

Maternal Effects Mediated by Antioxidants and the Evolution of Carotenoid-Based Signals in Birds

Clotilde Biard,^{1,*} Diego Gil,² Filiz Karadaş,^{3,4} Nicola Saino,⁵ Claire N. Spottiswoode,⁶ Peter F. Surai,³ and Anders P. Møller⁷

1. Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 7103, Laboratoire de Parasitologie Evolutive, Université Pierre et Marie Curie–Paris 6, 7 quai Saint Bernard, F-75252 Paris Cedex 05; and Konrad Lorenz Institute for Ethology, Austrian Academy of Sciences, Savoyenstraße 1a, A-1160 Wien, Austria; 2. Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, E-28006 Madrid, Spain; 3. Department of Biochemistry and Nutrition, Avian Science Research Centre, Scottish Agricultural College, Ayr KA6 5HW, United Kingdom; 4. Department of Animal Science, University of Yüzüncü Yıl, 65080, Van, Turkey; 5. Dipartimento di Biologia, Università degli Studi di Milano, via Celoria 26, I-20133 Milano, Italy; 6. Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom; and Department of Science and Technology/National Research Foundation Centre of Excellence at the Percy FitzPatrick Institute, University of Cape Town, Rondebosch 7701, South Africa; 7. CNRS UMR 8079, Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud, Bâtiment 362, 91405 Orsay Cedex, France

Submitted December 10, 2008; Accepted June 26, 2009; Electronically published September 29, 2009

Online enhancement: appendix.

ABSTRACT: Bright yellow to red signals used in mate choice or intrasexual competition are based on carotenoid pigments that are hypothesized to be traded between physiological functions and coloration. These signals have recently been shown to be influenced by maternal effects. Indeed, yolk-derived carotenoids are essential for embryos to develop efficient carotenoid metabolism in posthatching life. Maternal effects facilitate adaptation to environmental variability and influence the evolution of phenotypic traits such as secondary sexual signals. Here we propose that maternal investment in yolk carotenoids promotes the evolution of carotenoid-based ornaments. We conducted a comparative analysis of lipid-soluble antioxidants (carotenoids and vitamins A and E) in the eggs of 112 species of bird. Species with large clutch sizes deposited higher yolk concentrations of the three antioxidants. There was a significant positive relationship between yolk carotenoids and the expression of male carotenoid-based signals, but not between yolk carotenoids and sexual dichromatism in these signals. These relationships were specific to carotenoids, as they were not found for vitamins A and E. This provides evidence consistent with the hypothesis that maternal effects mediated by yolk carotenoids play a role in the evolution of carotenoid-based signals as a response to sexual selection, likely based on organizational effects of carotenoids during embryo development.

Keywords: carotenoids, egg composition, sexual dichromatism, sexual selection, vitamin A, vitamin E.

Introduction

Maternal (or parental) effects occur when the phenotype of a mother or the environment she experiences influences the phenotype and fitness of her offspring (Mousseau and Fox 1998b). Conditions experienced during early stages of development may have long-lasting effects on an individual's phenotype and fitness (Lindström 1999; Metcalfe and Monaghan 2001), and maternal effects can modulate these early developmental conditions (Mousseau and Fox 1998a; Price 1998). In particular, maternal effects may affect the development of sexual signals in the offspring (Strasser and Schwabl 2004; McGraw et al. 2005; Eising et al. 2006; Rubolini et al. 2006). Given the potential for maternal effects to influence the evolution of phenotypic traits and facilitate adaptation to environmental variability (Wade 1998; Wolf et al. 1998; Räsänen and Kruuk 2007), maternal effects might promote or constrain the evolution of secondary sexual traits. In addition, maternal effects on the development of condition-dependent secondary sexual characters provide another source of additive genetic variance that can reinforce genetic variation in such signals (Miller and Moore 2007). If genetically based, maternal effects that cause offspring to develop new or more intensely colored signals would evolve more readily than zygotic genes and might thereby promote comparatively rapid evolution of these signals (Brodie and Agrawal 2001). A recent comparative analysis provided the first evidence in line with these hypotheses, showing that the evolution of sexual dichromatism in birds was linked to maternal deposition of immune factors in eggs (Saino et al. 2007).

* Corresponding author. Present address: Laboratoire Ecologie-Evolution, Université Pierre et Marie Curie–Paris 6, CNRS UMR 7625, 7 quai Saint Bernard (case 237), F-75252 Paris Cedex 05, France; e-mail: clotilde.biard@snnv.jussieu.fr.

Bright yellow-red signals based on carotenoid pigments have repeatedly been shown to be used in mate choice or intrasexual competition (reviewed in Hill 1998; Møller et al. 2000; Hill and McGraw 2006*b*). Carotenoid pigments may be a limiting resource to vertebrates because of environmental variability in their availability, differences in foraging ability, or differences in absorption or metabolizing efficiency (reviewed in Olson and Owens 1998; Møller et al. 2000). The involvement of these pigments in functions related to self-maintenance through the immune and detoxification systems (reviewed in Bendich 1993; Edge et al. 1997; Møller et al. 2000; Surai 2002) has led to the hypothesis of a trade-off between physiological functions and signaling. Such a trade-off could ensure the reliability of sexual displays that depend on carotenoids as pigments for signal production (Lozano 1994; Shykoff and Widmer 1996; von Schantz et al. 1999). However, the mechanism for maintaining reliability in carotenoid-based traits based on the antioxidant properties of these pigments has recently been questioned. A meta-analysis of the association between carotenoid concentration and antioxidant function revealed no clear evidence of a major antioxidant effect (Costantini and Møller 2008). The advertising role of carotenoids has instead been proposed to reveal availability of more important nonpigmented antioxidants (such as antioxidant enzymes or vitamin E and C) that would protect carotenoids from oxidation and consecutive bleaching and thereby allow development of bright signals (Hartley and Kennedy 2004).

Apart from their role in development of sexual signals, carotenoids also play a central role in reproduction, especially during embryo development and at hatching (reviewed in Surai et al. 2001*b*). This period of intense growth is indeed associated with elevated oxidative stress, to which avian embryos are very susceptible (Surai et al. 1999; Surai 2002). Experiments in wild birds in natural and captive conditions have shown that yolk carotenoids efficiently protect embryos against oxidative stress (Blount et al. 2002; McGraw et al. 2005). Female birds allocate large amounts of lipid-soluble antioxidants, carotenoids, and vitamins A and E to their eggs, relative to their circulating levels and body stores (Blount et al. 2000). Thus, carotenoids have been hypothesized to mediate maternal effects through egg composition as well as costs of reproduction in females (Blount et al. 2000). Yolk carotenoid concentration decreases with laying order in several species (Royle et al. 2001, 2003; Saino et al. 2002), and it has been hypothesized that maternal deposition of carotenoids in eggs may be an adaptive strategy related to hatching asynchrony and/or brood reduction (Royle et al. 2001, 2003). Maternal deposition of carotenoids to egg yolk may be modulated as a function of the embryo sex (Verboven et al. 2005; Badyaev et al. 2006; but see Saino et al. 2003*b*; Groothuis

et al. 2006; Romano et al. 2008). Carotenoid concentration in egg yolk may vary as a function of female condition and male attractiveness (Saino et al. 2002; Williamson et al. 2006; Szigeti et al. 2007). Finally, yolk-derived carotenoids modulate offspring phenotypic traits, such as color (Hörak et al. 2000; Biard et al. 2005, 2007; McGraw et al. 2005; Isaksson et al. 2006) and immune response (Saino et al. 2003*a*; Berthouly et al. 2007; Biard et al. 2007; Koutsos et al. 2007; Romano et al. 2008). Therefore, there is growing evidence that maternal allocation of antioxidants, in particular carotenoids, to eggs is a conditional maternal strategy of adaptive value.

A particularly important consequence of maternal investment of carotenoids in eggs for the development of carotenoid-based signals is that the availability of yolk-derived carotenoids is essential for embryos to develop an ability to acquire and use carotenoids in posthatching life (Koutsos et al. 2003). Indeed, embryonic exposure to maternally derived carotenoids determines the subsequent efficiency of carotenoid metabolism and the capacity of offspring to assimilate dietary carotenoids and to use and accumulate these at the cellular level in different tissues, independently of actual availability of dietary carotenoids: in immune organs (Koutsos et al. 2003), epithelium (Koutsos et al. 2003; McGraw et al. 2005), and feathers (Hörak et al. 2000; Biard et al. 2005, 2007; Isaksson et al. 2006). Yolk carotenoid concentration may thus have long-term effects on expression of carotenoid-based signals in adulthood (McGraw et al. 2005). Embryonic exposure to yolk carotenoids also modulates the development of immune function (Biard et al. 2005, 2007; Koutsos et al. 2007) as well as the potential costs of immune response, because chicks hatched from carotenoid-poor eggs showed an impaired ability to regulate systemic inflammation (Koutsos et al. 2006). Similar long-term organizational effects of early developmental conditions on physiological functions are well documented for early nutrition, even when a shortage of dietary availability of nutrients occurs during a short period in crucial stages of the development (e.g., Birkhead et al. 1999; Ohlsson et al. 2002; Blount et al. 2003). As a consequence, embryos that develop under poor conditions (i.e., low yolk carotenoid content) may be less efficient in adulthood at acquiring and using carotenoids for physiological or signaling functions, or they may be more in need of carotenoids as a result of early deficiency. Conversely, maternally derived carotenoids increase the efficiency of carotenoid metabolism in posthatching life, and thus increased maternal investment of carotenoids in eggs may confer an advantage to offspring in development of carotenoid-based signals. Therefore, if such signals are under directional selection, a positive genetic covariance between the particular maternal effect of yolk carotenoid content and offspring performance due to the expression

of the signal may enhance the rate of evolution of carotenoid-based signals (Moore et al. 1998; Wolf et al. 1998).

In light of the long-term enhancing effects of maternally derived yolk carotenoids on the ability to develop carotenoid-based signals, we hypothesize that, through this mechanism, such maternal effects may promote the evolution of carotenoid-based ornaments as a response to sexual selection. Under this hypothesis, maternal effects should mediate a positive relationship between yolk carotenoid concentration and expression of carotenoid-based signals at the interspecific level. However, under the hypothesis that the effects of carotenoids on expression of carotenoid-based signals are mediated through or reveal the effects of antioxidants other than carotenoids per se (Hartley and Kennedy 2004), we would expect a positive relationship between carotenoid-based coloration and these other, more important antioxidants in eggs. We tested these predictions in a comparative analysis of the yolk biochemical composition of 112 bird species from 39 families and 13 orders, for which we collected eggs and determined antioxidant composition in terms of carotenoids and vitamins A and E.

Material and Methods

Egg Collection and Biochemical Analyses

With the help of colleagues we collected fresh eggs from nonincubated clutches during the breeding seasons 2000–2003 in Europe, North America, and South Africa ($n = 93$ species). For ethical reasons we restricted the number of clutches to the minimum that would allow quantification of the amount of variance within and among species. In addition, we gathered data already available from the literature ($n = 22$ species), for which biochemical analyses were conducted in the same laboratory and with the same protocol and equipment. A full list of species and number of eggs and clutches sampled is reported in table A1 in the online edition of the *American Naturalist*. Antioxidants were extracted from 0.100–0.200 g of yolk, and carotenoid, vitamin A, and vitamin E concentrations were determined according to previously published procedures (Surai 2000; H  rak et al. 2002). Mean ($\pm$ SE) antioxidant concentration ($\mu\text{g/g}$) for each species and details on the biochemical analyses are given in the appendix in the online edition of the *American Naturalist*.

Carotenoid-Based Color Scores

To score carotenoid-based colors of plumage and bare parts, we adapted the method of Yezerinac and Weatherhead (1995), using color plates and species descriptions from handbooks (Brown et al. 1982–2004; Cramp et al.

1982–1994; del Hoyo et al. 1992–2004; Poole and Gill 1992–2004). Two of the regions defined by Yezerinac and Weatherhead (1995) were subdivided to increase scoring precision, and the body was divided into the following 10 regions (corresponding percentage of body surface): bill (3%), eye and eye ring (1%), chin, cheeks, and above eye (8%), breast (23%), belly (10%), crown and nape (11%), back (15%), wing (20%), tail (6%), and legs and feet (3%). The proportion of the surface covered with carotenoid-based color was recorded for each body region, as were color hue and brightness.

Color hue was scored as 1 for green, excluding glossy and iridescent green structural colors, 2 for yellow, 3 for orange, and 4 for red colors. Color hue scores ranged from 1 to 4 for plumage and from 2 to 4 for bare parts. Score values increased from yellow to red hues to reflect an increase in the concentration of pigments deposited (Inouye et al. 2001; Saks et al. 2003; Andersson et al. 2007) and/or an increase in the proportion of red pigments metabolically derived from yellow dietary pigments (Hill 1996; Inouye et al. 2001; McGraw and Schuetz 2004; Andersson et al. 2007), both potentially leading to an increase in production costs of the signal. The last score estimated color brightness and saturation and was coded as 1 for dull/pale, 2 for medium, and 3 for bright/intense colors.

In the species included in this study, yellow, orange, and red feathers have always been found to be, at least partly, based on carotenoids (see appendix). Noniridescent green or olive-green feather colors correspond to carotenoprotein complexes (Ong and Tee 1992) and/or a combination of carotenoids and structural colors (Prum et al. 1999) or a combination of carotenoids and brown melanins (Hill and McGraw 2006a). In accordance with biochemical analyses (McGraw and Wakamatsu 2004; McGraw et al. 2004a, 2004c), red-brown, or “rufous,” feather colors were not scored as carotenoid based (i.e., *Aptenodytes* spp., *Gallus* spp., *Hirundo* spp., *Sialia sialis*, *Taeniopygia guttata*). In the case of bare parts, “fleshy” pinks and brown of legs and feet, as well as “horn” bill colors were not scored as carotenoid based, as these colors may mainly be determined by blood (Negro et al. 2006) and keratin, respectively. Yellow and orange skin colors may be produced by carotenoids alone, by a combination of carotenoids and structural color (Prum and Torres 2003), or by a combination of carotenoids and blood hemoglobin (Negro et al. 2006). Determination of eye color, however, is mechanistically more complex and is reported to be likely due to the presence of purin and pteridin pigments and hemoglobin, whereas the importance of carotenoids is unclear (Hill and McGraw 2006a). A review of information on the biochemical and structural nature of colors used for scoring the species included in this study is provided in table A2 in the online edition of the *American Naturalist*.

The carotenoid-based color score of each body region was calculated as the percentage of the surface colored by carotenoids, multiplied by hue and brightness-saturation scores. The final score was calculated as the sum of the scores of all body regions weighted by the corresponding proportion of the body surface covered (Yezerinac and Weatherhead 1995). Results presented here are based on color scores including feathers, skin, and bill but excluding eye color because of the uncertain role of carotenoids in its determination. Color scores are reported in table A1. Analyses based on color scores calculated on plumage color alone gave qualitatively similar results. Sexual dichromatism in carotenoid-based signals was calculated as the difference between male and female scores.

Carotenoid-based color scoring was done by C.B. blindly with respect to yolk antioxidant data. In order to test the reliability of the scoring method, all species were additionally scored by a naive observer. Repeatability of scores among observers, calculated as the intraclass correlation coefficient (Lessells and Boag 1987) was highly significant (male score: $r = 0.89$; female score: $r = 0.87$; sex difference in score: $r = 0.88$; all $P < .0001$). In order to check whether the results were dependent on the method used to score color and/or on using color plates in handbooks, the analyses were run with male carotenoid-based coloration and sexual dichromatism in carotenoid-based coloration coded as binary variables (Birkhead and Møller 1994). The results were qualitatively similar (data available from the authors upon request).

Male and female carotenoid-based color scores were strongly positively correlated ($r = 0.83$, $P < .0001$), preventing them from being used together as predictors in multiple-regression analyses because of problems of collinearity. Male color scores and sexual dichromatism were also positively correlated ($r = 0.61$, $P < .0001$). Therefore, variation in yolk antioxidant concentration was investigated in relation to the extent of carotenoid-based color by use of male color score or sexual dichromatism in carotenoid-based color scores.

Comparative Data

Data on female body mass and clutch size were collected either from handbooks (Brown et al. 1982–2004; Cramp et al. 1982–1994; del Hoyo et al. 1992–2004; Dunning 1992; Poole and Gill 1992–2004; Hockey et al. 2005) or from our own field data, and where several estimates were available, mean values were used (data are reported in table A1). Female body mass was used to control for allometric effects because information on egg or yolk mass was not available for all species and because female body mass and egg mass were strongly positively correlated ($r = 0.98$, $P < .0001$, $n = 102$). Animals cannot synthesize caroten-

oids, which are therefore obtained from the diet (Goodwin 1984). High prevalence of carotenoid-based color across families of birds has been shown to be associated with carotenoid-rich diets, although diet was not the best ecological predictor of interspecific variation in carotenoid-based color (Olson and Owens 2005). We did not find any indication of correlated evolution between yolk antioxidants and diet. Furthermore, including diet in the analyses did not change the conclusions of the study (these results are reported in the appendix). Any relationship between clutch size and yolk antioxidant concentration might reflect the underlying relationship between clutch size and latitude. Mean latitude was taken as the midpoint of the northernmost and southernmost limits of each species' geographical breeding range (Garamszegi et al. 2005b; table A1). Including latitude in all models yielded results qualitatively similar to those presented here (data available from the authors upon request).

Statistical Analyses

Patterns of antioxidant investment in egg yolk in relation to female body mass, clutch size, and carotenoid-based signals were first investigated via generalized linear models with log-transformed mean concentrations for species and the GLM procedure in SAS v8.2 (SAS Institute, Cary, NC). Tests of residuals for normality and homoscedasticity were used to check the validity of the model.

However, phenotypic mean values for species cannot be considered statistically independent observations because cases of convergent evolution are mixed with cases of similarity due to common ancestry (Felsenstein 1985; Harvey and Pagel 1991). Therefore, we used a comparative method based on a composite phylogeny compiled from recent molecular phylogenies. Sources of phylogenetic information are detailed in the appendix, and the topology of the phylogenetic tree is shown in figure A1 in the online edition of the *American Naturalist*. Information on branch lengths was unavailable for this composite phylogeny, and thus all distances between nodes and nodes and tips were set to 1. Using alternative phylogenies or branch lengths based on molecular data gave qualitatively similar results (appendix).

We tested for correlated evolution of continuous characters, using generalized least squares models implemented in the software Continuous (Pagel 1997, 1999). These models control for similarity due to common ancestry and estimate evolutionary changes along the phylogeny through variance components of traits (Pagel 1997). Hypothesis testing relies on likelihood ratio statistics, by comparing the fits of two nested models. Phylogenetic trees should be bifurcating for Continuous to fit these models optimally. Polytomies were therefore resolved to bifurcations by excluding three species from the phylogeny (*Va-*

nellus vanellus, *Turdus philomelos*, and *Cercotrichas coryphaeus*). Species to be excluded were chosen at random among those involved in a polytomy, if none had carotenoid-based coloration. If some species in a polytomy had carotenoid-based colors, species were chosen at random so that the resulting bifurcating node included one species with and one species without carotenoid-based color. We first constructed our models controlling for potentially confounding variables and assessed the contribution of scaling parameters. In the absence of information on branch length in the phylogeny, the branch length scaling parameter κ and the overall path length scaling factor δ were set to their default value of 1. Another reason for setting the path length scaling factor δ to 1 is that its estimation has been shown to be biased (Freckleton et al. 2002). The importance of phylogenetic relationships is estimated through the phylogeny scaling parameter λ (Pagel 1999). At its default value of 1, λ yields a constant-variance (Brownian motion) model of evolution, while a value of $\lambda = 0$ would suggest that the trait evolved as if species were independent. Values of $\lambda < 1$ indicate that the tree topology overestimates the covariance among species. The contribution of λ was assessed by comparing the log-likelihood ratio of a model in which λ was constrained to its default value with that of an alternative model in which λ was estimated and took its maximum likelihood value. The estimation of λ was used in the final model if doing so improved the log likelihood; otherwise, the default value of 1 was kept. We then tested for correlated evolution between traits by comparing the log likelihood of the model of independent evolution, in which covariances between traits are forced to 0, with that of a model allowing correlated evolution, in which covariances are estimated. In all analyses, the better model was that allowing for correlated evolution, which was thus subsequently used to estimate correlation and covariances between traits. From the correlation coefficients we calculated phylogenetic partial correlation coefficients, and we used their associated Student's t_s values to derive corresponding P values. According to our directional predictions, we used unilateral tests at $\alpha = 0.05$.

Sample sizes differ among tests because information was not available for all species on all three antioxidants, the number of clutches sampled, and clutch size (table A1).

Results

There was significantly more variation in carotenoid concentration among than within species ($F_{92,631} = 20.60$, $MS_{\text{within}} = 283.75$, $MS_{\text{among}} = 5,845.57$, $P < .0001$), and this difference accounted for 75% of the total variance in yolk carotenoids (this test does not include species for which data were collected from the literature and for which

only mean values were available; see table A1). Similarly, there was more variance in vitamin A and vitamin E concentration in egg yolk among than within species (vitamin A: $F_{92,547} = 5.08$, $MS_{\text{within}} = 1.79$, $MS_{\text{among}} = 9.08$, $P < .0001$, $R^2 = 0.46$; vitamin E: $F_{92,547} = 7.77$, $MS_{\text{within}} = 3,772.14$, $MS_{\text{among}} = 29,322.58$, $P < .0001$, $R^2 = 0.57$). In addition, for carotenoids and vitamins, respectively, there was 13 and three times as much variation in yolk antioxidant concentration among species as among females (clutches) within species (carotenoids: among species: $MS = 5,082.59$, $F_{71,335} = 22.42$, $P < .0001$, females within species: $MS = 394.23$, $F_{207,335} = 1.74$, $P < .0001$; vitamin A: among species: $MS = 7.59$, $F_{71,292} = 4.28$, $P < .0001$, females within species: $MS = 2.70$, $F_{166,292} = 1.52$, $P = .001$; vitamin E: among species: $MS = 23,517.77$, $F_{71,292} = 13.52$, $P < .0001$, females within species: $MS = 8,536.47$, $F_{166,292} = 4.91$, $P < .0001$). Therefore, the relatively low number of eggs that we could ethically or practically sample in some species should not have affected the outcome of the comparative analyses. Mean carotenoid and vitamin A and E concentrations for the species were used in subsequent analyses. Variance in mean yolk antioxidant concentration across species is illustrated in frequency distributions given in figure A2 in the online edition of the *American Naturalist*.

