samples were compared to positive and negative controls. Antibody titers were expressed as the $\log_2$ of the highest dilution of plasma containing hemagglutination (Lochmiller et al., 1993).

Phytohemagglutinin skin test for cell-mediated immune response

Phytohemagglutinin (PHA) is a known T-cell stimulant in passerine birds (Goto et al., 1978). Therefore, injection of this antigen results in swelling around the injection site within 24 h. A week after the completion of the SRBC challenge, the response of T lymphocytes in American goldfinches was tested by using the standard protocol for avian species (Goto et al., 1978). A 1-cm patch on the left mid-patagium was cleared of feathers. Two measures of thickness were taken by using a pressure-sensitive digital micrometer (accuracy = 0.05 mm). The bare skin was swabbed with alcohol, and 20 µg PHA in 20

μl PBS was injected subcutaneously by using a 27-gauge needle. Injection dosages were extrapolated according to weight from the amounts used in a variety of passerine species in a study by Smits and Williams (1999). Two measurements of wing-web thickness were taken after 24 h to assess swelling. For this study, only the left patagium was used, because this decreases the handling time and the probability of error compared with that of the injection and measurement of both the left and right side (Smits and Williams, 1999).

Inoculation and disease resistance

Mycoplasma gallisepticum (MG) is a well-known pathogen that causes respiratory disease in poultry and is the causative agent for a recent epidemic of mycoplasmal conjunctivitis in house finches (*Carpodacus mexicanus*) (Ley et al., 1996). American goldfinches with the disease are also observed in the wild (Hartup et al., 2001, 2000), and they develop conjunctivitis when exposed to MG in aviaries.

In late May (after completion of the PHA challenge), during the second half of molt, we inoculated all captive goldfinches intraorbitally with 5 μl of MG isolate cultured from wild house finches. MG was introduced to each eye in a drop-wise fashion (Farmer et al., 2002). After inoculation, all birds were caught and scored daily for degree of conjunctivitis on a seven-point scale: zero indicating no signs of conjunctivitis; seven, maximal swelling of the conjunctiva. All scoring was performed by the same investigator to minimize interobserver error. To test the effects of carotenoid limitation on immune function, we examined the progression of this disease for all birds within the treatments. When assessing the relationship between plumage coloration and disease resistance, we used time to an infection level of three, the maximum state of infection reached by all males, which is characterized by a red swollen conjunctiva.

Color measurements

We scored carotenoid-based pigmentation of plumage and bill of males both at the time of capture and after they had completed molt in captivity by using a Colortron reflectance spectrophotometer (Light Source, San Rafael, California, USA) (Hill, 1998). The Colortron provides tri-stimulus color descriptors (hue, saturation, and brightness), and we used saturation to compare the plumage coloration of males in the different treatment groups (see McGraw and Hill, 2000) because this is the color dimension that varies most among male goldfinches and is the aspect of color that appears to be the object of female choice (Johnson et al., 1993).

RESULTS

Males in different treatment groups did not differ in premolt plumage saturation (df = 2,57, $F = 2.60$, $p = .835$ ANOVA). By late June, all birds had molted into a characteristic yellow nuptial coloration, and males in the different treatment groups differed significantly in the saturation of both their plumage (df = 2,51, $F = 3.3$, $p = .04$ ANOVA) and bill coloration (df = 2,51, $F = 14.60$, $p < .01$ ANOVA). A posteriori paired comparisons indicated that the plumage of males in the low-carotenoid group was significantly less saturated than was the plumage of males in the medium- or high-carotenoid groups, but the plumage saturation of males in the medium and high group were not significantly different (low–medium: $p = .02$, low–high: $p = .02$, medium–high: $p = .96$ Fisher PLSD) (Figure 1A). In addition, the high-carotenoid group had significantly more saturated bills than did the medium- and the low-carotenoid groups (low–medium: $p = .14$, low-high: $p < .01$, medium–high: $p < .01$ Fisher PLSD) (Figure 1B).

