Trade-Offs between Immune Investment and Sexual Signaling in Male Mallards

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ABSTRACT: Allocation trade-offs between the immune system and sexual traits are central to current sexual selection hypotheses but remain contentious. Such trade-offs could be brought about by the dual action of testosterone that stimulates sexual signals but also suppresses immune functions and/or by competition for carotenoids that can be deposited in ornaments or used as antioxidants in support of immune functions. We investigated the trade-off between investment in immunity and maintenance of testosterone, carotenoids, and sexually selected, carotenoid-based bill color in male mallards. Following a nonpathogenic immune challenge, facultative immune investment resulted in a syndrome of changes in allocation. Plasma carotenoids disappeared from circulation proportional to antibody production. In addition, the reflectance spectrum of the bill was affected; greater antibody production was associated with an increase in relative UV reflectance. Although changes in bill reflectance and plasma carotenoids were related, the relationship appeared more complex than direct competition with immunity. Finally, maintenance of testosterone was affected by immune investment: testosterone levels declined substantially when males produced more antibodies. Because males with high testosterone are preferred by females, the decline in testosterone, in addition to carotenoid depletion and effects on bill reflectance, could constitute a significant cost of immune investment.

Keywords: immunocompetence, carotenoids, testosterone, sexual signal, handicap, immunization.

Males of many species show a variety of exaggerated ornamental traits that are selected through female mate choice. Why females should prefer to mate with more ornamented males and how variability in ornaments under intense directional sexual selection is maintained are central questions of sexual selection theory (Andersson 1994). In a seminal article, Hamilton and Zuk (1982) first suggested that the answers might lie in the ever-changing selection pressures exerted by parasites that lead to continuous evolution of optimal host resistance types. However, how superior immunocompetence could be reliably reflected in superior development of sexual ornaments is a contentious issue. Recently, two direct trade-offs between immune function and sexual signals have been proposed: competition for carotenoids (Lozano 1994; von Schantz et al. 1999) and testosterone-induced immune suppression (Folstad and Karter 1992).

Many colorful sexual ornaments contain carotenoids, pigments that animals cannot synthesize but must ingest with their food (Olson and Owens 1998). Carotenoids also have antioxidant and immunostimulant properties (Bendich 1993) and hence may be required not only for deposition in ornaments but also in support of immune functioning. It has been hypothesized that competition for carotenoids between ornaments and immune function may then enforce honesty on carotenoid-based sexual signals (Lozano 1994; von Schantz et al. 1999). Recently, the first evidence for this hypothesis has been presented. Experimental immune activation resulted in reduced expression of the carotenoid-dependent sexually selected bill color in blackbirds (*Turdus merula*; Faivre et al. 2003), and extra carotenoids added to the diet of zebra finches (*Taeniopygia guttata*) resulted in increased immune responsiveness and more attractive bill color (Blount et al. 2003).

Expression of many secondary sexual characters depends on the steroid hormone testosterone (Andersson 1994). However, testosterone can also suppress the immune system (for review, see Folstad and Karter 1992 and references below). Thus, Folstad and Karter (1992) proposed that immunosuppression as an unavoidable cost of testosterone-dependent traits would ensure that only males with a superior immune system could bear high testosterone associated with more developed sexual signals. Al-

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though the generality of this mechanism has been questioned (see, e.g., Evans et al. 2000), it has been demonstrated that potential immune suppression can enforce honesty on testosterone-dependent sexually selected traits such as size of the exposed black bib in house sparrows (*Passer domesticus*; Evans et al. 2000; Gonzalez et al. 2001), timing of molt into nuptial plumage in superb fairy wrens (*Malurus cyaneus*; Peters 2000; Peters et al. 2000), and song behavior in European starlings (*Sturnus vulgaris*; Duffy et al. 2000; Duffy and Ball 2002).

