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Availability of nonpigmentary antioxidant affects red coloration in gulls

Cristóbal Pérez,[a] Marta Lores,[b] and Alberto Velando[a]
[a]Departamento de Ecoloxía e Bioloxía Animal, Facultade de Bioloxía, Universidade de Vigo, As Lagoas-Marconsende s/n 36310 Vigo, Spain and [b]Departamento de Química Analítica, Nutrición e Bromatoloxía, Facultade de Química, Instituto de Investigacións e Análises Alimentarios, Universidade de Santiago de Compostela, Avenida, das Ciencias s/n 15782 Santiago de Compostela, Spain

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Red, orange, and yellow carotenoid-based colorations displayed by fishes and birds may function as honest sexual signals of the bearer's quality. However, the mechanisms underlying the expression of these traits and the information they convey are still controversial. Because carotenoids are antioxidants and carotenoid-based pigmentation is bleached as a consequence of oxidative processes, it has been suggested that the pigmentation may signal antioxidant status. We tested this hypothesis in the yellow-legged gull (*Larus michahellis*), a seabird that exhibits a carotenoid-based red spot on the lower mandible. The availability of a nonpigmentary antioxidant (i.e., vitamin E) to the gulls was modified before egg laying by means of a supplementary feeding experiment. During the incubation period, breeding pairs were captured to assess the intensity of the color and the size of the red bill spots. We measured the plasma level of lipid peroxidation, total antioxidant capacity, and carotenoids. We found that males that received vitamin E supplements had larger red spot than control birds but that color intensity was not affected by the supplements. Moreover, we found that only those plasma carotenoids involved in the red coloration were affected by the antioxidant supplementation, suggesting an active mechanism to increase red coloration. Overall, our results provide experimental evidence for the hypothesis that carotenoid-based coloration reflects the bearer's antioxidant status in male gulls.
Key words: carotenoids, oxidative stress, sexual selection, trade-off, vitamin E. *[Behav Ecol 19:967–973 (2008)]*

Many animals exhibit elaborate ornamental traits that have evolved as signals of the bearer's quality and can be evaluated by prospective mates or opponents (Andersson 1994). One of the major goals of animal communication studies has been to identify the information content of these signals and the entailed costs that may prevent cheating (Zahavi 1975; Grafen 1990; Espmark et al. 2000). However, the currency of these costs is still under debate and may depend on the nature of the signal involved.

The red, orange, and yellow carotenoid-based colorations displayed by fishes and birds can be considered as good examples of honest sexual signals (e.g., Olson and Owens 1998; Badyaev and Hill 2000; Pike et al. 2007). However, the information conveyed by these traits and the mechanisms underlying their expression are still controversial and have been subject of intensive research in recent decades. Vertebrates can transform carotenoids through different metabolic routes (Brush 1990; Møller et al. 2000) but cannot synthesize them de novo and, therefore, must acquire them from food. Several experimental studies involving carotenoid supplementation have shown that an increase in dietary carotenoids leads to enhanced carotenoid-based ornament expression (e.g., McGraw and Ardia 2003; Bertrand et al. 2006). The honesty of carotenoid-based signals may also be reinforced by the important physiological functions of carotenoids (Lozano 1994; von Schantz et al. 1999, Møller et al. 2000). One of the functions attributed to carotenoids is their antioxidant activity (Krinsky 2001; Young and Lowe 2001; Rao AV and Rao LG 2007). Accordingly, it has been suggested that oxidative stress is the proximate cause of the genuine information revealed to prospective females through male carotenoid-dependent traits (von Schantz et al. 1999).

Organisms produce reactive oxygen species (ROS) as by-products of physiological functions, which provoke oxidative damage to DNA, proteins, and lipids (Finkel and Holbrook 2000). To mitigate oxidative injury, organisms use endogenous enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, as well as extracellular antioxidants such as uric acid, vitamin E, vitamin C, and carotenoids (Godin and Garnett 1992; Fang et al. 2002; Rao AV and Rao LG 2007). In this context, it has been suggested that there is a trade-off between allocation of carotenoids to the sexual signal or to functions of antioxidant defense ("antioxidant trade-off" hypothesis; von Schantz et al. 1999). Thus, oxidative stress may also underlie the relationship between carotenoid-dependent expression and current immunological status (Faivre et al. 2003; Alonso-Alvarez et al. 2004; Velando et al. 2006). The activation of the immune system produces ROS that must be counteracted by the mobilization of bodily antioxidants, including carotenoids, to balance oxidative stress at the expense of the expression of sexual coloration (Blount et al. 2003; Faivre et al. 2003; Alonso-Alvarez et al. 2004; Grether et al. 2004).

