Carotenoid-Based Plumage Colors and Immune Function: Is There a Trade-Off for Rare Carotenoids?

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ABSTRACT: Theory suggests that carotenoid-based signals are used in animal communication because they contain specific information about parasite resistance or immunocompetence. This implies that honesty of carotenoid-based signals is maintained by a trade-off between pigmentation and immune function for carotenoids, assuming that the carotenoids used for coloration are also immunoenhancing. We tested this hypothesis by altering the diets of nestling great tits (*Parus major*) with supplementary beadlets containing the carotenoids that are naturally ingested with food or beadlets containing the carotenoids that are incorporated into the feathers; a control group received beadlets containing no carotenoids. We simultaneously immune challenged half of the nestlings of each supplementation group, using a two-factorial design. Activation of the immune system led to reduced color expression. However, only nestlings fed with the naturally ingested carotenoids and not with the carotenoids deposited in the feathers showed an increased cellular immune response. This shows that the carotenoids used for ornamentation do not promote the immune function, which conflicts with the trade-off hypothesis. Our results indicate that honesty of carotenoid-based signals is maintained by an individual's physiological limitation to absorb and/or transport carotenoids and by access to carotenoids, indicating that preferences for carotenoid-based traits in sexual selection or parent-offspring interactions select for competitive individuals, rather than specifically for immune function.

Keywords: lutein, zeaxanthin, β-carotene, ornamentation, immune function, carotenoid availability.

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Carotenoid-based signals are frequently used by animals in sexual selection (Hill 1991; Andersson 1994) and parent-offspring interactions (Saino et al. 2000). To honestly reflect an individual's quality and thus allow for preferences to evolve, ornaments must be costly to produce or maintain (Andersson 1994). However, determining which evolutionary mechanism maintains honesty of carotenoid-based traits and thus why the more intensely colored individuals are preferred is a matter of some controversy.

Studies carried out mainly in birds and fish agree that the amount of carotenoids ingested is an important determinant of the color intensity (Hudon et al. 1989; Hill 1992, 2000; Wedekind et al. 1998; Hill et al. 2002; Fitze et al. 2003*a*; Tschirren et al. 2003*a*; Alonso-Alvarez et al. 2004; but see Linville and Breitwisch 1997). However, recent studies suggest that honesty of carotenoid-based coloration is maintained not simply by limited access to carotenoids but rather by a trade-off between ornamentation and immune function for rare carotenoids (Blount et al. 2003; Faivre et al. 2003; McGraw and Ardia 2003). The basic idea is that the immunoenhancing carotenoids (e.g., Bendich 1991; Blount et al. 2003) that are used in an immune response can no longer be used for ornamentation. Consequently, individuals that are mounting an immune response will appear more drab, suggesting that the more intensely colored individuals are healthier and have superior immunocompetence. By favoring intensely colored individuals, parents or mate partners are thus selecting the better-adapted individuals. Carotenoid-based signals frequently consist of lutein, lutein derivatives, and zeaxanthin but rarely of β-carotene (Stradi 1998). Consequently, in species whose carotenoid-based ornaments do not consist of β-carotene, the suggested trade-off maintains honesty only if lutein or zeaxanthin have immunoenhancing effects. Evidence for immunoenhancing effects of lutein and zeaxanthin, however, is scant, while it is well established for β-carotene (Bendich 1991). In addition, a recently published experimental study provides further evidence against immunoenhancing effects of lutein and zeaxanthin (Navara and Hill 2003).

An alternative hypothesis suggests that the intensity of the carotenoid-based coloration depends (apart from the carotenoid availability) on the capacity to absorb and/or transport carotenoids (Surai 2002; Tschirren et al. 2003*a*; McGraw et al. 2005) and thus reflects an individual's nutritional condition. Carotenoids are absorbed and transported by lipoprotein complexes (Surai 2002) consisting of proteins and lipids (e.g., triglycerides; Stevens 1996). Since lipids are the main energy reserves, honesty of carotenoid-based coloration might be maintained by a trade-off between lipids being used for energy generation or for absorption and/or transportation of carotenoids (Surai 2002; Tschirren et al. 2003*a*; McGraw et al. 2005).