Through trade-offs with testosterone and/or carotenoids, a variety of traits may thus honestly signal male immune quality. We investigated these trade-offs in the mallard (*Anas platyrhynchos*), a species that possesses several ornamental traits. Males in nuptial plumage have a bright green head, white collar, dark brown breast, black tail curls (Cramp and Simmons 1977), and a bright yellow bill that contains carotenoid pigments (Lönnberg 1938). Male plumage and bill become brown in summer and return to their ornamental state in autumn, when courtship and pair formation occur (Williams 1983). Although plumage qualities appear to have little effect on pairing success, females show a strong preference for males with a more yellow bill (Omland 1996*a*, 1996*b*) and for males that have higher courtship activity (Bossema and Kruijt 1982; Kruijt et al. 1982) and higher testosterone (Klint 1985; Schmedemann and Haase 1985; Klint et al. 1989; Davis 2002*b*). In this study, we examine the relationship between investment in immunity and sexually selected traits and show that antibody production after an immune challenge affected maintenance of plasma carotenoids, bill reflectance, and circulating testosterone.

Methods

Subjects and Experimental Protocol

Mallard duck pairs were collected in the wild in Bavaria, Germany, in 2000. Offspring produced by those pairs were hatched in 2000 and 2001 and belonged to 13 families, each with one to five (median = 3) full brothers. Ducklings were raised in groups and then at times were housed in single pairs in isolation during July 2002 in three mixed-sex flocks (sex ratio = 1 : 1) in outdoor aviaries (60 m^2, 60 m^2, and 120 m^2 with 11, 12, and 17 males, respectively). In August 2002, all females were redistributed while males remained in their original aviaries. Water was provided in a concrete pond of 4 m^2 plus two bathing pools or a fenced section (70 m^2) of a lake. Duck food (Kaspar Faunafood) and wheat were provided ad lib. at five or more feeding stations per aviary, with regular addition of lettuce. Birds were habituated to daily human presence in their aviaries and were accustomed to regular capture.

On October 21 and 22, 2002, 36 males (13 hatched in 2000, and 23 hatched in 2001; four males were excluded because they had been immunized previously to determine when antibody titers peak) were immunized with sheep red blood cells (SRBC; Haemosan). All males had recently completed the molt into nuptial plumage (we monitored plumage state from August until November at weekly intervals). At the time of immunization, the pairing and courting season (Williams 1983) had started, and testosterone levels were in their October–November peak (Paulke and Haase 1978). All birds in one aviary were treated in one session, and time of capture and delay between capture and completion of blood sampling were noted. A preimmunization blood sample was collected in heparinized capillary tubes and immediately put on ice. Animals were immunized by intraperitoneal injection with 0.5 mL of 10% SRBC in phosphate buffered saline. One week later (October 28 and 29), when antibody titers reached their maximum (A. Peters, unpublished data), postimmunization blood samples were collected.

Plasma Analysis

Blood samples were centrifuged for 3 min in a hematocrit centrifuge, and plasma was separated from the packed cells and stored at −70°C until analysis. Antibody concentrations in 20 μL of plasma were determined within 3 wk after plasma collection in a standard haemagglutination titration assay (Hudson and Hay 1976) using SRBC from the same blood sample used for immunization (for details, see Peters 2000). Most ducks (28 of 36) produced antibodies (titers: range = 0–6, median = 2.5, mean ± SE = 2.3 ± 0.3).

Plasma carotenoids were determined by spectrophotometric analysis using a Palm-SPEC spectrophotometer (Ocean Optics). We added 15–30 μL of plasma sample to 100–110 μL acetone, centrifuged for 10 min at 1,500 *g*, and measured absorbance of the supernatant at 446 nm (Tella et al. 1998; Bortolotti et al. 2000; J. J. Negro, personal communication). Linearity was confirmed in a dilution series (1 : 3–1 : 20) of five plasma samples. The total protein level in 10 μL of plasma was analyzed colorimetrically using the Fluitest total protein kit (Biocon). Testosterone was determined by VetMedLabor (Ludwigsburg) in 50 μL of plasma using a direct double-antibody radioimmunoassay (RIA; DSL-4100, Diagnostic Systems Laboratories). This RIA shows high sensitivity (0.05 ng/mL) and high specificity for testosterone (relative cross-reactivity for 5α-dihydrotestosterone 6.6%, all other steroids <2%). Intraassay variability was estimated at 6.7%–8.1% (3 × 14 replicate samples) and interassay variability at 5.7%–10.5% (3 × 11 replicate assays). Testosterone levels were ln transformed to normalize residuals. Testosterone levels were not

