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Testosterone increases bioavailability of carotenoids: Insights into the honesty of sexual signaling

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Androgens and carotenoids play a fundamental role in the expression of secondary sex traits in animals that communicate information on individual quality. In birds, androgens regulate song, aggression, and a variety of sexual ornaments and displays, whereas carotenoids are responsible for the red, yellow, and orange colors of the integument. Parallel, but independent, research lines suggest that the evolutionary stability of each signaling system stems from tradeoffs with immune function: androgens can be immunosuppressive, and carotenoids diverted to coloration prevent their use as immunostimulants. Despite strong similarities in the patterns of sex, age and seasonal variation, social function, and proximate control, there has been little success at integrating potential links between the two signaling systems. These parallel patterns led us to hypothesize that testosterone increases the bioavailability of circulating carotenoids. To test this hypothesis, we manipulated testosterone levels of red-legged partridges *Alectoris rufa* while monitoring carotenoids, color, and immune function. Testosterone treatment increased the concentration of carotenoids in plasma and liver by >20%. Plasma carotenoids were in turn responsible for individual differences in coloration and immune response. Our results provide experimental evidence for a link between testosterone levels and immunoenhancing carotenoids that (*i*) reconciles conflicting evidence for the immunosuppressive nature of androgens, (*ii*) provides physiological grounds for a connection between two of the main signaling systems in animals, (*iii*) explains how these signaling systems can be evolutionary stable and honest, and (*iv*) may explain the high prevalence of sexual dimorphism in carotenoid-based coloration in animals.

honest signaling | immune function | immunocompetence handicap hypothesis | coloration

Parallel, but independent, lines of research indicate that both androgens and carotenoids play a major role in the development and expression of honest signals of individual quality among vertebrates, relevant in sexual and social contexts (1–14). In birds, androgens control song, aggression, and a variety of sexual ornaments and displays (1–4), and carotenoids are bright red and yellow pigments of showy integument (6–12). In addition to signaling functions, testosterone regulates male sexual maturation and sperm production (15), and carotenoids have antioxidant and immunoenhancing activity (8, 9, 12, 16, 17). Signaling theory posits that the stability of traits conveying information on individual quality relies on the costs associated with signal production (18, 19), which prevent cheating in poor-quality individuals (they are "honest" signals) (19). Following this reasoning, the immunocompetence handicap hypothesis (ICHH) (20) posits that the honesty of androgen-dependent sexual traits relies on the immunosuppressive action of testosterone (21, 22). Recent studies emphasize that carotenoid allocation to color displays imply diversion away from the immune and detoxification systems, once again suggesting an immunological basis for honest signaling (8–10, 12, 17). Despite the strong similarities, research on the roles of testosterone and carotenoids in signaling individual quality has followed independent lines, with little success at integrating potential proximate links. In birds, circulating levels of both testosterone and carotenoids show vernal elevations that coincide with the mating season, higher values occur in males compared with females, and lower levels occur in younger compared with older individuals (8, 11, 15, 17, 23–25). The parallel nature of these patterns led us to generate a sequence of hypotheses and tests (Table 1) aimed at connecting the two research frameworks into a single system that would allow honest signaling of testosterone- and carotenoid-dependent traits. We first hypothesize that testosterone, produced endogenously, facilitates the availability of circulating carotenoids, that cannot be synthesized *de novo* (6, 16). Demonstration of this causal link plus the dual function of carotenoids on ornament development and immune function led us to hypothesize that elevation of plasma carotenoids would buffer testosterone-mediated immunosupression, providing an explanation for contradictory evidence for the ICHH (26, 27). In addition, a causal link between circulating testosterone and carotenoids would reveal a more comprehensive system of signaling individual quality: only in high-quality individuals could an increase in carotenoids triggered by androgens be used for ornamentation, rather than compensation for testosterone-mediated immunosuppression. The existence of a link between androgen- and carotenoid-dependent traits is expected according to status signaling theory, because a "badge of status" (e.g., carotenoid coloration) has to be consistent with individual behavior (e.g., aggression) to avoid social costs (28).

