

Fecundity compromises attractiveness when pigments are scarce

Judith Morales,^{a,b} Alberto Velando,^b and Roxana Torres^a

^aLaboratorio de Conducta Animal, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, AP 70-275, México DF 04510, México and ^bDepartamento de Ecología e Biología Animal, Universidade de Vigo, 36310 Vigo, Spain

Theory predicts that the trade-off between ornamentation and fecundity limits female attractiveness. However, there is little evidence on this theoretical trade-off and its proximate background. Our aim was to study whether pigment availability modulates this potential relationship in blue-footed booby females. We supplemented females with dietary carotenoids after laying the first egg and assessed the change in foot color, a carotenoid-based sexually selected trait in both sexes. We measured the change in body mass and in the levels of plasma antioxidants and carotenoids. Also, we registered the mass and volume of eggs. Surprisingly, experimental females reduced zeaxanthin concentration in plasma, but not other carotenoids or total antioxidant levels. Conversely, they increased foot color intensity and laid heavier second eggs and larger second and third eggs than controls. Furthermore, under natural conditions (controls), ornamentation was negatively associated with the mass and volume of second eggs, but the association was reversed under conditions of high carotenoid availability (experimental females). Results suggest that carotenoid availability may mediate the theoretical trade-off between ornamentation and fecundity. We highlight that pigment limitation for females could represent an evolutionary pathway to male choosiness in the blue-footed booby. *Key words:* carotenoid pigments, fecundity, female ornamentation, life-history trade-offs, maternal effects, oxidative stress, sexual selection. [*Behav Ecol* 20:117–123 (2009)]

Because typically resources are limited, individuals have to optimize resource allocation among conflicting functions. Therefore, a major assumption of models in evolutionary biology is the occurrence of trade-offs between fitness-linked traits (Stearns 1992). For instance, life-history theory predicts that increased investment in reproduction may trade off against self-maintenance (Stearns 1992), whereas increased investment in sexual signaling may trade off against offspring quality (Kokko 1998). Understanding the proximate mechanisms and environmental circumstances that shape trade-offs may broaden our view about life-history evolution. However, these precise physiological mechanisms remain insufficiently known (for a review, Harshman and Zera 2007).

Antioxidants and, more specifically, carotenoids have become a target in the study of resource allocation trade-offs (Lozano 1994; Olson and Owens 1998; Hill 1999). Carotenoids are involved in immune (reviewed in Chew 1993) and antioxidant defenses (von Schantz et al. 1999), acting as free radical scavengers to protect DNA, proteins, and cells (Edge et al. 1997; Møller et al. 2000; Surai 2002; but see Costantini and Møller 2008). Furthermore, they are widely responsible for sexual coloration in fishes and birds (Andersson 1994). Vertebrates cannot synthesize them and have to acquire them from food (Surai 2002), which suggests that carotenoids could be scarce and may be a limiting resource in wild animals. That carotenoids can be limiting and important for functions related to reproduction and survival may have implications for the evolutionary theory of honest signaling, which posits that visual signals are costly to produce or maintain (Zahavi 1975). Indeed, experimental studies suggest that carotenoids allocated to sexual signaling can no longer be used for the immune and the detoxification systems (e.g., Alonso-Álvarez

et al. 2004; Martínez-Padilla et al. 2007; Pike et al. 2007; but see Navara and Hill 2003; Smith et al. 2007). The type of sexual ornament might determine how fast carotenoids are gathered and incorporated and the type of information conveyed (Lozano 1994). Dynamic traits based on carotenoids have the potential to indicate accurately current physical condition (Negro et al. 1998; Hill et al. 1999; Torres and Velando 2005). These traits allow flexible sexual signaling according to both carotenoid availability and to the need to signal in a specific moment of reproduction (Blount et al. 2003; Velando et al. 2006). Signaling would be needed most when, for instance, the bearer benefits from modifying the investment of its mate (Burley 1986) or from attracting extrapair mates. Therefore, species with facultative carotenoid-based ornaments provide a unique opportunity to examine life-history and context-dependent variation in the allocation of resources to conflicting functions (Badyaev and Duckworth 2003).

Most experiments have focused on males, and very little attention has been devoted to how females allocate carotenoids among different fitness-linked traits. This is surprising because several components of reproductive effort, including clutch size and egg quality (e.g., yolk carotenoid content), are under female control (Hill 2002). In birds, as in other oviparous animals, females must invest all the resources required for embryonic development in one self-contained package, and a key feature of a high-quality egg is yolk carotenoid content (Blount et al. 2000). Indeed, laying females fed with supplemental carotenoids laid eggs with reduced yolk susceptibility to lipid peroxidation *in vitro* (Surai and Speake 1998; Blount, Surai, Houston, and Møller 2002) and raised chicks with enhanced immunocompetence (Biard et al. 2005) and antioxidant capacity (Surai and Speake 1998). These results suggest that carotenoids might impose a limit on female fecundity (Blount et al. 2000). However, the picture becomes far more intricate when females express facultative carotenoid-based ornaments after pairing, so that the available carotenoids at laying must be allocated not only

Address correspondence to J. Morales. E-mail: jmorales@uvigo.es.

Received 5 May 2008; revised 21 August 2008; accepted 25 August 2008.

to the clutch and to self-maintenance processes but also to sexual signaling.

