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SHORT COMMUNICATION

Physiological stress links parasites to carotenoid-based colour signals

F. MOUGEOT*†, J. MARTÍNEZ-PADILLA‡, G. R. BORTOLOTTI§, L. M. I. WEBSTER‡ & S. B. PIERTNEY‡

*EEZA (CSIC), Almeria, Spain
†IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain
‡Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK
§Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada

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Abstract

Vertebrates commonly use carotenoid-based traits as social signals. These can reliably advertise current nutritional status and health because carotenoids must be acquired through the diet and their allocation to ornaments is traded-off against other self-maintenance needs. We propose that the coloration more generally reveals an individual's ability to cope with stressful conditions. We tested this idea by manipulating the nematode parasite infection in free-living red grouse (*Lagopus lagopus scoticus*) and examining the effects on body mass, carotenoid-based coloration of a main social signal and the amount of corticosterone deposited in feathers grown during the experiment. We show that parasites increase stress and reduce carotenoid-based coloration, and that the impact of parasites on coloration was associated with changes in corticosterone, more than changes in body mass. Carotenoid-based coloration appears linked to physiological stress and could therefore reveal an individual's ability to cope with stressors.

Introduction

The evolution and maintenance of exaggerated or brightly coloured plumage traits and ornaments have long fascinated evolutionary biologists. A central issue lies in understanding how such traits evolved as honest signals of quality and what aspects of individual quality are advertised (Andersson, 1994; Zahavi & Zahavi, 1997). In birds, ornaments often advertise a greater body mass, or health (absence of diseases or parasite infections) or a heritable ability to resist parasites (e.g. Hamilton & Zuk, 1982; Andersson, 1994), and could more generally reveal how individuals cope with stressors (Bortolotti *et al.*, 2009a).

Stress hormones can act as mediators of sexually selected, animal signals (Husak & Moore, 2008; Roulin *et al.*, 2008; Bortolotti *et al.*, 2009a), and there is growing interest in understanding the role of stress in social dominance and mate choice (Roberts *et al.*, 2007; Husak & Moore, 2008). Free-living animals experience many stressors (e.g. inclement weather, predators, parasites, social conflicts) that challenge their homeostasis (Romero, 2004). A major adaptation is the hypothalamic–pituitary–adrenal axis, which releases glucocorticoids in response to stressors, allowing individuals to recover homeostasis in the best condition (Wingfield *et al.*, 1998). However, chronic or inappropriate stress responses can have serious negative effects (Romero, 2004), such as immunosupression (Buchanan, 2000; Korte *et al.*, 2005). How an individual responds to or copes with stressors is likely a major determinant of its fitness, and it was recently suggested that ornaments could reveal how individuals cope with stressors (Bortolotti *et al.*, 2009a).

Carotenoids underpin the coloration of many animal ornaments (e.g. Hill & McGraw, 2006). There is considerable interest in understanding how these signals

Correspondence: Francois Mougeot, EEZA (CSIC), Almeria 04001, Spain.
Tel.: +34 926 29 54 50; fax: +34 926 29 54 51;
e-mail: francois.mougeot@eeza.csic.es