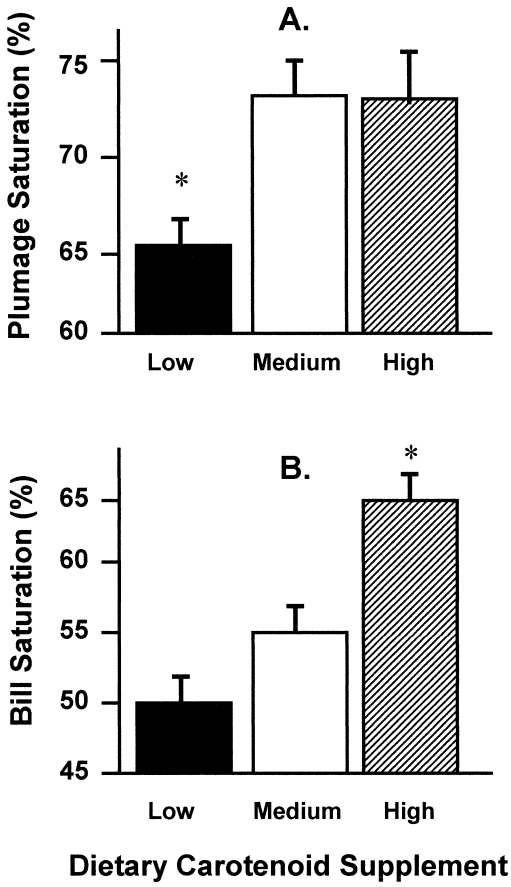

Figure 1
Effects of access to carotenoid pigments during molt on the mean ± SE saturation of yellow plumage (A) and orange bill (B) of captive male American goldfinches. Carotenoids (70% lutein and 30% zeaxanthin) embedded in starch gel beadlets were dissolved in the bird's drinking water at the following concentrations: low treatment, 0.01 g/L water; medium treatment, 0.1 g/L water; and high treatment, 1.0 g/L water.

We found no evidence that the immune systems of American goldfinches were carotenoid-limited. Male goldfinches maintained on the different carotenoid dietary treatments responded similarly to the PHA challenge (df = 2,62, $F = 0.57$, $p = .57$, repeated-measure ANOVA, power = 0.8 to detect an effect less than 0.05 mm) (Figure 2A). There was no indication that males in the low-carotenoid groups had a compromised T-cell response compared with that of males in the high-carotenoid group. Male in the different treatment groups also responded similarly to the SRBC antigen, regardless of dietary treatment (Figure 2B) (df = 2,36, $F = 0.91$, $p = .41$ ANOVA, power = 0.8 to detect a titer difference greater than 0.65). Birds from the high-carotenoid treatment had a slightly elevated antibody titer compared with the other treatments, but this difference was not statistically significant (low–high: $p = .27$, medium–high; $p = .21$ Fisher PLSD). The PHA response did not relate to the SRBC response in this study (df = 36, $F = 2.34$, $p = .14$).

Males in the different treatment groups also showed very similar and statistically indistinguishable course of infection after exposure to MG. At day 1 after inoculation, almost all birds showed signs of mild conjunctivitis. Within 5 days, most had progressed to a symptom score of five. By day 14, 36% of the birds showed signs of recovery from the disease. A repeated-measure ANOVA revealed a significant difference

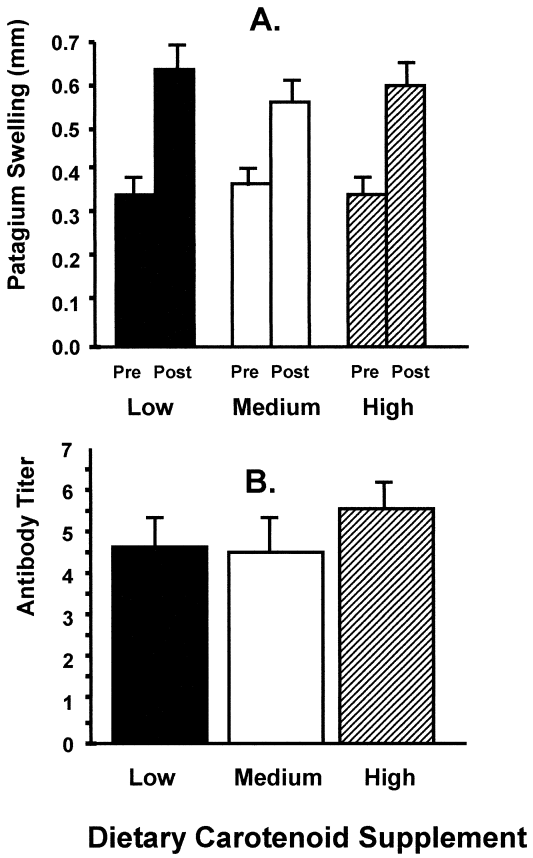

Figure 2
Immune responsiveness in relation to dietary intake of carotenoid pigments by captive male American goldfinches: cell-mediated immune response to injection with phytohemagglutinin (mean ± SE pre and postinjection patagium swelling) (A) and humoral immune response to SRBC injection (mean ± SE antibody titer) (B). Antibody titer was calculated as the $\log_2$ of the highest dilution of plasma containing hemagglutination during the hemagglutination assay. Concentrations of carotenoids in the three treatment groups are given in Figure 1.