As an additional goal of our study, upon testing the sequence of arguments presented above, we introduced a second set of hypotheses (Table 2) to explain potential proximate mechanisms for testosterone-induced elevation in plasma carotenoids. First, a potential increase in energy demands associated with higher testosterone levels may result in increased food intake. Because carotenoid availability in animals ultimately relies on dietary access, increased food intake could elevate circulating carotenoids. Second, the anabolic effects of testosterone could increase the production of reactive oxygen species and hence a requirement for antioxidants. As a result, carotenoids may be liberated from major body stores such as the liver and fat (29, 30) into circulation. A third possibility is that testosterone increases the absorption efficiency of ingested carotenoids (e.g., changing the activities of digestive enzymes or levels of lipoproteins), resulting also in elevated plasma levels.

We tested most of these hypotheses (Tables 1 and 2) by experimental manipulation of testosterone levels in captive

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Abbreviations: ICHH, immunocompetence handicap hypothesis; GLM, general linear model; CMI, T cell-mediated immune response.

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Table 1. Brief explanation of the main framework hypotheses (in bold) and predictions

Hypothesis	Framework hypotheses description/prediction
h1	**Testosterone elevates circulating levels of carotenoids** Circulating carotenoids will be higher in testosterone-treated birds compared with controls. Within testosterone-treated birds, circulating carotenoids will increase after an increase in the hormone.
h2	**Circulating carotenoids explain external coloration** Positive association between plasma carotenoids and integument color.
h3	**There are tradeoffs in the use of carotenoids for ornamentation and immune function** Positive association between residual carotenoids (i.e. circulating carotenoids not explained by external coloration) and immune response.
h4	**Testosterone levels decrease immune function (ICHH)** Immune function will be reduced in testosterone-treated birds compared with controls.
h5	**Testosterone-dependent elevations in plasma carotenoids buffer immunosuppression** Immune function will not be reduced in testosterone-treated birds compared controls, because the former will show elevated carotenoid levels (according to *h1*) with immunoenhacing activity (according to *h3*). Further predictions involve manipulation of testosterone among groups that differ in individual quality (carotenoids will be used for ornamentation rather than immune function only in the high-quality group).

red-legged partridges. In a first experiment, we predicted an elevation in circulating carotenoids outside the mating season in both males and females treated with exogenous testosterone (T-birds) but not among sham-implanted controls (C-birds). The timing of this experiment during the gonadal involution period allowed us to take advantage of a natural "seasonal castration" without interfering with the normal physiology of birds as would be the case with highly invasive methods such as surgical gonadectomy. In a second experiment performed during the breeding season, we studied the combined effects of testosterone and carotenoids on immune function and color display. Here we restricted hormone manipulations to males at mating in a context of sexual selection. In that experiment, we also analyzed the effects of testosterone on food intake and distribution of carotenoids among body stores, to investigate proximate mechanisms (Table 2).

Results and Discussion

Testosterone implants during the gonadal involution period (i.e., postbreeding season) elevated plasma androgen levels of T-birds above controls (Mann–Whitney U, day 10: $P < 0.001, n = 24$; day 25: $P < 0.001, n = 25$), and the increase was similar for males and females (repeated measures ANOVA: sex $F_{1,9} = 0.146$, $P = 0.711$; day $F_{1,9} = 13.451$, $P = 0.005$). Testosterone treatment produced a striking concomitant increase in plasma carotenoids above controls (t test, day 10: $t_{22} = 3.843$, $P = 0.001$; day 25: $t_{23} = 4.00$, $P = 0.001$), parallel to circulating androgens and independent of sex (repeated measures ANOVA: sex $F_{1,9} = 0.013, P = 0.913$; day $F_{1,9} = 27.318, P = 0.001$). Our manipulation generated plasma androgen and carotenoid levels similar to those found in unmanipulated birds at mating (25, 31) and confirmed our predictions regarding a cause–effect association.