Although commonly assumed, the importance of carotenoids in the trade-off between coloration and free radical scavenging remains controversial (Hõrak et al. 2006; Costantini et al. 2007; Isaksson et al. 2007). Thus, it has been suggested that antioxidant activity is not the main biological role of carotenoids (Hartley and Kennedy 2004) but that as they are bleached as a consequence of oxidative processes (Woodall et al. 1997) they may reflect the healthy functioning of systems that prevent their oxidation ("protection" hypotheses; Hartley and Kennedy 2004).

The common prediction implicit in both hypotheses (the antioxidant trade-off and the protection hypotheses) is that increasing the availability of other (nonpigmentary) antioxidants

Address correspondence to C. Pérez. E-mail: cristobal@uvigo.es.
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should favor the expression of carotenoid-based signals (Bertrand et al. 2006; Pike et al. 2007). Thus, individuals with greater access to other antioxidants may use carotenoids for coloration rather than for antioxidant defense purposes (von Schantz et al. 1999; Blount et al. 2000) or, alternatively, high levels of antioxidant defenses may diminish the oxidation of carotenoids (Hartley and Kennedy 2004).

In this study, we tested the prediction that antioxidant availability modulates carotenoid-based coloration in a wild population of the yellow-legged gull (*Larus michahellis*), a seabird in which both sexes show intense integumentary carotenoid-based coloration in legs, eye rings, gape flanges, gape, and bill spots (Cramp and Simmons 1983). In related gull species, red coloration reflects body condition (Kristiansen et al. 2006) and is related to carotenoid intake (Blount et al. 2002). We focused on the red spot area on the lower mandible because expression of this trait is very variable throughout the reproductive period and is enhanced during courtship (Cramp and Simmons 1983). Moreover, in a recent experimental study, we found that the red bill spot is used by individuals after pairing to modify the reproductive decisions of their mate (Morales J, Pérez C, Alonso C, Torres R, Serafino E, Velando A, unpublished data). During courtship period in established pairs, we modified the availability of nonpigmentary antioxidant by means of a supplemental vitamin E feeding study. We predicted that carotenoid supply may enhance the expression of the red bill spot. We also measured the effect of treatment on the levels of lipid peroxidation, total antioxidant capacity, and total carotenoids in plasma. Moreover, we differentiated the carotenoids present exclusively in the red bill spot from all other carotenoids. We expected increases in all plasma carotenoids and such increases related to their oxidation susceptibility (Woodall et al. 1997) if carotenoids are passively protected (Hartley and Kennedy 2004). In contrast, we expected an effect especially on red spot carotenoids if the antioxidant availability promotes an active allocation to the signal.

MATERIALS AND METHODS

Experimental procedure

This study was carried out in 2005 in the Illas Cíes (Ría de Vigo, Galicia, northwest of Iberian Peninsula). In mid-April, during the courtship period of the yellow-legged gull (*L. michahellis*), 40 nest sites in the central part of the colony were randomly allocated either to a feeding treatment ($n = 20$) or to a control group ($n = 20$). Food supplementation was begun 10.17 ± 4.62 days (range 3–19 days) before egg laying. The experimental pairs were fed daily with 198 mg of vitamin E (α-tocopherol acetate, Sigma-Aldrich, Madrid, Spain); an individual dose of 112 mg/kg of body weight, similar to dosages used in poultry (i.e., Grobas et al. 2002; Sahin et al. 2002). The daily amount of vitamin E supplied was within the estimated natural range of intake (2–757 mg of vitamin E per day), estimation based on daily food consumption of gulls (280.6 g of food; Munilla 1997; Hunt et al. 2005) and the amount of vitamin E in the main prey (0.2 mg/g in marine invertebrates and 2.7 mg/g in marine fish; Sikorski 1990). Indeed, the increase of Vitamin E in experimental gulls (see Results) was within the natural range reported in seabirds (i.e., Murvoll et al. 2007). Vitamin E was mixed with vegetable oil and placed on a slice of bread, which was hidden under vegetation and close to the nest (ca., 50 cm). This was done to prevent nontarget birds eating the bread. In a previous feeding experiment performed in the same way, the behavior of gulls around the territory was observed, and it was reported that no food item was stolen by other birds (Pérez et al. 2006). The control group was provided with the bread and vegetable oil, but without vitamin E. The period of supplementation prior to laying did not differ significantly between treatment groups ($t_{16} = 0.13$, $P = 0.89$). Supplemental feeding was halted 5 days after the first egg was laid.