Theoretically, in females, “the fecundity costs of sexually selected traits will constrain both the potency of sexual selection mechanisms and the degree of elaboration of sexually selected traits” (Fitzpatrick et al. 1995). This has been explored and supported in a recent theoretical model (Chenoweth et al. 2006). The assumption that female attractiveness is limited by the trade-off between allocation to fecundity and to female ornamentation is worth studying, as it may shed light on the adaptive dynamics of female signaling, a hitherto rather unattended topic in evolutionary biology (Cunningham and Birkhead 1998; Amundsen 2000). The availability of resources at laying and the ability of females to obtain them may constrain female ornament expression. However, females potentially benefit from ornamentation at this stage if it increases their chance of obtaining resources held by males or attracting extrapair mates (Amundsen 2000). The evolution of high-cost female ornamentation is expected in theory when the ornament accurately indicates condition or fecundity (Hooper and Miller 2008). Yet, it remains controversial whether ornamentation reflects fecundity (e.g., Nordeide et al. 2006, 2008), and there is very little evidence on the proximate background of this potential relationship (Doutrelant et al. 2008; Hegyi et al. 2008; reviewed in Hooper and Miller 2008).

Previous evidence that carotenoid availability mediates the trade-off between female facultative ornament expression and fecundity is mixed. Studies in fishes have shown that the association between female ornament expression and egg quality or egg number can be either negative (Nordeide et al. 2006) or positive (Svensson et al. 2006). Carotenoid supplementation weakly affected ornamentation while egg carotenoid content was strongly and positively affected (Grether et al. 2008). In birds, correlative studies have either found a positive association between facultative carotenoid-based female ornamentation and some aspect of reproductive success (Massaro et al. 2003; Kristiansen et al. 2006) or failed to find such an association (Velando et al. 2001). The few experimental studies performed in birds have found a positive effect of carotenoid supplementation on female integument pigmentation and on egg quality or egg number in *Larus fuscus*, the lesser black-backed gull (Blount, Surai, Houston, and Møller 2002), and in *Taeniopygia guttata*, the zebra finch (Bertrand et al. 2006). Yet, only the former study has been developed in the wild. Undoubtedly, more studies are needed to clarify whether carotenoids constrain female decisions at laying.

The aim of the present study was to contrast the hypothesis that pigment availability modulates female attractiveness and fecundity as expressed by egg quality. We performed a carotenoid supplementation experiment in female blue-footed boobies in the wild, after laying their first egg. In this socially monogamous species, both adults invest heavily in parental care and show a highly dynamic sexual trait, colorful feet, and displayed ostentatiously before and after pairing (Nelson 1978; Guerra and Drummond 1995). Foot color varies from turquoise green to dull blue, and turquoise green is preferred in both sexes (Torres and Velando 2003, 2005). Color can vary rapidly in relation to changes in nutritional status, and its expression depends on the combined effect of carotenoids and structural collagen arrays (Velando et al. 2006). Males supplied with dietary carotenoids enhanced foot color and cell-mediated immunity (Velando et al. 2006). Furthermore, an induced immune activation led to reduced male ornamentation and enhanced oxidative damage (Torres and Velando 2007). These findings suggest that carotenoids may be a limiting factor for both, ornamentation and self-maintenance (Velando et al. 2006). Mean foot brightness decreases in most females after laying (Espinosa I, Torres R, unpublished data).

However, investing in ornamentation at this stage might be beneficial, as signaling may induce a higher incubation effort by the male or may attract extrapair mates. Our experimental design enabled us to investigate the potential relationship between egg production and female ornamentation pre- and postlaying and whether carotenoid supplementation may mitigate any decline in ornamentation due to egg production. We predicted that carotenoid supplementation positively affected female allocation to ornamentation and to egg quality. Furthermore, carotenoid supplementation may positively affect changes in female body mass and plasma antioxidants.

MATERIALS AND METHODS

Experimental procedure

We carried out the study in the breeding colony of the blue-footed booby *Sula nebouxii* at Isla Isabel, Nayarit, México, from January to March 2007. On the laying day of the first egg, 48 females were captured by night-lighting and randomly assigned to receive a single doses given by means of a syringe of either 1 mL of drinking water (control group, $n = 22$) or a solution of 0.4 mg of carotenoids in 1 mL of drinking water (experimental group, $n = 26$). The experimental group received 0.2 mg of Xamacol (16 g/kg of lutein and 1 g/kg of zeaxanthin) and 0.2 mg of Canthacol (100 g/kg of canthaxanthin), CaroTech, Carotenoid Technologies S.A., IQF Group, Tarragona, Spain. Supplementation with the mixture of these 3 carotenoids resembled natural conditions. Prior to the experiment, we knew that the diet of blue-footed boobies was mainly based on fishes like anchovies, mackerels, and sardines (Nelson 1978), which provide birds with lutein, zeaxanthin, and canthaxanthin among other carotenoids (Shahidi et al. 1998). Indeed, these were the most commonly identified carotenoids in the plasma of blue-footed booby females prior to manipulation. Known carotenoids represented almost 60% of all carotenoids; we also found another 4 unidentified carotenoids in most samples. Prior to manipulation, the sum of concentrations of unknown carotenoids in plasma was positively associated with lutein concentration (adjusted $r^2 = 0.23$, $P = 0.005$), marginally with canthaxanthin concentration ($P = 0.093$) and was not related with zeaxanthin levels ($P = 0.5$). Although the carotenoid mainly responsible for pigment coloration is zeaxanthin (Velando and Torres, unpublished data), the biosynthetic pathways of plasma carotenoids in birds are little studied and it cannot be ruled out that they transform into one another (McGraw 2006) or have synergistic effects. Therefore, supplementation with a mixture of carotenoids is appropriate. The proportion used is recommended by the provider, widely employed for poultry feeds and commercially available (www.carotech.biz/natural_pigments.htm). A higher dose has been applied previously to male blue-footed boobies without any apparent adverse effects on the birds (Velando et al. 2006). In the latter study, supplemented males showed turquoise green feet a few hours after treatment, and we expect a similar change in females. This experimental design allows controlling for female investment before treatment (first egg quality).