evolved and are maintained as honest signals, by studying the proximate and ultimate causes of variability in carotenoid-based displays (Olson & Owens, 1998; Tschirren *et al.*, 2003; Tella *et al.*, 2004; Hill & McGraw, 2006) and the mechanisms constraining or enhancing carotenoid allocation to ornaments (Hill & McGraw, 2006). Carotenoid-based signals were first suggested to advertise a greater body mass because vertebrates cannot synthesize carotenoids *de novo*, but must ingest them (e.g. Hill & McGraw, 2006). Carotenoids are also immunostimulants, so individuals can alternatively use them as pigments or for combating parasites (Lozano, 1994; Blount *et al.*, 2003; Faivre *et al.*, 2003). A finite supply of carotenoids leads to trade-offs in how they are used (Olson & Owens, 1998; Hill *et al.*, 2002; Hill & McGraw, 2006). Carotenoid signalling has often been shown to be limited by parasites, consistent with a trade-off for using carotenoids to combat infections or pigment ornaments (e.g. Brawner *et al.*, 2000; Horak *et al.*, 2004; Martinez-Padilla *et al.*, 2007; Mougeot *et al.*, 2009). Carotenoid-based signals should be particularly sensitive to physiological stress for several reasons. First, food shortage and carotenoid limitation via diet acquisition is typically associated with both a reduced body mass and elevated stress hormone levels (Romero, 2004). Second, elevated stress hormone levels are associated with reduced immunocompetence (Buchanan, 2000; Korte *et al.*, 2005), implying a greater need for using carotenoids for self-maintenance rather than for pigmenting colourful ornaments, given the immunostimulant properties of carotenoids (Blount *et al.*, 2003). Finally, prolonged parasite infections that induce chronic stress (Romero, 2004) have been shown to reduce the expression of carotenoid signals (e.g. Brawner *et al.*, 2000; Horak *et al.*, 2004; Martinez-Padilla *et al.*, 2007; Mougeot *et al.*, 2007b).

A main limitation for understanding how stress may mediate the expression of ornaments or affect carotenoid allocation has been the lack of meaningful measures of how animals respond to stressors (Buchanan, 2000; Romero, 2004). The main avian glucocorticoid, corticosterone, can be quantified reliably in feathers (Bortolotti *et al.*, 2008, 2009a), providing an integrated measure of responses to stressors. The amount of corticosterone deposited in a feather parallels the amount produced in response to all the stressors experienced during the time over which the feather grows (see Bortolotti *et al.*, 2008, 2009b).

We investigated the potential role of parasites as stressors impacting the expression of a carotenoid-based signal in free-living male red grouse *Lagopus lagopus scotticus*. These birds display bright red supra-orbital combs, which are pigmented by carotenoids (Mougeot *et al.*, 2007a) and used in intra- and inter-sexual interactions (Bart & Earnst, 1999; Redpath *et al.*, 2006b). *Trichostrongylus tenuis* is a main nematode parasite of red grouse, which negatively affects body mass (Delahay *et al.*, 1995; Mougeot & Redpath, 2004) and reproduction (Hudson, 1986; Redpath *et al.*, 2006a). Previous work showed that *T. tenuis* parasites reduce circulating carotenoids and comb redness (Martinez-Padilla *et al.*, 2007; Mougeot *et al.*, 2007a), although the mechanism of these impacts was unknown. Here, we test whether parasites are responsible for modulating circulating carotenoids, body mass and physiological stress, and ultimately the expression of the carotenoid-based coloration of male combs. We manipulated *T. tenuis* parasites using an effective anthelminthic drug. At the treatment level, we expected parasite purging to (i) improve body mass, and increase circulating carotenoids and comb redness and to (ii) reduce feather corticosterone, either because parasites directly increase glucocorticoid production, or because they make males more vulnerable to other stressors, such as social conflicts (Mougeot *et al.*, 2005a) or predators (Hudson *et al.*, 1992). At the individual level, we tested whether changes in comb redness were associated with the effects of parasites on changes body mass or corticosterone levels.

Materials and methods

Experiment

In autumn 2006 (1 October ± 5 days), we caught 29 male red grouse on Catterick moor (North Yorkshire, UK). Upon initial capture (C1), birds were ringed and fitted with a radio-collar (TW3-necklace radio-tags; Biotrack, Wareham, Dorset, UK) to facilitate recapture. Birds were randomly assigned to one of two treatments: treated (parasite purged) or control. Males were given a 1 mL oral dose of water (controls; $n = 8$) or of levamisole hydrochloride (Nilverm Gold, Schering-Plough Animal Health, Welwyn Garden City, UK) to kill *T. tenuis* nematodes (treated males; $n = 21$). We choose to include more males within the treated than control group because we were interested in studying individual changes within this manipulated group and their mediation by corticosterone. We recaught males 13 ± 4 days after treatment (C2; see Table 1). Upon each capture (C1 and C2), we (i) weighed males; (ii) took a blood sample; (iii) collected a faecal sample for parasite counts; (iv) took a photograph of a comb on a standard grey card and (v) plucked a growing feather from the rump. Blood was centrifuged, and plasma kept frozen at −80 °C. Feathers were kept in individual paper envelopes until analysed.