Our second implant experiment performed during the mating season successfully created differences in hormone levels between groups (t test: $t_{26} = 3.519$, $P = 0.002$), with T-birds showing androgen concentrations above controls until the end of the experiment (T-birds: 1,515 pg/ml ± 530.7 SD; C-birds: 691 pg/ml ± 683.9 SD), but again, well within the normal range of the species at breeding (31). The concentration of carotenoids in the liver and plasma of T-birds were, respectively, 23.1% and 24.6% higher than those of C-birds, and no other significant difference was found (Table 3). Furthermore, plasma carotenoids increased by 22.1% within T-birds relative to preimplant levels (before implanting: 17.3 μg/ml ± 4.26 SD; after implanting: 21.1 μg/ml ± 3.97 SD, paired t test, $t_{12} = -2.64$, $P = 0.021$), whereas they remained virtually identical in C-birds (before: 17.4 μg/ml ± 3.71 SD; after: 17.5 μg/ml ± 3.08 SD, paired t test, $t_{14} = -0.147$, $P = 0.885$). To understand how the experimental differences in circulating carotenoids could affect fitness components, we analyzed color development and immune response. Because our model species displays external red coloration, we tested whether carotenoids in plasma and body stores predicted color in the bare integument extending from the lore to the ocular ring, a sexually dimorphic trait (32). Integument coloration was strongly associated with carotenoids in plasma (Pearson $r^2 = 0.42, P < 0.001, n = 28$), but not in the liver or fat (both $P > 0.1$). Color was not related to testosterone

Table 2. Brief explanation of the mechanistic hypotheses (in bold) and predictions

Hypothesis	Mechanistic hypotheses underlining *h1* description/prediction
h1.1	**Testosterone increases food intake rate (thereby elevating plasma carotenoids)** Food intake will be higher in testosterone-treated birds compared to controls. Food intake will increase within testosterone-treated birds following manipulation of hormone levels. In the long-term, high levels of plasma carotenoids will be maintained as long as food intake rate is elevated.
h1.2	**Testosterone increases mobilization of carotenoids from body stores (thereby elevating plasma carotenoids)** Carotenoid levels in the main body stores (fat and liver) will be lower in testosterone-treated birds compared to controls. In the long-term, testosterone-treated birds will deplete carotenoid stores, showing concomitant reductions of circulating carotenoids.
h1.3	**Testosterone increases absorption efficiency of ingested carotenoids (thereby elevating plasma carotenoids)** The elevation of plasma carotenoids in testosterone-treated birds cannot be explained by increased food intake or elevated carotenoid turnover among body stores. In the long term, high levels of plasma carotenoids will be maintained even if food intake rate remains constant.

We formulate the potential proximate mechanism explaining *h1*. Note that the formulation of the hypotheses comes after verification that testosterone had a positive effect on circulating carotenoids, and therefore, there is a hierarchy in the presentation and testing of the hypotheses. Note also that the hypotheses are complementary, not mutually exclusive.

Table 3. Effect of testosterone treatment on morphological and physiological measures

Measure	T-birds (n = 13), mean ± SE	C-birds (n = 15), mean ± SE	Test statistic*	P
Plasma carotenoids, μg/ml	21.1 ± 1.10	17.5 ± 0.79	2.696	0.012
Carotenoid concentration in fat, μg/g	6.2 ± 1.47	6.1 ± 1.27	0.068	0.946
Carotenoid concentration in liver, μg/g	10.6 ± 0.80	8.6 ± 0.46	2.208	0.036
Intensity of red coloration	211.1 ± 1.14	210.7 ± 0.94	0.301	0.766
Abdominal fat deposit, g†	1.5 ± 0.45	1.6 ± 0.36	−0.560	0.580
Liver mass, g†	7.2 ± 0.53	7.3 ± 0.42	−0.259	0.798
Body mass, g	486.9 ± 8.14	473.3 ± 7.30	1.247	0.224
Pectoral muscle thickness,mm	23.6 ± 0.21	24.0 ± 0.30	−0.924	0.364
Length of digestive tract, cm‡	86.2 ± 2.45	82.7 ± 2.04	1.122	0.273

*Between groups differences are shown as the result of two-tailed t tests.
†t test performed on log-transformed data.
‡T-birds n = 12; C-birds n = 14.