Mean carotenoid concentration ($\pm$ SE) in egg yolk was $40.43 \pm 3.82 \mu\text{g/g}$, ranging from 1.50 to $162.09 \mu\text{g/g}$ across 112 species. Mean vitamin A concentration was $1.66 \pm 0.16 \mu\text{g/g}$ (range 0.12– $7.07 \mu\text{g/g}$, $n = 104$), and mean vitamin E concentration was $97.31 \pm 9.28 \mu\text{g/g}$ (range 9.30– $387.57 \mu\text{g/g}$, $n = 110$).

Mean carotenoid and vitamin A concentrations were both positively correlated with mean vitamin E concentration (log-transformed data; $r = 0.30$, $P = .001$, $n = 110$ and $r = 0.27$, $P = .005$, $n = 104$, respectively), while carotenoid and vitamin A concentrations were not significantly correlated with each other (log-transformed data; $r = 0.06$, $P = .56$, $n = 104$). When similarity among species due to common descent was taken into account, all three egg components were significantly positively correlated (carotenoids and vitamin A, correlation at $\lambda = 0.763$: $r_{\text{phyl}} = 0.21$, $P < .05$, $n = 100$; carotenoids and vitamin E, correlation at $\lambda = 0.796$: $r_{\text{phyl}} = 0.40$, $P < .001$, $n = 106$; vitamins A and E, correlation at $\lambda = 0.671$: $r_{\text{phyl}} = 0.30$, $P < .005$, $n = 100$).

Antioxidants in Relation to Female Body Mass and Clutch Size

In an analysis based on species, yolk carotenoids and vitamin A were significantly positively related to clutch size after female body mass was controlled for, but that was not the case for vitamin E (table 1). When similarity

among species due to common descent was taken into account, yolk carotenoids (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.33$, $P < .001$, $n = 108$), vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.34$, $P < .001$, $n = 100$), and vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.35$, $P < .001$, $n = 106$) showed significant positive correlation with clutch size after female body mass was controlled for.

Yolk Antioxidants and Carotenoid-Based Signals

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: the model controlling for female body mass and clutch size explained 28% of the total variance (table 2; fig. 1A). In addition, carotenoid concentration increased with sexual dichromatism in carotenoid-based signals: the model controlling for female body mass and clutch size explained 17% of the variance (table 2). Vitamin A concentration was not significantly related to the extent of carotenoid-based color (table 2; fig. 1B) but also increased with sexual dichromatism, although less strongly than did carotenoid concentration (table 2). In contrast, vitamin E concentration was not significantly related to carotenoid-based color of feathers and bare parts (table 2; fig. 1C) or to sexual dichromatism in these signals (table 2). When similarity among species due to common descent was taken into account, models controlling for female body mass and clutch size confirmed the significant positive relations between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.36$, $P < .0005$, $n = 108$) and between yolk carotenoids and sexual dichromatism (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.18$, $P < .05$). When incorporating effects of phylogeny, models controlling for female body mass and clutch size confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.02$, $P > .05$, $n = 100$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.03$, $P > .05$, $n = 106$). Sexual dichromatism was confirmed to be positively related to vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.18$,

$P < .05$, $n = 100$) but not to vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.10$, $P > .05$, $n = 106$).

In a multiple regression of male carotenoid-based color score as a function of female body mass, clutch size, and the three antioxidants, only carotenoid concentration explained a significant part of the variance (table 3). The same result was found when similarity among species due to common descent was taken into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.40$, $P < .0005$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.0003$, $P > .05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.13$, $P > .05$; at $\lambda = 0.965$, $n = 100$). In a multiple-regression model based on species, sexual dichromatism in carotenoid-based color was positively related to yolk carotenoids and vitamin A but not to vitamin E (table 3). These results were confirmed only for yolk carotenoids when the effects of similarity due to common phylogenetic descent were controlled for (carotenoids, partial correlation: $r_{\text{phyl}} = 0.18$, $P < .05$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.16$, $P > .05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.02$, $P > .05$; at $\lambda = 1$, $n = 100$).

Among species that were sexually dichromatic for carotenoid-based signals, sexual dichromatism was strongly positively related to male color scores ($F_{1,21} = 40.72$, $P < .0001$, $R^2 = 0.67$, slope estimate $\pm$ SE = 0.80 ± 0.13). In order to determine whether the previously found relationship between sexual dichromatism and yolk carotenoids actually reflected an underlying relationship between male carotenoid-based color score and yolk carotenoids, we used the residuals from the phylogenetic regression as an index of the extent of sexual dichromatism, independent of male color, in analyses based on species and controlling for phylogeny. In analyses based on species and including female body mass and clutch size, residual sexual dichromatism did not explain significant variation in yolk carotenoids ($F_{1,18} = 2.24$, $P = .16$), vitamin A ($F_{1,17} = 0.45$, $P = .51$), or vitamin E ($F_{1,18} = 0.01$, $P = .92$; fig. 2). The same results were obtained after phylogeny was controlled for (carotenoids, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.014$, $n = 22$; vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.03$, $n = 21$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.007$, $n = 22$; all

Table 1: Regression models with log-transformed yolk antioxidant concentrations as dependent variables and clutch size as independent variable, with log-transformed female body mass controlled for, from species-specific data

Variable	Female body mass			Clutch size		
	Slope $\pm$ SE	F (df)	P	Slope $\pm$ SE	F (df)	P
Carotenoids	-.02 $\pm$.04	.15 (1, 108)	.70	.09 $\pm$.03	6.99 (1, 108)	.01
Vitamin A	.09 $\pm$.02	20.28 (1, 100)	<.0001	.04 $\pm$.02	6.83 (1, 100)	.01
Vitamin E	.004 $\pm$.034	.01 (1, 106)	.91	.01 $\pm$.03	.19 (1, 106)	.66

Note: See text for results of phylogenetic analyses (r_{phyl}).

Table 2: Regression models with log-transformed yolk antioxidant concentrations as dependent variables and log-transformed male carotenoid-based color scores or sexual dichromatism in carotenoid-based color as independent variables, with female body mass and clutch size controlled for

Variable	Male color			Sexual dichromatism		
	Slope $\pm$ SE	F (df)	P	Slope $\pm$ SE	F (df)	P
Carotenoids	1.18 $\pm$.21	32.01 (1, 107)	<.0001	1.31 $\pm$.35	13.45 (1, 107)	.0004
Vitamin A	.11 $\pm$.11	1.04 (1, 99)	.31	.36 $\pm$.17	4.21 (1, 99)	.04
Vitamin E	.06 $\pm$.20	.09 (1, 105)	.76	.28 $\pm$.31	.82 (1, 105)	.37

Note: Results are given for analyses performed on species-specific data. See text for results of phylogenetic analyses (r_{phyl}).

$P > .05$). The relationship between sexual dichromatism and egg carotenoids was therefore not supported when the effect of carotenoid-based color was controlled for. Indeed, in a multiple regression, none of the three antioxidants explained a significant part of the variation in residual sexual dichromatism (model including female body mass and clutch size; carotenoids: $F_{1,15} = 1.92$, $P = .07$; vitamin A: $F_{1,15} = 0.81$, $P = .43$; vitamin E: $F_{1,15} = 2.79$, $P = .12$). This result was confirmed when phylogeny was taken into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.20$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.10$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.27$; all $P > .05$; at $\lambda = 0.741$, $n = 21$).

Discussion

The main results of this comparative analysis of egg yolk antioxidants in birds were that concentration of all three egg components increased with clutch size across species and that there were significant positive relationships between yolk carotenoids, but not vitamins A or E, and the extent of carotenoid-based signals. Maternal deposition of antioxidants in eggs was not significantly related to the extent of sexual dichromatism in carotenoid-based colored signals.

Maternal deposition of all three antioxidants in eggs increased with clutch size, which is somewhat unexpected if they are limited resources for females. Clutch size has been found to coevolve with adult immunocompetence (ability to efficiently raise defenses against parasites) across species, reflecting natural selection due to parasite-induced mortality (Martin et al. 2001). Given the importance of early availability of antioxidants for development of the immune system, we might speculate that the increase in maternal deposition of antioxidants in eggs with increasing clutch size favors increased immunocompetence. Increased levels of antioxidants with clutch size might also reflect a maternal allocation strategy to reduce the costs of oxidative stress due to increased sibling competition in the early posthatching phase. At that time, nestlings still rely mainly on yolk-derived antioxidants, which have been

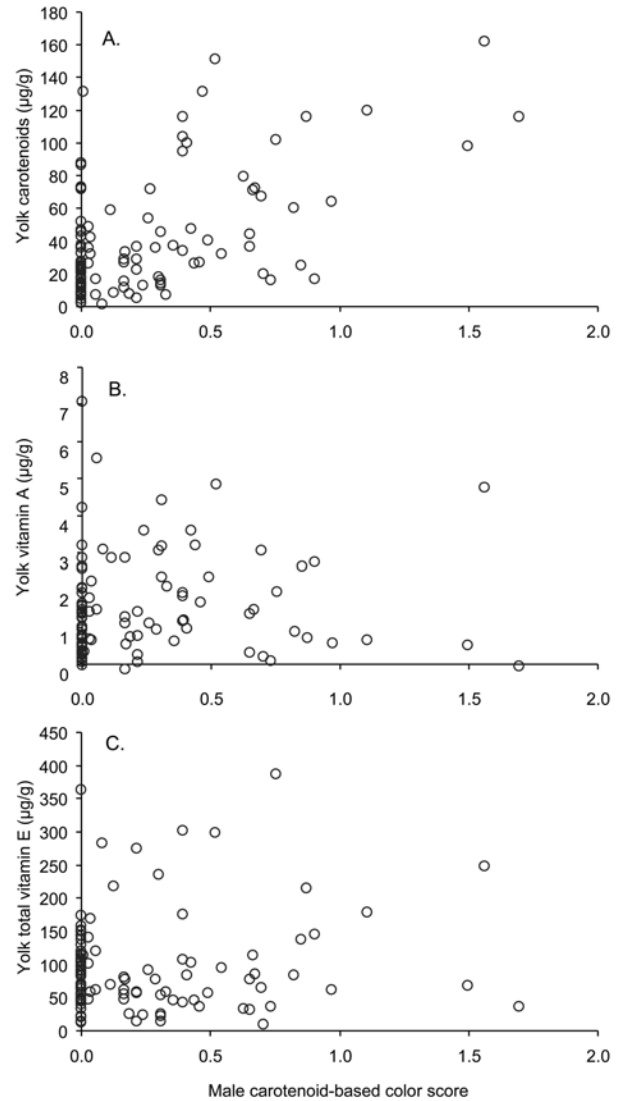


Figure 1: Mean concentrations of egg yolk antioxidants ($\mu\text{g/g}$) as a function of male carotenoid-based color scores presented on a log scale ($\log(\text{score} + 1)$): A, carotenoids ($n = 112$); B, vitamin A ($n = 104$); and C, vitamin E ($n = 110$).

Table 3: Multiple-regression model of male carotenoid-based color score and sexual dichromatism in carotenoid-based color scores as a function of yolk carotenoid and vitamin A and E concentrations, with female body mass and clutch size controlled for

Variable	Male color			Sexual dichromatism		
	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>
Female body mass	-.02 $\pm$.02	.74 (1, 97)	.39	-.03 $\pm$.01	4.67 (1, 97)	.03
Clutch size	-.02 $\pm$.01	2.88 (1, 97)	.09	-.01 $\pm$.01	2.89 (1, 97)	.09
Carotenoids	.21 $\pm$.04	30.44 (1, 97)	<.0001	.09 $\pm$.02	13.75 (1, 97)	.0003
Vitamin A	.1 $\pm$.08	1.53 (1, 97)	.242	.11 $\pm$.05	4.45 (1, 97)	.04
Vitamin E	-.08 $\pm$.05	2.63 (1, 97)	.11	-.03 $\pm$.03	.72 (1, 97)	.40

Note: All variables were log transformed except clutch size. Results are given for analyses performed on species-specific data. See text for results of phylogenetic analyses (r_{phy}).

shown to reduce oxidative stress during development (Blount et al. 2002; Surai 2002; McGraw et al. 2005).

We hypothesized that maternal effects might promote the evolution of carotenoid-based signals. A possible mechanism might be the long-term positive effects of embryonic exposure to yolk carotenoids on the ability to develop bright carotenoid-based signals. If that were the case, yolk carotenoid concentration should covary positively with expression of carotenoid-based signals across species. However, a positive relationship between color and antioxidants more important than carotenoids in eggs would instead be expected if the effects of carotenoids were indirect (as suggested by Hartley and Kennedy [2004]). Yolk carotenoid concentration increased with the extent of carotenoid-based signals, whereas that was not the case for the other two major antioxidants in egg yolk, vitamins A and E. This result is consistent with the hypothesis of a direct relationship between yolk carotenoids and development of carotenoid-based signals. In addition, the relationship between yolk carotenoids and carotenoid-based color was unaffected by inclusion of vitamins A and E in the analyses, contrary to what would be expected under the hypothesis that the relationship with carotenoids was mediated through or revealed by the role of other antioxidants (Hartley and Kennedy 2004). In fact, statistically controlling for levels of vitamins A and E in eggs even increased the strength of the relationship between yolk carotenoids and carotenoid-based color. This relationship was not confounded by any potential underlying relationship with diet or latitude and was also consistently found within lower phylogenetic taxa (see “Material and Methods” and the appendix).

Maternal investment of carotenoids in eggs may thus have evolved as an indirect result of selection for carotenoid-based signals in offspring when they reach adulthood. An increase in yolk carotenoid concentration enhances the ability of offspring to acquire and use carotenoids later in life (Hörak et al. 2000; Koutsos et al. 2003), thereby allowing offspring, when reaching adulthood, to develop bright carotenoid-based signals (McGraw

et al. 2005) that might increase fitness. Such a mechanism may also apply in an interspecific context and was specific to the properties of carotenoids, as vitamins A and E did not increase significantly with the extent of carotenoid-based signals across species. This may be due to the fact that vitamins A and E have physiological roles somewhat different from that of carotenoids, independent of their antioxidant functions. Carotenoids are involved in activation and regulation of the immune system (reviewed in Bendich 1993; Møller et al. 2000; Surai 2002), including inflammatory response relying on the production of reactive oxidative molecules (Rafi and Shafaie 2007; Kim et al. 2008), gap-junction and cell communication, and gene expression and protection against mutation (reviewed in Burri 2000; Surai 2002), and they are precursors of vitamin A (reviewed in Surai 2002). However, there is only limited evidence suggesting that they play a role *in vivo* as major antioxidants in birds (Costantini and Møller 2008). In contrast, vitamin A is involved in immune function, but to a lesser extent than carotenoids (Bendich 1993), and vitamin E is involved in membrane stabilization and cellular signaling (reviewed in Brigelius-Flohé and Traber 1999; Surai 2002). Carotenoids are simultaneously involved in various physiological and signaling functions, which is not the case for vitamins A and E. Carotenoids are thus potentially at the center of a multiple trade-off that might be modulated by maternal effects during early development. Maternal investment of these pigments in eggs has indeed been shown to exert long-lasting organizational effects (reviewed in “Introduction”). Maternally derived carotenoids increase the efficiency of carotenoid metabolism in posthatching life, through the ability of offspring to assimilate and use carotenoids, and thereby have long-lasting positive effects on the ability to develop bright carotenoid-based signals. Embryonic exposure to yolk carotenoids also modulates the development of immune function as well as the costs of its use in relation to regulation of systemic inflammation. Maternally derived carotenoids may therefore allow offspring to change the trade-off between sexual signaling and physiological func-

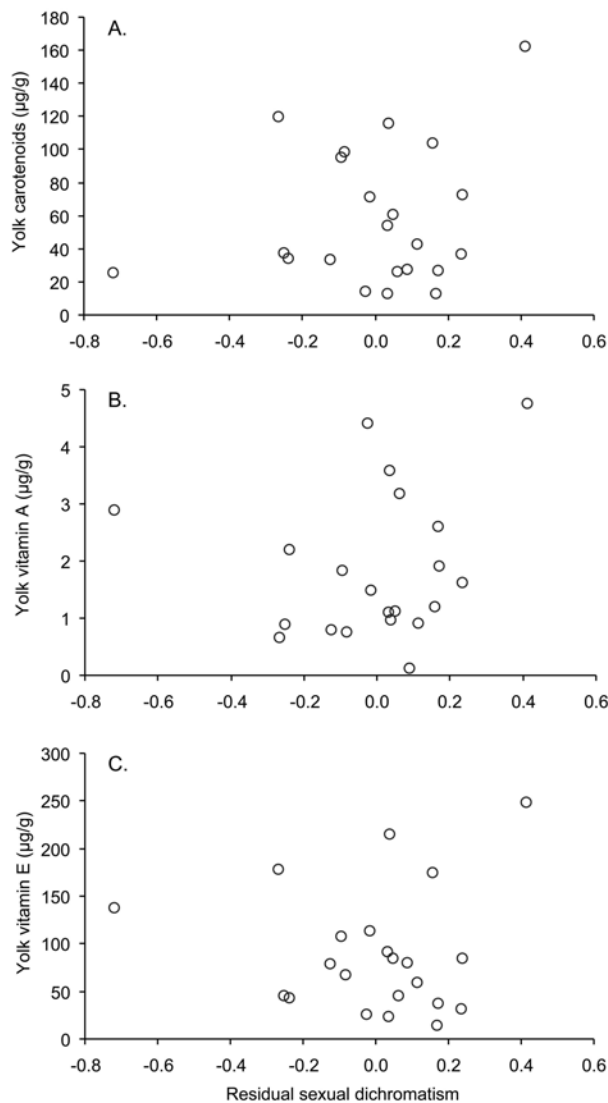


Figure 2: Mean concentrations of egg yolk antioxidants ($\mu\text{g/g}$) as a function of residual sexual dichromatism in carotenoid-based color scores: A, carotenoids ($n = 22$); B, vitamin A ($n = 21$); and C, vitamin E ($n = 22$).

tions, while the costs of maternal investment in eggs would maintain the reliability of signals. Furthermore, maternal effects increase phenotypic plasticity in offspring traits (Mousseau and Fox 1998b). Therefore, we speculate that maternal effects through yolk carotenoids might increase plasticity and potential for innovation in this metabolism that they influence, making more readily possible the use of newly available dietary carotenoids or the development of new metabolic pathways for transforming carotenoids. Variation among several tanager species (*Piranga* spp.) in metabolic pathways for producing colorful feathers and

their evolution in relation to dietary availability of pigments illustrates how phenotypic plasticity might drive the evolution of colored signals (Price et al. 2003; Grether 2005; Price 2006). Maternal effects through egg carotenoids may thus underlie the relatively greater evolutionary flexibility observed for carotenoid-based coloration, as compared to melanin- or structurally based coloration (Badyaev and Hill 2003; Hill and McGraw 2006b).

Comparative studies of sexual dichromatism and carotenoid-based coloration have shown that sexual dichromatism has a positive correlation with carotenoid-based plumage coloration but not with melanin-based or structural coloration, suggesting that sexual selection has driven the evolution of sexual dichromatism in carotenoid-based signals (in North American passerines: Gray 1996; in cardueline finches: Hill 1996; Badyaev and Hill 2000). We found a positive relationship between sexual dichromatism in carotenoid-based signals and the level of carotenoids deposited in eggs across species. However, this relationship was weak and appeared mainly to reflect that sexual dichromatism in carotenoid-based color strongly increased with the extent of carotenoid-based color. It should be noted that the number of species in our data set that were sexually dichromatic for carotenoid-based signals was relatively low (22 species). Such a sample size is sufficient to detect a strong effect (explaining 25% of the variance). The absence of a significant relationship between yolk antioxidants and sexual dichromatism thus indicates that there was no strong effect, but we cannot rule out the possibility that a weak effect (explaining 1% of the variance) might be detected with a larger sample size. Females did not invest more carotenoids in eggs in dichromatic species than in monochromatic species with the same level of male carotenoid-based color. In sexually dichromatic species, males would have elevated requirements for carotenoids to invest in development of carotenoid-based signals, while females that do not develop carotenoid-rich plumage would have elevated needs for carotenoids to invest in production of carotenoid-rich eggs. We hypothesize that these requirements in males and females may be of similar magnitude, potentially explaining the absence of a significant relationship between carotenoid concentration in eggs and sexual dichromatism in carotenoid-based coloration. The absence of significant covariation between sexual dichromatism and maternal investment of antioxidants in eggs raises a question about the evolution of female ornamentation that is still a subject of debate (Amundsen 2000). Female coloration may have evolved as a by-product of sexual selection due to a genetic correlation with male ornamentation or independently as a response to male mate choice or female contests (Amundsen 2000). The positive relationship between maternal investment of carotenoids in eggs and the devel-

opment of carotenoid-based colors suggests other mechanisms for the evolution of female ornamentation in relation to sex allocation. There is recent evidence that females can differentially allocate carotenoids to eggs as a function of embryo sex (Verboven et al. 2005; Badyaev et al. 2006), although the occurrence of sex allocation of carotenoids might not be a general pattern, even in closely related species (Saino et al. 2003b; Groothuis et al. 2006; Romano et al. 2008). In the case where females do not differentially allocate carotenoids to eggs as a function of embryo sex and requirements for carotenoids are equivalent in both sexes, then offspring of both sexes would benefit equally from maternally derived carotenoids, and carotenoid-based color would evolve in the same direction in females as in males, without necessarily implying a genetic correlation. In contrast, if females do invest carotenoids as a function of embryo sex and if males and females do not differ in development of carotenoid-based signals, this would suggest a differential rate of use of antioxidants by the two sexes, with males having higher turnover than females. For example, a differential rate of use of antioxidants by males may be related to increased susceptibility of males to parasites (Møller et al. 1998).