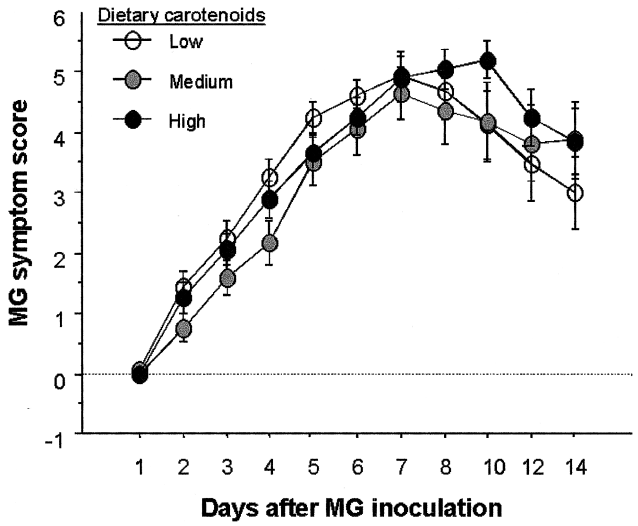

Figure 3
Effects of access to carotenoid pigments during molt on mean ± SE progression of mycoplasmal conjunctivitis in captive male American goldfinches. Symptoms of disease, from no swelling of the conjunctiva to maximum swelling in both eyes, was scored on a seven-level scale. Concentrations of carotenoids in the three treatment groups are given in Figure 1.

for disease progression over time for the entire group (df = 2,49, $F = 97.09$, $p < .01$), illustrating that the birds did, in fact, respond to the pathogen. However, there was no significant difference in disease progression between the dietary treatments (df = 2,49, $F = 0.51$, $p = .60$, power = 0.8 to detect a difference in disease level greater than 0.84) (Figure 3). The progression of this disease did not relate to either the humoral (df = 36, $F = 0.80$, $p = .38$) or the cell-mediated (df = 54, $F = 0.43$, $p = .52$) immune responses, and the two immune responses did not relate to one another (df = 36, $F = 2.33$, $p = .14$). These results suggest that we examined three distinct measures of immunocompetence in this experiment, and that none of these aspects of immunocompetence is affected by dietary access to carotenoid pigments.

To test directly for an ornament-immunocompetence trade-off, we compared the plumage brightness of males to each immune challenge. We compared immune responsiveness to three different measures of plumage coloration: coloration at the time of capture, coloration after captive molt on carotenoid treatment and exposure to a pathogen, and change in plumage coloration from time of capture to completion of captive molt. Because the color scores indicated carotenoid limitation within the low-carotenoid treatment group, we restricted our analysis to males on the low-carotenoid diet.

Within males in the low-carotenoid group, regardless of the plumage color measure that we used, we found no evidence for a trade-off between use of carotenoid pigments for ornament display and use of carotenoids for immune response. There was no significant relationship between cell-mediated immune response ($r_s = -.04$, $n = 18$, $p = .87$), humoral immune response ($r_s = -.34$, $n = 14$, $p = .23$), or resistance to infection by MG ($r_s = .13$, $n = 19$, $p = .59$) and plumage coloration at the time of capture (Figure 4). Likewise, plumage coloration after captive molt and change in plumage coloration between time of capture and completion of captive molt did not relate to cell-mediated immune response (post: $r_s = .21$, $n = 17$, $p = .40$; Δplumage: $r_s = -.18$, $n = 17$, $p = .48$, power = 0.8 to detect a difference greater than 0.7), humoral immune response (post: $r_s = -.20$, $n = 13$, $p = .49$; Δplumage: $r_s = -.27$, $n = 13$, $p = .79$, power = 0.8 to detect a titer difference greater than 1.0), or resistance to infection by MG (post: $r_s = .02$, $n = 18$, $p = .93$; Δplumage: $r_s = .18$, $n = 18$, $p = .53$, power = 0.8 to detect a disease level difference greater than 0.65) (Figure 4). Plumage color scores indicated that the low-carotenoid treatment group was carotenoid-limited, and it was in this group that we expected to see the clearest trade-off between use of carotenoids for ornament display and use of carotenoids for immune defense. We also made the same comparison between plumage coloration and immune response for males in the medium- and high-carotenoid supplement groups. We found no significant relationship between any measure of color and any measure of immune response.