affected either by the time between capture of an individual and start of capture in its aviary or by the time between capture and completion of blood sampling of an individual (all $P > .1$). Despite reports from the literature that testosterone concentration follows a diurnal pattern in mallards (Davis 2002*a*), no correlation was found between time of capture (range = 10 : 10–15 : 45) and testosterone level (pre- and postimmunization both $P > .4$).

Bill Color Measurement and Analysis

We determined the reflectance spectrum of the bill between 320 and 700 nm using an S-2000 spectroradiometer with a DH-2000-FHS deuterium-halogen light source (Ocean Optics, Eerbek). Inclusion of the ultraviolet (320–400 nm, UV) is necessary because ducks are sensitive to UV light (Parrish et al. 1981). A cylindrical plastic tube was mounted on the bifurcated fiber optic probe and positioned at a 90° angle to the bill on three standardized spots (11.3 mm^2) between 5 and 10 mm under the right nostril. Reflectance (R) was calculated relative to a white standard (WS-2) with the program Spectrawin 5.0 (Avantes). The three spectra obtained for each bird were averaged and summarized over 3-nm steps.

The reflectance spectrum is double peaked, with a prominent trough around 450 nm, the area of peak carotenoid absorption (fig. 1, *left*). The carotenoid content of the bill consists mainly of lutein, with more or less equal parts zeaxanthin and 3-de-hydrolutein (high-performance liquid chromatography analysis; S. Andersson and A. Johansson, unpublished data). Double-peaked spectra are typically difficult to describe in terms of hue and chroma, and we mathematically summarized the spectra using principal component analysis (PCA). Three principal components (PCs) summarized >99% of spectral variation. Their coefficients reflect the multipeaked nature of the spectrum, with reflectance at most wavelengths correlated with that at other wavelengths (fig. 1, *right*; for a detailed description of how to relate PCs through their coefficients to the original spectra, see Cuthill et al. 1999). As is typical for PCA of reflectance spectra, PC1 summarized most (93%) of the variance between spectra and represented achromatic brightness (correlation with brightness: $r > 0.99$, $P < .001$), which often contains little relevant information (Cuthill et al. 1999). The other PCs then describe spectral shape (color), typically a more informative characteristic of the spectrum (see Cuthill et al. 1999). PC2 (describing approximately 60% of spectral shape variation) is negatively correlated with plasma carotenoids (Peters et al. 2004) and with carotenoid chroma ($r = -0.9$, $P < .0001$, $n = 36$), an estimate of carotenoid absorbance that predicts lutein content of yellow feathers ($(R_{450\,nm} - R_{700\,nm})/R_{700\,nm}$; Örnborg 2002). PC3, describing approximately 40% of variation in spectral shape, indicates relative reflectance in the UV and is positively ($r = 0.5$, $P = .001$, $n = 36$) correlated with UV chroma (R_{UV}/R_{total}; Andersson et al. 1998). PC3 is negatively correlated with plasma carotenoids (Peters et al. 2004), and lower preimmunization PC3 scores are associated with higher antibody response to immunization and greater sperm velocity, a trait affecting fertility (Peters et al. 2004). PCs may differ depending on the data set analyzed, and we calculated the three PCs for pre- and postimmunization spectra separately as well as together. These analyses produced identical P values for all significant results, and we present only those including the PCs calculated separately.