The hypothesis that maternal effects through egg composition may influence the evolution of secondary sexual characters has recently been tested for two other important maternal effects, immune compounds and hormones (Garamszegi et al. 2007a; Gil et al. 2007; Saino et al. 2007). Although there was evidence of a positive relationship between maternal investment of lysozymes in eggs and sexual dichromatism in plumage brightness across species (Saino et al. 2007), no evidence for such a relationship was found when two yolk androgens, testosterone and androstenedione, were considered (Gil et al. 2007). However, maternal testosterone has been shown to impose constraints on the evolution of song characteristics (Garamszegi et al. 2007a). This suggests a differential effect of sexual selection on different aspects of maternal effects and that the role of particular maternal effects in development of sexual signals relies on specific physiological mechanisms and constraints. In addition, this result suggests complex maternal effects based on multiple pathways, which might have evolved independently to optimize a cocktail of specific egg components as a function of prevailing selective forces and physiological constraints.

In conclusion, a comparative analysis of the antioxidant contents in bird eggs revealed a positive relationship between yolk carotenoids and relative size of carotenoid-based sexual signals but not between yolk carotenoids and sexual dichromatism in these signals. These findings provide evidence consistent with the hypothesis, based on the specific role of carotenoids during embryo development, that maternal effects mediated by yolk carotenoids may

promote the evolution of carotenoid-based signals as a response to sexual selection. Maternal effects may therefore contribute to the evolution of secondary sexual characters and to the maintenance of additive genetic variation for these traits.

Acknowledgments

We gratefully acknowledge colleagues, amateurs, and nature conservation authorities who helped bring this project to completion. In particular, the following people provided egg samples: J. Avilés, A. Barbosa, J. Blount, F. de Lope, M. Martín-Vivaldi, C. Navarro, P. Ninni, J. L. Osorno, J. Soler, J. Viñuela, and R. Visagie. L. Z. Garamszegi greatly helped with advice on comparative analyses and on the use of Continuous. B. Chéron kindly accepted the task of scoring carotenoid-based colors. Two reviewers provided helpful comments on a previous version of the manuscript. C.B. was supported by a doctoral grant from the Ministère de l'Éducation et de la Recherche. P.E.S. was supported by the Scottish Executive Environment and Rural Affairs Department.

Literature Cited

- Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology & Evolution* 15:149–155.
- Andersson, S., M. Prager, and E. I. A. Johansson. 2007. Carotenoid content and reflectance of yellow and red nuptial plumages in widowbirds (*Euplectes* spp.). *Functional Ecology* 21:272–281.
- Badyaev, A. V., and G. E. Hill. 2000. Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. *Biological Journal of the Linnean Society* 69:153–172.
- . 2003. Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology, Evolution, and Systematics* 34:27–49.
- Badyaev, A. V., D. A. Acevedo Seaman, K. J. Navara, G. E. Hill, and M. T. Mendonça. 2006. Evolution of sex-biased maternal effects in birds. III. Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins. *Journal of Evolutionary Biology* 19:1044–1055.
- Bendich, A. 1993. Physiological roles of antioxidants in the immune system. *Journal of Dairy Science* 76:2789–2794.
- Berthouly, A., F. Helfenstein, and H. Richner. 2007. Cellular immune response, stress resistance and competitiveness in nestling great tits in relation to maternally transmitted carotenoids. *Functional Ecology* 21:335–343.
- Biard, C., P. F. Surai, and A. P. Møller. 2005. Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia* (Berlin) 144:32–44.
- . 2007. An analysis of pre- and post-hatching maternal effects mediated by antioxidants in the blue tit. *Journal of Evolutionary Biology* 20:326–339.
- Birkhead, T. R., and A. P. Møller. 1994. The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution* 48:1089–1100.
- Birkhead, T. R., F. Fletcher, and E. J. Pellatt. 1999. Nestling diet,

- secondary sexual traits and fitness in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences* 266:385–390.
- Blount, J. D., D. C. Houston, and A. P. Møller. 2000. Why egg yolk is yellow. *Trends in Ecology & Evolution* 15:47–49.
- Blount, J. D., P. F. Surai, R. G. Nager, D. C. Houston, A. P. Møller, M. L. Trewby, and M. W. Kennedy. 2002. Carotenoids and egg quality in the lesser black-backed gull (*Larus fuscus*): a supplemental feeding study of maternal effects. *Proceedings of the Royal Society B: Biological Sciences* 269:29–36.
- Blount, J. D., N. B. Metcalfe, K. E. Arnold, P. F. Surai, G. L. Devevey, and P. Monaghan. 2003. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences* 270:1691–1696.
- Brigelius-Flohé, R., and M. Traber. 1999. Vitamin E: function and metabolism. *FASEB Journal* 13:1145–1155.
- Brodie, E. D., III, and A. A. Agrawal. 2001. Maternal effects and the evolution of aposematic signals. *Proceedings of the National Academy of Sciences of the USA* 98:7884–7887.
- Brown, L. H., C. H. Fry, S. Keith, E. K. Urban, and K. Newman. 1982–2004. *The birds of Africa*. Academic Press, London.
- Burri, B. J. 2000. Carotenoids and gene expression. *Nutrition* 16:577–578.
- Costantini, D., and A. P. Møller. 2008. Carotenoids are minor antioxidants in birds. *Functional Ecology* 22:367–370.
- Cramp, S., K. E. L. Simmons, and C. M. Perrins. 1982–1994. *Handbook of the birds of Europe, the Middle East and North Africa*. Oxford University Press, Oxford.
- del Hoyo, J., A. Elliott, and J. Sagartal. 1992–2004. *Handbook of the birds of the world*. Lynx, Barcelona.
- Dunning, J. B. J. 1992. *CRC handbook of avian body masses*. CRC, Orlando, FL.
- Edge, R., D. J. McGarvey, and T. G. Truscott. 1997. The carotenoids as anti-oxidants: a review. *Journal of Photochemistry and Photobiology B* 41:189–200.
- Eising, C., W. Müller, and T. G. G. Groothuis. 2006. Avian mothers create different phenotypes by hormone deposition in their eggs. *Biology Letters* 2:20–22.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125:1–15.
- Freckleton, R. P., P. H. Harvey, and M. D. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist* 160:712–726.
- Garamszegi, L. Z., M. Eens, S. Hurtrez-Boussès, and A. P. Møller. 2005b. Testosterone, testes size, and mating success in birds: a comparative study. *Hormones and Behavior* 47:389–409.
- Garamszegi, L. Z., C. Biard, M. Eens, A. P. Møller, and N. Saino. 2007a. Interspecific variation in egg testosterone levels: implications for the evolution of bird song. *Journal of Evolutionary Biology* 20:950–964.
- Gil, D., C. Biard, A. Lacroix, C. Spottiswoode, M. Puerta, and A. P. Møller. 2007. Evolution of yolk androgens in birds: development, coloniality, and sexual dichromatism. *American Naturalist* 169:802–819.
- Goodwin, T. W. 1984. *The biochemistry of carotenoids*. Vol. 2. Animals. Chapman & Hall, London.
- Gray, D. A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. *American Naturalist* 148:453–480.
- Grether, G. F. 2005. Environmental change, phenotypic plasticity, and genetic compensation. *American Naturalist* 166:E115–E123.
- Groothuis, T. G. G., C. Eising, J. D. Blount, P. F. Surai, V. Apanius, C. Dijkstra, and W. Müller. 2006. Multiple pathways of maternal effects in black-headed gull eggs: constraint and adaptive compensatory adjustment. *Journal of Evolutionary Biology* 19:1304–1313.
- Hartley, R. C., and M. W. Kennedy. 2004. Are carotenoids a red herring in sexual display? *Trends in Ecology & Evolution* 19:353–354.
- Harvey, P. H., and M. D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford.
- Hill, G. E. 1996. Redness as a measure of the production cost of ornamental coloration. *Ethology Ecology and Evolution* 8:157–175.
- . 1998. Mate choice, male quality, and carotenoid-based plumage coloration. Pages 1654–1668 in N. J. Adams and R. Slowtov, eds. *Proceedings of the 22nd International Ornithological Congress*, Durban, South Africa. BirdLife South Africa, Randburg.
- Hill, G. E., and K. J. McGraw, eds. 2006a. *Bird coloration*. Vol. 1. Mechanisms and measurements. Harvard University Press, Cambridge, MA.
- , eds. 2006b. *Bird coloration*. Vol. 2. Function and evolution. Harvard University Press, Cambridge, MA.
- Hockey, P. A. R., W. R. J. Dean, and P. G. Ryan. 2005. *Roberts birds of southern Africa*. Trustees of the John Voelcker Bird Book Fund, Cape Town, South Africa.
- Hörak, P., H. Vellau, I. Ots, and A. P. Møller. 2000. Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. *Naturwissenschaften* 87:460–464.
- Hörak, P., P. F. Surai, and A. P. Møller. 2002. Fat-soluble antioxidants in the eggs of great tits *Parus major* in relation to breeding habitat and laying sequence. *Avian Science* 2:123–130.
- Inouye, C. Y., G. E. Hill, R. D. Stradi, and R. Montgomerie. 2001. Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *Auk* 118:900–915.
- Isaksson, C., T. Uller, and S. Andersson. 2006. Parental effects on carotenoid-based plumage coloration in nestlings great tits, *Parus major*. *Behavioral Ecology and Sociobiology* 60:556–562.
- Kim, J.-H., H.-J. Na, C.-K. Kim, J.-Y. Kim, K.-S. Ha, H. Lee, H.-T. Chung, H. J. Kwon, Y.-G. Kwon, and Y.-M. Kim. 2008. The non-provitamin A carotenoid, lutein, inhibits NF- κ B-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF- κ B-inducing kinase pathways: role of H₂O₂ in NF- κ B activation. *Free Radical Biology and Medicine* 45:885–896.
- Koutsos, E. A., A. J. Clifford, C. C. Calvert, and K. C. Klasing. 2003. Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). *Journal of Nutrition* 133:1132–1138.
- Koutsos, E. A., J. C. García López, and K. C. Klasing. 2006. Carotenoids from in ovo or dietary sources blunt systemic indices of the inflammatory response of growing chicks (*Gallus gallus domesticus*). *Journal of Nutrition* 136:1027–1031.
- . 2007. Maternal and dietary carotenoids interactively affect cutaneous basophil responses in growing chickens (*Gallus gallus domesticus*). *Comparative Biochemistry and Physiology B* 147:87–92.
- Lessells, C. M., and P. T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121.
- Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology & Evolution* 14:343–348.

- Lozano, G. A. 1994. Carotenoids, parasites, and sexual selection. *Oikos* 70:309–311.
- Martin, T. E., A. P. Møller, S. Merino, and J. Clobert. 2001. Does clutch size evolve in response to parasites and immunocompetence? *Proceedings of the National Academy of Sciences of the USA* 98:2071–2076.
- McGraw, K. J., and J. G. Schuetz. 2004. The evolution of carotenoid coloration in estrild finches: a biochemical analysis. *Comparative Biochemistry and Physiology B* 139:45–51.
- McGraw, K. J., and K. Wakamatsu. 2004. Melanin basis of ornamental feather colors in male zebra finches. *Condor* 106:686–690.
- McGraw, K. J., R. J. Safran, M. R. Evans, and K. Wakamatsu. 2004a. European barn swallows use melanin pigments to color their feathers brown. *Behavioral Ecology* 15:889–891.
- McGraw, K. J., K. Wakamatsu, S. Ito, P. M. Nolan, P. Jouventin, F. S. Dobson, R. E. Austic, et al. 2004c. You can't judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. *Condor* 106:390–395.
- McGraw, K. J., E. Adkins-Regan, and R. S. Parker. 2005. Maternally derived carotenoid pigments affect offspring survival, sex-ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften* 92:375–380.
- Metcalf, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution* 16:254–260.
- Miller, C. W., and A. J. Moore. 2007. A potential resolution to the lek paradox through indirect genetic effects. *Proceedings of the Royal Society B: Biological Sciences* 274:1279–1286.
- Møller, A. P., G. Sorci, and J. Erritzøe. 1998. Sexual dimorphism in immune defense. *American Naturalist* 152:605–619.
- Møller, A. P., C. Biard, J. D. Blount, D. C. Houston, P. Ninni, N. Saino, and P. F. Surai. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian and Poultry Biology Reviews* 11:137–159.
- Moore, A. J., J. B. Wolf, and E. D. Brodie III. 1998. The influence of direct and indirect genetic effects on the evolution of behavior: social and sexual selection meet maternal effects. Pages 22–41 in T. A. Mousseau and C. W. Fox, eds. *Maternal effects as adaptations*. Oxford University Press, Oxford.
- Mousseau, T. A., and C. W. Fox. 1998a. The adaptive significance of maternal effects. *Trends in Ecology & Evolution* 13:403–407.
- , eds. 1998b. *Maternal effects as adaptations*. Oxford University Press, Oxford.
- Negro, J. J., J. H. Sarasola, F. Fariñas, and I. Zorrilla. 2006. Function and occurrence of facial flushing in birds. *Comparative Biochemistry and Physiology A* 143:78–84.
- Ohlsson, T., H. G. Smith, L. Råberg, and D. Hasselquist. 2002. Pheasant sexual ornaments reflect nutritional conditions during early growth. *Proceedings of the Royal Society B: Biological Sciences* 269:21–27.
- Olson, V. A., and I. P. F. Owens. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution* 13:510–514.
- . 2005. Interspecific variation in the use of carotenoid-based coloration in birds: diet, life-history and phylogeny. *Journal of Evolutionary Biology* 18:1534–1546.
- Ong, A. S. H., and E. S. Tee. 1992. Natural sources of carotenoids in plants and oils. Pages 142–167 in L. Packer, ed. *Carotenoids*. Part A: chemistry, separation, quantitation, and antioxidation. *Methods in Enzymology* 213. Academic Press, London.
- Page, M. D. 1997. Inferring evolutionary processes from phylogenies. *Zoologica Scripta* 26:331–348.
- . 1999. Inferring the historical pattern of biological evolution. *Nature* 401:877–884.
- Poole, A., and F. Gill, eds. 1992–2004. *The birds of North America*. Academy of Natural Sciences, Philadelphia; and American Ornithologists' Union, Washington, DC.
- Price, T. D. 1998. Maternal and paternal effects in birds: effects on offspring fitness. Pages 202–226 in T. A. Mousseau and C. W. Fox, eds. *Maternal effects as adaptations*. Oxford University Press, Oxford.
- . 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. *Journal of Experimental Biology* 209:2368–2376.
- Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* 270:1433–1440.
- Prum, R. O., and R. Torres. 2003. Structural colouration of avian skin: convergent evolution of coherently scattering dermal collagen arrays. *Journal of Experimental Biology* 206:2409–2429.
- Prum, R. O., R. Torres, S. Williamson, and J. Dyck. 1999. Two-dimensional Fourier analysis of the spongy medullary keratin of structurally coloured feather barbs. *Proceedings of the Royal Society B: Biological Sciences* 266:13–22.
- Rafi, M. M., and Y. Shafaie. 2007. Dietary lutein modulates inducible nitric oxide synthase (iNOS) gene and protein expression in mouse macrophage cells (RAW 264.7). *Molecular Nutrition and Food Research* 51:333–340.
- Räsänen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales. *Functional Ecology* 21:408–421.
- Romano, M., M. Caprioli, R. Ambrosini, D. Rubolini, M. Fasola, and N. Saino. 2008. Maternal allocation strategies and differential effects of yolk carotenoids on the phenotype and viability of yellow-legged gull (*Larus michahellis*) chicks in relation to sex and laying order. *Journal of Evolutionary Biology* 21:1626–1640.
- Royle, N. J., P. F. Surai, and I. R. Hartley. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? *Behavioral Ecology* 12:381–385.
- . 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. *Functional Ecology* 17:472–481.
- Rubolini, D., M. Romano, R. Martinelli, B. Leoni, and N. Saino. 2006. Effects of prenatal yolk androgens on armaments and ornaments of the ring-necked pheasant. *Behavioral Ecology and Sociobiology* 59:549–560.
- Saino, N., V. Bertacche, R. P. Ferrari, R. Martinelli, A. P. Møller, and R. D. Stradi. 2002. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. *Proceedings of the Royal Society B: Biological Sciences* 269:1729–1733.
- Saino, N., R. P. Ferrari, M. Romano, R. Martinelli, and A. P. Møller. 2003a. Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. *Proceedings of the Royal Society B: Biological Sciences* 270:2485–2489.
- Saino, N., M. Romano, R. P. Ferrari, R. Martinelli, and A. P. Møller. 2003b. Maternal antibodies but not carotenoids in barn swallow eggs covary with embryo sex. *Journal of Evolutionary Biology* 16:516–522.

- Saino, N., R. Martinelli, C. Biard, D. Gil, C. Spottiswoode, P. F. Surai, and A. P. Møller. 2007. Maternal immune factors and the evolution of secondary sexual characters. *Behavioral Ecology* 18:513–520.
- Saks, L., K. J. McGraw, and P. Hőrak. 2003. How feather colour reflects its carotenoid content. *Functional Ecology* 17:555–561.
- Shykoff, J. A., and A. Widmer. 1996. Parasites and carotenoid-based signal intensity: how general should the relationship be? *Naturwissenschaften* 83:113–121.
- Strasser, R., and H. Schwabl. 2004. Yolk testosterone organizes behaviour and male plumage coloration in house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* 56:491–497.
- Surai, P. F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *British Poultry Science* 41:235–243.
- . 2002. Natural antioxidants in avian nutrition and reproduction. Nottingham University Press, Nottingham.
- Surai, P. F., B. K. Speake, R. C. Noble, and N. H. C. Sparks. 1999. Tissue-specific antioxidant profiles and susceptibility to lipid peroxidation of the newly hatched chick. *Biological Trace Element Research* 68:63–78.
- Surai, P. F., B. K. Speake, and N. H. C. Sparks. 2001b. Carotenoids in avian nutrition and embryonic development. 2. Antioxidant properties and discrimination in embryonic tissues. *Journal of Poultry Science* 38:117–145.
- Szigeti, B., J. Török, G. Hegyi, B. Rosivall, R. Hargitai, E. Szöllösi, and G. Michl. 2007. Egg quality and parental ornamentation in the blue tit *Parus caeruleus*. *Journal of Avian Biology* 38:105–112.
- Verboven, N., N. P. Evans, L. D’Alba, R. G. Nager, J. D. Blount, P. F. Surai, and P. Monaghan. 2005. Intra-specific interactions influence egg composition in the lesser black-backed gull (*Larus fuscus*). *Behavioral Ecology and Sociobiology* 57:357–365.
- von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist, and H. Wittzell. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society B: Biological Sciences* 266:1–12.
- Wade, M. J. 1998. The evolutionary genetics of maternal effects. Pages 5–21 in T. A. Mousseau and C. W. Fox, eds. *Maternal effects as adaptations*. Oxford University Press, Oxford.
- Williamson, K. A., P. F. Surai, and J. A. Graves. 2006. Yolk antioxidants and mate attractiveness in the zebra finch. *Functional Ecology* 20:354–359.
- Wolf, J. B., E. D. Brodie III, J. M. Cheverud, A. J. Moore, and M. J. Wade. 1998. Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution* 13:64–69.
- Yezerinac, S. M., and P. J. Weatherhead. 1995. Plumage coloration, differential attraction of vectors and haematzoa infections in birds. *Journal of Animal Ecology* 64:528–537.

Associate Editor: Elizabeth Adkins-Regan
Editor: Mark A. McPeck



Females of the pied crow (*inset*) lay carotenoid-poor eggs, whereas females of the vividly colored southern masked weaver lay eggs that are particularly rich in carotenoid pigments (photographs by Mark Anderson).

Appendix from C. Biard et al., “Maternal Effects Mediated by Antioxidants and the Evolution of Carotenoid-Based Signals in Birds” (Am. Nat., vol. 174, no. 5, p. 000)

Detailed Methods, Biochemical Analyses and Phylogenetic Information, Analyses Including Diet, the Data Set, and Information on Color

Detailed Methods for the Biochemical Analyses of Egg Yolks

Antioxidants were extracted from 0.100–0.200 g of yolk. Samples were homogenized with 0.7 mL NaCl (5%) and 1 mL ethanol, after which antioxidants were extracted via the addition 2 mL hexane and further homogenization, centrifugation, and collection of the hexane phase (extraction repeated twice). Hexane extracts were pooled and evaporated at 60°–65°C under nitrogen flow; the residue was then dissolved in 0.1 mL dichloromethane and 0.1 mL methanol. Carotenoid, vitamin A, and vitamin E concentrations were determined according to previously published procedures (Surai 2000; Hörak et al. 2002). Total carotenoid concentration was determined by HPLC in a Spherisorb type S5NH₂ reverse-phase column (25 cm × 4.6 mm; Phase Separation, Clwyd, United Kingdom) with a mobile phase of methanol and distilled water (97 : 3), at a flow rate of 1.5 mL min⁻¹. Lutein was used for calibration (Sigma, Poole, United Kingdom). Concentrations of vitamins A and E were determined by injection of samples onto a Spherisorb type ODS2 3- μ C18 reverse-phase column (15 cm × 4.6 mm; Phase Separation) with a mobile phase of methanol and distilled water (97 : 3), at a flow rate of 1.05 mL min⁻¹, and fluorescence detection by excitation and emission wavelengths of 295 and 330 nm, respectively, for vitamin E and 330 and 480 nm for vitamin A. Peaks of retinol and δ -, γ -, and α -tocopherol were identified by comparison with the retention time of standards of tocopherols (Sigma). All sampled eggs were analyzed for total carotenoids and vitamin A and E concentrations. Vitamin E, calculated as the summed concentrations of δ -, γ -, and α -tocopherol, was used in subsequent analyses. However, results obtained considering only the major antioxidant form, α -tocopherol (Surai 2002) were qualitatively similar. Concentrations and not quantities of antioxidants were used as the variable of interest in statistical analysis because concentration is the main factor determining physiological action of antioxidants at the level of tissues (Surai 2002). Mean ($\pm$ SE) antioxidant concentrations (μ g/g) for each species are given in table A1.

Topology of the Composite Phylogeny and Phylogenetic Information

We used a composite phylogeny compiled from Sibley and Ahlquist (1990), Barker et al. (2002, 2004), Alström et al. (2006), and Jønsson and Fjeldså (2006) for families and orders, combined with information on species in Sheldon and Winkler (1993), Blondel et al. (1996), Martin and Clobert (1996), Badyaev (1997), Leisler et al. (1997), Cibois and Pasquet (1999), Kimball et al. (1999), Crochet et al. (2000), Kennedy et al. (2000), Møller et al. (2001), Donne-Goussé et al. (2002), Gill et al. (2005), Ewen et al. (2006c), and Jønsson and Fjeldså (2006). The topology of the composite phylogeny is shown in figure A1. Information on branch lengths was not available for this composite phylogeny, and thus all distances between nodes and nodes and tips were set to 1 for the comparative analyses.

