Nematode parasites reduce carotenoid-based signalling in male red grouse

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Carotenoids determine the yellow–red colours of many ornaments, which often function as signals of quality. Carotenoid-based signalling may reliably advertise health and should be particularly sensitive to parasite infections. Nematodes are among the commonest parasites of vertebrates, with well-documented negative effects on their hosts. However, to date, little is known about the effects that these parasites may have on carotenoid-based signalling. Tetraonid birds (grouse) exhibit supra-orbital combs, which are bright integumentary ornaments pigmented by carotenoids. We tested the effect of the nematode parasite Trichostrongylus tenuis on signalling in free-living male red grouse Lagopus lagopus scoticus. We show that experimentally reduced nematode infection increases plasma carotenoid concentration and comb redness, demonstrating for the first time that nematodes can influence carotenoid-based signals.

**Keywords:** carotenoids; comb; nematodes; red grouse Lagopus lagopus scoticus; Trichostrongylus tenuis

1. INTRODUCTION

The brightly coloured ornaments of animals often function as reliable signals of quality, indicating better body condition or ability to resist parasites (Hamilton & Zuk 1982). Carotenoid-based signals are among the most familiar criteria for mate choice (Hill & McGraw 2006). Identifying limiting factors along the pathway from nutritional access to coloration is essential for the understanding of how carotenoid-based ornaments have evolved and are maintained as honest signals (Hill & McGraw 2006). Vertebrates cannot produce carotenoids, so carotenoid intake can limit ornament expression (Olson & Owens 1998). Carotenoids also have beneficial physiological functions, being immunostimulants and antioxidants (Møller et al. 2000). The trade-offs resulting from carotenoid use in self-maintenance versus ornamentation may further confer honesty (Fairevre et al. 2003).

Carotenoid-based signals should be particularly sensitive to parasites (Lozano 1994), though experimental evidence remains limited (e.g. Hill & McGraw 2006). For instance, coccidia can directly reduce carotenoid uptake (Hörak et al. 2004), and cestodes might negatively influence carotenoid signalling (Figueroa et al. 2005). Nematodes are common intestinal parasites of vertebrates, and often have profound effects on hosts (Wakelin 1978); however, their effects on circulating carotenoids and carotenoid-dependent ornamentation have never been tested experimentally.

We manipulated nematode parasites in male red grouse and investigated the effects on carotenoid-based signalling. Red grouse display red supra-orbital combs pigmented by carotenoids (Mougeot et al. 2007) that function in intra- and inter-sexual selection (Mougeot et al. 2004, 2007). Using an anthelmintic drug, we reduced infection by Trichostrongylus tenuis worms. This main parasite of red grouse negatively impacts condition, productivity and survival (Hudson 1986). We predicted a reduction in T. tenuis would increase plasma carotenoids and the pigmentation of grouse combs.

2. MATERIAL AND METHODS

(a) Experiment

In autumn 2005 (16 October–1 November), we caught 37 males on Edinglassie Estate, northeast Scotland (57°12’N–3°07’W). Each was ringed, fitted with a radio collar (TW3-necklace tag, Biotrack) and aged (young, i.e. hatched that summer or old). Males were randomly assigned to one of two treatments: dosed (parasite reduction) or control. After collecting faecal samples for parasite counts, control males were given 1 ml oral dose of water, and dosed males were given 1 ml of anthelmintic (levamisole hydrochloride, Nilverm Gold), a drug effective at reducing nematodes and cestodes might limit ornament expression (Olson & Owens 1998), though experimental evidence remains limited (e.g. Hill & McGraw 2006). For instance, coccidia can directly reduce carotenoid uptake (Hörak et al. 2004), and cestodes might negatively influence carotenoid signalling (Figueroa et al. 2005). Nematodes are common intestinal parasites of vertebrates, and often have profound effects on hosts (Wakelin 1978); however, their effects on circulating carotenoids and carotenoid-dependent ornamentation have never been tested experimentally.

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(b) Comb redness

High-resolution (2272×1704 pixels) pictures of the flattened comb were taken at a standard distance (50 cm) using the flash of the digital camera (Nikon Coolpix 4500). The same grey reference chip was placed beside the comb for each picture. We analysed digital images using Adobe Photoshop v. 7.0, measuring the optical density at 446 nm, the wavelength of maximal absorbance for lutein (Mínguez-Mosquera 1993), the most common circulating carotenoid in birds (Hill & McGraw 2006). We recaptured 30 males 18-1-4 days after treatment. Time between capture and recapture did not differ between groups (general linear model, F_{1,26}=0.22, p=0.643). At each capture, we took a blood sample from the wing vein and a digital photograph of the comb. Blood was centrifuged and plasma kept frozen at –20°C. Males were kept overnight in individual boxes to collect faecal samples.

(c) Plasma carotenoid concentration

Carotenoids were quantified by diluting 60 μl of plasma in acetone (1 : 10). The mixture was vortexed and centrifuged at 10 000 r.p.m. for 10 min. The supernatant was examined in a ShimadzuUV-1603 spectrophotometer and we determined the optical density at 446 nm, the wavelength of maximal absorbance for lutein (Minguez-Mosquera 1993), the most common circulating carotenoid in birds (Hill & McGraw 2006). This wavelength has been considered as a reliable index of total carotenoids (Bloom et al. 2003; McGraw et al. 2003). Plasma carotenoid concentration (μg ml−1) was calculated using a standard curve of lutein (Sigma Chemicals).