Parasite abundance estimates

We estimated *T. tenuis* abundance using caecal egg concentration. We kept males overnight in individual pens to collect fresh caecal samples produced by grouse early morning. Samples were taken to the laboratory, stored in a cold room at a constant temperature of 4 °C to inhibit parasite egg development and processed within 2 weeks of collection to ensure reliable estimates (Seivwright *et al.*, 2004). For each bird, a subsample

Table 1 Overview of the timing of the experiment, procedures and data sampling.

Captures	First capture (C1)	Second capture (C2) = C1 + 13 days
Procedure	Oral dose of anthelminthic (treated males) Oral dose of water (control males)	
Data sampling	*Trichostrongylus tenuis* parasites	*T. tenuis* parasites
	Plasma carotenoids	Plasma carotenoids
	Comb redness (photo)	Comb redness (photo)
	Body mass	Body mass
Corticosterone assay	Growing rump feather plucked	Growing rump feather plucked
	← Corticosterone deposited prior to C1	
	→ Corticosterone deposited between C1 and C2	

of approximately 0.2 g of faeces was diluted in 5 mL of saline water and mixed thoroughly. A subsample of this solution was placed in a MacMaster slide under a ×40 microscope to count eggs. The number of eggs per gram of caecal material was calculated by multiplying the average number of eggs counted under both grids by the total volume of caecal suspension contained in both chambers and then dividing this by the quantity of caecal droppings used in the suspension. Number of *T. tenuis* worms per grouse was calculated from caecal egg concentration using the equations provided in Seivwright *et al.*, 2004. Parasite egg counts were highly repeatable (see Martinez-Padilla *et al.*, 2007).

Plasma carotenoid concentration

We diluting 60 μL of plasma in acetone (1 : 10 dilution), vortexed and centrifuged the mixture at 7000 *g* for 10 min to precipitate flocculent proteins. The supernatant was examined in a Shimadzu UV-1603 spectrophotometer (Shimadzu, Kyoto, Japan), and we determined the optical density at 446 nm, the wavelength of maximal absorbance for lutein in acetone. Plasma carotenoid concentration (μg mL^{-1}) was calculated using a standard curve of lutein (Sigma Chemicals, St Louis, MO, USA). Repeatability was determined on a subsample measured twice (*repeatability* = 0.99, $F_{19,20}$ = 602.2; $P < 0.001$).

Comb colour measurements

We took photographs of the left comb with a Nikon Coolpix 4500 digital camera from a standard distance (50 cm) and using the camera flash. Using Adobe Photoshop 7.0, we measured for each picture the average component of red (R) for comb and grey reference using the RGB system. We used the 'magic tool' in this software, with a tolerance set at 45 for each comb. The grey reference was obtained from Dulux® (Ebony Mists 1–6) grid. The same reference (Ebony Mists 4) was measured for all comb photographs. Comb *R*-values were standardized using the grey reference *R*-values [residuals from a General Linear Model (GLM) of R-comb on the *R*-reference values, hereafter 'comb redness']. We evaluated the repeatability of comb redness by measuring twice a sample of males in two ways: repeatability (i) within the same picture and (ii) between two different pictures of the same combs, taken at the same time. Comb redness measurements were highly repeatable both within (*repeatability* = 0.98, $F_{14,29}$ = 111.09, $P < 0.001$) and between pictures (*repeatability* = 0.80, $F_{13,27}$ = 8.98, $P < 0.001$). More details are given in Martinez-Padilla *et al.* (2007).