However, in order to check that our results were not dependent on the assumption of equal branch lengths in our composite phylogeny, we ran the analyses using an alternative phylogeny based on Sibley and Ahlquist (1990) above the family level and completed within families with more recent information for genera and species (see references in preceding paragraph). Above the family level, we applied information on branch lengths based on DNA-DNA hybridization from the tapestry tree. Below the family level, we applied the convention that within families genera differed by 3.4 $\Delta T_{50}H$ units, and that species within genera differed by 1.1 $\Delta T_{50}H$ units (Sibley and Ahlquist 1990; Bennett and Owens 2002; Garamszegi et al. 2007b). The results were qualitatively

similar to those reported in the article. When we used an alternative phylogeny based on Hackett et al. (2008) and equal branch lengths, results were also qualitatively similar to those reported in the article.

In our data set, 60 out of 112 species were passerines (i.e., 54%). In the context of a comparative analysis in which we investigate patterns of coevolution among traits, this reflects quite well the natural diversity of birds: passerines form the largest order of birds and represent more than half of the extant avian species diversity (precisely 59%; Sibley and Monroe 1990; Barker et al. 2002). In order to check whether the conclusions of the study held not only across all birds but also within lower phylogenetic groups, we ran the analyses on restricted data sets corresponding to the two groups for which sufficient data were available: Passeriformes and Ciconiformes. However, although the sample size of 112 species allowed testing of evolutionary hypotheses on the overall data set, it allowed only limited investigation of patterns within subgroups. In particular, it was impossible to run reliable analyses with sexual dichromatism as a covariate or as a dependent variable, because the sample of dichromatic species in these two groups was too small. All other analyses involving carotenoid-based color yielded qualitatively similar results. Therefore, this suggests that the corresponding results obtained on the overall data set reflected general patterns within birds and were not driven by outlier species or taxa. Results based on alternative phylogenies and branch lengths and on restricted data sets are available from the authors upon request.

Analyses Including Diet

Information on diet (breeding diet was used when available) was collected by searching handbooks (Brown et al. 1982–2004; Cramp et al. 1982–1994; del Hoyo et al. 1992–2004; Poole and Gill 1992–2004). Diet was scored according to the six categories defined by Tella et al. (2004), but we did not make any assumptions regarding their potential carotenoid content. Diet categories were ranked from 1 to 6 according to their relative places in the food chain: (1) plants only, (2) invertebrates and plants, (3) omnivorous, (4) invertebrates only, (5) vertebrates and invertebrates, and (6) vertebrates only. Diet data are reported in table A1. In the analyses, diet was treated as a continuous variable, under the assumption that intermediate states are biologically meaningful (Bennett and Owens 2002; Garamszegi et al. 2005a, 2007a).

Results: Yolk Antioxidants and Diet

In an analysis based on species, yolk carotenoid concentration was weakly positively correlated with diet (log-transformed data: $r = -0.18$, $P = .05$, $n = 112$), while vitamin A and E concentrations were not significantly correlated with diet (log-transformed data: $r = 0.17$, $P = .07$, $n = 104$ and $r = 0.18$, $P = .06$, $n = 110$, respectively). When similarity among species due to common descent was taken into account, none of the three egg components was significantly positively correlated with diet, as there was no indication of correlated evolution (likelihood ratio tests [$df = 1$]: carotenoids at $\lambda = 0.907$, $n = 109$; vitamin A at $\lambda = 0.868$, $n = 100$; and vitamin E at $\lambda = 0.853$; $n = 106$; all $P > .05$).

Results: Yolk Antioxidants in Relation to Female Body Mass and Clutch Size

In an analysis based on species, yolk carotenoids and vitamin A were significantly positively related to clutch size after female body mass and diet were controlled for, but that was not the case for vitamin E (table A3). When similarity among species due to common descent was taken into account, yolk carotenoids (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.34$, $P < .001$, $n = 108$), vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.34$, $P < .001$, $n = 100$), and vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.35$, $P < .001$, $n = 106$) showed significant positive correlation with clutch size after female body mass and diet were controlled for.

Results: Yolk Antioxidants and Carotenoid-Based Signals

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: the model controlling for female body mass, clutch size, and diet explained 28% of the total variance (table A4). In addition, carotenoid concentration increased with sexual dichromatism in carotenoid-based signals: the model controlling for female body mass, clutch size, and diet explained 17% of the variance (table A4). Vitamin A concentration was not significantly related to the extent of carotenoid-based color (table A4) but also increased with sexual dichromatism, although less strongly than did carotenoid concentration (table A4). In

contrast, vitamin E concentration was not significantly related to carotenoid-based color of feathers and bare parts (table A4) or to sexual dichromatism in these signals (table A4). When similarity among species due to common descent was taken into account, models controlling for female body mass, clutch size, and diet confirmed the significant positive relations between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.37$, $P < .0005$, $n = 108$) and between yolk carotenoids and sexual dichromatism (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.18$, $P < .05$). When incorporating effects of phylogeny, models controlling for female body mass, clutch size, and diet confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.005$, $P > .05$, $n = 100$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.04$, $P > .05$, $n = 106$). Sexual dichromatism was confirmed to be positively related to vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.19$, $P < .05$, $n = 100$) but not to vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.11$, $P > .05$, $n = 106$).

In a multiple regression of male carotenoid-based color score as a function of female body mass, clutch size, diet, and the three antioxidants, only carotenoid concentration explained a significant part of the variance (table A5). The same result was found when similarity among species due to common descent was taken into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.41$, $P < .0005$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.0009$, $P > .05$; vitamin E, partial correlation: $r_{\text{phyl}} = 0.04$, $P > .05$; at $\lambda = 0.965$, $n = 100$). In a multiple-regression model based on species, sexual dichromatism in carotenoid-based color was positively related to yolk carotenoids and vitamin A but not to vitamin E (table A5). These results were confirmed only for yolk carotenoids when the effects of similarity due to common phylogenetic descent were controlled for (carotenoids, partial correlation: $r_{\text{phyl}} = 0.18$, $P < .05$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.16$, $P > .05$; vitamin E, partial correlation: $r_{\text{phyl}} = 0.03$, $P > .05$; at $\lambda = 1$, $n = 100$).

Among species that are sexually dichromatic for carotenoid-based signals, sexual dichromatism was strongly positively related to male color scores ($F_{1,21} = 40.72$, $P < .0001$, $R^2 = 0.67$, slope estimate $\pm$ SE = 0.80 ± 0.13). In order to determine whether the previously found relationship between sexual dichromatism and yolk carotenoids actually reflected an underlying relationship between male carotenoid-based color score and yolk carotenoids, we used the residuals from the phylogenetic regression as an index of the extent of sexual dichromatism, independent of male color, in analyses based on species and controlling for phylogeny. In analyses based on species and including female body mass, clutch size, and diet, residual sexual dichromatism did not explain significant variation in yolk carotenoids ($F_{1,17} = 1.89$, $P = .19$), vitamin A ($F_{1,16} = 0.08$, $P = .78$), or vitamin E ($F_{1,17} = 0.00$, $P = .99$). The same results were obtained after phylogeny was controlled for (carotenoids, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.17$, $n = 22$; vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.05$, $n = 21$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.003$, $n = 22$; all $P > .05$). The relationship between sexual dichromatism and egg carotenoids was therefore not supported when the effect of carotenoid-based color was controlled for. Indeed, in a multiple regression, none of the three antioxidants explained a significant part of the variation in residual sexual dichromatism (model including female body mass, clutch size, and diet; carotenoids: $F_{1,14} = 3.07$, $P = .10$; vitamin A: $F_{1,14} = 0.24$, $P = .63$; vitamin E: $F_{1,14} = 2.60$, $P = .13$). This result was confirmed when phylogeny was taken into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.10$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.15$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.20$; all $P > .05$; at $\lambda = 0.704$, $n = 21$).

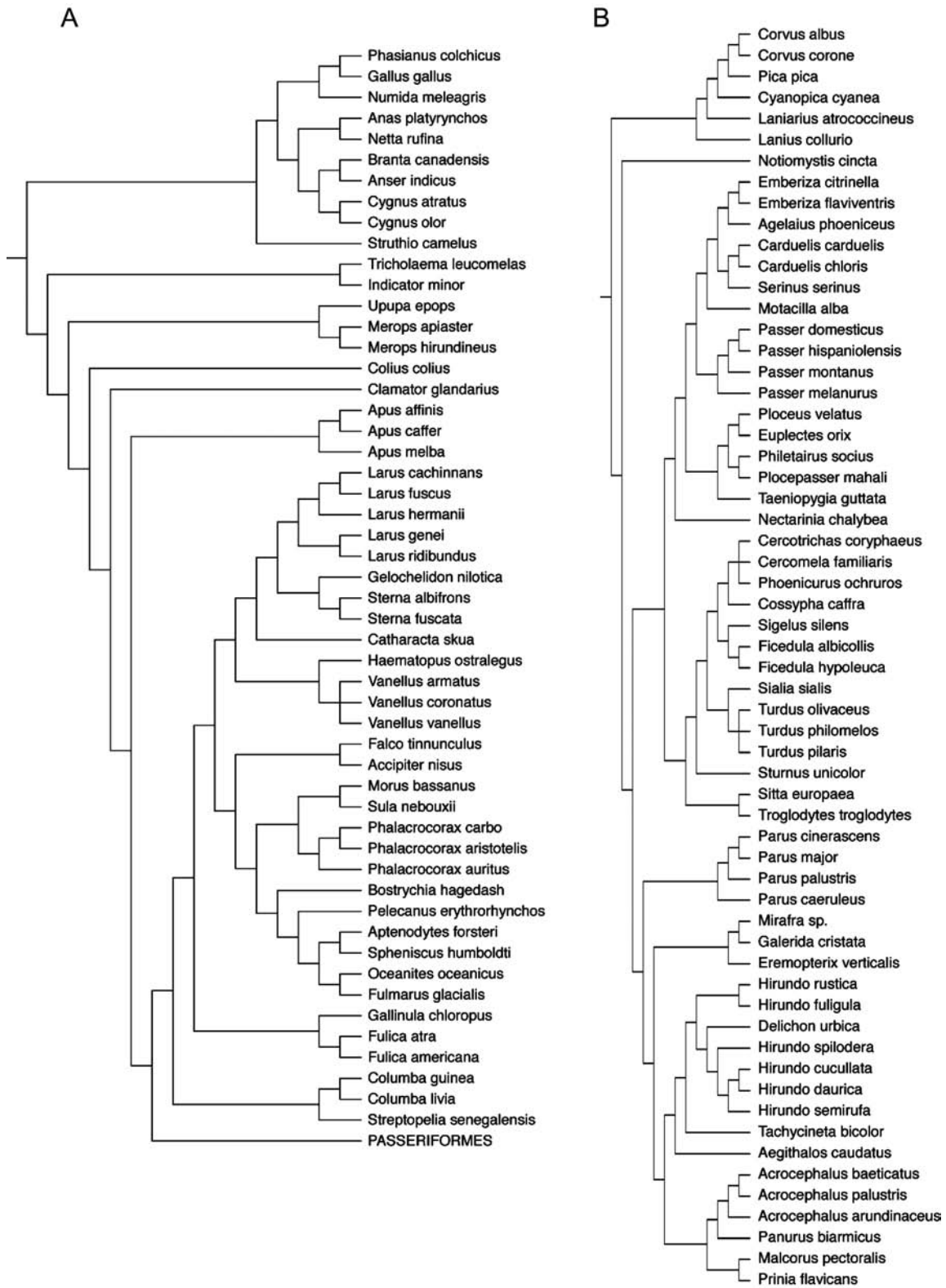


Figure A1: Topology of the composite phylogeny: A, non-Passeriformes; B, Passeriformes.

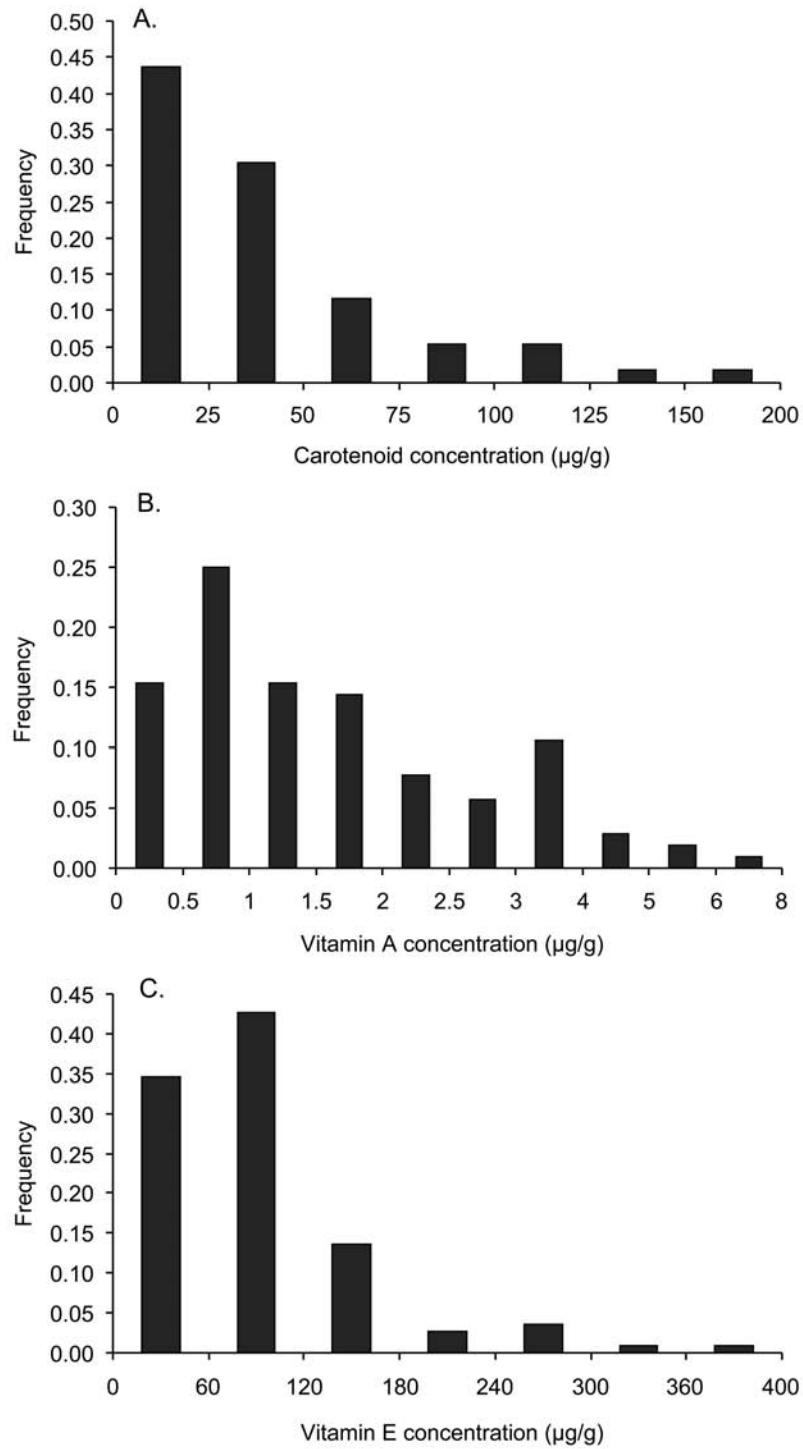


Figure A2: Frequency distributions of lipid-soluble antioxidant concentration in egg yolk ($\mu\text{g/g}$): A, carotenoids ($n = 112$); B, vitamin A ($n = 104$); and C, vitamin E ($n = 110$).

Table A1

Mean concentration (with SE) of total carotenoids, vitamin A, and vitamin E in egg yolk, female body mass, clutch size, diet, latitude, and male and female carotenoid-based color scores of the 112 species included in this study

Species	Family	N	N _F	Carotenoids (μg/g)	Vitamin A (μg/g)	Vitamin E (μg/g)	Female body mass (g)	Clutch size	Diet ^a	Latitude	Male color score	Female color score	Sample origin	References ^b
<i>Accipiter nisus</i>	Accipitridae	3	1	7.4 (.39)	.75 (.1)	25.69 (3.49)	318	4.5	6	50.5	.204	.204	Wild	1
<i>Acrocephalus arundinaceus</i>	Sylviidae	5	2	36.06 (2.09)	1.02 (.11)	129.59 (9.77)	29.5	4.7	2	48	0	0	Wild	1
<i>Acrocephalus baeticatus</i>	Sylviidae	7	4	48.89 (11.87)	.69 (.16)	47.37 (12.71)	10.3	2.7	4	−7	.03	.03	Wild	1
<i>Acrocephalus palustris</i>	Sylviidae	1	1	55.22	.61	93.44	12	4.5	2	49.5	.295	.295	Wild	1
<i>Aegithalos caudatus</i>	Aegithalidae	5	. . .	52.13 (3.27)	7.07 (.47)	96.43 (14.89)	8.3	10	2	52	0	0	Wild	1
<i>Agelaius phoeniceus</i>	Fringillidae	2	. . .	94.77 (18.49)	1.83 (.13)	107.77 (8.58)	41.5	3.3	2	39	.48	.36	Wild	1
<i>Anas platyrhynchos</i>	Anatidae	2, 10	2, ND	53.66 (4.51)	1.10 (.86)	91.37 (5.01)	1,010	9.7	3	52	.18	.12	Wild	1, 2
<i>Anser indicus</i>	Anseranatidae	1	1	45.44	. . .	22.62	3,095	5	1	42	.36	.36	Feral	3
<i>Aptenodytes forsteri</i>	Spheniscidae	6	. . .	8.6 ^c	. . .	218.2 (32.98)	34,000	1	5	−67.5	.135	.135	Wild	4
<i>Apus affinis</i>	Apodidae	19	8	2.17 (.48)	.37 (.18)	65.78 (18.46)	26.2	2.5	4	1	0	0	Wild	1
<i>Apus caffer</i>	Apodidae	4	2	4.33 (.11)	.26 (.04)	151.55 (23.54)	22.1	2	4	1.5	0	0	Wild	1
<i>Apus melba</i>	Apodidae	3	2	2.87 (.2)	.3 (.08)	67.64 (22.04)	89.75	2.6	4	−5	0	0	Wild	1
<i>Bostrychia hagedash</i>	Threskiornithidae	4	1	6.9 (.48)	2.1 (.18)	57.84 (3.33)	1,168	3.2	5	−7.5	.39	.39	Wild	1
<i>Branta canadensis</i>	Anatidae	5	. . .	22.1 (1.4)	. . .	84 (1.91)	3,314	5	1	53	0	0	Feral	5
<i>Carduelis carduelis</i>	Fringillidae	8	2	101.93 (10.66)	1.94 (.23)	387.57 (31.74)	15.4	5	2	44	1.128	1.128	Wild	1
<i>Carduelis chloris</i>	Fringillidae	5	2	103.71 (12.74)	1.19 (.07)	174.67 (22.48)	28.3	5	2	49.5	.485	.04	Wild	1
<i>Catharacta skua</i>	Laridae	5	3	12.7 (2.54)	4.2 (.19)	90.2 (5.25)	1,525	2	6	3.5	0	0	Wild	6
<i>Cercomela familiaris</i>	Muscicapidae	7	3	20.2 (2.65)	.3 (.09)	89.54 (8.79)	20.8	3.1	2	−11	0	0	Wild	1
<i>Cercotrichas coryphaeus</i>	Muscicapidae	6	2	22.81 (4.38)	.38 (.09)	49.35 (16.23)	21.4	2.8	2	−29.5	0	0	Wild	1
<i>Clamator glandarius</i>	Cuculidae	7	3	87.85 (5.76)	1.29 (.14)	105.49 (6.14)	130	16	4	12.5	0	0	Wild	1
<i>Colius colius</i>	Coliidae	3	1	4.87 (2.1)	.26 (.06)	14.7 (6.68)	41.4	3.5	1	−27	.24	.24	Wild	1
<i>Columba guinea</i>	Columbidae	2	1	99.71 (48.45)	.96 (.07)	83.36 (6.15)	330	2	2	−9.5	.504	.504	Wild	1
<i>Columba livia</i>	Columbidae	5	5	36.7 (18.37)	.76 (.11)	56.27 (15.52)	340	2	2	17	.24	.24	Wild	1
<i>Corvus albus</i>	Corvidae	9	3	10.68 (1.63)	.72 (.16)	45.64 (9.33)	476	4.1	3	−6.5	0	0	Wild	1
<i>Corvus corone</i>	Corvidae	10	. . .	72.1 (7.38)	2.63 (.17)	104.38 (7.24)	499	4.5	3	46.5	0	0	Wild	3
<i>Cossypha caffra</i>	Muscicapidae	2	1	36.97 (2.07)	1.01 (.25)	136.89 (5.53)	28	2.5	3	−15.5	0	0	Wild	1
<i>Cyanopica cyanea</i>	Corvidae	6	. . .	36.97 (3.84)	2.57 (.17)	96.41 (8.34)	71	6	3	45	0	0	Wild	1
<i>Cygnus atratus</i>	Anatidae	6	. . .	14.24 (1.6)	4.41 (.23)	25.6 (13.2)	5,095	5.1	1	−29.5	.36	.24	Captive	3
<i>Cygnus olor</i>	Anatidae	4	. . .	12.58 (2.58)	3.58 (.83)	23.64 (3.2)	8,850	6	3	8	.27	.144	Feral	3
<i>Delichon urbica</i>	Hirundinidae	5	. . .	14.71 (1.89)	3.19 (.71)	90.73 (10.06)	19.7	4	4	46	0	0	Wild	1
<i>Emberiza citrinella</i>	Fringillidae	4	1	70.94 (2.79)	1.47 (.49)	113.22 (36.46)	28.7	4	2	55	.948	.436	Wild	1

Table A1 (Continued)