treatment (t test: $t_{26} = 0.301$, $P = 0.766$), but differences should not be expected if the signal conveys reliable information on the quality of the individual, and quality did not differ between experimental groups before or after treatment. In fact, whereas testosterone manipulation did not affect condition estimates (i.e., mass, pectoral muscle or fat stores, see Table 3), individual changes in body mass along the experiment predicted final coloration regardless of treatment [i.e., birds increasing in body mass showed greater color development, general linear model (GLM): mass change $F_{1,25} = 6.763$, $P = 0.015$; treatment $F_{1,24} = 0.044$, $P = 0.836$; interaction $F_{1,23} = 0.617$, $P = 0.440$]. A condition dependence rather than androgen dependence of carotenoid allocation to display functions (33) may explain inconsistent results of previous experimental studies where the confounding effects of body condition and food intake were not considered (34, 35). Along this line, recent research suggests that the development of sexual traits may ultimately depend on the condition of the individual, which affects both androgen production and carotenoid use (33, 36, 37). Our results may also reconcile conflicting evidence for the immunosuppressive nature of androgens [i.e., ICHH (20)]. A recent review (27) shows that the immunosuppressive effect of testosterone disappears when the effect of multiple studies on the same species are taken into account. In addition, some studies report just the opposite effect of testosterone on immune function (i.e., testosterone may be immunoenhancing, e.g., ref. 26). However, because we show that increased levels of plasma androgens facilitate the physiological availability of carotenoids, the immunosuppressive effects of androgens may be buffered by the capacity of carotenoids to boost the immune system, and differences between experimental treatments should not necessarily be expected. Moreover, the tradeoff in carotenoid function between health and ornamentation (8–10, 12, 17) predicts that the fraction of plasma carotenoid levels not explained by coloration should be associated with immune response. To test this prediction, we calculated the carotenoid residuals from a reduced major axis regression with red coloration. T cell-mediated immune response (CMI) was unrelated to treatment or overall plasma carotenoids (GLM: carotenoids $F_{1,25} = 0.068$, $P = 0.797$; treatment $F_{1,26} = 1.517$, $P = 0.229$; interaction $F_{1,24} = 0.004$, $P = 0.951$; Fig. 1*A*). However, carotenoid residuals explained interindividual differences in immune response regardless of treatment (GLM: residual carotenoids $F_{1,26} = 5.768$, $P = 0.024$; treatment $F_{1,25} = 0.052$, $P = 0.821$; interaction $F_{1,24} = 0.745$, $P = 0.396$; Fig. 1*B*). This result confirms the immunomodulatory role of carotenoids and highlights the importance of considering their dual function (i.e., health and display) when testing effects on only one endpoint. In addition, it reveals the potentially confounding consequences of carotenoid increase when androgen titers are manipulated to test the ICHH. Because androgens play an essential role in male reproductive function across animal taxa, we suggest that the reported effect on carotenoid bioavailability may be an ancestral physiological mechanism selected to protect the immune system. The evolution of carotenoid-based signals of quality may have been derived secondarily from this association, in concordance with the ICHH, that good-quality individuals would allocate fewer carotenoids to compensate for the immunosuppressive actions of testosterone, providing grounds to advertise their quality by devoting the carotenoid surplus to ornaments. Our results also provide an explanation for the preponderance of sexual dichromatism in carotenoid-dependent coloration compared with melanin or struc-