Between 5 and 26 days after egg laying was completed (i.e., the third egg was laid), 10 control and 8 supplemented males were trapped. Head, bill, and tarsus length were measured (to the nearest 1 mm). Body mass was also determined (to the nearest 10 g). The tarsus length allowed confirmation of the sex of the birds by means of a discriminant function (Bosch 1996). The bill was photographed against a white standard, together with a standard red color and a millimetric scale, inside a black box, with a digital camera (Nikon Coolpix 5200). The distance from the lens to the bill (15 cm) was held constant. The red spot area was measured by the same person (C.P.) by use of image analysis software (analySIS FIVE) blindly with respect to treatment. Repeatability of the method performed 3 times on 6 randomly selected photographs was very high ($r = 0.98$, $F_{5,12} = 161.01$, $P < 0.001$). The intensity of color (redness) of the red spot was measured using 3 pixel values in RGB color space on the central part of the spot. A single redness intensity value was calculated according to Pike et al. (2007). Redness is expressed as the proportion of the *R* value to the red standard color.

A blood sample (about 1.5 mL) was taken from the brachial vein, with a 25-G heparinized needle. The blood was immediately transferred to plastic tubes and maintained on ice in cool boxes (4 °C) and then centrifuged in the laboratory at the end of the day. Plasma and blood cells (pellet) were frozen separately at −80 °C until analysis. Eggs were measured (to the nearest 0.01 mm) to calculate egg volume (volume = length × width^2 × 0.52; Hoyt 1979).

Biochemical assays

The carotenoids responsible for the red spot and orange bill pigmentation were identified by analysis of the bills of 3 gulls that were found dead, by high-performance liquid chromatography (HPLC). The red- and orange-pigmented layers were separated from the bill keratin with a scalpel and cut into small pieces, avoiding exposure of the samples to direct light and high temperatures. The tissue was placed in a tube and covered with absolute ethanol. The solution was mixed on a vortex mixer, sonicated for 15 min in an ultrasonic bath (BRANSON, model 5510) and subsequently centrifuged at 10 000 rpm for 10 min. The supernatant was collected in a new tube, dried under a nitrogen atmosphere, and diluted again in 200 μL of methanol. The carotenoids and vitamin E contained in the plasma samples were also measured by HPLC. Plasma samples (50 μL) were diluted in 250 μL of absolute ethanol in tubes, avoiding exposure of the plasma to high temperatures and direct light (Alonso-Alvarez et al. 2004). The solution was processed in the same way as described above.

Samples (20 μL) were injected into a HPLC system (JASCO Comparison Proven, model 1500) fitted with a SecurityGuard column and a C18 reverse phase analytical column (15 cm × 4.6 mm × 3 μm) (SphereClone type ODS(2), Phenomenex, Torrance, CA). 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, and 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 use of external standards (canthaxanthin, astaxanthin, and β-carotene; Dr Ehrenstorfer GmbH; Lutein, Sigma-Aldrich; zeaxanthin, echinenone, and β-cryptoxanthin; LGC Promochem SL, Barcelona, Spain). The calibration curves for the carotenoids present in the samples

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showed high correlation coefficients (in all cases $R^2 > 0.99$). The concentration of the unknown carotenoids was calculated in relation to the 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, $R^2 = 0.99$). Concentrations of carotenoids and vitamin E are expressed in micrograms per milliliter.