Species	Family	N	N _F	Carotenoids (μg/g)	Vitamin A (μg/g)	Vitamin E (μg/g)	Female body mass (g)	Clutch size	Diet ^a	Latitude	Male color score	Female color score	Sample origin	References ^b
<i>Emberiza flaviventris</i>	Fringillidae	2	1	119.62 (4.74)	.65 (.06)	177.72 (1.08)	19.8	2.4	2	−7	2.025	1.35	Wild	1
<i>Eremopterix verticalis</i>	Alaudidae	4	2	24.87 (8.51)	.54 (.11)	14.22 (8.86)	17	2.1	2	−19.5	0	0	Wild	1
<i>Euplectes orix</i>	Passeridae	7	1	162.09 (22.98)	4.75 (1.44)	247.9 (41.56)	15.5	3	2	−17	3.768	0	Wild	1
<i>Falco tinnunculus</i>	Falconidae	4	1	58.68 (10.8)	2.85 (.29)	69.61 (7.92)	168	5	5	18.5	.12	.12	Wild	1
<i>Ficedula albicollis</i>	Muscicapidae	6	1	45.76 (2.41)	.78 (.27)	173.3 (6.52)	14.7	5.5	4	47.5	0	0	Wild	1
<i>Ficedula hypoleuca</i>	Muscicapidae	5	. . .	27.25 (2.75)	1.39 (.08)	109.11 (5.09)	14.3	6.5	2	51	0	0	Wild	1
<i>Fulica americana</i>	Rallidae	10	10	131 (6)	. . .	. . .	540	9.2	3	30	.6	.6	Wild	7
<i>Fulica atra</i>	Rallidae	1	1	131.27	.34	113.51	678	7.5	2	10.5	.009	.009	Wild	1
<i>Fulmarus glacialis</i>	Procellariidae	1	1	7.28	5.55	120.44	578.5	1	5	65	.06	.06	Wild	3
<i>Galerida cristata</i>	Alaudidae	4	1	73.18 (1.05)	1.02 (.12)	150.4 (3.56)	43.2	4	2	31.5	0	0	Wild	1
<i>Gallinula chloropus</i>	Rallidae	6, 10	ND, 10	40.06 (5.59)	2.36 (.12)	57.61 (11.21)	287.7	7	3	14	.636	.636	Wild	1, 7
<i>Gallus gallus</i>	Phasianidae	5, 10	. . .	26.17 (3.22)	3.18 (.42)	45.46 (2.63)	767	5.5	2	12.5	.552	.192	Feral	1, 2
<i>Gelochelidon nilotica</i>	Laridae	1	1	16.86	3.11	115.76	238	2.3	5	28.5	0	0	Wild	1
<i>Haematopus ostralegus</i>	Charadriidae	7	3	34.17 (14.6)	2.18 (1.04)	43.23 (12.85)	526	3	4	54	.48	.51	Wild	1
<i>Hirundo cucullata</i>	Hirundinidae	6	4	26.02 (6.3)	1.89 (.47)	102.44 (25.74)	27.2	3	2	−19.5	0	0	Wild	1
<i>Hirundo daurica</i>	Hirundinidae	4	1	13.82 (1.61)	1.25 (.1)	86.24 (15.96)	19.9	4.5	4	16	0	0	Wild	1
<i>Hirundo fuligula</i>	Hirundinidae	2	. . .	2.6 (.91)	.23 (.01)	12.05 (2.02)	17.1	2.5	4	−.5	0	0	Wild	1
<i>Hirundo rustica</i>	Hirundinidae	5	. . .	46.58 (3.16)	2.33 (.44)	97.97 (10.02)	19	4.5	4	45	0	0	Wild	1
<i>Hirundo semirufa</i>	Hirundinidae	5	2	13.73 (2.02)	1.18 (.22)	70.34 (15.3)	29.7	4	4	−9	0	0	Wild	1
<i>Hirundo spilodera</i>	Hirundinidae	9	4	23.47 (5.23)	.93 (.11)	113.38 (15.6)	20.7	4.5	4	−28	0	0	Wild	1
<i>Indicator minor</i>	Indicatoridae	1	1	45.19	.4	43.78	25.6	. . .	2	−7	0	0	Wild	1
<i>Laniarius atrococcineus</i>	Corvidae	2	1	115.99 (23.61)	.21 (.11)	35.55 (10.88)	27.8	2.7	2	−22	4.44	4.44	Wild	1
<i>Lanius collurio</i>	Laniidae	7	3	22.2 (1.83)	.7 (.09)	82.8 (7.84)	31	5	5	49	0	0	Wild	1
<i>Larus argentatus</i> <i>cachinnans</i>	Laridae	6	6	35.98 (8.41)	1.2 (.54)	77.3 (6.44)	1,043	3	5	51	.336	.336	Wild	1
<i>Larus fuscus</i>	Laridae	20	20	71.6 (6.5)	. . .	. . .	708	3	3	55.5	.306	.306	Wild	7
<i>Larus genei</i>	Laridae	1	1	63.99	.81	61.66	235.5	2	5	34.5	1.64	1.64	Wild	1
<i>Larus hermannii</i>	Laridae	2	2	15.99 (4.16)	3.43 (.18)	53.64 (8.5)	500	1.5	5	31.5	.36	.36	Wild	1
<i>Larus ridibundus</i>	Laridae	57	20	31.76 (1.33)	. . .	94.49 (3.42)	254	3	3	52	.72	.72	Wild	8
<i>Malcorus pectoralis</i>	Cisticolidae	3	1	16.82 (1.44)	1.72 (.72)	61.69 (9.06)	9.6	3.8	2	−26.5	.06	.06	Wild	1
<i>Merops apiaster</i>	Meropidae	8	5	67.25 (10.01)	3.3 (.5)	65.3 (10.41)	52.9	6	4	9	1.006	1.006	Wild	1
<i>Merops hirundinaceus</i>	Meropidae	4	1	19.57 (5.07)	.45 (.11)	9.3 (4.04)	19.8	3	4	−5.5	1.022	1.022	Wild	1
<i>Mirafra</i> sp.	Alaudidae	2	1	22.96 (4.79)	.58 (.53)	22.39 (1.3)	27.3	2.5	2	. . .	0	0	Wild	1
<i>Morus bassanus</i>	Sulidae	4	4	17.69 (1.84)	3.3 (.31)	235 (15.49)	3,004	1	6	60	.348	.348	Wild	6

Table A1 (Continued)

Species	Family	N	N _F	Carotenoids (μg/g)	Vitamin A (μg/g)	Vitamin E (μg/g)	Female body mass (g)	Clutch size	Diet ^a	Latitude	Male color score	Female color score	Sample origin	References ^b
<i>Motacilla alba</i>	Passeridae	5	1	42.63 (4.67)	1.49 (.1)	70.74 (7.33)	19.9	5.6	4	47	0	0	Wild	1
<i>Nectarinia chalybea</i>	Nectariniidae	1	1	36.58	1.61	31.12	7.8	2.3	2	−28	.92	0	Wild	1
<i>Netta rufina</i>	Anatidae	4	. . .	12.58 (.57)	2.59 (.15)	14.24 (.71)	1,108	9	3	45.5	.36	0	Captive	3
<i>Notiomystis cincta</i>	Meliphagidae	5	5	26.69 (4.9)	1.91 (.25)	36.73 (4.41)	30.3	4	2	−37.5	.582	.04	Wild	9
<i>Numida meleagris</i>	Numididae	10	. . .	79.18 (6.88)	. . .	33.65 (2.08)	1,575	9	3	−8	.876	.876	Feral	2
<i>Oceanites oceanicus</i>	Procellariidae	20	20	7.36 (1.23)	.72 (.11)	44.82 (5.31)	38	1	4	−52.5	0	0	Wild	1
<i>Panurus biarmicus</i>	Sylviidae	32	10	27.04 (2.08)	.12 (.01)	79.87 (6.95)	14.9	5.2	2	48	.18	.06	Wild	1
<i>Parus caeruleus</i>	Paridae	100	50	16.59 (.6)	3.01 (.62)	144.74 (8.74)	10.8	11.4	2	47.5	1.47	1.47	Wild	1
<i>Parus cinerascens</i>	Paridae	14	4	12.13 (2.34)	1.25 (.24)	86.55 (19.21)	19.9	4	4	−21	0	0	Wild	1
<i>Parus major</i>	Paridae	67	34	25.18 (1.84)	2.88 (.18)	137.27 (8.59)	17.3	10	2	30.5	1.346	1.478	Wild	1
<i>Parus palustris</i>	Paridae	4	2	16.86 (2.2)	1.83 (.28)	95.69 (29.33)	11.1	9.2	2	42.5	0	0	Wild	1
<i>Passer domesticus</i>	Passeridae	7	2	7.88 (2.23)	1.26 (.16)	363.54 (158.37)	29.9	4	2	8	0	0	Wild	1
<i>Passer hispaniolensis</i>	Passeridae	9	4	13.19 (1.38)	2.3 (.14)	49.12 (7.73)	26.7	4.9	2	38	0	0	Wild	1
<i>Passer melanurus</i>	Passeridae	11	5	13.72 (3.47)	.81 (.21)	46.76 (14.31)	20.3	3.5	2	−23	0	0	Wild	1
<i>Passer montanus</i>	Passeridae	9	. . .	17.74 (1.35)	2.82 (.22)	157.94 (8.44)	22.1	4.5	2	29.5	0	0	Wild	1
<i>Pelecanus erythrorhynchos</i>	Pelecanidae	8	8	150.9 (23.97)	5.1 (.21)	299.2 (36.24)	6,130	2	6	43.5	.684	.684	Wild	6
<i>Phalacrocorax aristotelis</i>	Phalacrocoracidae	4	. . .	32.16 (1.06)	2.49 (.46)	169.58 (41)	1,598	3.1	6	49	.036	.036	Wild	1
<i>Phalacrocorax auritus</i>	Phalacrocoracidae	21	21	115.7 (4.58)	1.4 (.09)	302.3 (22.84)	1,567	4	6	40	.48	.48	Wild	6
<i>Phalacrocorax carbo</i>	Phalacrocoracidae	6	. . .	22.69 (3.03)	1.66 (.25)	275.39 (20.76)	2,049	3.5	6	11.5	.24	.24	Wild	1
<i>Phasianus colchicus</i>	Phasianidae	10	. . .	72.61 (8.22)	. . .	84.77 (4.77)	1,016	11.7	3	49.5	.96	0	Wild	2
<i>Philetairus socius</i>	Passeridae	62	21	26.15 (1.64)	.74 (.06)	39.61 (3.22)	27.3	4.5	2	−20	0	0	Wild	1
<i>Phoenicurus ochruros</i>	Muscicapidae	5	3	42.81 (11.41)	.81 (.22)	104.36 (22.67)	15.5	4.8	2	46	0	0	Wild	1
<i>Pica pica</i>	Corvidae	10	. . .	86.58 (9.55)	1.62 (.33)	143.54 (14.22)	199.5	6	3	50	0	0	Wild	1
<i>Plocepasser mahali</i>	Passeridae	7	3	9.21 (1.55)	.77 (.19)	63.53 (9.33)	40.5	3	2	−10	0	0	Wild	1
<i>Ploceus velatus</i>	Passeridae	9	6	98.01 (17.96)	.75 (.14)	67.2 (15.08)	28.5	4	2	−17.5	3.46	1.71	Wild	1
<i>Prinia flavicans</i>	Cisticolidae	7	3	36.89 (15.88)	.88 (.23)	45.21 (22.53)	8.2	3.5	4	−21.5	.43	.499	Wild	1
<i>Serinus serinus</i>	Fringillidae	4	. . .	115.86 (16.42)	.97 (.06)	214.85 (12.69)	11.8	3.5	2	44.5	1.392	.512	Wild	1
<i>Sialia sialis</i>	Muscicapidae	6	2	71.88 (8.1)	.61 (.13)	91.53 (4.76)	30	4.4	2	29	0	0	Wild	1
<i>Sigelus silens</i>	Muscicapidae	3	1	8.02 (1.08)	1.82 (1.39)	32.67 (7.03)	26.9	3	2	−28	0	0	Wild	1
<i>Sitta europaea</i>	Sittidae	3	1	16.75 (2.4)	2.16 (.28)	95.57 (4.74)	22.8	6.9	2	45	0	0	Wild	1
<i>Spheniscus humboldti</i>	Spheniscidae	4	. . .	1.5 (.08)	3.33 (.16)	283.14 (14.15)	5,000	2	5	−22.5	.084	.084	Captive	3
<i>Sterna albifrons</i>	Laridae	1	1	33.31	.79	77.93	51.8	2	5	11.5	.186	.276	Wild	1
<i>Sterna fuscata</i>	Laridae	2	. . .	21.5 (5.43)	.3 (.12)	53.54 (2.43)	180	1	5	−1.5	0	0	Wild	1

Table A1 (Continued)

Species	Family	N	N _F	Carotenoids (μg/g)	Vitamin A (μg/g)	Vitamin E (μg/g)	Female body mass (g)	Clutch size	Diet ^a	Latitude	Male color score	Female color score	Sample origin	References ^b
<i>Streptopelia senegalensis</i>	Columbidae	3	2	28.58 (11.55)	.32 (.23)	59.2 (17.52)	90	2	2	1	.24	.24	Wild	1
<i>Struthio camelus</i>	Struthionidae	5	. . .	28.9 (5.95)	3.11 (.23)	48.07 (4.62)	100,000	8	2	−.5	.18	.18	Captive	1
<i>Sturnus unicolor</i>	Sturnidae	5	1	42.44 (3.31)	.9 (.16)	58.63 (8.85)	87.5	4.3	5	36	.036	0	Wild	1
<i>Sula neboxii</i>	Sulidae	1	1	24.08	1.72	81.89	1,801	2	6	8.5	0	0	Wild	1
<i>Tachycineta bicolor</i>	Hirundinidae	9	3	17.19 (2.65)	1 (.07)	115.66 (13.27)	20.4	5.5	2	48.5	0	0	Wild	1
<i>Taeniopygia guttata</i>	Passeridae	6	. . .	60.52 (5.95)	1.12 (.19)	83.71 (6.46)	12	5	2	−22.5	1.278	.45	Captive	1
<i>Tricholaema leucomelas</i>	Lybiidae	9	2	44.02 (10.39)	.55 (.07)	76.73 (12.43)	30.15	2.9	1	−22	.918	.918	Wild	1
<i>Troglodytes troglodytes</i>	Certhiidae	2	. . .	26.09 (1.3)	2.03 (.11)	100.68 (11.78)	8.6	6.5	3	44	.03	.03	Wild	1
<i>Turdus olivaceus</i>	Muscicapidae	4	2	15.71 (3.25)	.33 (.11)	36.43 (9.32)	71	1.5	3	−8	1.08	1.08	Wild	1
<i>Turdus philomelos</i>	Muscicapidae	5	1	36.03 (5.89)	1.68 (.09)	140.29 (15.11)	66.7	4	2	53.5	.03	.03	Wild	1
<i>Turdus pilaris</i>	Muscicapidae	5	1	11.5 (2.48)	1.53 (.1)	55.6 (10.54)	104	5	2	58.5	.18	.18	Wild	1
<i>Upupa epops</i>	Upupidae	4	2	17.27 (2.06)	.43 (.12)	120.44 (17.74)	63.1	7	5	13	0	0	Wild	1
<i>Vanellus armatus</i>	Charadriidae	3	1	32.47 (7.23)	1.51 (.24)	57.57 (8.36)	167	3	4	−17	0	0	Wild	1
<i>Vanellus coronatus</i>	Charadriidae	4	2	47.24 (8.67)	3.85 (.59)	102.12 (19.44)	163	2.5	4	−11	.528	.528	Wild	1
<i>Vanellus vanellus</i>	Charadriidae	4	1	6.18 (1.06)	1.66 (.33)	20.19 (6.71)	221	3.8	3	53	0	0	Wild	1

Note: N is the number of eggs collected, and N_F is the number of different females (clutches) sampled, where the information was available. When there are two values in these columns, the values are, in order, the sample sizes for the two references shown. ND = no data.

^a 1 = plants only; 2 = invertebrates and plants; 3 = omnivorous; 4 = invertebrates only; 5 = vertebrates and invertebrates; 6 = vertebrates only.

^b 1 = this study; 2 = Karadaş et al. 2005; 3 = A. L. Fidgett and P. F. Surai, unpublished data; 4 = Speake et al. 1999b; 5 = Speake et al. 1999a; 6 = Surai et al. 2001c; 7 = Surai et al. 2001a; 8 = Groothuis et al. 2006; 9 = Ewen et al. 2006b.

^c SE value not given.

Table A2

Review of information on the biochemical or structural nature of green and yellow to red coloration of bird plumage, bill, or bare skin of species included in this study

Family, species	Details	References ^a
Accipitridae:		
<i>Accipiter nisus</i>	Yellow orbital ring, cere, and legs: likely presence of carotenoids	
Aegithalidae:		
<i>Aegithalos caudatus</i>	Yellow upper orbital ring: likely presence of carotenoids	
Alaudidae:		
<i>Eremopterix verticalis</i>	No green or yellow to red colors	
<i>Galerida cristata</i>	No green or yellow to red colors	
<i>Mirafra</i> sp.	No green or yellow to red colors	
Anatidae:		
<i>Anas platyrhynchos</i>	Yellow bill and legs: likely presence of carotenoids	8, 9
<i>Branta canadensis</i>	No green or yellow to red colors	
<i>Cygnus atratus</i>	Red bill, eye, and orbital ring: likely presence of carotenoids	8
<i>Cygnus olor</i>	Orange bill: likely presence of carotenoids	8
<i>Netta rufina</i>	Red bill: likely presence of carotenoids	
Anseranatidae:		
<i>Anser indicus</i>	Yellow bill and legs: likely presence of carotenoids	8, 9
Apodidae:		
<i>Apus affinis</i>	No green or yellow to red colors	
<i>Apus caffer</i>	No green or yellow to red colors	
<i>Apus melba</i>	No green or yellow to red colors	
Certhiidae:		
<i>Troglodytes troglodytes</i>	Yellow lower mandible: likely carotenoids	
Charadriidae:		
<i>Haematopus ostralegus</i>	Red bill, orbital ring, and legs: likely carotenoids	
<i>Vanellus armatus</i>	No green or yellow to red colors	
<i>Vanellus coronatus</i>	Orange to red bill and legs: likely presence of carotenoids	
<i>Vanellus vanellus</i>	Orange to red legs: likely presence of carotenoids	
Cisticolidae:		
<i>Malcorus pectoralis</i>	No green or yellow to red colors	
<i>Prinia flavicans</i>	No green or yellow to red colors	
Coliidae:		
<i>Colius colius</i>	Red-pink tarsi: likely presence of carotenoids together with hemoglobin	
Columbidae:		
<i>Columba guinea</i>	Red-pink tarsi and orbital skin: likely presence of carotenoids together with hemoglobin	
<i>Columba livia</i>	Red-pink tarsi: likely presence of carotenoids together with hemoglobin	
<i>Streptopelia senegalensis</i>	Rufous and brown-pinkish feathers: probably melanin	
Corvidae:		
<i>Corvus albus</i>	No green or yellow to red colors	
<i>Corvus corone</i>	No green or yellow to red colors	
<i>Cyanopica cyanea</i>	No green or yellow to red colors	
<i>Laniarius atrococcineus</i>	Red feathers: likely presence of carotenoids	
<i>Pica pica</i>	No green or yellow to red colors	
Cuculidae:		
<i>Clamator glandarius</i>	No green or yellow to red colors	
Falconidae:		
<i>Falco tinnunculus</i>	Yellow tarsus, cere, and lores of <i>Falco sparverius</i> : carotenoids; brown and red-brown eye: unlikely based on carotenoids in <i>F. sparverius</i>	10, 11

Table A2 (Continued)

Family, species	Details	References ^a
Fringillidae:		
<i>Agelaius phoeniceus</i>	Red upper marginal coverts: carotenoids and melanin (eu- and phaeomelanin); yellow upper lesser coverts: melanin (phaeomelanin)	7
<i>Carduelis carduelis</i>	Red head feathers and yellow remiges: carotenoids	2, 9
<i>Carduelis chloris</i>	Yellow remiges: carotenoids; green feathers: carotenoids and melanin	2, 9, 12; Lucas and Stettenheim 1972 in 7
<i>Emberiza citrinella</i>	Yellow breast feathers of <i>Icteria virens</i> : carotenoids	4
<i>Emberiza flaviventris</i>	Yellow breast feathers of <i>I. virens</i> : carotenoids	4
<i>Serinus serinus</i>	Yellow feathers: carotenoids	2
Hirundinidae:		
<i>Delichon urbica</i>	No green or yellow to red colors	
<i>Hirundo cucullata</i>	No green or yellow to red colors; rufous-brown feathers of <i>Hirundo rustica</i> : melanin	13, 14
<i>Hirundo daurica</i>	No green or yellow to red colors; rufous-brown feathers of <i>H. rustica</i> : melanin	13, 14
<i>Hirundo fuligula</i>	No green or yellow to red colors	
<i>H. rustica</i>	No green or yellow to red colors; rufous-brown feathers: melanin	13, 14
<i>Hirundo semirufa</i>	No green or yellow to red colors; rufous-brown feathers of <i>H. rustica</i> : melanin	13, 14
<i>Hirundo spilodera</i>	No green or yellow to red colors; rufous-brown feathers of <i>H. rustica</i> : melanin	13, 14
<i>Tachycineta bicolor</i>	No green or yellow to red colors	
Indicatoridae:		
<i>Indicator minor</i>	Green back and wing feathers: likely presence of carotenoids	
Laniidae:		
<i>Lanius collurio</i>	No green or yellow to red colors	
Laridae:		
<i>Catharacta skua</i>	No green or yellow to red colors	
<i>Gelochelidon nilotica</i>	No green or yellow to red colors	
<i>Larus argentatus cachinnans</i>	Yellow bill and tarsus, orange-red bill spot, gape flanges, and orbital ring: carotenoids (as in <i>Larus fuscus</i>)	15
<i>L. fuscus</i>	Yellow bill and tarsus, orange-red bill spot, gape flanges, and orbital ring: carotenoids (enhanced by dietary carotenoid supplementation)	15
<i>Larus genei</i>	Red tarsus, bill, gape flanges, and orbital ring: carotenoids (as in <i>L. fuscus</i>); rosy flush on the breast: carotenoids	15, 16, 17
<i>Larus hermanni</i>	Red tarsus, bill, gape flanges, and orbital ring: carotenoids (as in <i>L. fuscus</i>)	15
<i>Larus ridibundus</i>	Red tarsus, bill, gape flanges, and orbital ring: carotenoids (as in <i>L. fuscus</i>)	15
<i>Sterna albifrons</i>	Yellow bill and yellow-orange legs: likely presence of carotenoids	
<i>Sterna fuscata</i>	No green or yellow to red colors	
Lybiidae:		
<i>Tricholaema leucomelas</i>	Yellow and red feathers: likely presence of carotenoids	
Meliphagidae:		
<i>Notiomystis cincta</i>	Yellow feathers: carotenoids	18
Meropidae:		
<i>Merops apiaster</i>	Yellow throat and blue-green breast feathers: likely presence of carotenoids; red feathers of <i>Merops nubicus</i> : carotenoids (enhanced by dietary carotenoid supplementation)	6
<i>Merops hirundinaceus</i>	Yellow throat and green feathers: likely presence of carotenoids; red feathers of <i>M. nubicus</i> : carotenoids (enhanced by dietary carotenoid supplementation)	6