Statistical Analyses

We initially controlled for family- or aviary-based differences by including aviary and family as random effects, using residual maximum likelihood regression models. Consequences of antibody production on maintenance of sexual signals and condition were investigated by modeling effects of titer on changes (postimmunization − preimmunization) in condition (body mass, hematocrit, plasma protein), plasma carotenoids, testosterone (ln transformed), and the three PCs describing bill reflectance. Age (1.5 or 2.5 yr old) was also included in the models. Nonsignificant terms were eliminated one by one in order of smallest effect size by examining the change in deviance, which follows a χ^2 distribution. We present significance details for tests when excluding a term from the final model, which contains all terms with $P < .1$. Because antibody production did not vary for males of different ages ($\chi^2 = 0.4$, df = 1, $P = .5$) and because age had no significant effect in any model (all $P > .2$), we do not report details of individual tests of age. Similarly, family membership did not affect immune responses ($F = 0.9$, df = 12, 23, $P = .6$), and no significant contribution of family as a random effect was detected in any mixed model (all $P > .2$). Aviary membership did not significantly affect the proportion of males that produced antibodies (10/13, 10/12, and 8/11, respectively; $\chi^2 = 0.4$, df = 2, $P = .8$) or antibody titers (mean ± SEM = 2.5 ± 0.4, 2.6 ± 0.5, and 2.0 ± 0.3, respectively; $F = 1.7$, df = 2, 33, $P = .2$). Unless specifically mentioned otherwise, inclusion of aviary as a random effect made no significant contribution to the models (all $P > .2$). Excluding nonsignificant random effects never had qualitative effects on significance or conclusions from models. Means ± SE of difference as predicted by the final model are presented in the figures and text. Because all terms were either continuous or factors with two levels, for all tests df = 1, and we do not repeat this for each test separately. Descriptive data are

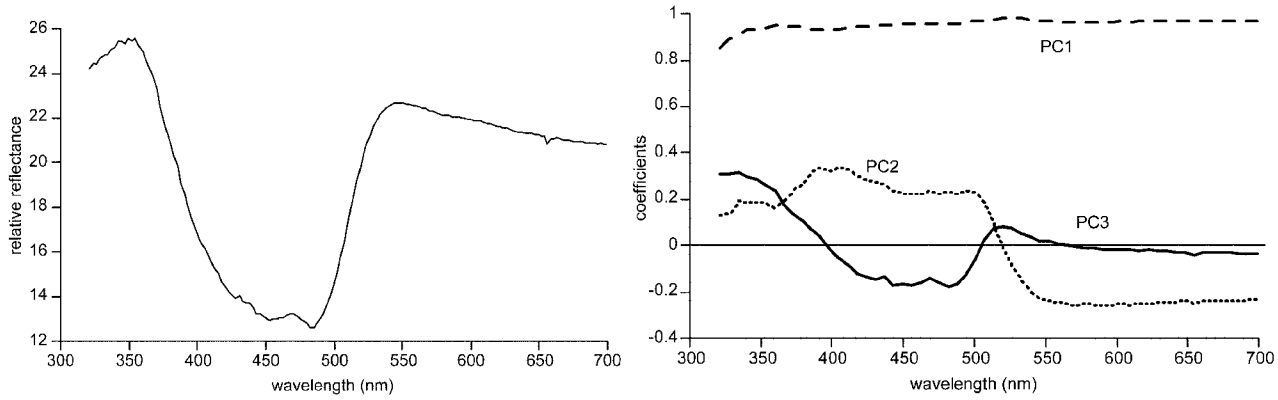

Figure 1: *Left*, mean reflectance spectrum of the yellow bill of the male mallard ($n = 36$ males, preimmunization). The prominent trough is a consequence of absorption of intermediate wavelengths by carotenoids. *Right*, coefficients relating the three principal components (PC1–3) to the original reflectance spectra (see "Methods" for details).

presented as means ± SEM unless indicated otherwise. Data were analyzed using Genstat (2002).