Negative results are only convincing if the tests conducted have adequate power for statistical comparisons to indicate significant differences if such differences exist. "High" power is a subject of discussion, but a convention of 0.8 has been suggested in the literature (Cohen, 1992). The effect sizes detected with a power of 0.8 for each of the tests are included in the individual analyses above. Although current literature does not clearly define an effect size that would be biologically

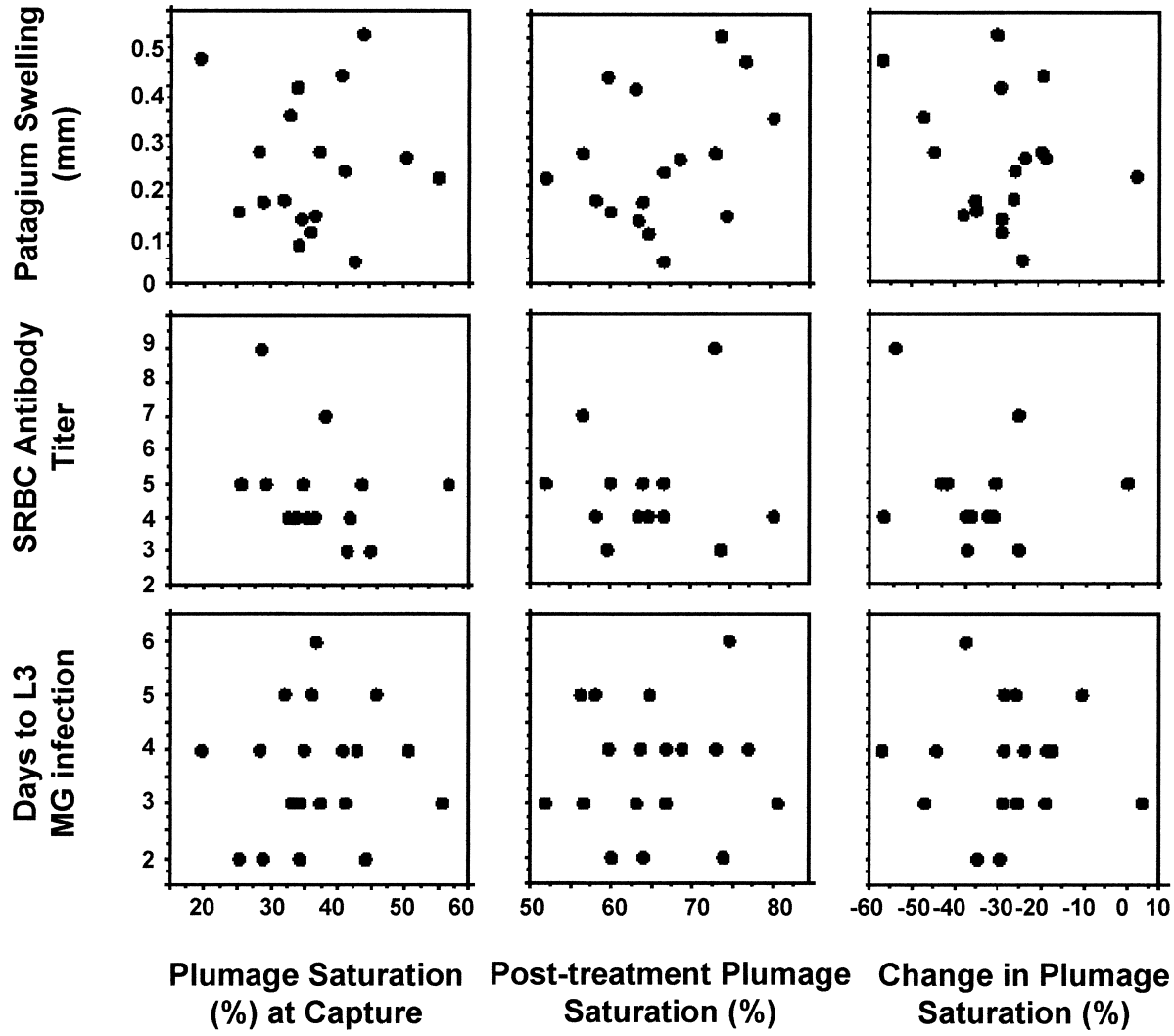

Figure 4
The relationship between saturation of yellow plumage coloration and immune response in captive male American goldfinches maintained on low-carotenoid diets (see Figure 1 for types and concentrations of carotenoids). Immune defenses were measured as days to level three mycoplasmal conjunctivitis infection (column 1), humoral immune response (antibody titer) to SRBC (column 2), and cell-mediated immune response (patagium swelling) to phytohemagglutinin (column 3). Three measures of plumage saturation were examined: plumage saturation at capture (natural ornamental coloration) (row 1), posttreatment plumage saturation (row 2), and the change in plumage saturation from capture to postmolt (row 3).

meaningful for each of these immunological challenges, the effect sizes presented here are not large in the context of these specific immunological parameters, and our conclusions are rather conservative.

DISCUSSION

The hypothesis that carotenoid-based color display reflects a trade-off between use of carotenoid pigments for immune response and use of carotenoid pigments for integumentary display hinges on two key assumptions. First, it requires that the immune systems of animals with extensive carotenoid-based ornamental traits are carotenoid-limited. Second, it requires that individuals trade off the use of the carotenoids for color display versus use of carotenoids for immune response, such that highly ornamented males are signaling their lower dependency on immune-bolstering carotenoids and, hence, the superior nature of their immune function. We failed to find support for either of these assumptions.

First, we found no evidence that the immune systems of male American goldfinches were carotenoid-limited during molt of yellow ornamental feathers. Goldfinches in the low-carotenoid treatment group were carotenoid-limited in terms of the carotenoids needed for ornament display, as indicated by the brighter plumage developed by males in the high- and medium- versus low-carotenoid treatment groups. Nevertheless, individuals in the three carotenoid treatment groups did not differ significantly in antibody response to the SRBC challenge (humoral immune response), in wing-web swelling in response to PHA skin challenge (cell-mediated immune response), or in progression of mycoplasmal conjunctivitis after exposure to MG. Because carotenoids have been implicated as stimulants of both B- and T-cell proliferation (Bendich, 1989; Bendich and Shapiro, 1986; Chew, 1993, 1996; Olson and Owens, 1998; Ziegler, 1989), if American goldfinches have immune systems that are carotenoid-limited, then in groups supplied with more dietary carotenoids, we would have expected to see more antibodies produced in

response to a foreign antigen and more swelling, indicative of a larger cell-mediated immune response. The lack of differences among groups indicates that B- and T-cell proliferation or function and the ability to fend off a pathogen is not carotenoid-limited in this species.