Corticosterone assays

We extracted corticosterone from feathers collected at C1 and C2 using a methanol-based extraction technique. The feather material without the calamus was first cut into pieces of < 5 mm^2 with scissors. We then added 10 mL of methanol (HPLC grade; VWR International, Mississauga, ON, Canada) and placed the samples in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a shaking water bath. The methanol was then separated from feather material by vacuum filtration, using a plug of synthetic polyester fibre in the filtration funnel. The feather remnants, original sample vial and filtration material were washed twice with approximately 2.5 mL of additional methanol; the washes were added to the original methanol extract. The methanol extract was placed in a 50 °C water bath and subsequently evaporated in a fume hood. Evaporation of the samples was completed within a few hours, and the extract residues were reconstituted in a small volume of the phosphate buffer system (PBS; 0.05 M, pH 7.6) used in the corticosterone radioimmunoassay. The filtration step was generally found to be sufficient to remove feather particulates but further particulate material could be removed, if needed, by centrifugation of the PBS-reconstituted samples. Reconstituted samples were frozen at −20 °C until analysed for corticosterone. We assessed the efficiency of the methanol extraction by including feather samples spiked with a small amount (approximately 4000 dpm) of ^{3}H-corticosterone in each extraction. More than 90% of the radioactivity was recoverable in the reconstituted samples. Data values are expressed as pg corticosterone per mm of feather, which gives a valid estimate of corticosterone per unit time of feather growth (Bortolotti *et al.*, 2008, 2009a,b).

Statistical analyses

Body mass, carotenoid and corticosterone concentrations were log-transformed to achieve a normal distribution (the distribution of log-transformed values did not differ from a normal distribution; Wilk–Shapiro tests; all $P > 0.20$). These log-transformed variables, as well as comb redness (normally distributed), were fitted to models using a normal error distribution (GLM or Mixed procedures; SAS, 2001). *Trichostrongylus tenuis* abundance (count data; dependent variable) was fitted to models using a Poisson error distribution and log-link function (Genmod or Glimmix procedures). We tested for treatment effects on changes over time (first capture, before treatment, vs. second capture, after treatment) in study parameters using generalized linear mixed models (Mixed and Glimmix procedures in SAS) that included 'individual' as a random effect, to account for repeated measures. We used mixed models because not all parameters could always be measured before and after treatment (unbalanced data set as a result of the lack of sampling material for some parasite counts or carotenoid assays). We used GLMs to test for associations between study parameters prior to treatment (un-manipulated males). In these analyses *T. tenuis* abundance was log-transformed when included as an explanatory variable. Changes in study parameters (body mass, comb redness, feather corticosterone and plasma carotenoid concentration) were calculated as the difference between final and initial values, corrected for initial values (residuals from a GLM of the difference on the initial value).

Results

Before treatment, feather corticosterone levels (GLM; $F_{1,21} = 0.24$; $P = 0.631$; slope: 0.012 ± 0.023 SE) and body mass ($F_{1,27} = 0.72$; $P = 0.402$; slope: 1.018 ± 1.197 SE) were not related to parasite abundance. Comb redness was not significantly related to plasma carotenoid concentration ($F_{1,23} = 0.12$; $P = 0.727$; slope: −2.784 ± 7.893 SE) or corticosterone levels ($F_{1,27} = 0.08$; $P = 0.778$; slope: −2.275 ± 7.986 SE), but was negatively correlated with *T. tenuis* parasite abundance ($F_{1,21} = 11.60$; $P = 0.0027$; slope: −2.486 ± 0.729 SE). Plasma carotenoid concentration was negatively related to corticosterone levels ($F_{1,23} = 14.93$; $P = 0.008$; slope: −0.680 ± 0.176 SE).