Results

Condition

Condition was not affected by immune investment. Antibody production was not related to changes in body mass ($\chi^2 = 0.4$, $P = .5$; controlling for an aviary effect: $\chi^2 = 17$, $P < .001$), hematocrit ($\chi^2 = 0.3$, $P = .6$), or plasma protein levels ($\chi^2 = 0.3$, $P = .6$).

Carotenoids

Carotenoid concentration was affected by immune investment (fig. 2*A*). The more antibodies males produced, the greater a decline in plasma carotenoids they experienced ($\chi^2 = 7.87$, $P = .005$; controlling for an aviary effect: $\chi^2 = 5.21$, $P = .02$). Changes in carotenoid level were independent of individual changes in condition; although there was a tendency that carotenoid concentration declined more when males lost more mass ($\chi^2 = 2.72$, $P = .099$), changes in carotenoids were independent of changes in plasma protein ($\chi^2 = 0.1$, $P = .7$), hematocrit ($\chi^2 = 2.4$, $P = .12$), or testosterone ($\chi^2 = 1.2$, $P = .3$). Preimmunization carotenoid concentration did not affect antibody production ($\chi^2 = 1.2$, $P = .3$). The decline in carotenoids was substantial, ranging on average from 0.0 μg/mL in nonresponders to -2.1 μg/mL in males that produced maximum titers (model predictions; see also fig. 2*A*), which equates to a difference of 1.1 SD (preimmunization mean = 4.07, SD = 1.87 μg/mL).

Bill Reflectance

The shape of the bill reflectance spectrum was affected by investment in antibody production as reflected by the third PC (fig. 2*B*). PC1 (brightness) was not significantly affected by immune investment (titer: $\chi^2 = 0.1$, $P = .8$) or by changes in condition (mass: $\chi^2 = 0.03$, $P = .9$; plasma protein: $\chi^2 = 0.7$, $P = .4$; hematocrit: $\chi^2 = 0.7$, $P = .4$) or testosterone level ($\chi^2 = 0.00$, $P = .99$), although PC1 tended to decrease with increasing decline in plasma carotenoids ($\chi^2 = 2.72$, $P = .099$). Similarly, PC2 was not significantly affected by titer ($\chi^2 = 0.5$, $P = .5$) or by the change in testosterone ($\chi^2 = 0.2$, $P = .7$) or condition (mass: $\chi^2 = 0.02$, $P = .9$; plasma protein: $\chi^2 = 0.03$, $P = .9$; hematocrit: $\chi^2 = 0.1$, $P = .7$). Although the change in PC2 was negatively related to the change in carotenoids, this relationship was not significant ($\chi^2 = 2.45$, $P = .1$). In accord with the negative correlation between PC2 and carotenoid chroma, the latter was also not affected by antibody production (titer: $\chi^2 = 0.00$, $P = .99$) but was positively related to the change in plasma carotenoid ($\chi^2 = 3.55$, $P = .06$). Scores for PC3 increased significantly with increasing antibody titer ($\chi^2 = 7.45$, $P = .006$; fig. 2*B*). PC3 was not affected by changes in condition (mass: $\chi^2 = 0.02$, $P = .9$; plasma protein: $\chi^2 = 0.1$, $P = .7$; hematocrit: $\chi^2 = 0.04$, $P = .8$; testosterone: $\chi^2 = 0.06$, $P = .8$). However, there was a positive relationship between change in PC3 and change in plasma carotenoids ($\chi^2 = 6.0$, $P = .014$); that is, a greater increase in PC3 was associated with a smaller decline in carotenoids. An increase in PC3 represents an increase in relative UV reflectance (fig. 1). Accordingly, there were positive correlations between change in UV chroma and titer ($\chi^2 = 10.7$, $P = .001$) and change in plasma carotenoids ($\chi^2 = 3.13$, $P = .077$).