Table A2 (Continued)

Family, species	Details	References ^a
Muscicapidae:		
<i>Cercomela familiaris</i>	No green or yellow to red colors; rufous and brown feathers: probably melanin	
<i>Cercotrichas coryphaeus</i>	No green or yellow to red colors	
<i>Cossypha caffra</i>	No green or yellow to red colors; rufous and brown feathers: probably melanin	
<i>Ficedula albicollis</i>	No green or yellow to red colors	
<i>Ficedula hypoleuca</i>	No green or yellow to red colors	
<i>Phoenicurus ochruros</i>	No green or yellow to red colors; rufous and brown feathers: probably melanin	
<i>Sialia sialis</i>	Yellow flanges: likely presence of carotenoids; rufous-brown feathers: melanin	14
<i>Sigelus silens</i>	No green or yellow to red colors	
<i>Turdus olivaceus</i>	Yellow-orange bill, orbital ring, and tarsi: likely presence of carotenoids; yellow to orange bill in <i>Turdus merula</i> : carotenoids; breast and belly feathers: likely presence of carotenoids	19
<i>Turdus philomelos</i>	Yellow lower mandible and orbital ring: likely presence of carotenoids; yellow to orange bill in <i>T. merula</i> : carotenoids	19
<i>Turdus pilaris</i>	Yellow-orange bill and orbital ring: likely presence of carotenoids; yellow to orange bill in <i>T. merula</i> : carotenoids	19
Nectariniidae:		
<i>Nectarinia chalybea</i>	Red breast band: probably carotenoids	8
Numididae:		
<i>Numida meleagris</i>	Red lores and flanges: likely carotenoids; blue flanges: structural	20, 21
Paridae:		
<i>Parus caeruleus</i>	Yellow breast and belly feathers: carotenoids; green back feathers: likely presence of carotenoids	1
<i>Parus cinerascens</i>	No green or yellow to red colors	
<i>Parus major</i>	Yellow breast and belly feathers: carotenoids; green back and wing feathers: likely presence of carotenoids	1
<i>Parus palustris</i>	No green or yellow to red colors	
Passeridae:		
<i>Euplectes orix</i>	Red feathers: carotenoids	5, 8, 9
<i>Motacilla alba</i>	No green or yellow to red colors	
<i>Passer domesticus</i>	No green or yellow to red colors	
<i>Passer hispaniolensis</i>	No green or yellow to red colors	
<i>Passer melanurus</i>	No green or yellow to red colors	
<i>Passer montanus</i>	No green or yellow to red colors	
<i>Philetairus socius</i>	No green or yellow to red colors	
<i>Plocepasser mahali</i>	No green or yellow to red colors	
<i>Ploceus velatus</i>	Yellow to orange feathers: carotenoids	5, 8, 9
<i>Taeniopygia guttata</i>	Orange-red bill and legs: carotenoids (enhanced by dietary carotenoid supplementation); orange-brown cheek feathers: melanin	3, 22, 23
Pelecanidae:		
<i>Pelecanus erythrorhynchos</i>	Yellow to orange bill, lores, and legs: likely presence of carotenoids	
Phalacrocoracidae:		
<i>Phalacrocorax aristotelis</i>	Yellow flanges and bill: likely presence of carotenoids	
<i>Phalacrocorax auritus</i>	Yellow flanges and bill and skin around eye: likely presence of carotenoids	
<i>Phalacrocorax carbo</i>	Yellow flanges and bill and skin around eye: likely presence of carotenoids	

Table A2 (Continued)

Family, species	Details	References ^a
Phasianidae:		
<i>Phasianus colchicus</i>	Red lores: likely presence of carotenoids; rufous and brown feathers: melanin	9
<i>Gallus gallus</i>	Red cere, lores, and comb: carotenoids; rufous and brown feathers: melanin	9, 14
Procellariidae:		
<i>Fulmarus glacialis</i>	No green or yellow to red colors	
<i>Oceanites oceanicus</i>	Yellow skin between metatarsi: likely presence of carotenoids	
Rallidae:		
<i>Fulica americana</i>	Greenish-yellow legs and red forehead patch: likely carotenoids	
<i>Fulica atra</i>	Greenish-yellow and red legs: likely carotenoids	
<i>Gallinula chloropus</i>	Greenish-yellow legs and yellow and red bill: likely carotenoids	
Sittidae:		
<i>Sitta europaea</i>	No green or yellow to red colors; rufous and brown feathers: probably melanin	
Sphenicidae:		
<i>Aptenodytes forsteri</i>	Yellow and orange-brown feathers of <i>Aptenodytes patagonicus</i> : melanin and other undetermined yellow pigments likely to be pterins	14
<i>Spheniscus humboldti</i>	Pink skin around bill and eye: probably mainly blood determined	21
Struthionidae:		
<i>Struthio camelus</i>	Red-pink bill and flanges: likely presence of carotenoids; pink head, neck, and legs: probably mainly blood determined	21
Sturnidae:		
<i>Sturnus unicolor</i>	Yellow bill and orange-red legs: likely carotenoids	24
Sulidae:		
<i>Morus bassanus</i>	No green or yellow to red colors	
<i>Sula neboxii</i>	No green or yellow to red colors; blue legs and feet: structural	20
Sylviidae:		
<i>Acrocephalus arundinaceus</i>	No green or yellow to red colors	
<i>Acrocephalus baeticatus</i>	No green or yellow to red colors	
<i>Acrocephalus palustris</i>	Yellow lower mandible and yellow-olive breast and belly feathers: likely carotenoids	
<i>Panurus biarmicus</i>	Yellow-orange bill: likely carotenoids	
Threskiornithidae:		
<i>Bostrychia hagedash</i>	Red upper mandible: likely presence of carotenoids	8
Upupidae:		
<i>Upupa epops</i>	No green or yellow to red colors; rufous-brown feathers: melanin	

Note: When biochemical or structural analyses were unavailable for the particular species, the most likely nature of the color is inferred from biochemical or structural analyses available for a closely related species or from indirect determination (i.e., color change after dietary carotenoid supplementation). Our data set did not contain any species belonging to Psittaciformes, Trochilidae, or Musophagidae, in which yellow, orange, or red color hues are known or suspected to be produced by pigments other than carotenoids or structurally (Hudon and Brush 1992). In studies of other groups, yellow, orange, and red feathers have always been found to be (at least in part) based on carotenoids (yellow and orange colors: Troy and Brush 1983; Ford and Simpson 1987; Partali et al. 1987; Hudon and Brush 1992; Stradi et al. 1995; McGraw et al. 2002b; Mays et al. 2004; McGraw and Schuetz 2004; Andersson et al. 2007; red colors: Dierenfeld and Sheppard 1996; Stradi et al. 1995, 1996, 1997, 2001; Inouye et al. 2001; McGraw and Schuetz 2004; McGraw et al. 2004b; Blanco et al. 2005; Andersson et al. 2007).

^a 1 = Partali et al. 1987; 2 = Stradi et al. 1995; 3 = McGraw and Schuetz 2004; 4 = Mays et al. 2004; 5 = Andersson et al. 2007; 6 = Dierenfeld and Sheppard 1996; 7 = McGraw et al. 2004b; 8 = Olson and Owens 2005; 9 = Hill and McGraw 2006a; 10 = Casagrande et al. 2006; 11 = Bortolotti et al. 2003; 12 = Saks et al. 2003; 13 = McGraw et al. 2004a; 14 = McGraw et al. 2004c; 15 = Blount et al. 2002; 16 = Hudon and Brush 1990; 17 = McGraw and Hardy 2006; 18 = Ewen et al. 2006a; 19 = Faivre et al. 2003; 20 = Prum and Torres 2003; 21 = Negro et al. 2006; 22 = McGraw et al. 2002a; 23 = McGraw and Wakamatsu 2004; 24 = Wydoski 1964.

Table A3

Regression models with log-transformed yolk antioxidant concentrations as dependent variables and clutch size as independent variable, with log-transformed female body mass and diet controlled for, from species-specific data

Variable	Female body mass			Clutch size		
	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>
Carotenoids	-.005 $\pm$.04	.01 (1, 107)	.91	.08 $\pm$.03	4.95 (1, 107)	.03
Vitamin A	.09 $\pm$.02	14.27 (1, 99)	.0003	.04 $\pm$.02	7.13 (1, 99)	.01
Vitamin E	-.02 $\pm$.04	.48 (1, 105)	.49	.03 $\pm$.03	.98 (1, 105)	.32

Note: See text for results of phylogenetic analyses (r_{phyt}).

Table A4

Regression models with log-transformed yolk antioxidant concentrations as dependent variables and log-transformed male carotenoid-based color scores or sexual dichromatism in carotenoid-based color as independent variables, with female body mass, clutch size, and diet controlled for

Variable	Male color			Sexual dichromatism		
	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>
Carotenoids	1.16 $\pm$.21	29.95 (1, 106)	<.0001	1.25 $\pm$.36	11.94 (1, 106)	.0008
Vitamin A	.13 $\pm$.11	1.32 (1, 98)	.25	.40 $\pm$.18	5.13 (1, 98)	.03
Vitamin E	.13 $\pm$.20	.48 (1, 104)	.49	.43 $\pm$.31	1.97 (1, 104)	.16

Note: Results are given for analyses performed on species-specific data. See text for results of phylogenetic analyses (r_{phyt}).

Table A5

Multiple-regression model of male carotenoid-based color score and sexual dichromatism in carotenoid-based color scores as a function of yolk carotenoid and vitamin A and E concentrations, with female body mass, clutch size, and diet controlled for

Variable	Male color			Sexual dichromatism		
	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>	Slope $\pm$ SE	<i>F</i> (df)	<i>P</i>
Female body mass	-.005 $\pm$.02	.08 (1, 96)	.78	-.01 $\pm$.01	1.29 (1, 96)	.26
Clutch size	-.03 $\pm$.01	3.82 (1, 96)	.05	-.02 $\pm$.01	4.82 (1, 96)	.03
Diet	-.03 $\pm$.03	1.62 (1, 96)	.20	-.04 $\pm$.02	4.77 (1, 96)	.03
Carotenoids	.20 $\pm$.04	26.90 (1, 96)	<.0001	.08 $\pm$.02	10.82 (1, 96)	.001
Vitamin A	.10 $\pm$.08	1.56 (1, 96)	.21	.12 $\pm$.05	4.67 (1, 96)	.03
Vitamin E	-.06 $\pm$.05	2.66 (1, 96)	.20	-.01 $\pm$.03	.11 (1, 96)	.74

Note: All variables were log transformed except clutch size and diet. Results are given for analyses performed on species-specific data. See text for results of phylogenetic analyses (r_{phyt}).

Literature Cited Only in Appendix

- Alström, P., P. G. P. Ericson, U. Olsson, and P. Sundberg. 2006. Phylogeny and classification of the avian superfamily Sylvioidea. *Molecular Phylogenetics and Evolution* 38:381–397.
- Badyaev, A. V. 1997. Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behavioral Ecology* 8:675–690.
- Barker, F. K., G. F. Barrowclough, and J. G. Groth. 2002. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. *Proceedings of the Royal Society B: Biological Sciences* 269:295–308.

- Barker, F. K., A. Cibois, P. Schikler, J. Feinstein, and J. Cracraft. 2004. Phylogeny and classification of the largest avian radiation. *Proceedings of the National Academy of Sciences of the USA* 101:11040–11045.
- Bennett, P. M., and I. P. F. Owens. 2002. *Evolutionary ecology of birds*. Oxford University Press, Oxford.
- Blanco, G., O. Frías, J. Garrido-Fernández, and D. Hornero-Méndez. 2005. Environmental-induced acquisition of nuptial plumage expression: a role of denaturation of feather carotenoproteins? *Proceedings of the Royal Society B: Biological Sciences* 272:1893–1900.
- Blondel, J., F. Catzefflis, and P. Perret. 1996. Molecular phylogeny and the historical biogeography of the warblers of the genus *Sylvia* (Aves). *Journal of Evolutionary Biology* 9:871–891.
- Bortolotti, G. R., J. E. Smits, and D. M. Bird. 2003. Iris colour of American kestrels varies with age, sex, and exposure to PCBs. *Physiological and Biochemical Zoology* 76:99–104.
- Casagrande, S., D. Csermely, E. Pini, V. Bertacche, and J. Tagliavini. 2006. Skin carotenoid concentration correlated with male hunting skill and territory quality in the kestrel *Falco tinnunculus*. *Journal of Avian Biology* 37:190–196.
- Cibois, A., and E. Pasquet. 1999. Molecular analysis of the phylogeny of 11 genera of the Corvidae. *Ibis* 141: 297–306.
- Crochet, P.-A., F. Bonhomme, and J.-D. Lebreton. 2000. Molecular phylogeny and plumage evolution in gulls (*Larini*). *Journal of Evolutionary Biology* 13:47–57.
- Dierenfeld, E. S., and C. D. Sheppard. 1996. Canthaxanthin pigment does not maintain color in carmine bee-eaters. *Zoo Biology* 15:183–185.
- Donne-Goussé, C., V. Laudet, and C. Hänni. 2002. A molecular phylogeny of Anseriformes based on mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution* 23:339–356.
- Ewen, J. G., P. F. Surai, R. D. Stradi, A. P. Møller, B. Vittorio, R. Griffiths, and D. P. Armstrong. 2006a. Carotenoids, colour and conservation in an endangered passerine, the hihi or stitchbird (*Notiomystis cincta*). *Animal Conservation* 9:229–235.
- Ewen, J. G., R. Thorogood, F. Karadaş, A. C. Pappas, and P. F. Surai. 2006b. Influences of carotenoid supplementation on the integrated antioxidant system of a free living endangered passerine, the hihi (*Notiomystis cincta*). *Comparative Biochemistry and Physiology A* 143:149–154.
- Ewen, J. G., I. Flux, and P. G. P. Ericson. 2006c. Systematic affinities of two enigmatic New Zealand passerines of high conservation priority, the hihi or stitchbird *Notiomystis cincta* and the kokako *Callaeas cinerea*. *Molecular Phylogenetics and Evolution* 40:281–284.
- Faivre, B., A. Grégoire, M. Préault, F. Cézilly, and G. Sorci. 2003. Immune activation rapidly mirrored in a secondary sexual trait. *Science* 300:103.
- Ford, J., and I. W. Simpson. 1987. Pigment and morphometric variation in the bluff-rumped thornbill. *Emu* 87: 53–56.
- Garamszegi, L. Z., M. Eens, J. Erritzøe, and A. P. Møller. 2005a. Sexually size dimorphic brains and song complexity in passerine birds. *Behavioral Ecology* 16:335–345.
- Garamszegi, L. Z., C. Biard, M. Eens, A. P. Møller, N. Saino, and P. F. Surai. 2007b. Maternal effects and the evolution of brain size in birds: overlooked developmental constraints. *Neuroscience and Biobehavioral Reviews* 31:498–515.
- Gill, F. B., B. Slikas, and F. H. Sheldon. 2005. Phylogeny of titmice species (Paridae). II. Species relationship based on sequences of the mitochondrial cytochrome-*b* gene. *Auk* 122:121–143.
- Hackett, S. J., R. T. Kimball, S. Reddy, R. C. K. Bowie, E. L. Braun, M. J. Braun, J. L. Chojnowski, et al. 2008. A phylogenetic study of birds reveals their evolutionary history. *Science* 320:1763–1768.
- Hudon, J., and A. H. Brush. 1990. Carotenoids produce flush in the elegant tern plumage. *Condor* 92:798–801.
- . 1992. Identification of carotenoid pigments in birds. Pages 312–321 in L. Packer, ed. *Carotenoids*. Part A: chemistry, separation, quantitation, and antioxidation. *Methods in Enzymology* 213. Academic Press, London.
- Jönsson, K. A., and J. Fjeldså. 2006. A phylogenetic supertree of oscine passerine birds (Aves: Passeri). *Zoologica Scripta* 35:149–186.
- Karadaş, F., N. A. R. Wood, P. F. Surai, and N. H. C. Sparks. 2005. Tissue-specific distribution of carotenoids and vitamin E in tissues of newly hatched chicks from various avian species. *Comparative Biochemistry and Physiology A* 140:506–511.
- Kennedy, M., R. D. Gray, and H. G. Spencer. 2000. The phylogenetic relationships of the shags and cormorants:

- can sequence data resolve a disagreement between behavior and morphology? *Molecular Phylogenetics and Evolution* 17:345–359.
- Kimball, R. T., E. L. Braun, P. W. Zwartjes, T. M. Crowe, and J. D. Ligon. 1999. A molecular phylogeny of the pheasants and partridges suggests that these lineages are not monophyletic. *Molecular Phylogenetics and Evolution* 11:38–54.
- Leisler, B., P. Heidrich, K. Schulze-Hagen, and M. Wink. 1997. Taxonomy and phylogeny of reed warblers (genus *Acrocephalus*) based on mtDNA sequences and morphology. *Journal of Ornithology* 138:469–496.
- Lucas, A. M., and P. R. Stettenheim. 1972. *Avian anatomy: integument*. USDA, Washington, DC.
- Martin, E., and J. Clobert. 1996. Nest predation and avian life-history evolution in Europe versus North America: a possible role of humans? *American Naturalist* 147:1028–1046.
- Mays, H. L., Jr., K. J. McGraw, G. Ritchison, S. Cooper, V. N. Rush, and R. S. Parker. 2004. Sexual dichromatism in the yellow-breasted chat *Icteria virens*: spectrophotometric analysis and biochemical basis. *Journal of Avian Biology* 35:125–134.
- McGraw, K. J., and L. S. Hardy. 2006. Astaxanthin is responsible for the pink plumage flush in Franklin’s and ring-billed gulls. *Journal of Field Ornithology* 77:29–33.
- McGraw, K. J., E. Adkins-Regan, and R. S. Parker. 2002a. Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. *Comparative Biochemistry and Physiology B* 132:811–818.
- McGraw, K. J., G. E. Hill, R. D. Stradi, and P. G. Parker. 2002b. The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. *Comparative Biochemistry and Physiology B* 131:261–269.
- McGraw, K. J., K. Wakamatsu, A. B. Clark, and K. Yasukawa. 2004b. Red-winged blackbirds *Agelaius phoeniceus* use carotenoid and melanin pigments to color their epaulets. *Journal of Avian Biology* 35:543–550.
- Møller, A. P., S. Merino, C. R. Brown, and R. J. Robertson. 2001. Immune defense and host sociality: a comparative study of swallows and martins. *American Naturalist* 158:136–145.
- Partali, V., S. Liaaen-Jensen, T. Slagsvold, and J. T. Lifjeld. 1987. Carotenoids in food chain studies. II. The food chain of *Parus* spp. monitored by carotenoid analysis. *Comparative Biochemistry and Physiology B* 87:885–888.
- Sheldon, F. H., and D. W. Winkler. 1993. Intergeneric phylogenetic relationships of swallows estimated by DNA-DNA hybridization. *Auk* 110:798–824.
- Sibley, C. G., and J. E. Ahlquist. 1990. *Phylogeny and classification of birds: a study in molecular evolution*. Yale University Press, New Haven, CT.
- Sibley, C. G., and B. L. Monroe Jr. 1990. *Distribution and taxonomy of the birds of the world*. Yale University Press, New Haven, CT.
- Speake, B. K., P. F. Surai, R. C. Noble, J. V. Beer, and N. A. R. Wood. 1999a. Differences in egg lipid and antioxidant composition between wild and captive pheasants and geese. *Comparative Biochemistry and Physiology B* 124:101–107.
- Speake, B. K., F. Decrock, P. F. Surai, and R. Groscolas. 1999b. Fatty acid composition of the adipose tissue and yolk lipids of a bird with a marine-based diet, the emperor penguin (*Aptenodytes forsteri*). *Lipids* 34:283–290.
- Stradi, R. D., G. Celentano, E. Rossi, G. Rovati, and M. Pastore. 1995. Carotenoids in bird plumage. I. The carotenoid pattern in a series of palearctic *Carduelinae*. *Comparative Biochemistry and Physiology B* 110:131–143.
- Stradi, R. D., E. Rossi, G. Celentano, and B. Bellardi. 1996. Carotenoids in bird plumage: the pattern in three *Loxia* species and in *Pinicola enucleator*. *Comparative Biochemistry and Physiology B* 113:427–432.
- Stradi, R. D., G. Celentano, M. Boles, and F. Mercato. 1997. Carotenoids in bird plumage: the pattern in a series of red-pigmented *Carduelinae*. *Comparative Biochemistry and Physiology B* 117:85–91.
- Stradi, R. D., E. Pini, and G. Celentano. 2001. Carotenoids in bird plumage: the complement of red pigments in the plumage of wild and captive bullfinch (*Pyrrhula pyrrhula*). *Comparative Biochemistry and Physiology B* 128:529–535.
- Surai, P. F., B. K. Speake, N. A. R. Wood, J. D. Blount, G. R. Bortolotti, and N. H. C. Sparks. 2001a. Carotenoid discrimination by the avian embryo: a lesson from wild birds. *Comparative Biochemistry and Physiology B* 128:743–750.
- Surai, P. F., G. R. Bortolotti, A. L. Fidgett, J. D. Blount, and B. K. Speake. 2001c. Effects of piscivory on the

- fatty acid profiles and antioxidants of avian yolk: studies of the gannet, skua, pelican and cormorant. *Journal of Zoology (London)* 255:305–312.
- Tella, J. L., J. Figuerola, J. J. Negro, G. Blanco, R. Rodríguez-Estrella, M. G. Forero, M. C. Blázquez, A. J. Green, and F. Hiraldo. 2004. Ecological, morphological and phylogenetic correlates of interspecific variation in plasma carotenoid concentration in birds. *Journal of Evolutionary Biology* 17:156–164.
- Troy, D. M., and A. H. Brush. 1983. Pigments and feather structure of the redpolls, *Carduelis flammea* and *C. hornemanni*. *Condor* 85:443–446.
- Wydoski, R. S. 1964. Seasonal changes in the color of starling bills. *Auk* 81:542–550.