To disentangle these two hypotheses, we performed an experimental field study on great tit nestlings (*Parus major*). The great tit is a small hole-nesting passerine (Gosler 1993) whose diet contains the carotenoids lutein, zeaxanthin, and β-carotene (Partali et al. 1987). It shows a yellow plumage coloration, which consists of the carotenoids lutein and zeaxanthin but not β-carotene (Partali et al. 1987). Since immunoenhancing effects of β-carotene are known but those of lutein and zeaxanthin are not, it is first necessary to test whether the carotenoids deposited in the plumage (lutein and zeaxanthin) have immunoenhancing effects and thus whether the assumptions of the trade-off hypothesis are fulfilled. Consequently, we fed nestlings three different diets starting during the early stages of feather development and ending when the development of plumage feathers was almost finished. The first group of nestlings was fed with carotenoids that are deposited in the feathers (lutein and zeaxanthin: CarotF group) to test whether the carotenoids, which are responsible for the yellow plumage coloration, have immunostimulating effects. The second group was supplemented with the carotenoids that are ingested with the natural food (lutein, zeaxanthin, and β-carotene: CarotN group). Since immunoenhancing effects of β-carotene are known, nestlings of this second group were expected to show increased immune activity, thus allowing the alternative hypothesis to be tested. A third group of nestlings was supplied with no additional carotenoids (control group), allowing detection of the effects of the carotenoid supplementation treatments.

To test for the trade-off between immune function and ornamentation, we immune challenged half of the nestlings of each carotenoid supplementation group before the feathers appeared; the remaining nestlings received control injections. The carotenoid supplementation and the immune challenge were carried out simultaneously to ensure that the individuals faced the suggested trade-off situation where they might have to invest in both immune function and ornamentation at the same time (Svensson et al. 1998; Fitze et al. 2003*a*). If the carotenoids deposited in the feathers promoted immune responses, nestlings of both carotenoid-supplementation groups (CarotN and CarotF groups) would show increased immune function. Alternatively, if only β-carotene promoted immune responses, the immune function of nestlings of the CarotN group, but not the CarotF group, should have been increased. According to the trade-off hypothesis, nestlings with limited access to carotenoids (control group) were expected to develop duller plumage coloration when mounting an immune response (Olson and Owens 1998) because the limited carotenoids would be used up by immune function and thus no longer incorporated into the feathers. Further, the plumage coloration of carotenoid-supplemented nestlings (CarotN and CarotF groups) would be less or not affected by immunization, since nestlings were less or not carotenoid limited, and thus carotenoids could be used for both immune function and incorporation into plumage. Alternatively, if honesty of carotenoid-based ornaments was maintained by limited absorption and/or transportation capacity of carotenoids, experimental activation of the immune function should have led to reduced color expression regardless of the carotenoid treatment.

Material and Methods

Field Experiment

The experiment was conducted in 2001 in the Forst, a forest near Bern (46°54′N, 7°17′E/46°57′N, 7°21′E), Switzerland. To test for the trade-off hypothesis, we carried out a carotenoid supplementation treatment consisting of three treatment groups and an immune challenge consisting of two treatment groups, using a two-factorial design. The three treatment groups were dosed with a combination of beadlets (lutein/zeaxanthin, β-carotene, or control; Hoffmann–La Roche, Basel, Switzerland), which differed only in carotenoid content. We supplemented two randomly chosen nestlings per nest with beadlets containing the carotenoids that occur in their natural diet (CarotN group; Partali et al. 1987). They were supplemented with 17 mg ($\pm$0.25 mg) lutein/zeaxanthin beadlets (containing 5.58% lutein and 0.44% zeaxanthin) and 2.6 mg ($\pm$0.25 mg) β-carotene beadlets containing 8% β-carotene. Another two nestlings of the same nest were fed with 17 mg ($\pm$0.25 mg) lutein/zeaxanthin beadlets per feeding, the carotenoids present in the feathers (CarotF group; Partali et al. 1987), and 2.6 mg beadlets containing no carotenoids. A control group (control group) of two nestlings per nest received 19.6 mg ($\pm$0.25 mg) beadlets containing no carotenoids per feeding. The supplemented amount of carotenoids and the carotenoid ratios were chosen according to previous experiments (Tschirren et al. 2003*a*). All nestlings were fed six times every other day

with the beadlets corresponding to their supplementation treatment. The feeding treatment started 4 days posthatching and ended 14 days posthatching. In great tits, the first breast feathers usually break through the skin 7–8 days posthatching. The carotenoid supplementation thus started before the first breast feathers appeared (Winkel 1970; B. Tschirren and P. S. Fitze, personal observations), and it ended at the time when most body feathers were fully developed and feather carotenoid content thus could no longer be increased (Fitze et al. 2003*a*). The beadlets were directly inserted into the throat of the nestlings. Thereafter, a small bee larva of standard size was inserted into the throat to ensure the swallowing of the beadlets (Tschirren et al. 2003*a*).