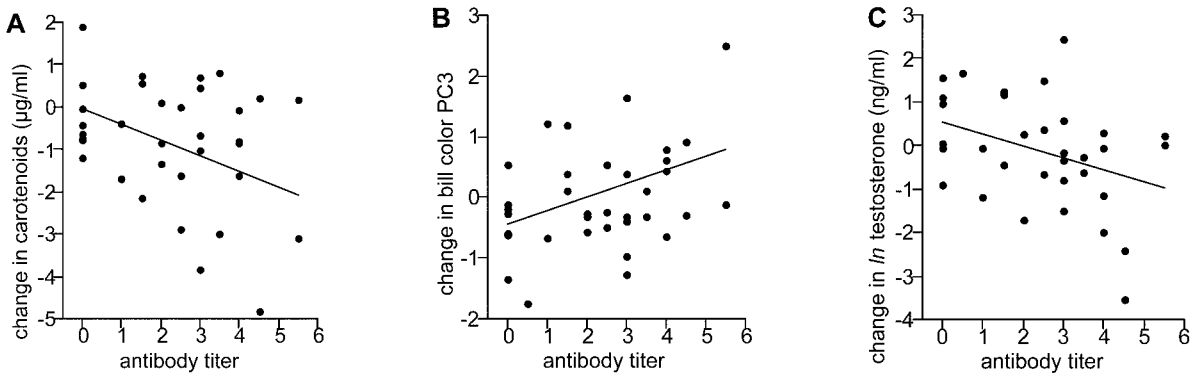

Figure 2: Consequences of immune investment. Depicted are changes (postimmunization − preimmunization) as a function of titer, primary antibody production after a single immunization with SRBC. *A*, Plasma carotenoid levels disappear from circulation ($\chi^2 = 7.87$, $P = .005$). *B*, Bill reflectance is affected: PC3 increases ($\chi^2 = 7.45$, $P = .006$). *C*, Maintenance of testosterone level is impaired ($\chi^2 = 6.06$, $P = .014$). Circles show observed values. The lines are predicted by linear (mixed) models.

Testosterone

Maintenance of testosterone was negatively affected by antibody production (fig. 2*C*). Testosterone level decreased more in males that produced more antibodies ($\chi^2 = 6.06$, $P = .014$; controlling for a marginally greater decrease when males lost more mass: $\chi^2 = 3.67$, $P = .055$). Testosterone maintenance was not affected by changes in other measures of condition (plasma protein: $\chi^2 = 0.6$, $P = .5$; hematocrit: $\chi^2 = 0.2$, $P = .7$) or plasma carotenoids ($\chi^2 = 0.02$, $P = .9$). The preimmunization testosterone level did not affect antibody production ($\chi^2 = 1.8$, $P = .2$). There was no overall (seasonal) change in testosterone (mean change $= -0.093$, paired $t = -0.453$, $P = .65$, $n = 36$). However, when males produced maximum titers, ln testosterone changed by -0.98 compared with $+0.52$ in nonresponders (model predictions; see also fig. 2*C*). The magnitude of this difference, 1.50 units, equates to 1.1 SD of preimmunization testosterone levels (mean = 0.79 ng/mL; ln-transformed mean $= -0.8$, SD = 1.30).

Discussion

We found evidence for trade-offs between production of an immune response and maintenance of sexually selected traits. Immunization with SRBC, invoking a T-cell dependent antibody response without any concomitant effects of disease, allowed us to estimate the isolated costs of antibody production (Hudson and Hay 1976). A single challenge induced a syndrome of changes proportional to the magnitude of immune investment; although condition (body mass, plasma protein, and hematocrit) was not affected, investment in antibody production affected maintenance of testosterone, plasma carotenoid levels, and bill reflectance (spectral shape). The observations emphasize the proportional nature of the trade-off; males that produced more antibodies showed greater changes in testosterone, plasma carotenoids, and bill reflectance compared with nonresponders (fig. 2). Other studies have demonstrated alternative costs of SRBC immunization, for example, increased metabolic rate and mass loss (Ots et al. 2001), reduced growth (Fair et al. 1999), and depressed protein deposition and increased fat deposition (Henken and Brandsma 1982). Few effects on sexual signals have been described: decreased attractiveness of the male's odor to females in mice and Siberian hamsters (*Phodopus sungorus*; Moshkin et al. 2001) and a duller bill in male blackbirds (Faivre et al. 2003). Our study highlights that antibody production can simultaneously affect various traits important in sexual signaling.