**MATERNAL EFFECTS MEDIATED BY ANTIOXIDANTS AND THE EVOLUTION OF CAROTENOID-
BASED SIGNALS IN BIRDS**

Clotilde Biard*, Diego Gil, Filiz Karadas, Nicola Saino, Claire N. Spottiswoode,
Peter F. Surai and Anders P. Møller

The American Naturalist 174 (5), November 2009

Additional Information for Review

A. Analyses based on an alternative phylogeny with branch lengths based on molecular data

B. Analyses based on restricted data sets: Passeriformes and Ciconiiformes

C. Analyses using male carotenoid-based color and sexual dichromatism in carotenoid-based color coded as binary variables

D. Analyses including latitude

Literature cited

* corresponding author: clotilde.biard@upmc.fr

A. ANALYSES BASED ON AN ALTERNATIVE PHYLOGENY WITH BRANCH LENGTHS BASED ON MOLECULAR DATA

In order to check that our results were not dependent on the assumption of equal branch lengths in our composite phylogeny, we ran the analyses using an alternative phylogeny based above the family level on Sibley and Ahlquist (Sibley and Ahlquist 1990) and completed within families with recent information for genera and species (see main text for references). The phylogeny is shown in Fig. A1. We therefore applied information on branch lengths from the tapestry tree based on DNA-DNA hybridisation, above the family level. Below the family level, we applied the convention that within families genera differed by 3.4 ΔT_{50H} units, and that species within genera differed by 1.1 ΔT_{50H} units (Bennett and Owens 2002; Sibley and Ahlquist 1990; Garamszegi et al. 2007b).

The importance of branch lengths was estimated through the branch length scaling parameter κ as implemented in Continuous. At its default value of 1, the branch length scaling factor implies that traits change linearly with branch length (gradualism). A value of $\kappa > 1$ indicates that traits change more in longer than in shorter branches, when a value of $\kappa = 1$ would suggest that traits evolve independently of branch lengths (punctuational model) (Pagel 1999). The contribution of κ was assessed by comparing the log-likelihood ratio of a model in which κ was constrained to its default value to that of an alternative model in which κ was estimated and took its maximum likelihood value. The estimation of κ was used in the final model if this improved the log-likelihood, otherwise the default value of 1 was kept. The statistical methods were otherwise identical to those described in the Methods section.

RESULTS

When similarity among species due to common descent was taken into account, all three yolk antioxidants were significantly positively correlated (carotenoids and vitamin A: correlation at $\kappa = 0.225$ and $\lambda = 0.575$: $r_{\text{phyl}} = 0.20$, $p < 0.05$, $n = 100$; carotenoids and vitamin E: correlation at $\kappa = 0.273$ and $\lambda = 0.656$: $r_{\text{phyl}} = 0.39$, $p < 0.001$, $n = 106$; vitamins A and E: correlation at $\kappa = 0.105$ and $\lambda = 0.441$: $r_{\text{phyl}} = 0.29$, $p < 0.02$, $n = 100$).

ANTIOXIDANTS IN RELATION TO FEMALE BODY MASS AND CLUTCH SIZE

When similarity among species due to common descent was taken into account, all three yolk antioxidants were significantly positively correlated with clutch size after controlling for female body mass (carotenoids: partial correlation at $\kappa = 0.603$ and $\lambda = 0.912$: $r_{\text{phyl}} = 0.30$, $p < 0.02$, $n = 108$; vitamin A: partial correlation at $\kappa = 0.556$ and $\lambda = 0.884$: $r_{\text{phyl}} = 0.32$, $p < 0.002$, $n = 100$; vitamin E: partial correlation at $\kappa = 0.519$ and $\lambda = 0.814$: $r_{\text{phyl}} = 0.32$, $p < 0.001$, $n = 106$).

YOLK ANTIOXIDANTS AND CAROTENOID-BASED SIGNALS

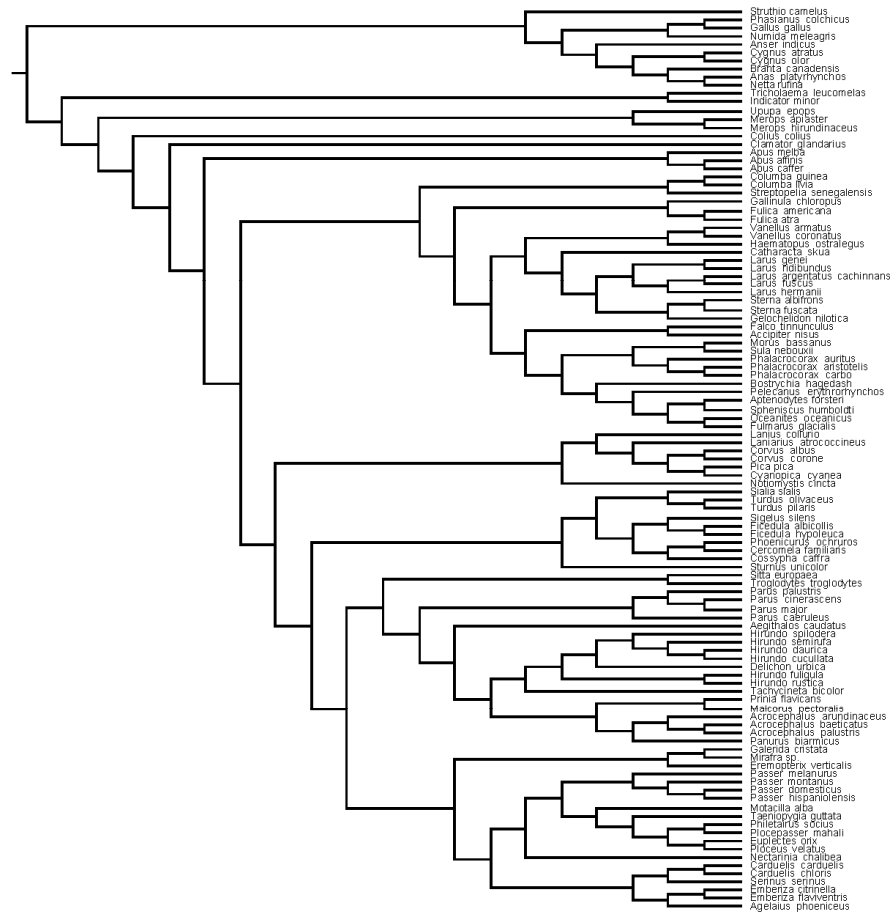
When similarity among species due to common descent was taken into account, models controlling for female body mass and clutch size confirmed the significant positive relation between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\kappa = 0.498$ and $\lambda = 0.875$: $r_{\text{phyl}} = 0.37$, $p < 0.0005$, $n = 108$) and sexual dichromatism (partial correlation at $\kappa = 0.619$ and $\lambda = 0.921$: $r_{\text{phyl}} = 0.20$, $p < 0.02$). The absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color was also confirmed (vitamin A, partial correlation at $\kappa = 0.476$ and $\lambda = 0.853$: $r_{\text{phyl}} = 0.03$, $p > 0.05$, $n = 100$; vitamin E, partial correlation at $\kappa = 0.447$ and $\lambda = 0.848$: $r_{\text{phyl}} = 0.001$, $p > 0.05$, $n = 106$).

Sexual dichromatism was not confirmed to be positively related to vitamin A (partial correlation at $\kappa = 0.559$ and $\lambda = 0.898$: $r_{\text{phyl}} = 0.16$, $p > 0.05$, $n = 100$), and was not significantly related to vitamin E (partial correlation at $\kappa = 0.542$ and $\lambda = 0.889$: $r_{\text{phyl}} = 0.08$, $p > 0.05$, $n = 106$).

In a phylogenetic multiple regression of male carotenoid-based color score, female body mass, clutch size and the three antioxidants, only carotenoid concentration was significantly correlated with male color (carotenoids, partial correlation: $r_{\text{phyl}} = 0.41$, $p < 0.0005$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.02$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.13$, $p > 0.05$; at $\kappa = 0.402$ and $\lambda = 0.794$, $n = 100$). Likewise, sexual dichromatism in carotenoid-based color was positively correlated with yolk carotenoids but not with vitamin A or vitamin E (carotenoids, partial correlation: $r_{\text{phyl}} = 0.23$, $p < 0.02$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.13$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.06$, $p > 0.05$; at $\kappa = 0.465$ and $\lambda = 0.833$, $n = 100$).

Among species that were sexually dichromatic for carotenoid-based signals, there was no evidence of correlated evolution between sexual dichromatism and yolk antioxidants in models of phylogenetic multiple correlations controlling for female body mass and clutch size (carotenoids, Likelihood Ratio test at $\kappa = 0.275$ and $\lambda = 0.832$: $p = 0.74$; vitamin A, Likelihood Ratio test at $\kappa = 0.514$ and $\lambda = 0.896$: $p = 0.54$; vitamin E, Likelihood Ratio test at $\kappa = 0.345$ and $\lambda = 0.783$: $p = 0.54$). The relationship between sexual dichromatism and egg carotenoids was therefore not supported when controlling for the effect of carotenoid-based color. In a phylogenetic multiple regression, residual sexual dichromatism was not significantly correlated with any of the three yolk antioxidant (carotenoids, partial correlation: $r_{\text{phyl}} = 0.26$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.03$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.27$; all $p > 0.05$; at $\kappa = 0$ and $\lambda = 0.467$, $n = 21$).

Fig. A1. Alternative phylogeny based on Sibley and Ahlquist. See above for further details.



B. ANALYSES BASED ON RESTRICTED DATA SETS: PASSERIFORMES AND CICONIFORMES

In order to check whether the conclusions of the study held not only across all birds but also within phylogenetic groups, we ran the analyses on restricted data sets corresponding to the two phylogenetic groups for which sufficient data were available: Passeriformes and Ciconiformes. However, it was impossible to run the analyses with sexual dichromatism as covariate or dependent variable, as splitting the data set resulted in too few sexually dichromatic species in each group: 14 sexually dichromatic species out of 60 (58 available for phylogenetic analyses) in Passeriformes, and 2 sexually dichromatic species out of 26 (25 available for phylogenetic analyses) in Ciconiformes. In our data set, Ciconiformes were represented by Charadriidae, Laridae, Falconidae, Accipitridae, Sulidae, Phalacrocoracidae, Threskiornidae, Pelicanidae, Spheniscidae and Procelariidae. Passerines were represented by Laniidae, Corvidae, Muscicapidae, Sturnidae, Sitidae, Certhiidae, Paridae, Aegithalidae, Hirundinidae, Cisticolidae, Sylviidae, Alaudidae, Passeridae, Nectariniidae, Fringilidae and Meliphagidae.

1. Passerine species

Mean carotenoid and vitamin A concentrations were both positively correlated with mean vitamin E concentration ($r = 0.43$, $p = 0.001$ and $r = 0.39$, $p = 0.002$, respectively, $n = 60$, on log-transformed data), while carotenoid and vitamin A concentration were not significantly correlated ($r = 0.10$, $p = 0.44$, $n = 60$, on log-transformed data). The same was true when similarity among species due to common descent was taken into account (carotenoids and vitamin A: correlation at $\lambda = 0.319$: $r_{\text{phyl}} = 0.04$, $p > 0.05$, $n = 58$; carotenoids and vitamin E:

correlation at $\lambda = 0.424$: $r_{\text{phyl}} = 0.34$, $p < 0.01$, $n = 58$; vitamins A and E: correlation at $\lambda = 0.0$: $r_{\text{phyl}} = 0.40$, $p < 0.002$, $n = 58$).

YOLK ANTIOXIDANTS IN RELATION TO FEMALE BODY MASS AND CLUTCH SIZE

In an analysis based on species, yolk vitamin A and E were significantly positively related to clutch size after controlling for female body mass, but that was not the case for carotenoids (Table B1-a). When similarity among species due to common descent was taken into account, vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.47$, $p < 0.001$, $n = 58$) and vitamin E (partial correlation at $\lambda = 0.849$: $r_{\text{phyl}} = 0.46$, $p < 0.001$, $n = 58$), but not yolk carotenoids (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.17$, $p > 0.05$, $n = 58$), showed a significant positive correlation with clutch size after controlling for female body mass.

YOLK ANTIOXIDANTS AND CAROTENOID-BASED SIGNALS

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: The model controlling for female body mass and clutch size explained 29% of total variance (Table B2-a; Fig. B2). Vitamin A and vitamin E concentration were not significantly related to the extent of carotenoid-based color (Table B2-a; Fig. B2). When similarity among species due to common descent was taken into account, models controlling for female body mass and clutch size confirmed the significant positive relation between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.32$, $p < 0.01$, $n = 58$). When incorporating effects of phylogeny, models controlling for female body mass and clutch size confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.13$, $p > 0.05$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.08$, $p > 0.05$; $n = 58$).

In a multiple regression of male carotenoid-based color score as a function of female body mass, clutch size and the three antioxidants, only carotenoid concentration explained a significant part of the variance (Table B3-a). The same result was found when taking similarity among species due to common descent into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.46$, $p < 0.0005$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.21$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.11$, $p > 0.05$; at $\lambda = 0.785$, $n = 58$).

2. Ciconiformes

Mean vitamin A and vitamin E concentrations were positively correlated ($r = 0.46$, $p = 0.03$, $n = 23$, on log-transformed data), while carotenoid concentration was not significantly correlated with vitamin A or vitamin E concentration ($r = -0.03$, $p = 0.81$, $n = 23$, and $r = 0.22$, $p = 0.28$, $n = 25$, respectively on log-transformed data). When similarity among species due to common descent was taken into account, only vitamins A and E were significantly positively correlated (carotenoids and vitamin A: correlation at $\lambda = 0.0$: $r_{\text{phyl}} = 0.05$, $p > 0.05$, $n = 23$; carotenoids and vitamin E: correlation at $\lambda = 0.399$: $r_{\text{phyl}} = 0.33$, $p > 0.05$, $n = 24$; vitamins A and E: correlation at $\lambda = 0.252$: $r_{\text{phyl}} = 0.50$, $p < 0.02$, $n = 23$).

ANTIOXIDANTS IN RELATION TO FEMALE BODY MASS AND CLUTCH SIZE

In an analysis based on species, yolk carotenoids, vitamin A and vitamin E were not significantly positively related to clutch size after controlling for female body mass (Table B1-b). When similarity among species due to common descent was taken into account, vitamin A (partial correlation at $\lambda = 0.586$: $r_{\text{phyl}} = -0.06$, $p > 0.05$, $n = 23$), vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.09$, $p > 0.05$, $n = 24$) or yolk carotenoids (partial correlation at λ

= 1: $r_{\text{phyl}} = 0.14$, $p > 0.05$, $n = 25$) did not show a significant positive correlation with clutch size after controlling for female body mass.

YOLK ANTIOXIDANTS AND CAROTENOID-BASED SIGNALS

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: The model controlling for female body mass and clutch size explained 32% of total variance (Table B2-b, Fig. B3). Vitamin A and vitamin E concentrations were not significantly related to the extent of carotenoid-based color (Table B2-b, Fig. B3). When similarity among species due to common descent was taken into account, models controlling for female body mass and clutch size confirmed the significant positive relation between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.42$, $p < 0.02$, $n = 25$). When incorporating effects of phylogeny, models controlling for female body mass and clutch size confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 0.283$: $r_{\text{phyl}} = -0.07$, $p > 0.05$, $n = 23$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.19$, $p > 0.05$, $n = 24$).

In a multiple regression of male carotenoid-based color score as a function of female body mass, clutch size and the three antioxidants, only carotenoid concentration explained a significant part of the variance (Table B3-b). The same result was found when taking similarity among species due to common descent into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.49$, $p < 0.01$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.08$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.18$, $p > 0.05$; at $\lambda = 0.092$, $n = 23$).

Table B1. Regression models with log-transformed yolk antioxidant concentrations as dependent variables and clutch size as independent variable, controlling for log-transformed female body mass, based on species-specific data (see main text for results of phylogenetic analyses).

Variable	Female body mass			Clutch size		
	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>
(a) Passeriformes						
Carotenoids	-0.02 $\pm$ 0.12	0.02 _{1,57}	0.88	-0.01 $\pm$ 0.05	0.05 _{1,57}	0.83
Vitamin A	0.01 $\pm$ 0.05	0.08 _{1,57}	0.78	0.11 $\pm$ 0.02	22.90 _{1,57}	<0.0001
Vitamin E	0.02 $\pm$ 0.10	0.05 _{1,57}	0.82	0.12 $\pm$ 0.04	8.44 _{1,57}	0.005
(b) Ciconiiformes						
Carotenoids	0.03 $\pm$ 0.13	0.04 _{1,23}	0.84	0.22 $\pm$ 0.17	1.55 _{1,23}	0.23
Vitamin A	0.16 $\pm$ 0.06	6.89 _{1,20}	0.01	-0.05 $\pm$ 0.07	0.52 _{1,20}	0.48
Vitamin E	0.31 $\pm$ 0.07	17.47 _{1,22}	0.0004	-0.08 $\pm$ 0.10	0.55 _{1,22}	0.49

Table B2. Regression models with log-transformed yolk antioxidant concentrations as dependent variables and log-transformed male carotenoid-based color scores as independent variable, controlling for female body mass and clutch size. Results are given for analyses performed on species-specific data (see main text for results of phylogenetic analyses).

Variable	Male color		
	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>
(a) Passeriformes			
Carotenoids	1.01 $\pm$ 0.21	23.31 _{1,56}	< 0.0001
Vitamin A	0.06 $\pm$ 0.10	0.32 _{1,56}	0.57
Vitamin E	0.27 $\pm$ 0.19	1.95 _{1,56}	0.17
(b) Ciconiformes			
Carotenoids	2.08 $\pm$ 0.73	8.17 _{1,22}	0.01
Vitamin A	-0.16 $\pm$ 0.37	0.18 _{1,19}	0.68
Vitamin E	0.31 $\pm$ 0.50	0.37 _{1,21}	0.55

Table B3. Multiple regression model of male carotenoid-based color score as a function of yolk carotenoid, vitamin A and E concentrations, controlling for female body mass and clutch size (all variables were log-transformed except clutch size). Results are given for analyses performed on species-specific data (see main text for results of phylogenetic analyses).

Variable	Male color		
	slope $\pm$ s.e.	F _{d.f.}	p
(a) Passeriformes			
Female body mass	-0.08 $\pm$ 0.06	2.01 _{1,54}	0.16
Clutch size	-0.01 $\pm$ 0.03	0.07 _{1, 54}	0.79
Carotenoids	0.31 $\pm$ 0.07	20.57 _{1, 54}	< 0.0001
Vitamin A	0.03 $\pm$ 0.15	0.03 _{1, 54}	0.87
Vitamin E	-0.07 $\pm$ 0.09	0.54 _{1, 54}	0.46
(b) Ciconiformes			
Female body mass	0.05 $\pm$ 0.05	1.16 _{1,17}	0.30
Clutch size	-0.03 $\pm$ 0.04	0.45 _{1,17}	0.51
Carotenoids	0.15 $\pm$ 0.05	7.15 _{1,17}	0.02
Vitamin A	-0.04 $\pm$ 0.13	0.09 _{1,17}	0.78
Vitamin E	-0.07 $\pm$ 0.10	0.56 _{1,17}	0.46

Legends to figures

Fig. B1. Mean concentrations of egg yolk antioxidants ($\mu\text{g/g}$) as a function of male carotenoid-based color scores in passerine species ($n = 60$), presented on a log-scale ($\log[\text{score} + 1]$): (A) carotenoids; (B) vitamin A; and (C) vitamin E.

Fig. B2. Mean concentrations of egg yolk antioxidants ($\mu\text{g/g}$) as a function of male carotenoid-based color scores in ciconiforme species, presented on a log-scale ($\log[\text{score} + 1]$): (A) carotenoids ($n = 26$); (B) vitamin A ($n = 23$); and (C) vitamin E ($n = 25$).