Trichostrongylus tenuis abundance did no differ significantly between groups prior to treatment (GENMOD; $\chi^2_{1,22} = 1.35$; $P = 0.33$; estimate: −0.683 ± 0.709 SE). Changes in *T. tenuis* abundance differed between treatment groups, decreasing more in purged males than in control males (Fig. 1a; Table 2). After treatment, control males had on average 222 worms at C2, whereas treated males had no worms at C2, indicating that the purging had been effective.

Changes over time in feather corticosterone levels significantly differed between treatment groups (Fig. 1b;

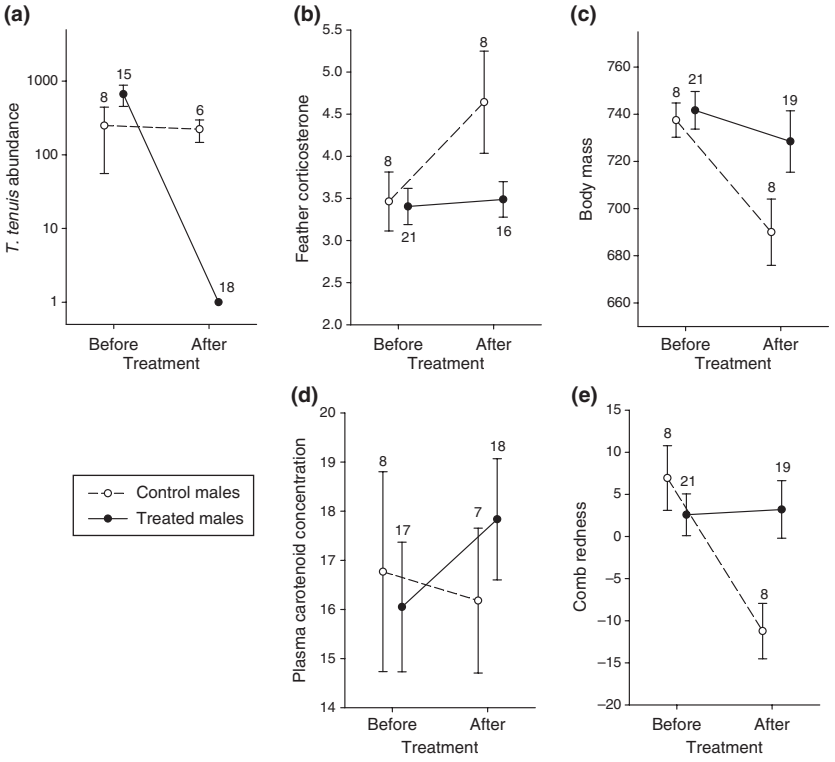

Fig. 1 Mean ± SE changes over time in (a) *Trichostrongylus tenuis* abundance (worms per male), (b) feather corticosterone (ng mm^{-1}), (c) body mass (g), (d) carotenoid concentration ($\mu g\ mL^{-1}$) and (e) comb redness (adjusted *R*-value; see Methods) according to treatment. Sample size: number of males.

Table 2 Effect of treatment on *Trichostrongylus tenuis* abundance, feather corticosterone, body mass, carotenoid concentration and comb redness.

Fixed effects		Time		Treatment		Time × Treatment		
Dependent	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	Parameter estimates ± SE‡
T. tenuis abundance*	1.17	13.91	0.002	0.62	0.443	91.37	< 0.001	Intercept: 0.045 ± 0.417 Time: 5.794 ± 0.485 Treatment: 4.661 ± 0.793 Time × Treatment: −8.336 ± 0.872
Corticosterone levels†	1.22	5.66	0.026	1.61	0.218	4.74	0.041	Intercept: 1.471 ± 0.065 Time: −0.135 ± 0.074 Treatment: 0.258 ± 0.114 Time × Treatment: −0.288 ± 0.132
Body mass†	1.25	29.73	< 0.001	1.95	0.174	9.01	0.006	Intercept: 6.589 ± 0.012 Time: 0.019 ± 0.009 Treatment: −0.053 ± 0.022 Time × Treatment: 0.048 ± 0.016
Carotenoids†	1.20	0.31	0.585	0.07	0.799	1.16	0.293	Intercept: 2.884 ± 0.066 Time: −0.074 ± 0.050 Treatment: −0.078 ± 0.125 Time × Treatment: 0.098 ± 0.091
Comb redness†	1.25	13.52	0.001	0.04	0.850	5.15	0.032	Intercept: −2.426 ± 2.89 Time: 5.013 ± 3.814 Treatment: −8.801 ± 5.235 Time × Treatment: 16.163 ± 7.125