Before carotenoid supplementation, the color of females' left foot was measured with a portable spectrophotometer (MINOLTA CM-2600d, Minolta Co. Ltd, Osaka, Japan). Reflectance spectra for each foot were automatically produced as means of 3 sequential measures on the foot web. The spectrophotometer covers from 360 to 700 nm wavelength at 10-nm intervals. As the maximum visual sensitivity in this species occurs between 460 and 620 nm (Reed 1987), we analyzed the chroma of foot color in this range (hereafter green chroma), estimated as the sum of the reflectance between

460 and 620 nm divided by the sum of total reflectance between 360 and 700 nm. Females were weighed (accuracy 25 g), and their ulna length was measured (accuracy 1 mm). They were sampled for blood (100–500 μ L) from the brachial vein with heparinized capillary tubes and individually marked with a numbered metal leg band. All samples were kept in ice (between 1 and 4 h) until centrifugation (10 min at 10 000 rpm). Cellular and plasma components were separated, and plasma samples were preserved immediately on liquid nitrogen for later analyses (concentration of carotenoids, vitamin E, and total antioxidant capacity).

On average, 4 days after treatment (3.9 ± 0.2 days), most females were recaptured (17 controls and 19 experimentals). They were again sampled for blood, weighed, and their foot color was measured. The birds were captured and manipulated at night in all captures to reduce perturbation. Handling time per bird was less than 5 min. In order to estimate egg quality, eggs were not collected but weighed and measured. The experiment adhered to the ABS/ASAB ethical guidelines (*Animal Behaviour* 2006). Twelve females abandoned between first and second capture (25%, 4 controls, and 8 experimentals). During incubation, on average, roughly 30% of clutches are abandoned. Thus, the proportion of abandoned clutches in our experiment is within the natural range. Close to hatching of the first egg (40 ± 0.6 days between second and third capture), females were captured for a third time (17 controls and 18 experimentals), weighed, and sampled for blood, in order to test if plasma parameters were affected in the long term.

Nests were inspected daily until the second egg was laid. Females lay a mode of 2 eggs with an average interval of 4 days, and egg mass predicts hatching probability (D'Alba and Torres 2007). On the day they were laid, eggs were marked with permanent paint marker, weighed (accuracy 0.1 g), and their length and width were measured (accuracy 0.1 mm). Egg volume was estimated as $(0.51 \times \text{length} \times \text{width}^2)/1000$. Only 9 females laid a third egg (2 controls and 7 experimentals; treatments did not differ in the probability to lay a third egg: Pearson chi-square $\chi^2 = 2.29$, $P = 0.13$). Frequency of nest inspection decreased after second eggs were laid in order to avoid excessive disturbance. Thus, third eggs were measured but not weighed (egg mass is subject to rapid changes and must be measured on the day of laying, whereas egg volume can be measured at any time). We calculated the sum of volume of second and third eggs as an estimation of the total female investment after manipulation.

Measurement of plasma carotenoids, vitamin E, and total antioxidant capacity

Because zeaxanthin seems to be the main carotenoid responsible for feet pigmentation in the blue-footed booby (Velando and Torres, unpublished data), we have focused on this specific carotenoid in data analysis. Additionally, we calculated the sum of concentrations of other carotenoids (hereafter “other carotenoids”), which includes lutein, canthaxanthin, and other unidentified plasma carotenoids. We identified and quantified carotenoids and vitamin E following the protocol described by Pérez et al. (2008). In summary, the protocol consists of a high-performance liquid chromatography (JASCO Comparison Proven, Madrid, Spain, model 1500) fitted with a Security Guard column and a C18 reverse-phase analytical column (15 cm \times 4.6 mm \times 3 μ m) (SphereClone type ODS(2), Phenomenex, Torrance, CA) at room temperature. The mobile phase was methanol–milliQ water (90:10 v/v) in gradient elution (gradient: 0–21 min 90:10 v/v, 21–25 min 100:0 v/v, 25–35 min 90:10 v/v) and the flow rate, 1.5 mL/min. Carotenoids were determined at 445 nm with a UV detector (JASCO Comparison Proven, model UV-1570) and quantified by the use of

external standards (canthaxanthin, astaxanthin, and β -carotene, Dr Ehrenstorfer GmbH; Lutein, Sigma-Aldrich, Madrid, Spain; zeaxanthin, echinenone, and β -cryptoxanthin, LGC Promochem S.L., Barcelona, Spain). The concentration of unknown carotenoids was calculated in relation to lutein standard. Vitamin E (α -tocopherol) was simultaneously determined from the same extract with the same column, mobile phase, gradient, and flow rate but with a fluorescence detector (JASCO Comparison Proven, model FP-1520). The excitation and emission wavelengths used were 295 and 330 nm, respectively. Concentrations were calculated in relation to the vitamin E standard (α -tocopherol, Sigma-Aldrich; calibration curve, $r^2 = 0.99$). Extractions were performed with a dilution of 50 μ L of plasma in 250 μ L of absolute ethanol. Carotenoid and vitamin E concentrations are reported as micrograms per milliliters. Samples were injected 3 consecutive times, and the mean areas of carotenoids and vitamin of each sample were calculated from the last 2 peaks (repeatability $r > 0.98$ in all cases).

The total antioxidant capacity was measured using a colorimetric reaction method described by Erel (2004). Basically, it consists in the use of the molecule 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), which is decolorized by antioxidants according to their concentration and antioxidant capacity. The change in color after 5 min is measured as the change in absorbance at 655 nm. The assay is calibrated with a Trolox standard.

Statistical analyses

All statistical analyses (general linear models [GLM], with normal error distributions) were performed with Statistica 7.0 with an alpha level set at 0.05. In the GLMs, all main effects and 2-way interactions with treatment were included in the initial models. Final models were obtained using a backward elimination procedure. Differences in sample sizes reflect missing values due to, for instance, reduced blood volume to assess carotenoid concentration. Data are expressed as mean \pm standard error, unless otherwise stated. We report results for main effects and significant interactions.