Lipid peroxidation was quantified with a lipid peroxidation assay kit (Calbiochem, catalog no. 437634. VWR International Eurolab, S.L. Barcelona, Spain). This method measures malondialdehyde and 4-hydroxyalkenals, which are end products derived from peroxidation of polyunsaturated fatty acids and related esters.

The total antioxidant capacity was measured by the method described by Erel (2004). Basically, it consists in the use of the molecule 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS*+), which is decolorized by antioxidants according to their concentration and antioxidant capacity. The change in color is measured as the change in absorbance at 415 nm (Microplate reader 550. Bio-Rad Laboratories, S.A. Barcelona, Spain). The antioxidant capacity is expressed as millimoles of Trolox equivalent per liter

Data analyses

Student's t-tests were used for comparison between groups when data met the assumptions of homoscedasticity and normality; otherwise, Mann–Whitney U test was used. The effects of the vitamin E supplementation on red spot size, spot redness, and plasma concentrations of vitamin E, carotenoids, lipid peroxidation products and total antioxidant capacity were analyzed using general linear models. In the models, the experimental treatment was included as factor and laying date, number of days elapsed from laying to capture, tarsus length, and bill size (in the red bill spot model) as covariates. The full models are reported, as recommended by Whittingham et al. (2006). When it was necessary (plasma carotenoids), data were transformed with Box–Cox transformation in order to meet model requirements (normality and homoscedasticity variance structure). Data are expressed as means ± standard errors.

RESULTS

Carotenoids present in bill and plasma

The HPLC analysis revealed that 10 different carotenoids were present in the orange and red bill coloration (Table 1) and that 5 of these were exclusively present in the red bill spot (hereafter called "red spot carotenoids"; Table 1). Red spot carotenoids were present in plasma of male gulls, with lutein predominating (Table 1). Another 2 carotenoids were also found in plasma (Table 1, hereafter referred to together as "other carotenoids").

Vitamin E supplementation experiment

The body size did not differ between experimental groups (tarsus length, $t_{16} = 1.42$, $P = 0.18$; bill size, $t_{16} = 0.02$, $P = 0.98$), nor the time elapsed from laying to capture ($Z = 0.45$, $P = 0.65$). Moreover, vitamin E supplementation did not affect body mass ($t_{16} = 0.65$, $P = 0.53$), clutch size ($Z = 0.41$, $P = 0.68$), or egg volume ($t_{16} = 0.25$, $P = 0.81$).

Table 1

Carotenoids present in samples of bill and plasma from control and vitamin E–supplemented yellow-legged gulls (*Larus michahellis*), as determined by HPLC analysis

Carotenoid	Retention time	Bill[a]	Plasma concentration	
			Control	Vitamin E
Astaxanthin	6.58	R	0.25 ± 0.0	0.72 ± 0.85
Unknown 1	9.21	R	3.09 ± 0.05	3.26 ± 0.26
Unknown 2	9.60	R	3.45 ± 0.20	3.58 ± 0.35
Lutein	10.07	R	4.31 ± 0.62	6.28 ± 3.98
Zeaxanthin	10.51	R	0.75 ± 0.31	1.20 ± 0.45
Canthaxanthin	14.59	O, R	0.31 ± 0.09	0.30 ± 0.08
Unknown 3	17.30	—	3.51 ± 1.40	3.75 ± 1.75
β-Cryptoxanthin	19.70	O, R	—	—
Unknown 4	21.33	O, R	—	—
Unknown 5	22.45	O, R	—	—
β-carotene	25.14	O, R	—	—

Carotenoids exclusively present in the red bill spot are shown in bold type. The retention time is expressed in minutes. Plasma concentration (mean ± standard error) is expressed in micrograms per milliliter.

[a] Presence of carotenoids in the orange area (O) and red spot area (R) of the bill.

Interestingly, vitamin E supplementation affected the size of the red bill spot (Table 2), so that the red spot of supplemented male gulls was 9% larger than that of controls (Figure 1a). The size of the red bill spot was related to the bill width (Table 2). In contrast, the redness of the bill spot did not differ in the different groups ($F_{1,16} = 0.30$, $P = 0.59$).