We should be clear that these observations are not evidence that passerine birds such as the American goldfinch do not use carotenoid pigments as antioxidants and benefit from the free-radical scavenging properties of these molecules. Rather, our observations indicate that the immune systems of male goldfinches are not carotenoid-limited, likely because high levels of carotenoid pigments are mobilized in the bodies of the birds for ornamental displays (Hill, 1999). If very small amounts of carotenoids are needed to bolster immune function, there will be no limitation in animals with an abundance of circulating plasma carotenoids.

The second critical assumption of the carotenoid-immunocompetence trade-off hypothesis is that there is a relationship between immune response and plumage coloration. We tested this assumption in three ways because the predictions relative to male plumage coloration and immune response are somewhat ambiguous. Moreover, we limited our analyses to males in the low-carotenoid treatment group because differences in plumage color indicate that this is group in which the males would be the most carotenoid-limited and in which the clearest trade-off would be expected. First we looked at the relationship between coloration of males at the time of capture—the natural ornamentation of males—and immune response. The carotenoid-immunocompetence trade-off hypothesis posits that males are signaling their immunocompetence through their carotenoid-based color display, so males with brighter plumage in the wild should have better innate immune systems than drabber males. We found no support for this idea. Males that had brighter premolt plumage coloration did no better than did drab males by any measure of immune response. Second, we looked at the plumage coloration that males produced during molt on the low-carotenoid treatment. Assuming that all males are dealing with the same challenges and abilities, we predicted that drabber males would have higher immune responses than would brighter males because the latter group have traded off immune protection for ornament display. We found no evidence for this trade-off. Finally, because we suspected that males comprising the flocks differed in their innate immune abilities and hence their need for carotenoid enhancement of their immune systems, we subtracted the preexperiment plumage scores of males (a measure of quality) from their postexperiment scores and looked at how the change in plumage score compared with immune response. Again, we found no relationship indicating that even when we adjusted for male quality, individuals did not trade off use of carotenoids for immune defense and use of carotenoids for ornamental coloration. We chose to supplement these goldfinches with two yellow xanthophylls, lutein and zeaxanthin, based on the composition of thistle seeds (a major dietary component). Although the diets of goldfinches are by no means limited to these carotenoids, these two are extremely prevalent in a variety of plants and are the primary precursor carotenoids for the yellow pigments that are responsible for the bright yellow throat-patch in male goldfinches (McGraw et al. 2002). Therefore, these are the most relevant pigments to examine when looking for an ornament-immunocompetence trade-off in American goldfinches.