Four days after hatching, one randomly chosen nestling of each supplementation group was immune challenged with an intramuscular injection to prime the immune response, and the other nestling served as a control for the immunization. The injection consisted of 50 μL human diphtheria-tetanus (DT) vaccine (Kinder, Merieux) and 50 μL 5% rabbit red blood cells in phosphate-buffered saline (PBS) in the immune-challenged group and 100 μL PBS in the control group. This challenge first induces a T cell–mediated immune response, which then activates the adaptive B cell–mediated immune response (e.g., Goldsby et al. 2003). B cell–mediated immune responses need more time to become detectable than T cell–mediated immune responses (e.g., Goldsby et al. 2003). It is also important to appreciate that the adaptive (B cell–mediated) and the innate (T cell–mediated) immune responses do not operate independently and that the order of their interplay is fixed (Goldsby et al. 2003). The response to the immune challenge that we induced can typically be quantified (*a*) by measuring the humoral immune response and thus the antibody (ab) titers of the antigen-specific immunoglobulins or (*b*) by the faster responding cell-mediated immune response. Challenging half of the nestlings and comparing them with control-injected nestlings thus allows for testing the effect of mounting an immune response on the expression of the carotenoid-based plumage coloration. There were no differences in body weight between treatment groups at the start of the experiment (feeding treatment: $F = 0.07$, $\mathrm{df} = 2, 216$, $P = .93$; immunization: $F = 0.14$, $\mathrm{df} = 2, 216$, $P = .71$; interaction: $F = 0.32$, $\mathrm{df} = 2, 216$, $P = .72$). To assess both the effect of carotenoid supplementation and the effect of the simultaneous immune stimulation on the activation of the immune system, we measured the in vivo cell-meditated immune response. As pointed out above, the immune challenge carried out 4 days posthatching first activates the cell-mediated immune system and thus should promote the cell-mediated immune response. To check whether and under which circumstances the immune challenge activated the immune system, we injected phytohemagglutinin (PHA) into the left wing web of each nestling 14 days posthatching. We subcutaneously injected 0.1 mg of PHA-P (Sigma, Germany) dissolved in 0.02 mL of sterile PBS in the center of the left wing web (patagium; Tschirren et al. 2003*b*). PHA induces a cell-mediated, nonspecific mitogenic immune response of T lymphocytes that results in a swelling. Twenty-four hours (± 1 h) after injection, this swelling was measured with a micrometer (Mitotuyo 2046FB-60) to the nearest 0.01 mm (Smits et al. 1999; Blount et al. 2003). The wing web index was calculated as the difference between the thickness of the patagium before and 24 h after injection (Tschirren et al. 2003*b*). The wing web index was always measured by the same person (B. Tschirren), and it was highly repeatable ($r = 0.99$, $P < .001$; Lessells and Boag 1987). Fifteen days posthatching, nestling plumage coloration was quantified under standardized conditions with a digital camera (Fitze et al. 2003*b*). Nestlings were placed in a box covered with a photographic filter lens. The bird's eye was protected from the flash by a thin cardboard at one end of this box. This box was placed in fixed position inside a larger opaque camera box. Two flashes (Nikon SB26) were mounted inside the camera box. Pictures were taken using a digital camera (Nikon E2 with a 105-mm, f/2.8 Nikkor objective) whose front lens was 50 cm above the bird's plumage. The digital camera used measured colors within the spectrum range visible to humans. Since the carotenoid pigments reflect only light in the human-visible range (e.g., Hill 1998, 2002; O'Neil et al. 2001), this method reliably quantifies the carotenoid content of the feathers (see also Tschirren et al. 2003*a*). Standard white chips (Kodak Color Control Patches) were fixed to each side of the filter for calibration of the equipment during the color analysis. Using this protocol, we were able to take photos with standardized light exposure, photographic angle, and object-objective distance. To analyze the coloration, the pictures were imported into Adobe Photoshop. A virtual second layer, holding 10 square measurement areas of 400 pixels each, was placed over the photograph. The program calculated the mean RGB (red, green, blue) values per square (filter-blur-average). Thereafter these values were converted to hue, saturation, and brightness values by the algorithm described by Foley and van Dam (1984). The repeatability of the carotenoid-based nestling plumage coloration was high (hue: $r = 0.80$, $F = 9.11$, $\mathrm{df} = 1, 12$, $P = .01$; saturation: $r = 0.842$, $F = 11.618$, $\mathrm{df} = 1, 12$, $P = .01$; brightness: $r = 0.824$, $F = 10.374$, $\mathrm{df} = 1, 12$, $P = .01$). Because previous studies found that the plumage saturation (but not plumage brightness and not always plumage hue) reflects the carotenoid content of the feathers (Saks et al. 2003; Tschirren et al. 2005), we restricted our analyses to plumage saturation.