As expected, plasma vitamin E concentration was significantly higher in male gulls that received the vitamin E diet than in control gulls (Table 2, Figure 1b). Vitamin E–supplemented males also showed a significantly higher concentration (22%) of plasma carotenoids than control gulls (Table 2, Figure 1c). These differences were due to red spot carotenoids present at higher levels in plasma from supplemented male gulls than in plasma from controls (Table 2, Figure 2). Nevertheless, the concentrations of other carotenoids did not differ between groups ($F_{1,16} = 0.66$, $P = 0.43$; Figure 2). The antioxidant capacity of gulls fed the vitamin E diet was twice that of control gulls, although the difference was not significant ($F_{1,16} = 3.09$, $P = 0.10$; Figure 1d). Plasma levels of lipid peroxidation markers did not differ between treatments ($F_{1,16} = 0.02$, $P = 0.88$).

DISCUSSION

We found that male gulls supplied with a nonpigmentary antioxidant (vitamin E) had higher levels of vitamin E and total carotenoids in plasma than control males, specifically those carotenoids responsible for the red spot coloration. Moreover, we found that vitamin E–supplemented males had larger red bill spot than control males. Because the saturation of red spot did not differ, the results suggest that a greater proportion of carotenoids were used to enlarge red spot area in antioxidant-supplemented males. The results provide the first evidence under field experimental conditions supporting the hypothesis that antioxidant availability modulates carotenoid-based coloration.

The role of oxidative stress as a mechanism mediating the expression of sexual signals has been the focus of several studies in recent years (e.g., Moreno and Osorno 2003; Alonso-Alvarez et al. 2004; Kurtz et al. 2006; Torres and Velando 2007). Our findings are consistent with the results of 2 recent experimental studies with captive vertebrates, which showed

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Table 2

General linear models showing treatment effects with all covariates (full models) and the model that retained only variables that caused a significant increase in deviance (minimal adequate models)

Dependent variable	Variables	Degrees of freedom	Parameter estimate	Full model		Minimal model	
				F	*P*	*F*	*P*
Bill spot size	Treatment	1,12	23.501	6.30	**0.03**	7.36	**0.02**
	Laying date		0.143	0.02	0.88		
	Days until capture		0.035	0.00	0.96		
	Bill width		18.234	8.37	**0.01**	4.82	**0.04**
	Tarsus		−6.421	3.96	0.07	9.91	**0.01**
Vitamin E	Treatment	1,13	13.602	23.45	**<0.01**	20.49	**<0.001**
	Laying date		0.116	0.15	0.70		
	Days until capture		0.447	4.12	0.06		
	Tarsus		−1.535	2.54	0.13		
Total carotenoids	Treatment	1,13	0.015	6.47	**0.02**	8.53	**0.01**
	Laying date		0.000	0.23	0.64		
	Days until capture		0.001	1.37	0.26		
	Tarsus		0.000	0.06	0.81		
Red spot carotenoids	Treatment	1,13	0.022	4.13	**0.04**	6.33	**0.02**
	Laying date		0.000	0.04	0.84		
	Days until capture		0.000	0.33	0.57		
	Tarsus		−0.002	0.24	0.63		

Significant values are represented in bold type.

that supplementation with nonpigmentary antioxidants enhances expression of carotenoid-based sexual colorations in the stickleback, *Gasterosteus aculeatus* (Pike et al. 2007), and in the zebra finch, *Taeniopygia guttata* (Bertrand et al. 2006). Overall, the results of these and the present study provide experimental evidence supporting the hypothesis that carotenoid-based coloration is an honest signal of the availability of antioxidants in an individual (von Schantz et al. 1999; Hartley and Kennedy 2004).