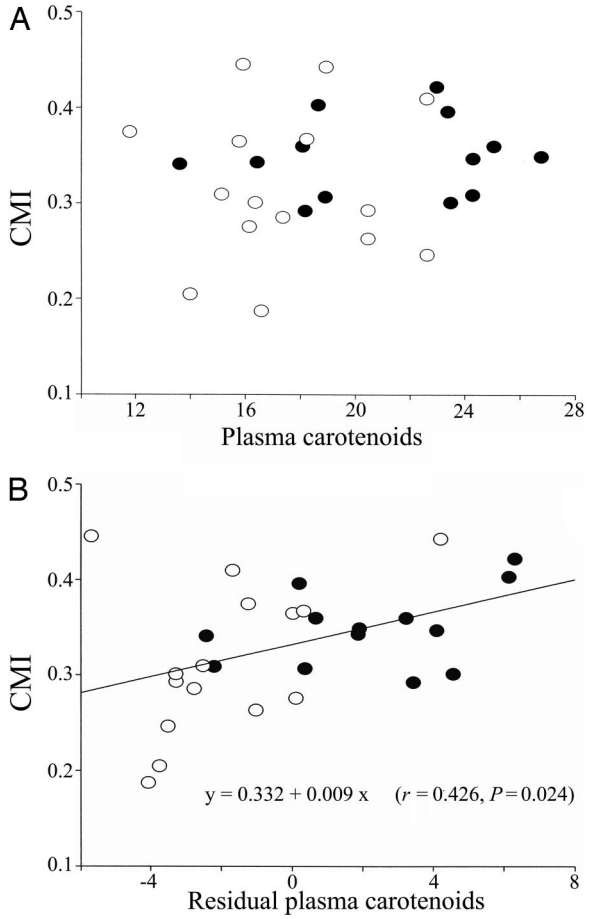

Fig. 1. CMI as a function of total plasma carotenoids μg/ml (*A*) and residual carotenoids from the regression with integument coloration (*B*). ○, C-birds (n = 15); ●, T-birds (n = 13).

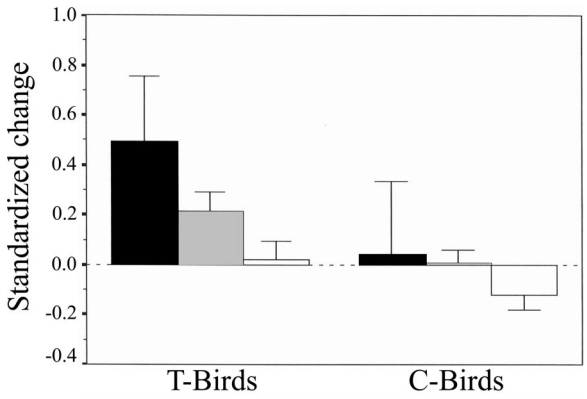

Fig. 2. Standardized change in plasma androgens (black bars), plasma carotenoids (gray bars), and food intake rate (white bars) after treatment with testosterone (T-Birds) or sham-implants (C-Birds). Error bars represent 1 ±SEM.

turally derived colors (38). We encourage further tests of our hypothesis in other animal models, including species without carotenoid-based color display, and involving different measures of immune function. The manipulation of testosterone levels under variable doses of dietary carotenoids will be especially valuable, because high doses could mask any difference in immune response whereas low doses may better reveal testosterone-mediated immunosuppression.

With regard to the proximate mechanisms responsible for the effect of testosterone on circulating carotenoids (i.e., Table 2), T-birds maintained their intake rate at preimplant level (before: 27.2 g per day ± 4.68 SD; after: 27.7 g per day ± 5.19 SD). The magnitude of the slight increase in food consumption within T-birds (i.e., 1.8%), regardless of the lack of statistical significance (paired t test: $t_{10} = -0.247$, $P = 0.810$), cannot alone explain the 22.1% increase in circulating carotenoids recorded within this treatment. In fact, any difference in food intake between groups after treatment (t test: $t_{26} = 1.734$, $P = 0.096$) was caused by the nonsignificant trend of C-birds to decrease food consumption along the experiment (before: 26.9 g per day ± 5.84 SD; after: 23.6 g per day ± 4.44 SD, paired t test: $t_{13} = 2.054$, $P = 0.061$). The marginal decrease in food intake among controls averaged −11.9%, whereas circulating carotenoids remained virtually identical (i.e., <0.7%; see Fig. 2). Although we do not reject the hypothesis that food intake could play a role in testosterone-mediated elevations of carotenoid levels, it alone would seem to be insufficient in accounting for our results. The hypothesis that testosterone increased the liberation of carotenoids from major body stores into circulation was also not supported by our experiment. Contrary to our predictions, the concentration of carotenoids in fat deposits and the liver were either the same or higher in T-birds compared with controls (Table 3). With regard to the hypothesis that testosterone increases the absorption efficiency of ingested carotenoids, we did not find any difference between treatments in the length of the digestive tract (Table 3), a trait showing considerable phenotypic plasticity in galliforms (39). Carotenoid absorption, however, could also be enhanced through increases in plasma lipoproteins or changes in the activity of digestive enzymes, all of which are affected by testosterone (40–42).