Fig. B1

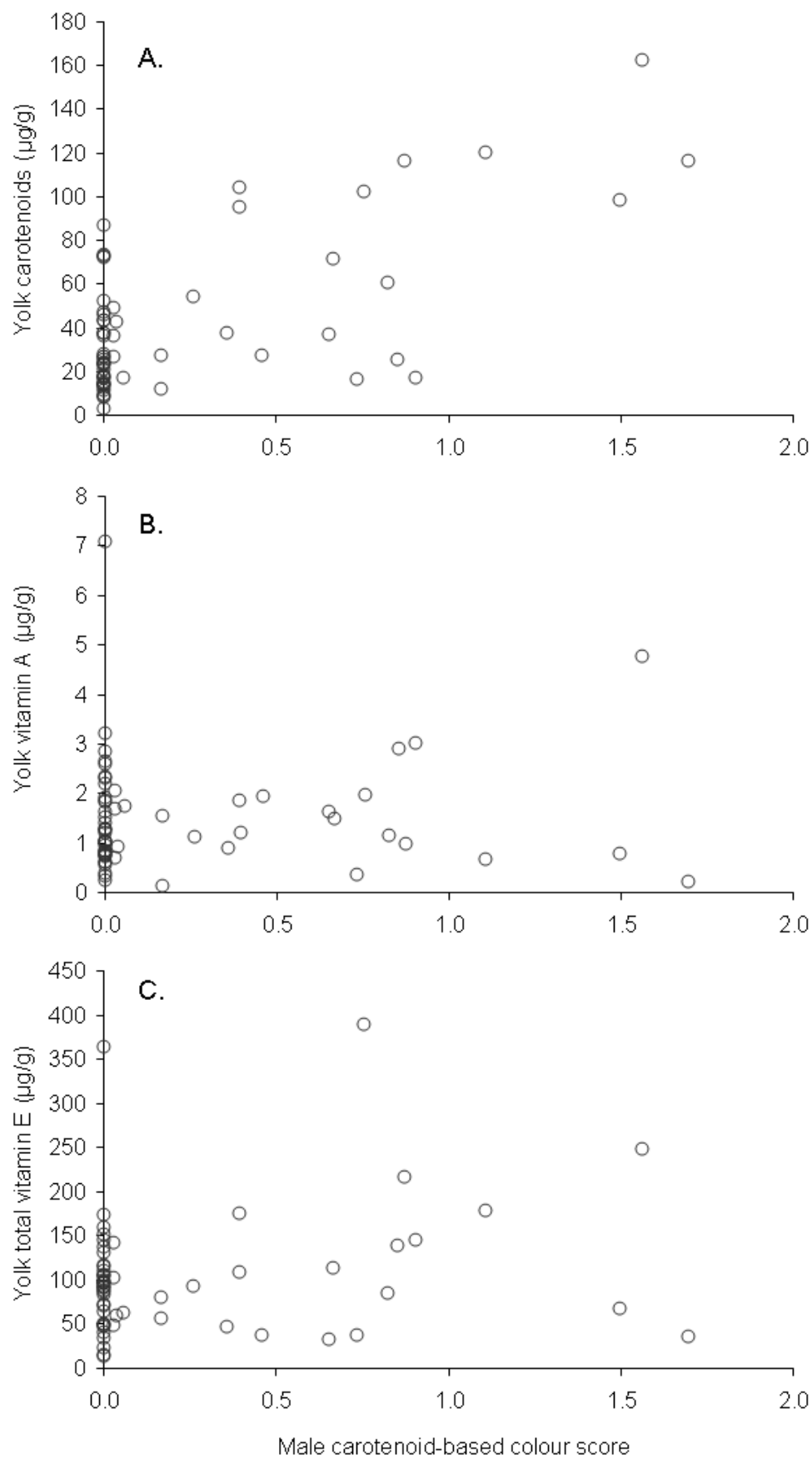
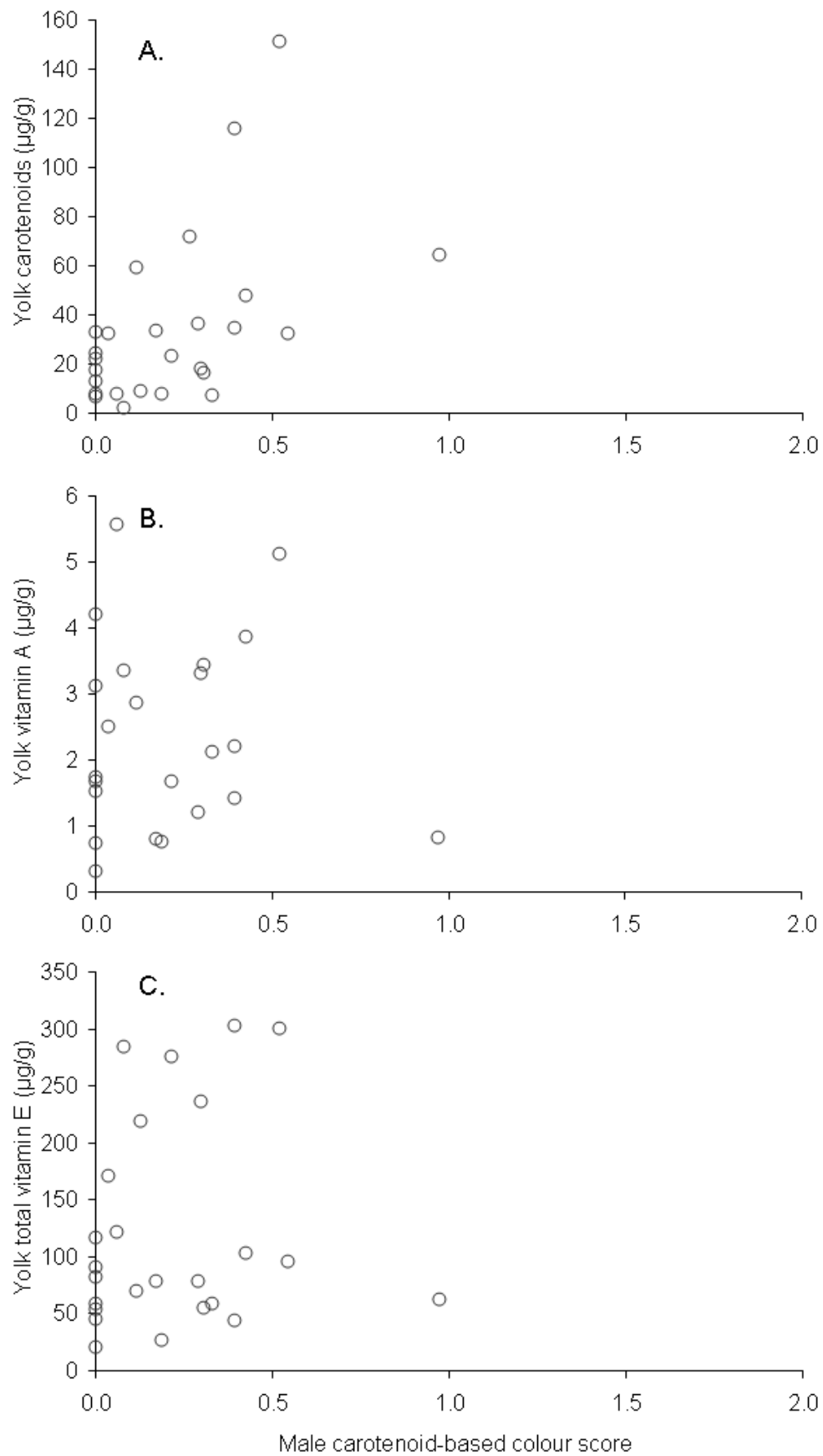


Fig. B2



C. ANALYSES USING MALE CAROTENOID-BASED COLOR AND SEXUAL DICHROMATISM IN CAROTENOID-BASED COLOR CODED AS BINARY VARIABLES

In order to check whether our results were dependent on the method used to score color and/or on color scores derived from color plates in handbooks, we ran the analyses using male carotenoid-based coloration and sexual dichromatism in carotenoid-based coloration coded as binary variables (absence/presence), following (Birkhead and Møller 1994). In the phylogenetic analyses in Continuous, color was treated as a continuous variable, under the assumption that intermediate states are biologically meaningful (Bennett and Owens 2002; Garamszegi et al. 2005a; Garamszegi et al. 2007a).

YOLK ANTIOXIDANTS AND CAROTENOID-BASED SIGNALS

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: The model controlling for female body mass and clutch size explained 19% of total variance (Table C1). In addition, carotenoid concentration increased with sexual dichromatism in carotenoid-based signals: the model controlling for female body mass and clutch size explained 12% of the variance (Table C1). Vitamin A concentration was not significantly related to the extent of carotenoid-based color or to the extent of sexual dichromatism (Table C1). Vitamin E concentration was not significantly related to carotenoid-based color of feathers and bare parts, or to sexual dichromatism in these signals (Table C1). When similarity among species due to common descent was taken into account, models controlling for female body mass and clutch size confirmed the significant positive relation between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.21$, $p < 0.02$, $n = 108$) but not with sexual dichromatism (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.13$, $p > 0.05$). When incorporating effects of phylogeny, models controlling for

female body mass and clutch size confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.10$, $p > 0.05$, $n = 100$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.05$, $p > 0.05$, $n = 106$). Sexual dichromatism was not significantly related to vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.03$, $p > 0.05$, $n = 100$) or vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.06$, $p > 0.05$, $n = 106$).

In a logistic multiple regression of male carotenoid-based color score as a function of female body mass, clutch size and the three antioxidants, only carotenoid concentration significantly predicted the probability of males in a species to display carotenoid-based color (Table C2). The same result was found when taking similarity among species due to common descent into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.26$, $p < 0.005$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.09$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.15$, $p > 0.05$; at $\lambda = 0.957$, $n = 100$). In a logistic multiple regression model based on species, the probability of a species to show sexual dichromatism in carotenoid-based color was positively related to yolk carotenoids and vitamin E but not to vitamin A (Table 5). These results were confirmed for yolk carotenoids only when the effects of similarity due to common phylogenetic descent were controlled (carotenoids, partial correlation: $r_{\text{phyl}} = 0.23$, $p < 0.01$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.007$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.15$, $p > 0.05$; at $\lambda = 0.961$, $n = 100$).

Table C1. ANCOVA models with log-transformed yolk antioxidant concentrations as dependent variables and log-transformed male carotenoid-based color scores or sexual dichromatism in carotenoid-based color as independent variables, controlling for female body mass and clutch size. Results are given for analyses performed on species-specific data (see main text for results controlling for phylogeny).

Variable	Male color			Sexual dichromatism		
	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>
Carotenoids	0.68 $\pm$ 0.17	16.96 _{1,107}	< 0.0001	0.52 $\pm$ 0.21	6.49 _{1,107}	0.01
Vitamin A	0.06 $\pm$ 0.08	0.46 _{1,99}	0.50	0.08 $\pm$ 0.10	0.71 _{1,99}	0.40
Vitamin E	-0.03 $\pm$ 0.15	0.03 _{1,105}	0.86	-0.12 $\pm$ 0.17	0.472 _{1,105}	0.49

Table C2. Logistic multiple regression modelling the probability that male carotenoid-based color score and sexual dichromatism in carotenoid-based color scores are equal to 1, as a function of yolk carotenoid, vitamin A and E concentrations, controlling for female body mass and clutch size (all variables were log-transformed except clutch size). Results are given for analyses performed on species-specific data (see main text for results controlling for phylogeny).

Variable	Male color			Sexual dichromatism		
	Estimate $\pm$ s.e.	χ^2 d.f.	<i>p</i>	Estimate $\pm$ s.e.	χ^2 d.f.	<i>p</i>
Female body mass	0.41 $\pm$ 0.15	7.61 _{1,97}	0.006	-0.10 $\pm$ 0.15	0.44 _{1,97}	0.51
Clutch size	-0.10 $\pm$ 0.10	0.99 _{1,97}	0.32	0.06 $\pm$ 0.11	0.27 _{1,97}	0.60
Carotenoids	1.16 $\pm$ 0.04	13.16 _{1,97}	0.0003	1.54 $\pm$ 0.47	10.85 _{1,97}	0.001
Vitamin A	0.61 $\pm$ 0.62	0.97 _{1,97}	0.32	0.10 $\pm$ 0.72	2.34 _{1,97}	0.13
Vitamin E	-0.59 $\pm$ 0.37	2.57 _{1,97}	0.11	-1.34 $\pm$ 0.50	7.12 _{1,97}	0.008

D. ANALYSES INCLUDING LATITUDE

In order to check that the relationships between yolk antioxidant concentration and clutch size were not confounded by latitude, this variable was included as a covariate in the analyses. Information on mean latitude was collected searching handbooks (Cramp et al. 1982-1994; Brown et al. 1982-2004; del Hoyo et al. 1992-2004; Poole and Gill 1992-2004). Mean latitude was taken as the midpoint of the northernmost and southernmost limits of each species' geographical breeding range (Garamszegi et al. 2005b).

RESULTS

YOLK ANTIOXIDANTS IN RELATION TO FEMALE BODY MASS, CLUTCH SIZE AND LATITUDE

In an analysis based on species, after controlling for female body mass, none of the three yolk antioxidants were significantly related to clutch size, and vitamin A and vitamin E were significantly positively related to latitude (Table D1). When similarity among species due to common descent was taken into account and in models controlling for female body mass, all three yolk components were significantly positively related with clutch size (carotenoids: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.28$, $p < 0.005$, $n = 107$, vitamin A: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.34$, $p < 0.001$, $n = 99$, vitamin E: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.21$, $p < 0.05$, $n = 105$). In the same models, only vitamin E was significantly related to latitude (carotenoids: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.05$, $p > 0.05$, vitamin A: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.08$, $p > 0.05$, vitamin E: partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.26$, $p < 0.01$).

YOLK ANTIOXIDANTS AND CAROTENOID-BASED SIGNALS

Across species, yolk carotenoid concentration was significantly positively related to the extent of carotenoid-based color: the model controlling for female body mass, clutch size and latitude explained 33% of total variance (Table D2). In addition, carotenoid concentration increased with sexual dichromatism in carotenoid-based signals: the model controlling for female body mass, clutch size and latitude explained 21% of the variance (Table D2). Vitamin A concentration was not significantly related to the extent of carotenoid-based color (Table D2) but also increased with sexual dichromatism, although less strongly than carotenoids (Table D2). In contrast, vitamin E concentration was not significantly related to carotenoid-based color of feathers and bare parts (Table D2), or to sexual dichromatism in these signals (Table D2). When similarity among species due to common descent was taken into account, models controlling for female body mass, clutch size and latitude confirmed the significant positive relation between yolk carotenoids and extent of carotenoid-based signals (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.38$, $p < 0.0005$, $n = 107$) and sexual dichromatism (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.19$, $p < 0.05$, $n = 107$). When incorporating effects of phylogeny, models controlling for female body mass, clutch size and latitude confirmed the absence of a significant relationship between vitamin A or vitamin E and extent of carotenoid-based color (vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.004$, $p > 0.05$, $n = 99$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.06$, $p > 0.05$, $n = 105$). Sexual dichromatism was confirmed to be positively related to concentration of vitamin A (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.21$, $p < 0.05$, $n = 99$) but not to concentration of vitamin E (partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.12$, $p > 0.05$, $n = 105$).

In a multiple regression of male carotenoid-based color score as a function of female body mass, clutch size, latitude and the three antioxidants, only carotenoid concentration explained a significant part of the variance (Table D3). The same result was found when taking similarity among species due to common descent into account (carotenoids, partial

correlation: $r_{\text{phyl}} = 0.42$, $p < 0.0005$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.03$, $p > 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = 0.04$, $p > 0.05$; at $\lambda = 0.923$, $n = 99$). In a multiple regression model based on species, sexual dichromatism in carotenoid-based color was positively related to concentration of yolk carotenoids and vitamin A but not to vitamin E (Table D3). These results were confirmed when the effects of similarity due to common phylogenetic descent were controlled (carotenoids, partial correlation: $r_{\text{phyl}} = 0.20$, $p < 0.02$; vitamin A, partial correlation: $r_{\text{phyl}} = 0.19$, $p < 0.05$; vitamin E, partial correlation: $r_{\text{phyl}} = 0.04$, $p > 0.05$; at $\lambda = 0.946$, $n = 99$).

Among species that are sexually dichromatic for carotenoid-based signals, sexual dichromatism was strongly positively related to male color scores ($F_{1,21} = 40.72$, $p < 0.0001$, $R^2 = 0.67$, slope estimate $\pm$ s.e. 0.80 ± 0.13). In order to determine whether the relationship between sexual dichromatism and yolk carotenoids previously found was actually reflecting the underlying relationship between male carotenoid-based color score and yolk carotenoids, we used the residuals from the phylogenetic regression as an index of the extent of sexual dichromatism independent of male color in the analyses based on species and controlling for similarity due to common phylogenetic descent, respectively. In analyses based on species and including female body mass, clutch size and latitude, residual sexual dichromatism did not explain significant variation in yolk carotenoids ($F_{1,17} = 2.85$, $p = 0.11$), vitamin A ($F_{1,16} = 0.27$, $p = 0.61$) or vitamin E ($F_{1,17} = 0.09$, $p = 0.77$). The same results were obtained after controlling for similarity due to common phylogenetic descent (carotenoids, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = 0.14$, $n = 22$; vitamin A, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.05$, $n = 21$; vitamin E, partial correlation at $\lambda = 1$: $r_{\text{phyl}} = -0.05$, $n = 22$; all $p > 0.05$). The relationship between sexual dichromatism and egg carotenoids was therefore not supported when controlling for the effect of carotenoid-based color. Indeed, in a multiple regression, none of the three antioxidant explained a significant part of the variation in residual sexual

dichromatism (carotenoids: $F_{1,14} = 3.41$, $p = 0.09$; vitamin A: $F_{1,14} = 0.48$, $p = 0.50$; vitamin E: $F_{1,14} = 2.26$, $p = 0.15$; model including female body mass, clutch size and latitude). This result was confirmed when phylogeny was taken into account (carotenoids, partial correlation: $r_{\text{phyl}} = 0.20$; vitamin A, partial correlation: $r_{\text{phyl}} = -0.09$; vitamin E, partial correlation: $r_{\text{phyl}} = -0.18$; all $p > 0.05$; at $\lambda = 0.746$, $n = 21$).

Table D1. Regression models with log-transformed yolk antioxidant concentrations as dependent variables and female body mass (log-transformed), clutch size and latitude as independent variable, based on species-specific data (see main text for results of phylogenetic analyses).

Variable	slope $\pm$ s.e.	F _{d.f.}	<i>p</i>
(a) Carotenoids			
Female body mass	-0.002 $\pm$ 0.04	0.28 _{1,106}	0.60
Clutch size	0.07 $\pm$ 0.03	3.61 _{1,106}	0.06
Latitude	0.005 $\pm$ 0.003	1.86 _{1,56}	0.07
(b) Vitamin A			
Female body mass	0.09 $\pm$ 0.02	17.87 _{1,98}	<0.0001
Clutch size	0.03 $\pm$ 0.01	3.19 _{1,98}	0.08
Latitude	0.003 $\pm$ 0.001	3.85 _{1,21}	0.05
(b) Vitamin E			
Female body mass	-0.01 $\pm$ 0.03	0.03 _{1,104}	0.87
Clutch size	-0.02 $\pm$ 0.03	0.50 _{1,104}	0.48
Latitude	0.007 $\pm$ 0.002	3.07 _{1,21}	0.003

Table D2. Regression models with log-transformed yolk antioxidant concentrations as dependent variables and log-transformed male carotenoid-based color scores or sexual dichromatism in carotenoid-based color as independent variables, controlling for female body mass, clutch size and latitude. Results are given for analyses performed on species-specific data (see main text for results of phylogenetic analyses).

Variable	Male color			Sexual dichromatism		
	slope (s.e.)	F _{d.f.}	<i>p</i>	slope (s.e.)	F _{d.f.}	<i>p</i>
Carotenoids	1.25 ± 0.20	37.32 _{1,105}	< 0.0001	1.42 ± 0.35	16.32 _{1,105}	0.0001
Vitamin A	0.13 ± 0.11	1.51 _{1,97}	0.22	0.41 ± 0.17	5.62 _{1,97}	0.02
Vitamin E	0.11 ± 0.19	0.31 _{1,103}	0.58	0.40 ± 0.30	1.72 _{1,103}	0.19

Table D3. Multiple regression model of male carotenoid-based color score and sexual dichromatism in carotenoid-based color scores as a function of yolk carotenoid, vitamin A and E concentrations, controlling for female body mass, clutch size and latitude (all variables were log-transformed except clutch size and latitude). Results are given for analyses performed on species-specific data (see main text for results of phylogenetic analyses).

Variable	Male color			Sexual dichromatism		
	slope (s.e.)	F _{d.f.}	<i>p</i>	slope (s.e.)	F _{d.f.}	<i>p</i>
Female body mass	-0.01 ± 0.02	0.57 _{1,95}	0.45	-0.02 ± 0.01	4.15 _{1,95}	0.04
Clutch size	-0.02 ± 0.01	1.26 _{1,95}	0.26	-0.01 ± 0.01	0.97 _{1,95}	0.33
Latitude	-0.002 ± 0.001	4.34 _{1,95}	0.04	-0.002 ± 0.001	6.17 _{1,95}	0.01
Carotenoids	0.22 ± 0.04	33.31 _{1,95}	< 0.0001	0.10 ± 0.02	15.60 _{1,95}	0.0002
Vitamin A	0.12 ± 0.08	2.19 _{1,95}	0.14	0.13 ± 0.05	5.90 _{1,95}	0.02
Vitamin E	-0.06 ± 0.05	1.33 _{1,95}	0.25	-0.01 ± 0.03	0.05 _{1,95}	0.83

Literature cited

- Bennett, P.M. and I.P.F. Owens. 2002. *Evolutionary ecology of birds*. Oxford University Press, Oxford, U.K.
- Birkhead, T. R., and A. P. Møller. 1994. The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution* 48:1089-1100.
- Brown, L. H., C. H. Fry, S. Keith, E. K. Urban, and K. Newman. 1982-2004. *The birds of Africa*. London, Academic Press.
- Cramp, S., K. E. L. Simmons, and C. M. Perrins. 1982-1994. *Handbook of the birds of Europe, the Middle East and North Africa*. Oxford, Oxford University Press.
- del Hoyo, J., A. Elliott, and J. Sagartal. 1992-2004. *Handbook of the birds of the world*. Barcelona, Lynx.
- Garamszegi, L. Z., C. Biard, M. Eens, A. P. Møller, and N. Saino. 2007a. Interspecific variation in egg testosterone levels: implications for the evolution of bird song. *Journal of Evolutionary Biology* 20:950-964.
- Garamszegi, L. Z., C. Biard, M. Eens, A. P. Møller, N. Saino, and P. F. Surai. 2007b. Maternal effects and the evolution of brain size in birds: overlooked developmental constraints. *Neuroscience and Biobehavioral Reviews* 31:498-515.
- Garamszegi, L. Z., M. Eens, J. Erritzøe, and A. P. Møller. 2005a. Sexually size dimorphic brains and song complexity in passerine birds. *Behavioral Ecology* 16:335-345.
- Garamszegi, L. Z., M. Eens, S. Hurtrez-Boussès, and A. P. Møller. 2005b. Testosterone, testes size, and mating success in birds: a comparative study. *Hormones and Behaviour* 47:389-409.
- Pagel, M. D. 1999. Inferring the historical pattern of biological evolution. *Nature* 401:877-884.

Poole, A., and F. Gill. 1992-2004. The birds of North America. Philadelphia, The birds of North America.

Sibley, C. G., and J. E. Ahlquist. 1990, Phylogeny and classification of birds. A study in molecular evolution. Yale University Press, New Haven, CT.