Quantification of the Humoral Immune Response

We measured the humoral immune response against DT vaccine by the enzyme-linked immunosorbent assay (ELISA) sandwich technique, following the protocol of Svensson et al. (1998). A blood sample (60 μL) of each nestling was taken before applying the experimental treatments 4 days posthatching to obtain a baseline measurement. Fourteen days posthatching (10 days postinjection), before PHA injection, a second blood sample was taken in order to detect increased ab titers. Blood samples were taken 10 days postinjection because ab titers in adult tits peaked 10–15 days postinjection (Svensson et al. 1998; also see end of next paragraph) and handling nestlings later than 14 days posthatching increases the risk of early fledging.

As a positive control for the ELISA assay, we collected blood in winter 2002 from vaccinated adult great tits from the same population. Adult great tits were injected with the same DT vaccine as the nestlings, and blood (60 μL) was collected before vaccination and afterward in 5-day intervals to measure the development of the immune response. For the analysis of the ab titers, we initially coated microtiter plates (8 × 12 well format, Fisher-Labosi) with 100 μL of tetanus toxin (3 μg dL^{-1}; AbCys 8750-2004, Paris). Plates were incubated overnight at room temperature and then saturated with 200 μL of PBS containing 1.5% bovine serum albumin. After 2 h, plates were washed three times with PBS, and 100 μL of diluted sample were added (1 : 100). Plates were then incubated 2 h and thereafter washed with PBS. One hundred μL of peroxidase-conjugated rabbit antichicken immunoglobulin G (dilution 1 : 3000; Sigma A-9046) were then added and incubated for 2 h. Plates were washed again, and 100 μL of peroxidase substrate (0.4 mg mL^{-1} *o*-phenylenediamine dihydrochloride; Sigma) were added. The reaction was left for 5 min in the dark and then stopped, using 50 μL HCl (1 M). The optical density (OD) value of each cone was read at 492 nm with a spectrophotometer. This measurement was used as a value of ab titers against tetanus. The ab titers against tetanus of adults peaked 10–15 days postinjection (Λoptical density [ΛOD = OD sample − OD baseline]: 1.23 ± 0.08 SE). The titers of 42 blood samples were measured twice to assess the repeatability of our method (Lessells and Boag 1987). The repeatability was acceptable ($r = 0.82$; $F = 4.32$, df = 41, 42, $P < .001$).

Statistics

For the analysis of plumage coloration, the plumage saturation was corrected for differences between photos (estimated with standard white chips [Kodak Color Control Patches]; Tschirren et al. 2003*a*). We conducted mixed-model ANOVAs with the nest as a random effect (blocking factor) and carotenoid supplementation and immunization and their interaction as fixed effects. For the analysis of differences between immunized and control-injected nestlings within a given carotenoid supplementation group, we used individual contrasts.

Results

Measuring the T-cell-mediated immune response against PHA revealed significant differences between carotenoid supplementation groups in immunized nestlings ($F = 3.49$, df = 2, 83, $P = .036$). Immunized nestlings in the CarotN group, but not in the CarotF and control groups, mounted a significantly larger response against PHA (individual contrasts; CarotN vs. CarotF: $F = 5.95$, df = 1, 83, $P = .017$; CarotN vs. control: $F = 4.56$, df = 1, 83, $P = .036$; CarotF vs. control: $F = 0.10$, df = 1, 83, $P = .759$). When analyzing both immunized and control-injected nestlings together, there was a significant interaction effect between the carotenoid supplementation and the immunization treatments (table 1; fig. 1). Immunized nestlings of the CarotN group mounted a significantly higher response against PHA (fig. 1), but there were no significant differences between immunized and control-injected nestlings in the CarotF group and the control group (fig. 1).