Until methods for the direct estimation of carotenoid absorption are applied (e.g., using labeled carotenoids to trace assimilation from diet to blood), serial measurements of plasma levels combined with different doses of testosterone may help to tease apart alternative explanations. If testosterone causes increased uptake of carotenoids in the gut, we predict that carotenoid levels should remain elevated as long as testosterone levels remain high, and that there should also be a positive dose-dependent effect. Alternatively, if testosterone causes transient elevations in carotenoid levels because of mobilization from body stores, we predict that blood carotenoids should decline over time as stores become depleted. In any case, mechanistic hypotheses investigating the role of testosterone in the regulation of plasma carotenoids deserve experimental studies designed specifically for that purpose. Because testosterone may also promote increased carotenoid intake by facilitating behavioral dominance over access to food, individual food intake should always be controlled for.

Materials and Methods

On July 4, 2003, during the involution phase of the gonadal cycle of the red-legged partridge [i.e., when endogenous levels of sexual steroids are minimal (31, 43)], 13 male and 12 female partridges were randomly assigned to experimental treatment and implanted with testosterone-filled (T-birds, $n = 11$) or empty (C-birds, $n = 14$) 30-mm-long s.c. capsules. Silastic implants (inner diameter 1.47 mm, outer diameter 1.97 mm; Dow Corning, Midland, MI) were sealed at both ends and filled with 20 μg of crystalline testosterone (ICN Biomedicals, Irvine, CA) or left empty. One week before manipulation all birds had low androgen levels (<96 pg·ml^{-1}) and circulating carotenoids were higher in males than in females regardless of experimental group (ANOVA treatment $F_{1,22} = 0.100$, $P = 0.755$; sex $F_{1,22} = 6.542$, $P = 0.018$). This species had not been previously subjected to hormone manipulations, so to explore the amount of exogenous testosterone needed to elevate plasma androgens within natural levels of males at mating (31) we used one, two, or three testosterone implants equally distributed between sexes. After manipulation, blood samples were collected on July 14 and 29, 2003. All of the T-birds elevated circulating androgens well above any control bird, within the natural range reported for the species [i.e., range <500 pg/ml to >5,000 pg/ml, (31)], and according to the number of implants (mean pg/ml ± SD: one implant, 899 ± 493.7; two implants, 1,220 ± 1,066.2; three implants, 2,221 ± 923.5). Birds were housed in individual pens (1 × 0.5 × 0.4 m) visually isolated from each other, at ambient temperature and natural photoperiod in Ciudad Real, Spain, and fed ad libitum a 1:1 mixture of commercial pelleted food (20% protein, 4.5% fat, 3.7% cellulose) and wheat.

On March 7, 2004, 28 male partridges not previously used in any experiment were randomly assigned to treatment and implanted with either one testosterone-filled (T-birds, $n = 13$) or an empty capsule (C-birds, $n = 15$). The timing of this experiment coincided with the mating period of the species, when gonadal size and endogenous production of sex steroids reach maximum seasonal values (31, 43). Housing conditions and location were exactly the same as in the previous year, although ambient temperature and photoperiod corresponded to natural spring values. We used a single 30-mm-long implant (20 μg of crystalline testosterone) because it was the minimal dose able to elevate plasma androgens in our previous experiment. One week before hormone manipulations, we collected a blood sample to determine initial levels of circulating androgens and carotenoids, and we also measured body mass, pectoral muscle thickness (with a USM22B ultrasonic meter; Krautkramer, Lewistown, PA), tarsus length, head width, and head length. Digital color pictures of the heads were taken under standardized conditions, and the initial intensity of red coloration of the bare lore and eye ring area was measured following Villafuerte and Negro (32). This procedure combines customized software with the use of digital imaging and commercial photofinishing software. Colors are quantified in a continuous scale of the conventional red-green-blue color model, amenable for statistical analyses. This method has previously revealed sexual dimorphism undetectable to the human eye for the same species and color trait that we used in our study (32). Preimplant daily food consumption was estimated by offering a known amount of food to 25 experimen-