A variety of parasites have been shown to depress expression of carotenoid-based ornaments, and in some cases, animals expressing more extensive carotenoid-based ornamentation have lower parasite loads and higher measures of immune function (Bletner et al., 1966; Ruff et al., 1974). Furthermore, carotenoids have been found to have direct effects on immune function in a variety of species (Bendich, 1989; Bendich and Shapiro, 1986; Chew, 1993, 1996; Olson and Owens, 1998; Ziegler, 1989). However, with a few exceptions, evidence is lacking to show that variation in immunocompetence among males is responsible for the variation in expression of carotenoid ornamentation.

The carotenoid-immunocompetence trade-off hypothesis is seductive because it links parasites, carotenoids, and immunocompetence. In previous studies, positive relationships have been observed between degree of carotenoid-based ornamentation and various measures of immune function (Dufva and Allander, 1995; Figuerola et al., 1999; Lindström and Lundström, 2000), and this evidence suggests that carotenoid-based ornaments are condition-dependent. However, this condition dependence does not require a direct immune-ornament trade-off. Although the current literature demonstrates links between carotenoids and the immune system as well as between parasite loads and carotenoid-based ornamentation, the present study is the first direct test of an actual trade-off between use of carotenoids for ornamentation and use for bolstering the immune system.

Finally, most of the data directly illustrating the role of carotenoids in immune function come from animals that lack carotenoid-based ornamental display (Bendich, 1989; Chew, 1993; Haq et al., 1996; Lawlor and O'Brien, 1995a; McWhinney and Bailey, 1989; Saino et al., 1999; Tengerdy et al., 1990). Many of these animals naturally have diets with much smaller quantities of carotenoids than do animals with carotenoid-based color displays (Gray, 1996; Hill, 1999) and, hence, have much lower levels of circulating carotenoid pigments (Hill, 1999). Indeed, the amount of carotenoids that we provided to male goldfinches in our low-carotenoid treatment would have been a substantial carotenoid supplement for many mammals (Hill, 1999 and references therein). Thus, although carotenoids may provide a beneficial immunoenhancing effect for all vertebrates, and some taxa with low-carotenoid diets may be limited in immune ability by lack of carotenoids, our observations indicate that the immune systems of American goldfinches, a species with extensive carotenoid-based color displays and high-carotenoid diets, are not carotenoid-limited, and we find no support for the idea of an ornament-immunocompetence trade-off within this species.

We thank J. Ariail and Tyler Smith for assistance with aviary work; S. Roberts, K. Farmer, M. Beck, L. Siefferman, and P. Nolan for assistance with SRBC, PHA, and MG immune challenges; and M. Mendonça, K. McGraw, A. Badyaev, and the Hill laboratory for their comments and input on both the experimental design and the manuscript. This work was supported by the National Science Foundation grants IBN9722172 and DEB0077804. Birds were collected under a State of Alabama permit (no. 12) and federal permit (no. 784373), and the treatment of captive birds was approved by the Institutional Animal Care and Use Committee (PRN no. 0201-R-2443).

REFERENCES

Bendich A, 1989. Carotenoids and the immune response. J Nutr 119:112–115.

Bendich A, Shapiro SS, 1986. Effect of β-carotene and canthaxanthin on the immune response of the rat. J Nutr 116:2254–2262.

Birkhead TR, Fletcher F, Pellatt EJ, 1998. Sexual selection in the zebra finch *Taeniopygi guttata*: condition, sex traits and immune capacity. Behav Ecol Sociobiol 44:179–191.