*Parasite abundance (number of *T. tenuis* worms per host) was fitted to models using a Poisson error distribution (GLIMMIX procedure; SAS).
†Dependent variables were log-transformed so that their distribution did not differ significantly from a normal distribution and were fitted to models using a normal error distribution (MIXED procedure; SAS).
‡Parameter estimate values are given for the following: Time: before treatment; Treatment: Control group; Time × Treatment: Before treatment and Control group. Other parameter estimates can be deducted from these.

Table 2). Corticosterone levels in feathers grown during the experiment, i.e. between C1 and C2, were higher in control than in treated males (Fig. 1b; GLM; $F_{1,22} = 5.62$; $P = 0.027$; estimate: 0.243 ± 0.102 SE). Males lost weight during experiment, control males losing more weight than treated males (Fig. 1c; Table 2). Changes over time in plasma carotenoids did not differ between treatment groups (Fig. 1d; Table 2), but changes in comb redness did. Comb redness decreased during the experiment, but less so in treated than in control males (Fig. 1e; Table 2).

At the individual level, changes in body mass (ΔBM) were related to changes in corticosterone (ΔCORT) depending on the treatment (ΔCORT: $F_{1,21} = 0.56$; $P = 0.464$; Treatment: $F_{1,21} = 6.45$; $P = 0.020$; ΔCORT × Treatment: $F_{1,21} = 11.56$; $P = 0.003$; Mean Square: 0.085). ΔBM was negatively related to ΔCORT in control males ($F_{1,6} = 10.36$; $P = 0.018$; slope: −16.575 ± 5.149 SE; Mean Square: 0.096; Fig. 2a) but not in treated males ($F_{1,13} = 3.11$; $P = 0.101$; slope: +10.587 ± 5.999 SE; Mean Square: 0.311; Fig. 2a). Changes in comb redness were associated with ΔCORT ($F_{1,21} = 6.63$; $P = 0.018$; slope: −4.923 ± 1.911 SE; Mean Square: 0.151; Fig. 2b), but not associated with ΔBM ($F_{1,20} = 1.25$; $P = 0.278$; slope: +0.112 ± 0.100 SE; Mean Square: 0.313) and did not differ between treatment groups ($F_{1,20} = 0.77$; $P = 0.392$; Mean Square: 0.209) when ΔCORT was also included in the model. Changes in plasma carotenoids were also associated with ΔCORT ($F_{1,17} = 9.01$; $P = 0.008$; slope: −1.135 ± 0.378 SE; Mean Square: 0.110; Fig. 2c), but not with ΔBM ($F_{1,16} = 1.19$; $P = 0.291$; slope: +0.024 ± 0.022 SE; Mean Square: 0.231) and did not differ between treatment groups ($F_{1,16} = 0.12$; $P = 0.720$; Mean Square: 0.263) when ΔCORT was also included in the model.

Discussion

Our experiment revealed that carotenoid signalling is intimately linked to physiological stress, which was itself influenced by infection by a nematode known to negatively impact on red grouse fitness (Hudson, 1986). Moreover, changes in carotenoid levels and comb coloration were associated with changes in stress levels rather than changes in body mass.