tal birds and collecting the remains 24 h later. None of the reported preimplant measurements showed a statistically significant difference between experimental groups (all t tests $P > 0.140$). On March 22, 2004, we measured postimplant daily food consumption by offering a known amount of food and collecting the remains 48 h later. On March 26, 2004, we subjected all birds to a phytohemagglutinin (PHA) skin test to estimate T lymphocyte responsiveness following Smits *et al.* (44). The right wing web was injected s.c. with 0.05 mg of the mitogen PHA-P in 0.1 ml of PBS. Three measures of skin thickness were taken with a digital micrometer (to the nearest 0.01 mm) immediately before the injection and again 24 h later. CMI is presented as the logarithm of the change in mean wing web thickness. On April 8, 2004, we collected a second blood sample to determine postimplant levels of circulating androgens and carotenoids, measured body mass and pectoral muscle thickness, and took a second set of digital pictures. Finally, on April 13, 2004, all birds were euthanized humanely by cervical translocation. The liver and abdominal fat deposits were removed, weighed with a digital balance to the nearest 0.01 g, and frozen at −80 C to further determine carotenoid body stores. Abdominal fat was by far the largest body adipose deposit detected during necropsies and is the best indicator of overall body fat stores in galliforms (45). The gut and cecae were extended on a flat surface to measure total length to the nearest 1 mm. To avoid unnecessary suffering or pain to study subjects we followed protocols in concert with Spanish laws and the veterinarian staff of the Instituto de Investigación en Recursos Cinegéticos.

Sample Processing and Determination of Androgens and Carotenoids. All blood samples were taken within 3 min after the removal of a bird from its cage. After centrifugation (7,000 × g, 10 min), plasma was stored at −80 C until analysis. Plasma androgen levels were measured by RIA as described by Bortolotti *et al.* (11). Steroid extraction efficiency was >95%, and samples were analyzed in duplicate in five separate assays. Intraassay and interassay coefficients of variation were 5.3% and 8.0%, respectively. The minimum detectable androgen level was 96 $pg \cdot ml^{-1}$. To be conservative, nondetectable androgen samples (corresponding to unmanipulated birds outside the breeding season and 8 of 56 samples during breeding) were assumed to be equal to the minimum detectable dose (11). Total carotenoid concentrations were determined by spectrophotometry at 476 nm (11), using a standard curve of lutein (α-carotene-3,3′-diol, Sigma, Oakville, Canada) and after verification through HPLC that lutein was the predominant carotenoid pigment present in the samples (J.B. and J. Garrido, unpublished data). Plasma aliquots (50 μl) were diluted in 1000 μl of acetone and mixed, and the flocculent protein was precipitated by centrifuging the sample at 11,000 × g for 5 min. Fat and liver samples (0.2–2.0 g) were homogenized in 20 ml of diethyl ether, sonicated for 1 min, and left covered overnight. The solution was filtered and the residue was rinsed twice with 20 ml of ether, collecting extracted carotenoids in a glass flask. Extracts were evaporated under a N_2 gas stream and reconstituted in hexane. Optical density was determined in a Beckman-DU-7400 spectrophotometer (Beckman, Mississauga, Canada).

Statistical Analyses. Univariant comparisons were performed by means of t tests and paired t tests when the independent variable was normally distributed or Mann–Whitney U tests when normality could not be achieved by transformation. Multivariant tests were performed by means of GLM where the least significant factors, covariates, or interactions were sequentially removed following standard backward procedures or by means of repeated measures ANOVA. Parametric tests were applied after verification of normality and homocedascity of the dependent variables. All tests were two-tailed with an α level of 0.05.

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