Effect of treatment on female ornamentation, female body mass, and plasma parameters

To investigate the effect of treatment on the change in foot green chroma, we performed a GLM that included treatment as a factor and the initial foot green chroma, laying date, the initial body mass, and the initial zeaxanthin concentration as covariates. The initial zeaxanthin was included in this and other analyses because we were interested in the potential effect that the initial levels of this carotenoid, responsible for foot coloration, may exert on resource allocation to different functions by females. Initial body mass was included in all models because, at least for males, the nutritional status is related to foot color (Velando et al. 2006). In addition, we explored if treatment affected the relationship between the change in foot green chroma and the changes in body mass and in the plasma parameters studied from first to second capture. We performed 3 independent GLMs, as collinearity between the covariates should be avoided (Kiers and Smilde 2007): the change in female body mass (first model), the change in the concentration of zeaxanthin, other carotenoids, and total antioxidant capacity (second model), and the change in vitamin E (third model). In all models, we looked for the interaction between treatment and the variable analyzed.

To investigate the effect of treatment on the change in body mass and the change in the concentration of plasma parameters, we performed independent GLMs that included treatment as a factor and laying date, initial body mass, and initial zeaxanthin concentration as covariates. Ulna length was included as a covariate of the change in body mass.

Effect of treatment on egg mass and volume

Effect on the relationships between egg mass and volume and ornamentation. To explore if treatment affected the relationship between egg quality variables and the change in foot green chroma, we analyzed independently second egg mass, second egg volume, and clutch volume after manipulation (sum of volume of second and third eggs) using GLMs. The following variables were included as covariates: the mass or volume of first eggs in the corresponding GLM, green chroma change, initial zeaxanthin concentration, and initial body mass. The latter may affect egg quality as has been found for other colonial breeding seabirds (Wendelin 1997).

Effect on the relationships between egg mass and volume and changes in body mass and in plasma parameters. We explored if treatment affected the association between second egg mass, second egg volume, and clutch volume after manipulation (dependent variables) and the changes in body mass and in the plasma parameters studied from first to second capture (covariates). To avoid collinearity among the parameters studied, we performed 3 independent GLMs in the same way as explained above.

RESULTS

Prior to manipulation, the initial values of mass and volume of first eggs, female foot green chroma, body mass, ulna length, the concentration of plasma parameters, and laying date did not differ between experimental treatments (*t*-test, all $P > 0.10$).

Effect of treatment on female ornamentation

Treatment had a positive effect on female ornamentation. From first to second capture, foot green chroma increased in experimental females, whereas control females showed a decrease in foot green chroma (experimental females: 0.0023 ± 0.0031 ; controls: -0.0025 ± 0.0032 ; $F_{1,32} = 4.96$, $P = 0.033$; Figure 1). Also, females with higher initial green chroma showed a smaller green chroma change between captures (initial green chroma: $\beta = -0.61$, $F_{1,32} = 16.89$, $P < 0.001$). Laying date, initial body mass, and initial plasma zeaxanthin concentration did not have a significant effect on the change of green chroma (all $P > 0.28$). Second egg mass and volume did not affect female ornamentation (both $P > 0.40$); thus, controlling for the quality of second eggs led to similar results.

From first to second capture, the change in foot green chroma was not related to the change in body mass or the change in the concentration of plasma parameters ($P > 0.15$).

Effect of treatment on female body mass and plasma parameters

From first to second capture, the changes in body mass and in the concentration of plasma parameters were not affected by treatment (all $P > 0.14$). Surprisingly, from first to third capture (i.e., 40 days after treatment), carotenoid-supplemented females had reduced plasma zeaxanthin concentration compared with controls (experimental females: $-1.02 \pm 0.37 \mu\text{g}/\text{mL}$; controls: $0.33 \pm 0.66 \mu\text{g}/\text{mL}$; $F_{1,23} = 4.96$, $P = 0.036$; initial zeaxanthin concentration: $\beta = -0.64$, $F_{1,23} = 19.36$, $P < 0.001$). Yet, the changes in body mass and in the concentration of plasma parameters were not reduced from first to third capture (all $P > 0.22$), neither from second to third capture (all $P > 0.1$).

Effect of treatment on egg mass and volume

Effect on the relationship between egg mass and volume and ornamentation

Clutch volume after manipulation (sum of volume of second and third egg) of carotenoid-supplemented females was higher

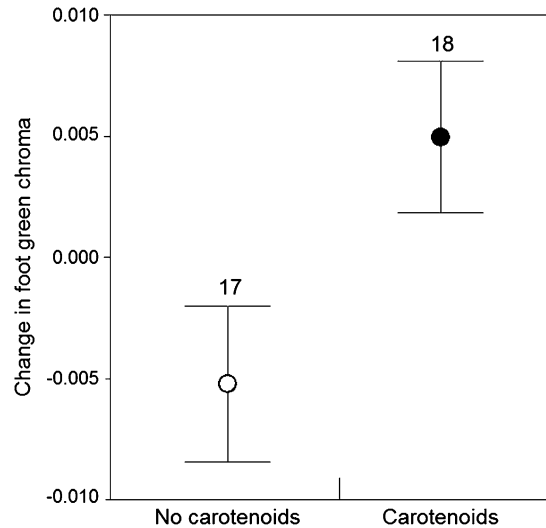


Figure 1

Effect of carotenoid supplementation on the change of female foot green chroma between the first and the second capture, controlled by the initial foot green chroma. Values are least square means \pm standard errors. Concise title: Effect of treatment on the change of female foot green chroma

than that of controls, controlling for the initial plasma zeaxanthin concentration (experimental females: $78.22 \pm 5.47 \text{ mm}^3$; controls: $61.11 \pm 4.93 \text{ mm}^3$; $F_{1,26} = 5.69$, $P = 0.025$; Figure 2A). Treatment did not affect clutch size ($P = 0.92$); hence, the effect on clutch volume after manipulation does not reflect variation in the number of eggs. Initial zeaxanthin concentration was positively associated with the clutch volume after manipulation ($\beta = 0.36$, $F_{1,26} = 4.76$, $P = 0.038$; Figure 2B). The changes in foot green chroma, laying date, body mass, and the volume of the first egg were not significant (all $P > 0.09$).