In unmanipulated males, feather corticosterone levels were not related to *T. tenuis* parasite abundance. Corticosterone deposition in feathers grown prior to experiment did not differ between treatment groups, but corticosterone deposition in feathers grown during experiment was lower in purged males than in controls, indicating that *T. tenuis* infection was 'stressful' (being associated with a higher corticosterone accumulation in feathers collected at C2). Although it is difficult to

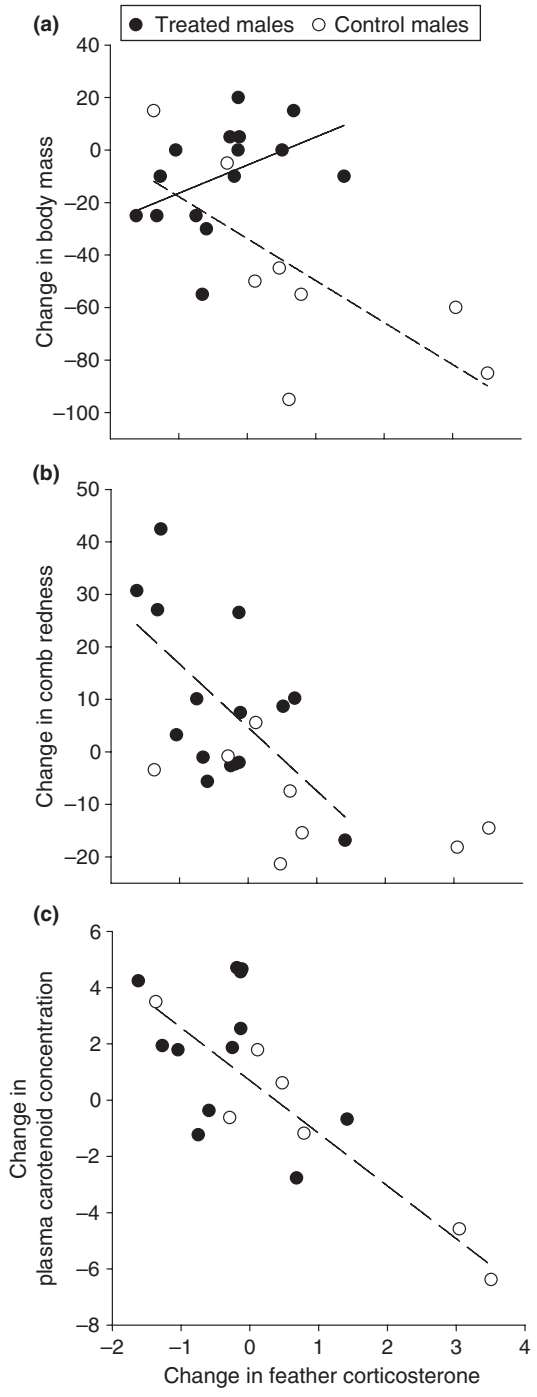

Fig. 2 Relationships between the change in feather corticosterone (pg mm^{-1}) and the changes in (a) body mass (g), (b) carotenoid concentration ($\mu g\ mL^{-1}$) and (c) comb redness. Treated males: black dots; control males: open dots. Regression lines are shown for control and treated males separately for changes in body mass (a) because the relationship differed between treatment groups, and for control and treated males altogether for changes in comb redness and circulating carotenoids (b and c), because the relationships did not differ between treatment groups (see Result section).

compare the corticosterone deposition levels before and after experiment (because different feathers were analysed), corticosterone deposition appear to have increased in control birds, but not in treated males (Fig. 1b). This could be explained by an increase in aggressiveness and social interactions at this time of year (autumn), when males establish and fight for territories (Mougeot *et al.*, 2005b), activities that are most likely stressful (Creel, 2001). This would also be consistent with the observation that control males lost weight during experiment. In contrast, corticosterone deposition was lower in males purged of *T. tenuis* worms, a parasite also know to limit aggressiveness and influence social interactions (Mougeot *et al.*, 2005a). Parasites can influence the corticosterone production directly or indirectly, by making males more exposed to the myriad of stressors they face in nature. For instance, males infected with *T. tenuis* are also less dominant (Fox & Hudson, 2001; Mougeot *et al.*, 2005a), which could also explain higher corticosterone levels via a lower dominance status (Creel, 2001) during autumn fights. Another possibility is that a higher predation risk increased stress levels in infected grouse, because grouse infected by *T. tenuis* parasites are more smelly and more easily detected by mammalian predators (Hudson *et al.*, 1992).