(d) Parasite abundance

We used faecal egg concentrations to estimate coccidia and T. tenuis abundance. Samples were stored at 4°C to inhibit egg development and analysed within 5 days of collection to ensure reliable estimates (Seivwright et al. 2004), and cestodes might negatively influence carotenoid signalling (Figueroa et al. 2005). Nematodes are common intestinal parasites of vertebrates, and often have profound effects on hosts (Wakelin 1978); however, their effects on circulating carotenoids and carotenoid-dependent ornamentation have never been tested experimentally.

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(e) Statistical analyses

We used SAS v. 9.1. Counts of coccidia eggs and T. tenuis worms were fitted to generalized linear mixed models (GLMMs) using a Poisson error distribution. Plasma carotenoid concentration and comb redness were fitted to GLMMs using a normal distribution
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(Shapiro–Wilks tests, NS). When testing for treatment effects, we included ‘individual’ as a random effect, to account for repeated measures. We tested for differences between treatment groups in changes over time of variables by including ‘recapture’ (before versus after treatment), treatment (dosed versus control), age (old and young) and their interactions as fixed effects. *Trichostrongylus tenuis* and coccidia abundances were log transformed when included as explanatory variables. For analyses of comb redness, all models including the explanatory variables d.f. Fp 1,33.8 0.17 0.686 1,15.6 8.59 0.010 1,24 4.37 0.046 1,24 14.50 0.019

**3. RESULTS**

Before treatment, comb redness positively correlated with circulating carotenoids (F2.13 = 3.17, p = 0.084; figure 1a), significantly so when parasites were included as covariates (F1.29 = 6.67, p = 0.015; *T. tenuis*, p = 0.011 and coccidia; F1.29 = 2.29, p = 0.141). Circulating carotenoids tended to decrease with increasing coccidia and *T. tenuis* intensities (F1.31 = 3.41, p = 0.074 and F1.31 = 2.53, p = 0.122, respectively), significantly so when comb redness was a covariate (coccidia, F1.29 = 6.13, p = 0.019 and *T. tenuis*, F1.29 = 4.99, p = 0.033; redness, F1.29 = 6.67, p = 0.015; figure 1). Comb redness correlated negatively with *T. tenuis* abundance (F1.29 = 7.49, p = 0.010; figure 1b). These relationships did not differ between age groups (all p > 0.38).

Before treatment, *T. tenuis* and coccidia prevalences were 89.2 and 100%, respectively. Old males had more *T. tenuis* than young grouse (table 1). *Trichostrongylus tenuis* abundance decreased significantly more in dosed than in control males (table 1; figure 2a; see also table S1 in electronic supplementary material) independently of bird age. Coccidia abundance was not affected by treatment (table 1). Young males had more coccidia than old males before treatment, but changes over time in abundance did not differ between treatment groups (table 1), in both old and young birds.

Prior to treatment, plasma carotenoid concentration did not differ between treatment and age groups (both p > 0.46). Circulating carotenoids increased significantly more in dosed than in control birds (table 1, figure 2b), in both young and old males. Comb redness also increased significantly more in dosed than in control birds (table 1; figure 2c and electronic supplementary material) in both young and old males. Coccidia abundance did not influence redness after controlling for treatment effects (F1.17 = 0.29, p = 0.669).

**4. DISCUSSION**

In untreated males, comb redness increased with circulating carotenoids, significantly so when parasites were taken into account. Thus, the relationship between ornament coloration and circulating carotenoids, which has been found in several other species...
Hill & McGraw 2006), was only revealed when parasites provided the appropriate context. Similarly, the negative relationships between carotenoid levels and parasites were better understood when comb colour was taken into account. The negative relationships between both coccidia and T. tenuis parasites and carotenoid levels suggested that both parasites limited carotenoid-based signalling.

Our treatment was effective at reducing T. tenuis worms, increasing circulating carotenoids and ultimately enhancing ornamental coloration. It is known that other intestinal parasites, particularly coccidia (McGraw & Hill 2000; Hörak et al. 2004) influence carotenoid-based signals in captive birds. Our anthelmintic treatment reduced nematode infection without significantly affecting coccidia parasites. Our experimental results were also consistent with the correlative results, and both indicated that T. tenuis nematodes reduce circulating carotenoids and redness of the comb. We are thus confident that our results indicate a negative effect of nematodes on plasma carotenoids and on carotenoid-based ornamentation. Despite a high prevalence, T. tenuis abundance was low in our study, compared with the range observed in red grouse (up to 30 000 worms, Hudson 1986). Thus, even subtle variations in nematode infection can affect ornamentation.

Nematodes can affect carotenoid signals in several, non-exclusive ways. The thickening of the gut epithelium caused by coccidiosis has been shown to constrain carotenoid absorption (Allen 1987). Adult nematodes inhabit the caeca of red grouse (Seivwright et al. 2004) and cause significant damage to epithelial tissues. Grouse have particularly long caeca to maximize digestion and absorption of plant nutrients. Although we do not know if carotenoid absorption takes place in the caeca, the caecal damage caused by T. tenuis worms could constrain absorption and explain the negative effect of nematodes on circulating carotenoids. Trichostrongylus tenuis worms might also reduce the production of high-density lipoproteins and their incorporation into ornaments (McGraw et al. 2006) or directly compete with the bird for carotenoids (Mawson & Wakabongo 2002). Finally, nematodes can also have other systemic effects on carotenoid availability (Hill et al. 2004) as carotenoids may be diverted to boost the immune system against nematodes instead of being displayed in ornaments (Møller et al. 2000; Blount et al. 2003).

Nematodes are among the commonest parasites of vertebrates (Wakelin 1978), and have the potential to reduce plasma carotenoid availability and carotenoid use for ornamentation, as demonstrated by our experiment. This should stimulate more experiments on wild and captive animals, and more detailed investigation of the mechanisms by which nematode parasites influence carotenoid signals.

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