Carotenoids

Investment in the immune response was associated with a decline in plasma carotenoid reserves, irrespective of initial levels. Although carotenoid concentration declined markedly in males that produced large antibody titers, nonresponders showed no reduction in carotenoid concentration (fig. 2*A*). Carotenoids have important roles as antioxidants and free-radical scavengers (Mortensen et al. 1997) and offer protection against negative effects of reactive metabolites produced by metabolic processes (oxidative stress; von Schantz et al. 1999). Immunoenhancing effects of carotenoid supplementation are known from medicine (for review, Bendich 1993), poultry husbandry (Sklan et al. 1994), and ecology (Fenoglio et al. 2002; Blount et al. 2003). The logical reverse—carotenoid de-

pletion as a consequence of immune activation—is less well described, but some evidence exists; free radicals generated by infection can destroy carotenoids (Allen 1997), although most of the reduction in plasma carotenoids was ascribed to direct effects of the parasite (Allen and Fetterer 2002). To our knowledge, no study has previously shown that antibody production per se results in a decline in plasma carotenoids. However, a linear relationship between titer and carotenoid reduction is in agreement with carotenoids being destroyed (Vershinin 1999) while scavenging free radicals formed in the process of—and presumably proportional to—antibody production.

Bill Reflectance

In this study, we demonstrate a relationship between immune investment and maintenance of bill coloration as shown by changes in the shape of the reflectance spectrum. Greater production of antibodies was associated with increased PC3 scores (fig. 2*B*), that is, increased relative reflectance in UV compared with intermediate wavelengths (cf. fig. 1, *right*). No studies in mallards or other birds have related reflectance of fleshy ornaments to female mate choice and male qualities. Although it is clear in mallards that female mate choice is based in part on bill coloration (Omland 1996*a*, 1996*b*) and although mallards can perceive UV (Parrish et al. 1981), we currently do not know whether females target relative UV reflectance or whether higher or lower PC3 values would be more attractive. However, we argue that the most parsimonious interpretation is that in mallard ducks, an increase in PC3 constitutes a decline in bill color quality. Although other reflectance characteristics (PC1, PC2) are unrelated to male qualities, males with lower PC3 scores have faster-swimming sperm and respond more strongly to immunization (Peters et al. 2004). Thus, the same component of bill reflectance that predicts the strength of an immune reaction is negatively affected by the magnitude of immune investment.

The changes in bill reflectance were unrelated to changes in testosterone. Although systematic studies of individual variation in testosterone and bill reflectance are lacking, bill color is unlikely to be a testosterone-dependent trait in mallards because castrated males permanently develop a colorful plumage and a yellow bill (Haase 1993). Thus, the absence of a testosterone-based link between bill reflectance and immunity is unsurprising. Carotenoids are more likely to provide such a link, and our data provide some evidence for this, although the nature of the relationship requires further clarification.

The reflectance spectrum of the bill is strongly influenced by carotenoid absorption (fig. 1, *left*), and the bill contains the same carotenoids as we measured in plasma (see "Methods"). Nonetheless, the relationships observed between immune investment, maintenance of bill reflectance, and carotenoid depletion are not straightforward. First, changes in PC2 were not directly related to immune investment. However, in the absence of a direct link to antibody production, PC2 might be affected by changes in plasma carotenoid levels. We would have predicted an increase in PC2 with greater depletion of carotenoids because PC2 has high positive coefficients for wavelengths of peak carotenoid absorption (fig. 1, *right*) and is negatively correlated with carotenoid chroma (which reflects carotenoid content in feathers; Örnborg 2002). Indeed, the change in plasma carotenoids was negatively related to the change in PC2 and positively related to the change in carotenoid chroma, although these relationships fell just short of statistical significance. Second, PC3 was affected by immune investment and changes in plasma carotenoids. However, a greater increase in PC3 was associated with greater antibody production (fig. 2*B*) and a smaller decline in carotenoids, opposite to that expected under direct competition for carotenoid use between bill maintenance and immunity. The relationships among bill reflectance, immune investment, and circulating and deposited carotenoids are presumably complex because not all available carotenoids are necessarily deposited (McGraw et al. 2003), not all carotenoids have equal antioxidant capacity (Olson and Owens 1998), and keratin structures per se are sensitive to oxidative stress (von Schantz et al. 1999). Experimental data are required to elucidate these relationships, such as supplementing ducks with carotenoids present in the bill and/or others that are not deposited, and to observe the effects on bill reflectance and antibody production.