The carotenoid-based plumage coloration was significantly different between the carotenoid supplementation groups (table 2; fig. 2). Individual contrasts revealed that there were significant differences between the CarotN and control groups ($F = 126.93$, df = 1, 131, $P \ll .01$) and between the CarotF and control groups ($F = 170.82$, df = 1, 136, $P \ll .01$) but not between the CarotN and CarotF groups ($F = 2.93$, df = 1, 138, $P = .09$). Additionally, there was a significant interaction effect between the carotenoid supplementation and immunization treatment (table 2). Immunized birds in the CarotN group showed reduced plumage coloration compared to control-injected nestlings of the CarotN group (fig. 2), while there were no color differences between immunized and control-injected individuals in the CarotF and control groups (fig.

Table 1: Effects of carotenoid supplementation and immunization on the PHA responses

Factor	df	*F*	*P*	% variance explained
Carotenoid supplementation	2, 216	.84	.434	.34
Immune challenge	1, 216	.48	.492	<.001
Interaction	2, 216	3.58	.029	1.44

Note: Results of a mixed-model ANOVA with nest as a random effect are shown.

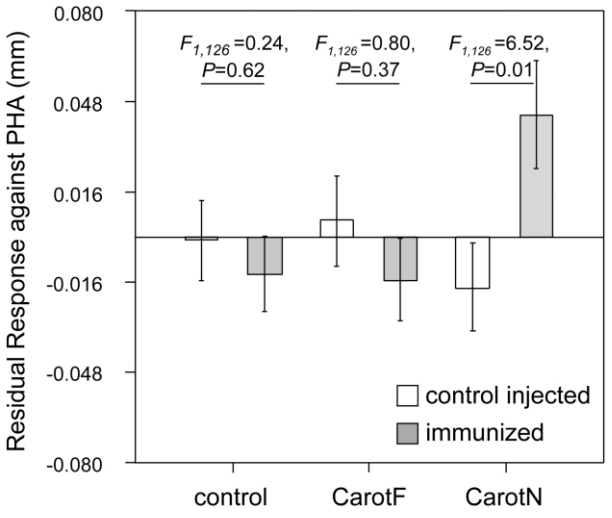

Figure 1: Effects of carotenoid supplementation (CarotN = supplementation with the carotenoids naturally ingested with the food: β-carotene, lutein, zeaxanthin; CarotF = supplementation with the carotenoids deposited in the feathers: lutein and zeaxanthin; control = no carotenoid supplementation) and immunization on PHA responses (residuals [mean ± SE] of the model including only the nest effect; see "Material and Methods"). The statistics of the individual contrasts between the two immunization groups of each carotenoid supplementation group are presented.

2). Individual contrasts further revealed that the plumage coloration of immunized birds of the CarotN group was significantly different from immunized ($F = 37.10$, df = 1, 39, $P \ll .01$) and control-injected birds ($F = 48.10$, df = 1, 40, $P \ll .01$) of the control-fed group.

We could not detect increased ab titers against tetanus in nestlings that were immune challenged (ΔOD [OD 14 days posthatching − OD 4 days posthatching] = 0.027 ± 0.002 SE), although our ELISA protocol allowed for the quantification of ab titers up to five times smaller than those measured in adults 10 days postinjection (minimal measurable ΔOD [95% limit] = 0.28 OD). This suggests that nestling great tits are not able to produce the same quantity of immunoglobulins within the same time span as adults do (Ros et al. 1997).

Discussion

Theoretical studies showed that ornamental traits, including carotenoid-based coloration, have to be costly to produce in order to honestly reflect an individual's status (Zahavi 1975; Grafen 1990), and recent work has suggested that many ornaments, ranging from coloration to morphological structures and exaggerated behaviors, serve as specific advertisements of health and immune state (Møller et al. 1999). In many cases, however, it is unclear how the ornamental traits reveal health.