Bletner JK, Mitchell RP, Tugwell RL, 1966. The effect of *Eimeria maxima* on broiler pigmentation. Poultry Sci 45:689–694.

Bortolotti G, Negro JJ, Tella JL, Marchant TA, Bird DM, 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. Proc R Soc Lond B 263:1171–1176.
Brawner WR 3rd, Hill GE, Sundermann CA, 2000. Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. Auk 117:952–963.
Burton GW, 1989. Antioxidant action of carotenoids. J Nutr 119:109–111.
Chew BP, 1993. Role of carotenoids in the immune response. J Dairy Sci 76:2804–2811.
Chew BP, 1996. Importance of antioxidant vitamins in immunity and health in animals. Anim Feed Sci Technol 59:103–114.
Christiansen R, Glette J, Lie O, Torrissen OJ, Waagbo R, 1995. Antioxidant status and immunity in Atlantic salmon, *Salmo salar* L., fed semi-purified diets with and without astaxanthin supplementation. J Fish Dis 18:317–328.
Cohen J, 1992. A power primer. Psychol Bull 112:155–159.
Dufva R, Allander K, 1995. Intraspecific variation in plumage coloration reflects immune response in grat tit (*Parus major*) males. Funct Ecol 9:785–789.
Farmer KL, Hill GE, Roberts SR, 2002. Susceptibility of a naive population of house finches to *Mycoplasma gallisepticum*. J Wildl Dis 38:282–286.
Figuerola J, Munoz E, Gutierrez R, Ferrer D, 1999. Blood parasites, leucocytes and plumage brightness in the cirl bunting, *Emberiza cirlus*. Funct Ecol 13:594–601.
Frischknecht M, 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. Evol Ecol 7:439–450.
Goodwin TW, 1984. The biochemistry of carotenoids, 2nd ed. New York: Chapman and Hall.
Goto N, Kodama H, Okada K, Fujimoto Y, 1978. Suppression of phytohaemagglutinin skin response in thymectomized chickens. Poultry Sci 57:246–250.
Gray DA, 1996. Carotenoids and sexual dichromatism in North American passerine birds. Am Nat 148:453–480.
Grether GF, Hudon J, Millie DF, 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proc R Soc Lond 266:1317–1322.
Haq A, Bailey CA, Chinnah A, 1996. Effect of β-carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. Poultry Sci 75:1092–1097.
Hartup BK, Dhondt AA, Sydenstricker KV, Hochachka WM, Kollias GV, 2001. Host range dynamics of mycoplasmal conjunctivitis among birds in North America. J Wildl Dis 37:72–81.
Hartup BK, Kollias GV, Ley DH, 2000. Mycoplasmal conjunctivitis in songbirds from New York. J Wildl Dis 36:257–264.
Hay L, Hudson FC, 1989. In: Practical immunology, 3rd ed. Oxford: Blackwell; 252–254.
Hill GE, 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. Auk 109:1–12.
Hill GE, 1998. An easy, inexpensive means to quantify plumage colouration. J Field Ornithol 69:353–363.
Hill GE, 1999. Is there an immunological cost to carotenoid-based ornamental coloration? Am Nat 154:589–595.
Hill GE, 2000. Energetic constraints on expression of carotenoid-based plumage coloration. J Avian Biol 31:559–566.
Hill GE, 2002. A red bird in a brown bag: the function and evolution of colorful plumage in the house finch. New York: Oxford University Press.
Hill GE, Inouye CY, Montgomerie R, 2002. Dietary carotenoids predict plumage coloration in wild house finches. Proc R Soc Lond B 269:1119–1124.
Hill GE, Montgomerie R, 1994. Plumage colour signals nutritional condition in the house finch. Proc R Soc Lond B 258:47–52.
Houde AE, 1997. Sex, color, and mate choice in guppies. Princeton, New Jersey: Princeton University Press.
Houde AE, Torio AJ, 1992. Effect of parasitic infection on male color pattern and female choice in guppies. Behav Ecol 3:346–351.
Johnson K, Dalton R, Burley N, 1993. Preferences of female American goldfinches (*Carduelis tristis*) for natural and artificial male traits. Behav Ecol 4:138–143.
Jyonouchi H, Zhang L, Gross MR, Tomita Y, 1994. Immunomodulating actions of carotenoids: enchancement of *in vivo* and *in vitro* antibody production to T-dependent antigens. Nutr Cancer 21: 47–58.
Lawlor SM, O'Brien NM, 1995a. Astaxanthin: antioxidant effects in chicken embryo fibroblasts. Nutr Res 15:1695–1704.