Two principal mechanisms have been proposed as underlying the honesty of carotenoid-based coloration as a signal of the individual's antioxidant status. The antioxidant trade-off

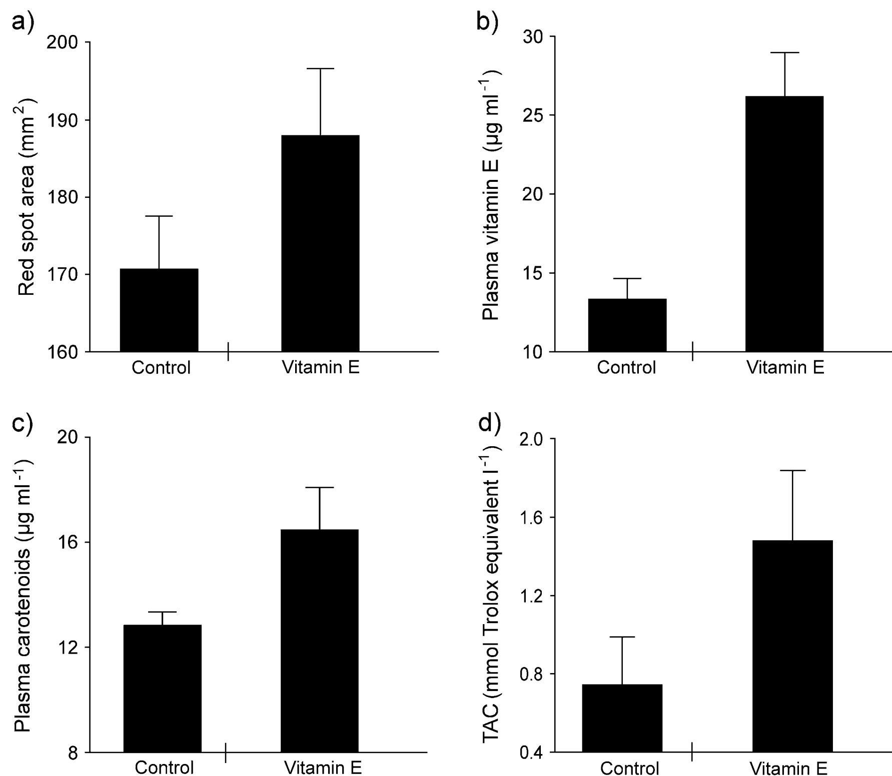

Figure 1
Effect of vitamin E supplementation on (a) size of the red bill spot; (b) plasma levels of vitamin E; (c) levels of total carotenoids in plasma; and (d) levels of total antioxidant capacity in plasma. Values are expressed as means ± standard errors.

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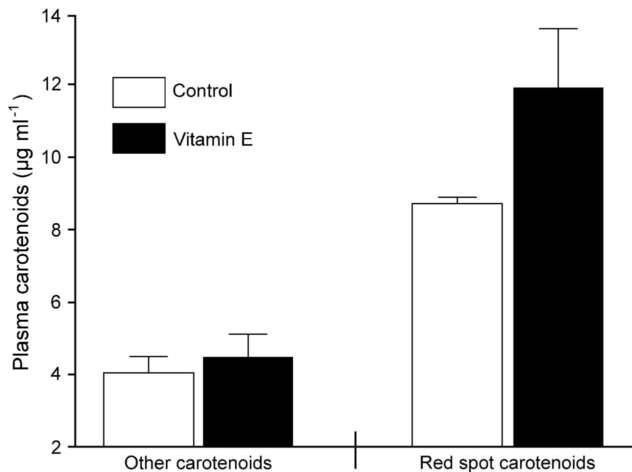

Figure 2
Effect of vitamin E supplementation on plasma carotenoids exclusively associated with the red bill coloration (red spot carotenoids) and on other carotenoids. Values are expressed as means ± standard errors.

hypothesis suggests that only individuals with good antioxidant defenses (or with low levels of ROS) can afford to divert carotenoids away from the detoxification system instead of allocating them to sexual signaling (von Schantz et al. 1999; Blount et al. 2000). Alternatively, it has been proposed that carotenoid-based coloration is an index of nonpigmentary antioxidants (protection hypothesis; Hartley and Kennedy 2004). This hypothesis is based on the idea that carotenoid coloration is altered and destroyed by oxidation (Woodall et al. 1997; Siems et al. 1999) and that therefore only individuals with high levels of antioxidants can prevent carotenoid-based coloration from diminishing.