Testosterone

Maintenance of testosterone was negatively correlated with immune investment; irrespective of initial levels, testosterone declined as males produced more antibodies (fig. 2*C*). Although there was no overall decline in testosterone and nonresponders showed an increase in testosterone (fig. 2*C*), our data cannot unequivocally demonstrate that investment in antibody production is the cause of the decline in testosterone. Testosterone levels are highly variable between individuals (Paulke and Haase 1978), and it might be that males whose testosterone levels were declining naturally were able to mount a stronger immune response. These alternatives could be distinguished in future experiments by comparing testosterone maintenance in immunized males with a sham-immunized control group.

A direct trade-off between immunity and testosterone is a central argument of one of the most debated hypotheses on the functional and evolutionary significance of

exaggerated ornamental traits (Folstad and Karter 1992). This hypothesis poses that when female mate choice is based on testosterone-dependent sexual signals, concomitant unavoidable immune-suppressive effects of testosterone impose honesty on such signals. Although immunosuppressive effects of testosterone were initially largely known from the mammalian literature (Folstad and Karter 1992), such effects, although not universal (Hasselquist et al. 1999), have now been described in a variety of bird species (Duffy et al. 2000; Evans et al. 2000; Peters 2000; Casto et al. 2001). This immune-suppressive effect of testosterone would lead one to predict that increased allocation to immunity should result in decreased allocation to testosterone. Indeed, in chicken lines selected for high and low antibody responsiveness to SRBC, Verhulst et al. (1999) demonstrated that lines selected for high responsiveness to SRBC not only were more responsive to many antigens but also had lower testosterone level and smaller combs (comb size is a sexually selected signal). Such allocation trade-offs, however, have not previously been demonstrated at an individual level, although they seem likely. For example, interleukins and other cytokines—produced by immune cells during an antibody response—can inhibit testosterone production in vitro (Besedovsky and Delrey 1996), while treatment with a cytokine caused a rapid, transient decrease in serum testosterone in rats and humans (Mealy et al. 1990; van der Poll et al. 1993). Simple immunization with antigens such as SRBC can induce substantial changes in circulating hormones such as corticosterone (Besedovsky and Delrey 1996; Parmentier et al. 2002) but also insulin, growth hormone, and thyroxine (Gabrilovac et al. 1982). Thus, it seems plausible that a trade-off between testosterone maintenance and antibody production, as we have demonstrated here, might be fairly general.

A decline in testosterone, as a cost of greater antibody production, could affect male mallards in autumn. Males were immunized during the autumnal pairing season (Williams 1983), and testosterone-dependent male cues are important in mate choice at that time (Klint 1985). There is a positive correlation between courtship activity and natural testosterone levels (Davis 2002*b*), and testosterone treatment increases the frequency of male displays (Schmedemann and Haase 1985). Females show strong preferences for males that direct more displays toward them (Bossema and Kruijt 1982; Kruijt et al. 1982), and females preferentially court males with higher testosterone levels (Klint et al. 1989). In view of such strong sexual selection on elevated autumn testosterone level, a reduction of up to 1 SD in males that produced high antibody titers to SRBC (fig. 2*C*) could comprise a substantial (mating) cost. This hypothesis could be tested by observing female preferences and display activity of immunized males.

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