There is considerable evidence that increased corticosterone levels are associated with a greater susceptibility to parasites (e.g. Buchanan, 2000; Romero, 2004), but a better understanding of the trade-off needs to demonstrate that parasites cause increased corticosterone levels, which has been rarely shown (Boughton *et al.*, 2006; Kowalski *et al.*, 2006). In red grouse, higher corticosterone levels are associated with a greater susceptibility to *T. tenuis* (Bortolotti *et al.*, 2009a), so the interaction between corticosterone and *T. tenuis* parasite is two-way: parasites cause more stress, and more stress cause greater parasite susceptibility.

Comb redness correlated negatively with parasite abundance. Males purged of *T. tenuis* maintained comb redness whereas infected control males reduced coloration (Fig. 1e), consistent with previous experiments (Martinez-Padilla *et al.*, 2007; Mougeot *et al.*, 2007b). Parasite purging improved body mass (Fig. 1b), but did not significantly affect circulating carotenoid levels, unlike in a previous experiment (Martinez-Padilla *et al.*, 2007). Males might have been more carotenoid-limited and kept carotenoids for needs other than coloration (control males lost colour and experienced increased corticosterone during the experiment); although males purged of parasites maintained comb colour, they may not have been able to increase circulating carotenoids. The relationship between carotenoid-based signals and body mass could also vary depending on how individuals solve allocation trade-offs; for instance, increasing signal output as a terminal effort could result in a positive association between parasite load and signal expression (e.g. Polak & Starmer, 1998; Candolin, 2000). This could

explain why changes in body mass did not appear to mediate the impact of parasite purging upon carotenoid-based signalling. Within individuals, changes in carotenoid levels and coloration were associated with physiological stress. Specifically, in un-manipulated males, carotenoid levels negatively correlated with feather corticosterone levels; in experimental birds, changes over time in circulating carotenoids and comb redness were associated with changes in corticosterone production (reduced physiological stress) and unrelated to changes in body mass. This suggested a stress-dependent link rather than body mass-dependent link between parasites and carotenoid-based ornamentation. In treated males, an increase in corticosterone was in fact associated with a tendency to improve body mass, opposite to control males (Fig. 2).

These negative consequences of parasitism, via increased physiological stress, can maintain honest carotenoid-based signalling. Free-living animals constantly face environmental perturbations or stressors (e.g. predators, parasites, food shortages, social conflicts), and carotenoid-based ornaments, intimately related to stress, could reveal how individuals cope with these stressors altogether. From a signaller/appraiser perspective, what matters is whether individuals managed to avoid or to cope well with these stressors (for instance, by being better able to access food resources or combat parasite infections), and this could be the quality generally revealed by carotenoid-based ornaments under different selection regimes (contrasted environmental conditions; Bortolotti *et al.*, 2009a). Our results on the role of stress are of correlative nature, so in future experiments, stress hormone levels could be manipulated to directly test the impact of stress hormones on ornament expression. However, this should be performed with care, given the nature of stress hormone responses, to mimic natural changes in stress hormone levels and obtain biologically meaningful insights (Romero, 2004). By measuring corticosterone in feathers, measured over days rather than instantaneously (as performed through blood sampling), we were uniquely able to link physiological stress, parasites and carotenoid-based signals. This new way of looking at the physiology of birds will shed new light on the evolution and maintenance of animal social signals and, more generally, on the role and biological significance of stress in evolutionary trade-offs.

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