The enlargement of the red spot area caused by vitamin E supplementation may be consistent with both hypotheses. According to the protection hypothesis, an increase in the antioxidant defenses should protect carotenoids from bleaching. Therefore, the protective effect of vitamin E may be higher in those carotenoids that are more susceptible to oxidation than in other carotenoids. We found that vitamin E supplementation increased the concentration of plasma carotenoids responsible for the red spot coloration, but not the others. This result would be consistent with the protection hypothesis if red spot carotenoids were more vulnerable to oxidation than other carotenoids. However, this is not the case, as, for example, the experiment affected to a red spot carotenoid, astaxanthin ($Z = 2.05$, $P = 0.04$), but not to canthaxanthin ($Z = 0.47$, $P = 0.64$) a carotenoid that was not exclusive to the red spot, even though both carotenoids present similar bleaching rates when are exposed to a free radical attack (Woodall et al. 1997). Thus, the results of the experiment suggest that rather than passive protection of carotenoids (Hartley and Kennedy 2004), an increase in vitamin E may promote an active mechanism to increase the amount of carotenoids responsible for the red spot coloration. Further experimental studies should confirm this idea.

Our findings may be consistent with the antioxidant trade-off hypothesis, as vitamin E supplementation may have allowed the male gulls to divert specific carotenoids from the detoxification system in order to allocate them to red coloration (von Schantz et al. 1999; Blount et al. 2000). Indeed, in other bird species, experimental activation of the immune system resulted in an increase of oxidative damage, which in turn caused a parallel decrease in carotenoid-based coloration (Alonso-Alvarez et al. 2004; Torres and Velando 2007). Overall, these results suggest that oxidative stress is one of the principal mechanisms underlying the trade-off between carotenoid coloration and self-maintenance (von Schantz et al. 1999).

It is also possible that specific carotenoid coloration is produced through metabolic pathways that modify dietary carotenoids (Fox and Hopkins 1966; Stradi et al. 2001; Hill and McGraw 2006; Hudon et al. 2007), thereby producing a release in ROS, such as those catalyzed by cytochrome P450 (Paolini et al. 1999; Lewis 2002). The cytochrome P450 reaction cycle produces different active oxygen species, specifically superoxide and peroxide (Lewis 2002), which can cause cellular damage leading to oxidative stress and its toxic consequences (De Groot and Sies 1989; Goeptar et al. 1995). Under this scenario, only individuals with a high-antioxidant status may be able to activate oxidative pathways for carotenoid transformation, explaining why carotenoids responsible for red colorations are more costly to produce (Hill 1996; Andersson et al. 2007).

In our experiment, vitamin E supplementation did not affect the levels of lipid peroxidation products, which suggests that extra antioxidants were not used to combat oxidation injury. This supports the idea that vitamin E–supplemented birds transformed carotenoids into those needed for red coloration and were thus able to afford the costs associated with carotenoid transformation. Consequently, vitamin E supply may have been used to inactivate the ROS produced in this transformation and to some extent explain the lack of significant differences in the antioxidant capacity of total plasma. The oxidative cost of carotenoid transformation may also fit with the observed results in previous experiments with captive animals (Bertrand et al. 2006; Pike et al. 2007). Thus, in the experimental study on sticklebacks, fishes received the same amount of dietary (yellow and red) carotenoids, but red coloration increased in males fed on a high-antioxidant diet (Pike et al. 2007). This may indicate that sticklebacks with high-antioxidant availability were able to transform dietary (from yellow to red) carotenoids. In other study, Bertrand et al. (2006) manipulated the availability of carotenoids and of melatonin, a colorless antioxidant, in zebra finches. They found that the concentration of plasma carotenoids did not change in the birds that received melatonin supplements but that these birds presented redder bills than controls (Bertrand et al. 2006). However, antioxidant supply in zebra finches may have affected specific red carotenoids (which were not measured) in the bill due to the transformation of dietary carotenoids.

Although our experimental design does not allow us to unravel the mechanism underlying the honesty of carotenoid-based coloration, the results of our study appear to be consistent with the idea that carotenoid allocation to sexual signals is costly and only individuals with high-antioxidant status are able to afford these costs. Further studies are needed to tease out apart the 2 hypotheses on the cost of carotenoid allocation into sexual signals (the antioxidant trade-off or the transformation hypothesis).

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