The volume of the second egg varied with treatment and the change in foot green chroma, so that control females that produced more voluminous second eggs decreased foot green chroma ($\beta = -0.28$); in contrast, carotenoid-supplemented females that laid more voluminous second eggs increased foot green chroma ($\beta = 0.21$) (interaction treatment \times green chroma change; Table 1). Also, females that laid more voluminous first eggs produced more voluminous second eggs (Table 1). Laying date, body mass, and the initial plasma zeaxanthin concentration were not significant (all $P > 0.4$).

Similarly, the mass of the second egg varied with treatment and the change in foot green chroma ($\beta = -0.26$ and 0.27 in control and carotenoid-supplemented females, respectively; interaction treatment \times green chroma change; Table 2, Figure 3). Moreover, carotenoid-supplemented females breeding late in the season laid heavier second eggs ($\beta = 0.51$) (interaction treatment \times laying date; Table 2). Also, females with higher initial plasma zeaxanthin concentration and females that laid heavier first eggs produced heavier second eggs (Table 2). Body mass was not significant ($P = 0.54$).

Effect on the relationships between egg mass and volume and changes in body mass and in plasma parameters

The changes in body mass and in the concentration of other carotenoids, vitamin E, and total antioxidant capacity from first to second capture had no effect on the clutch volume after manipulation (all $P > 0.3$). Interestingly, control, but not experimental, females that increased plasma zeaxanthin concentration from first to second capture reduced clutch volume after manipulation (treatment \times zeaxanthin change

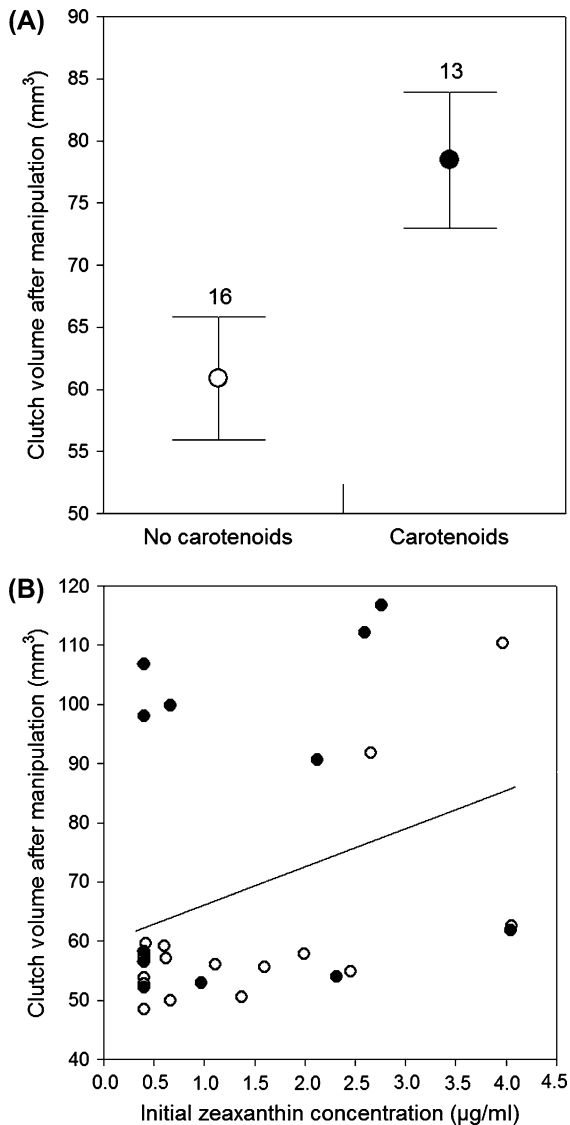


Figure 2
 (A) Effect of carotenoid supplementation on the clutch volume after manipulation, controlled by the initial zeaxanthin concentration. Values are least square means \pm standard errors of the sum of volume of second and third egg. Concise title: Effect of treatment on clutch volume after manipulation. (B) Association between the clutch volume after manipulation and the initial zeaxanthin concentration. Although treatment had no effect in this relationship, we have represented carotenoid-supplemented blue-footed booby females and controls by full and empty dots, respectively. Concise title: Association between the clutch volume after manipulation and the initial zeaxanthin concentration.

from first to second capture: $F_{1,22} = 4.51$, $P = 0.045$; all other P values > 0.1).

The mass of the second egg was not associated with the change in body mass or with the change in the plasma parameters measured from first to second capture (all $P > 0.15$).

DISCUSSION

Increased pigment availability at laying allowed blue-footed booby females to boost foot color within 3 days from the start of the experiment, confirming that the ornament is highly dynamic. Sexual signaling at laying may serve a function in pair

Table 1
Egg volume versus female foot color (green chroma)

	Beta	F	P
Treatment	0.06	0.26	0.615
First egg volume (mm ³)	0.75	45.54	<0.001
Green chroma change	0.10	0.48	0.494
Treatment \times green chroma change	-0.32	5.23	0.031

The analysis was performed using a GLM with the volume of the second egg as dependent variable and treatment, changes in foot green chroma, laying date, volume of the first egg, and the initial plasma zeaxanthin concentration as independent variables ($n = 29$ females). All main factors and 2-way interactions with treatment were included in the initial model, and the final model was obtained using a backward elimination procedure.

bonding or in promoting higher parental investment by the male (Roulin 1999). Interestingly, in the blue-footed booby, half of the females copulate with extrapair males during their fertile period, that is, 1–5 days before laying each egg (Osorio-Beristain and Drummond 1998). Hence, besides increasing parental effort, investing in sexual signaling at this stage could be a female strategy to attract extrapair males (Torres and Velando 2005).