For carotenoid-based coloration, one recently stated hypothesis suggests that the carotenoids used for pigmentation are also beneficial for the immune function and thus that a trade-off in the use of rare carotenoids occurs between ornamentation and the immune system. This suggests that only healthy individuals can afford to invest the carotenoids in ornamentation (Blount et al. 2003; McGraw and Ardia 2003; Hill et al. 2004). The basic assumption of this hypothesis is that the carotenoids used for pigmentation, that is, lutein and zeaxanthin, have immunoenhancing effects. Here we test this assumption and the existence of the proposed trade-off in nestling great tits. We experimentally show that during the time when the carotenoid content of the feathers can be modified, immunized nestlings supplemented with the carotenoids that they naturally ingest with the food (i.e., β-carotene, lutein, and zeaxanthin; CarotN group) mounted higher responses against PHA than did immunized nestlings of the control-fed (control group) and lutein/zeaxanthin (CarotF) groups. Further, the responses against PHA by the nestlings of the CarotF group (fed with the carotenoids responsible for yellow plumage coloration) and the immunized nestlings of the control group were not significantly different. This indicates that in nestling great tits, only β-carotene or β-carotene in combination with lutein and zeaxanthin (but not lutein and zeaxanthin alone; e.g., Bendich 1991; Navara and Hill 2003; but see Kim et al. 2000; Blount et al. 2003) have immunoenhancing properties. Immunoenhancing effects of lutein and zeaxanthin alone are rarely documented in literature (Kim et al. 2000; Blount et al. 2003; McGraw and Ardia 2003) and they could not be confirmed by our and by Navara and Hill's (2003) studies (see also Alonso-Alvarez et al. 2004). This result therefore suggests that in contrast to β-carotene (see, e.g., Bendich 1991 for a review), lutein and zeaxanthin do not have immunoenhancing effects, in general. Because positive effects of β-carotene alone are well understood (see, e.g., Bendich 1991 for a review), the positive effects on the cellular immune response of the immunized nestlings in the CarotN group are most likely due to β-carotene

Table 2: Effects of carotenoid supplementation and immunization on plumage saturation

Factor	df	F	P	% variance explained
Carotenoid supplementation	2, 216	101.26	<.001	30.11
Immune challenge	1, 216	.48	.49	.07
Interaction	2, 216	4.47	.01	1.33

Note: Results of a mixed-model ANOVA with nest as a random effect are shown.

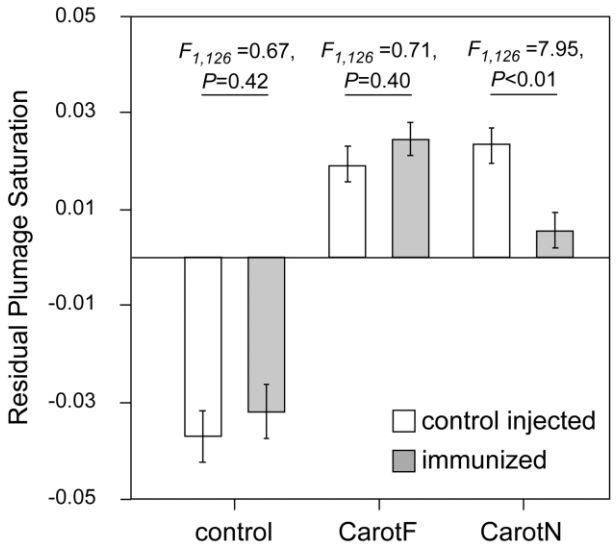

Figure 2: Plumage coloration in relation to carotenoid supplementation (CarotN = supplementation with the carotenoids naturally ingested with the food: β-carotene, lutein, zeaxanthin; CarotF = supplementation with the carotenoids deposited in the feathers: lutein and zeaxanthin; control = no carotenoid supplementation) and immunization (residuals [mean ± SE] of the model including only the nest effect; see "Material and Methods"). The statistics of the individual contrasts between the two immunization groups of each carotenoid supplementation group are presented.

alone, rather than the combination of the three carotenoids.

Since there were no significant differences in the cell-mediated immune responses between the control-injected nestlings of the CarotN group and the nestlings of the CarotF and control groups (individual contrast; CarotN group and control group vs. CarotF and control group: $F = 0.01–0.98$, df $= 1, 216$, $P = .32–.93$) and since the carotenoids were supplemented until the day when we measured the nestlings' cell-mediated immune responses (14 days posthatching), our results show that none of the naturally ingested carotenoids directly affected the T cell–meditated immune response (Gossage et al. 2000; Navara and Hill 2003; but see Blount et al. 2003; McGraw and Ardia 2003, 2005). However, immunized nestlings of the CarotN group showed enhanced responses against PHA, showing that β-carotene (but not lutein and zeaxanthin) primed the immune system and suggesting that it stimulates humoral immune responses.