Lawlor SM, O'Brien NM, 1995b. Modulation of oxidative stress by beta carotene in chicken embryo fibroblasts. Br J Nutr 73:841–850.
Ley DH, Berkhoff JE, McLaren JM, 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Dis 40:480–483.
Lindström, K, Lundström J, 2000. Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rates. Behav Ecol Sociobiol 48:44–51.
Lochmiller RL, Vestey MR, Boren JC, 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. Auk 110:503–510.
Lozano GA, 1994. Carotenoids, parasites, and sexual selection. Oikos 70:309–311.
Machlin LJ, Bendich A, 1987. Free radical tissue damage: protective role of antioxidant nutrients. FASEB 1:441–445.
McGraw K, Hill GE, Stradi R, Parker RS, 2002. The effect of carotenoid access in the diet on sexual dichromatism and plumage pigment composition in the American goldfinch. Comp Biochem Physiol B 131:261–269.
McGraw KJ, Hill GE, 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc R Soc Lond 267:1525–1531.
McGraw KJ, Hill GE, 2001. Carotenoid access and intraspecific variation in plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). Funct Ecol 15:732–739.
McWhinney SLL, Bailey CA, 1989. Immunoenhancing effect of β-carotene in chicks. Poultry Sci 68(Suppl 1):94(Abstr.).
Merilä J, Sheldon BC, Lindstrom K, 1999. Plumage brightness in relation to haematozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. Ecoscience 6:12–18.
Milinski M, Bakker TCM, 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature 344:330–333.
Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai P, 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poultry Sci Rev 11:137–159.
Møller AP, Dufva R, Erritzoe J, 1998. Host immune function and sexual selection in birds. J Evol Biol 11:703–719.
Mortensen A, Skibsted L, Sampson J, Rice-Evans CA, Everett SA, 1997. Comparative mechanisms and rates of free radical scavenging by carotenoid antioxidants. FEBS Lett 418:91–97.
Olson VA, Owens IPF, 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514.
Pyle P, Howell SNG, Yunick RP, DeSante DF, 1987. Identification guide to North American passerines. Bolinas, California: Slate Creek Press.
Ruff MD, Reid WM, Johnson JK, 1974. Lowered blood carotenoid levels in chickens infected with coccidia. Poultry Sci 53:1801–1809.
Saino N, Stradi R, Ninni P, Møller AP, 1999. Carotenoid plasma concentration, immune profile and plumage ornamentation of male barn swallows (*Hirundo rustica*). Am Nat 154:441–448.
Seutin G, 1994. Plumage redness in redpoll finches does not reflect hemoparasitic infection. Oikos 70:280–286.
Shykoff JA, Widmer A, 1996. Parasites and signal brightness: how general should the relationship be? Naturwissenschaften 83:113–121.
Skarstein F, Folstad I, Liljedal S, 2001. Whether to reproduce or not: immune suppression and costs of parasites during reproduction in the arctic charr. Can J Zool 79:271–278.
Smits JE, Williams TD, 1999. Validation of immunotoxicology techniques in passerine chicks exposed to oil sands tailings water. Ecotoxicol Environ Safety 44:105–112.
Tengerdy RP, Lacetera NG, Nockels CF, 1990. Effect of beta carotene on disease protection and humoral immunity in chickens. Avian Dis 24:848–854.
Thompson CW, Hillgarth N, Leu M, McClure HE, 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. Am Nat 149:270–294.
Völker O, 1938. The dependence of lipochrome-formation in birds on plant carotenoids. Proc Int Orthinol Congress 8:425–426.

Weatherhead PJ, 1990. Secondary sexual traits, parasites, and polygyny in red-winged blackbirds. Behav Ecol 1:125–130.
Weatherhead PJ, Merz KJ, Bennet GF, Irwin RE, 1993. Parasites faunas, testosterone and secondary sexual traits in male red-winged blackbirds. Behav Ecol Sociobiol 75:13–23.
Ziegler RG, 1989. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 199:116–122.
Zuk M, Kim T, Robinson SI, Johnsen TS, 1998. Parasites influence social rank and morphology, but not mate choice, in female red junglefowl, *Gallus gallus*. Anim Behav 56:493–499.