Pigment supply enhanced the mass of second eggs and the clutch volume after manipulation. The fact that treatment affected the mass of second eggs only in late experimental females may indicate that late breeders suffered harder conditions that constrained egg quality (Torres and Drummond 1997; D’Alba and Torres 2007). Previous studies in birds have shown that carotenoid-supplied females transfer more carotenoids to the yolk than controls (e.g., Surai and Speake 1998; Blount, Surai, Nager, et al. 2002; Biard et al. 2005) and that this has important consequences for offspring performance (Surai and Speake 1998; Biard et al. 2005). In our study, we are unable to conclude that experimental females transferred more zeaxanthin to the yolk, as we did not collect the eggs. However, results suggest that plasma zeaxanthin was allocated to egg production because control females that laid more voluminous eggs experienced reduced levels of zeaxanthin compared with experimental females. Besides, independently of treatment, the initial zeaxanthin concentration positively affected the mass of the second egg.

Table 2
Egg mass versus female foot color (green chroma)

	Beta	F	P
Treatment	2.32	7.27	0.014
Laying date	0.32	4.31	0.050
First egg mass (g)	0.57	22.35	<0.001
Green chroma change	0.05	0.10	0.758
Initial zeaxanthin concentration (µg/mL)	0.28	4.73	0.041
Treatment \times laying date	-2.43	7.59	0.012
Treatment \times green chroma change	-0.34	4.46	0.047

The analysis was performed using a GLM with the mass of the second egg as dependent variable and treatment, changes in foot green chroma, laying date, mass of the first egg, and the initial plasma zeaxanthin concentration as independent variables ($n = 29$ females). All main factors and 2-way interactions with treatment were included in the initial model, and the final model was obtained using a backward elimination procedure.

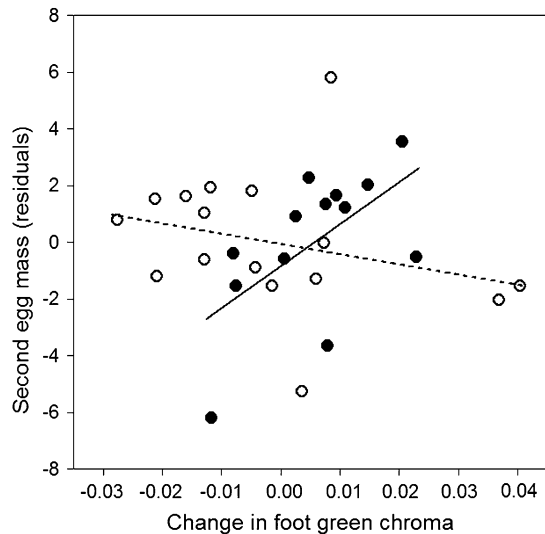


Figure 3

Effect of the interaction of treatment by change in foot green chroma from first to second capture on the mass of the second egg. Values of second egg mass are the residuals of the model performed without the interaction term. Full dots and solid line are carotenoid-supplemented blue-footed booby females and empty dots and dotted line are controls. Concise title: Interaction of treatment by change in foot green chroma on the second egg mass.

Unexpectedly, carotenoid-supplemented females reduced their levels of plasma zeaxanthin from first to third capture. Experimental females could have suffered increased susceptibility to oxidative stress due to a higher investment in reproduction and, therefore, demanded more plasma antioxidants than controls. However, this is unlikely, as we did not detect any reduction in total antioxidant capacity or vitamin E concentration; the latter a much more potent free radical scavenger than zeaxanthin (Hartley and Kennedy 2004). Our results best fit with the hypothesis that experimental females continued allocating plasma zeaxanthin to maintain ornamentation after second capture or to the formation of third eggs. Carotenoid-supplemented females might afford diverting zeaxanthin away from the detoxification system and instead allocate them to sexual signaling (von Schantz et al. 1999) or to egg formation. Measuring female foot green chroma at third capture and carotenoid content of eggs might have allowed us to test this idea.

Even though the existence of a trade-off between female ornamentation and egg mass and volume cannot be definitely concluded in this study, results provide, to our knowledge, the first experimental evidence that pigment availability may affect the association between these traits. Under natural conditions (control females), the association was negative, suggestive of a trade-off. Nonetheless, under conditions of abundant resources (experimental females), the association was reversed. The fact that there were supplemented birds showing low ornamentation and light second eggs could be due to initial differences in quality among experimental females that affected their ability to use them or their allocation decision. Oxidative stress could be the source of the conflict between attractiveness and fecundity (von Schantz et al. 1999) and has been proposed as a mechanism underlying life-history trade-offs (Alonso-Álvarez et al. 2006 and references therein). Conversely, carotenoid availability did not affect the potential associations between female antioxidant status and egg quality or between female antioxidant status and ornament expression. The results suggest that there is an invariable investment

in antioxidant-dependent processes to ensure survival during reproduction, and the conflict about how to allocate carotenoids might arise mainly among reproductive traits.