Besides assuming that the carotenoids used for pigmentation bear immunoenhancing effects, the trade-off hypothesis predicts reduced carotenoid-based coloration in individuals with an activated immune system. Indeed, carotenoid-based plumage coloration was affected by immunization in the group where the immune system was primed—the immunized CarotN group. This reveals that the timing of the immune challenge was early enough to provoke effects on plumage coloration (Fitze et al. 2003*a*). Since we have no evidence that lutein and zeaxanthin—the two carotenoids used for pigmentation—were significantly sequestered for immune tasks and since immune challenged and control-injected nestlings of the CarotN group received the same amount of carotenoids, no difference in plumage coloration between the immunized and the control-injected nestlings would have been expected. Consequently, our results do not provide evidence for the existence of the suggested trade-off between immune function and ornamentation for rare carotenoids (Blount et al. 2003; Faivre et al. 2003) but are evidence for the hypothesis that the limited absorption and/or transportation capacity of the carotenoids maintains honesty of carotenoid-based ornaments (Surai 2002; Tschirren et al. 2003*a*; McGraw et al. 2005). The fact that nestlings of the immune-challenged CarotN group showed reduced plumage coloration suggests that, due to the increased immune function, more energy and thus more lipids were consumed (see Demas 2004 for a review). As a consequence, fewer lipids were available for absorption and/or transportation of the ingested carotenoids (Surai 2002), which in turn may have caused the reduced ornamentation (Surai 2002; Tschirren et al. 2003*a*; McGraw et al. 2005).

Alternatively, our results might be explained by a trade-off between the uptake of β-carotene and the uptake of lutein and/or zeaxanthin due to limited absorption capacity (Kiessling et al. 2003). The uptake of β-carotene might be actively or passively favored (shown by McGraw et al. 2004 and McGraw and Gregory 2004 for zeaxanthin) due to increased β-carotene need during immune response (Bieri and Farrell 1976; Surai 2002). As a result, less lutein and/or zeaxanthin could be transported, which would result in decreased plumage coloration. In this study, we cannot distinguish between the two hypotheses. But, since both hypotheses assume that plumage coloration is reduced due to limited lipoprotein complexes used for carotenoid transportation, our study supports the idea that honesty of carotenoid-based coloration is maintained by the limited absorption and/or transportation capacity of carotenoids.

It is, however, important to note that the magnitude of the absorption and/or transportation limitation is relatively small, since it accounts only for 1.33% (table 2) of the observed variation of the carotenoid-based plumage coloration (see also Tschirren et al. 2003*a*). Much more important is the access to carotenoids, which explained 30.1% of the observed variation in plumage coloration.

None of the studies that support the existence of a trade-

off between pigmentation and immune function for rare carotenoids (Blount et al. 2003; Faivre et al. 2003; McGraw and Ardia 2003) showed that under a carotenoid-restricted food regime, an immune challenge led to a more reduced ornamental coloration than under a less carotenoid-restricted regime, and thus, it is difficult to judge what is the relative importance of carotenoid availability and immune function for ornamentation. However, what most of the studies dealing with carotenoid-based ornaments have in common is the fact that experimental carotenoid feeding leads to increased coloration (Hudon et al. 1989; Hill 1992, 2000; Wedekind et al. 1998; Hill et al. 2002; Blount et al. 2003; Fitze et al. 2003*a*; McGraw and Ardia 2003; Tschirren et al. 2003*a*). This and the finding in this study that the plumage coloration was only slightly limited by the absorption and/or transportation capacity of the ingested carotenoids, indicate that honesty of these traits is mainly guaranteed by the restricted access to carotenoids. Thus, differences between individuals in territory quality and food-searching abilities seem to determine most importantly the expression of the carotenoid-based traits (e.g., Slagsvold and Lifjeld 1985; Fitze et al. 2003*b*). This indicates that by favoring the most exaggerated ornaments, females and/or parents may select for food-searching abilities and individual territory quality and thus for the most competitive individuals, rather than specifically for immune function.

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