Theoretically, the fecundity costs of sexually selected traits in females may constrain the degree of elaboration of ornamental traits and the intensity of sexual selection (Fitzpatrick et al. 1995; Chenoweth et al. 2006). Our results seem to support the previous idea and stress the potential key role of pigment availability in this theoretical trade-off, probably fundamental in species with biparental investment and high frequency of extrapair copulations. Pigment limitation may thus represent an evolutionary pathway to male choosiness and, potentially, to the evolution of sexual dimorphism (Bonduriansky 2001). Our findings may contribute to generate predictions about male preferences on female attractiveness and have implications for the theory on the evolution of female ornamentation. Male preferences may be stabilizing, but when females differ markedly in their access to limiting resources, sexual selection on females could be directional (Chenoweth et al. 2006). Male mate choice may exert a stronger sexual selective pressure on female reproductive decisions than is generally acknowledged.

FUNDING

Universidad Nacional Autónoma de México (PAPIIT IN211406); Consejo Nacional de Ciencia y Tecnología (47599) of México; Ministerio de Educación y Ciencia (MEC; CGL2006-10347-C02-01/BOS) of Spain; Postdoctoral and Ramón y Cajal fellowships from MEC to J.M. and A.V. Research grant from the Association for the Study of Animal Behaviour.

We are grateful to Juan Moreno and 2 anonymous reviewers for comments on the manuscript; to René Beamonte-Barrientos, Fabrice Dentressangle, Emma Villaseñor, and Natalia Lifshitz for help in the field; and to Cristóbal Pérez for help in the laboratory. We thank the Armada de México, the staff from Parque Nacional Isla Isabel, and the fisherman from San Blas and Camichín for logistic support. During the experiment, we adhered to our institutional guidelines for animal husbandry and experiments. SEMARNAT gave working permissions and approved the research.

REFERENCES

- Alonso-Álvarez C, Bertrand S, Devevey G, Gaillard M, Prost J, Faivre B, Sorci G. 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am Nat.* 164:651–659.
- Alonso-Álvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Chastel O, Sorci G. 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution.* 60:1913–1924.
- Amundsen T. 2000. Why are female birds ornamented? *Trends Ecol Evol.* 15:149–155.
- Andersson M. 1994. *Sexual selection*. Princeton: Princeton University Press.
- Badyaev AV, Duckworth RA. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. *J Evol Biol.* 16:1065–1076.
- Bertrand S, Alonso-Álvarez C, Devevey G, Faivre B, Prost J, Sorci G. 2006. Carotenoids modulate the trade-off between egg production and resistance to oxidative stress. *Oecologia.* 147:576–584.
- Biard C, Surai PF, Møller AP. 2005. Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia.* 144:32–44.
- Blount JD, Metcalfe NB, Birkhead TR, Surai PF. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science.* 300:125–127.
- Blount JD, Møller AP, Houston DC. 2000. Why egg yolk is yellow. *Trends Ecol Evol.* 15:47–49.
- Blount JD, Surai PF, Houston DC, Møller AP. 2002a. Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. *Funct Ecol.* 16:445–453.

- Blount JD, Surai PF, Nager RG, Houston DC, Møller AP, Trewby ML. 2002b. Carotenoids and egg quality in the lesser blackbacked gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proc R Soc Lond B Biol Sci.* 269:29–36.
- Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev.* 76:305–339.
- Burley N. 1986. Sexual selection for aesthetic traits in species with biparental care. *Am Nat.* 127:415–445.
- Chenoweth SF, Doughty P, Kokko H. 2006. Can non-directional male mating preferences facilitate honest female ornamentation? *Ecol Lett.* 9:179–184.
- Chew BP. 1993. Role of carotenoids in the immune response. *J Dairy Sci.* 76:2804–2811.
- Costantini D, Møller AP. 2008. Carotenoids are minor antioxidants for birds. *Funct Ecol.* doi: 10.1111/j.1365-2435.2007.01366.x.
- Cunningham EJA, Birkhead TR. 1998. Sex roles and sexual selection. *Anim Behav.* 56:1311–1321.
- D'Alba L, Torres R. 2007. Seasonal egg-mass variation and laying sequence in a bird with facultative brood reduction. *Auk.* 124:643–652.
- Doutrelant C, Grégoire A, Grnac N, Gómez D, Lambrechts MM, Perret P. 2008. Female coloration indicates female reproductive capacity in blue tits. *J Evol Biol.* 21:226–233.
- Edge R, McGarvey DJ, Truscott TG. 1997. The carotenoids as antioxidants—a review. *J Photochem Photobiol B.* 41:189–200.
- Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem.* 37:277–285.
- Fitzpatrick S, Berglund A, Rosenqvist G. 1995. Ornaments or offspring: costs to reproductive success restrict sexual selection processes. *Biol J Linn Soc.* 55:251–260.
- Grether GF, Kolluru GR, Lin K, Quiroz MA, Robertson G, Snyder AJ. 2008. Maternal effects of carotenoid consumption in guppies (*Poecilia reticulata*). *Funct Ecol.* 22:294–302.
- Guerra M, Drummond H. 1995. Reversed sexual size dimorphism and parental care: minimal division of labor in the blue-footed booby. *Behaviour.* 132:479–496.
- Harshman LG, Zera AJ. 2007. The cost of reproduction: the devil in details. *Trends Ecol Evol.* 22:80–86.
- Hartley RC, Kennedy MW. 2004. Are carotenoids a red herring in sexual display? *Trends Ecol Evol.* 19:353–354.
- Hegyi G, Rosivall B, Szöllösi E, Hargitai R, Eens M, Török J. 2008. Phenotypic plasticity in a conspicuous female plumage trait: information content and mating patterns. *Anim Behav.* 75:977–989.
- Hill GE. 1999. Is there an immunological cost to carotenoid-based ornamental coloration? *Am Nat.* 154:589–595.
- Hill GE. 2002. A red bird in a brown bag. Oxford: Oxford University Press.
- Hill JA, Enstrom DA, Ketterson ED, Nolan V, Ziegenfuss G. 1999. Mate choice based on static versus dynamic secondary sexual traits in the dark-eyed junco. *Behav Ecol.* 10:1–96.
- Hooper PL, Miller GF. 2008. Mutual mate choice can drive costly signaling even under perfect monogamy. *Adapt Behav.* 16:53–70.
- Kiers HAL, Smilde AK. 2007. A comparison of various methods for multivariate regression with highly collinear variables. *Stat Methods and Appl.* 16:193–228.
- Kokko H. 1998. Should advertising parental care be honest? *Proc R Soc Lond B Biol Sci.* 265:1871–1878.
- Kristiansen KO, Bustnes JO, Folstad I, Helberg M. 2006. Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. *J Avian Biol.* 37:6–12.
- Lozano GA. 1994. Carotenoids, parasites, and sexual selection. *Oikos.* 70:309–311.
- Martínez-Padilla J, Mougeot F, Pérez-Rodríguez L. 2007. Nematode parasites reduce carotenoid-based signalling in male red grouse. *Biol Lett.* 3:161–164.
- Massaro M, Davis LS, Darby JT. 2003. Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*). *Behav Ecol Sociobiol.* 55:169–175.
- McGraw KJ. 2006. Mechanics of carotenoid-based colouration. In: Hill EG, McGraw KJ, editors. *Bird colouration I: Mechanisms and measurements*. Cambridge (MA): Harvard University Press. p. 177–242.
- Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult Biol Rev.* 11:137–159.
- Navara KJ, Hill GE. 2003. Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behav Ecol.* 14:909–916.
- Negro JJ, Bortolotti GR, Tella JL, Fernier KL, Bird DM. 1998. Regulation of integumentary colour and plasma carotenoids in American kestrels consistent with sexual selection theory. *Funct Ecol.* 12:307–312.
- Nelson B. 1978. The Sulidae: Gannets and Boobies. Oxford: Oxford University Press.
- Nordeide JT, Mohus Å, Nicolaisen O, Volden R, Egeland ES. 2008. Offspring or ornaments? Is carotenoid-based ornamentation in female Arctic charr, *Salvelinus alpinus* (L.), condition-dependent and traded off against offspring? *Ecol Freshwat Fish.* 17:328–339.
- Nordeide JT, Rudolfson G, Egeland ES. 2006. Ornaments or offspring? Female sticklebacks (*Gasterosteus aculeatus* L.) trade off carotenoids between spines and eggs. *J Evol Biol.* 19:431–439.
- Olson VA, Owens IPF. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol Evol.* 13:510–514.
- Osorio-Berstein M, Drummond H. 1998. Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behav Ecol Sociobiol.* 43:307–315.
- Pérez C, Lores M, Velando A. 2008. The availability of nonpigmentary antioxidant affects red coloration in gulls. *Behav Ecol.* 19:967–973.
- Pike TW, Blount JD, Linström J, Metcalfe V. 2007. Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornament. *Biol Lett.* 3:353–356.
- Reed JR. 1987. Scotopic and photopic spectral sensitivities of boobies. *Ethology.* 76:33–55.
- Roulin A. 1999. Nonrandom pairing by male barn owls *Tyto alba* with respect to a female plumage trait. *Behav Ecol.* 10:688–695.
- Shahidi F, Metusalach A, Brown JA. 1998. Carotenoid pigments in seafoods and aquaculture. *Crit Rev Food Sci.* 38:1–67.
- Smith HG, Råberg L, Ohlsson T, Granbom M, Hasselquist D. 2007. Carotenoid and protein supplementation have differential effects on pheasant ornamentation and immunity. *J Evol Biol.* 20:310–319.
- Stearns SC. 1992. The evolution of life histories. Oxford: Oxford University Press.
- Surai PF. 2002. Natural antioxidants in avian nutrition and reproduction. Nottingham (UK): Nottingham University Press.
- Surai PF, Speake BK. 1998. Distribution of carotenoids from the yolk to the tissues of the chick embryo. *J Nutr Biochem.* 9:645–651.
- Svensson PA, Pélabon C, Blount JD, Surai PF, Amundsen T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens*, Gobiidae)? *Funct Ecol.* 20:689–698.
- Torres R, Drummond H. 1997. Female-biased mortality in nestlings of a bird with size dimorphism. *J Anim Ecol.* 66:859–865.
- Torres R, Velando A. 2003. A dynamic trait affects continuous pair assessment in the blue-footed booby (*Sula nebouxi*). *Behav Ecol Sociobiol.* 55:65–72.
- Torres R, Velando A. 2005. Male preference for female foot colour in the socially monogamous blue-footed booby, *Sula nebouxi*. *Anim Behav.* 69:59–65.
- Torres R, Velando A. 2007. Male reproductive senescence: the price of immune induced oxidative damage on sexual attractiveness in the blue-footed booby. *J Anim Ecol.* 76:1161–1168.
- Velando A, Beamonte-Barrientos R, Torres R. 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia.* 149:535–542.
- Velando A, Lessells CM, Márquez JC. 2001. The function of female and male ornaments in the Inca tern: evidence for links between ornaments expression and both adult condition and reproductive performance. *J Avian Biol.* 32:311–318.
- von Schantz TV, Bensch S, Grahn M, Hasselquist D, Wittzell H. 1999. Good genes oxidative stress and condition-dependent sexual signals. *Proc R Soc Lond B Biol Sci.* 266:1–12.
- Wendelin H. 1997. Body mass of female common terns (*Sterna hirundo*) during courtship: relationships to male quality, egg mass, diet, laying date and age. *Colon Waterbirds.* 20:235–243.
- Zahavi A. 1975. Mate selection—selection for a handicap. *J Theor Biol.* 53:205–214.