Do carotenoids and spleen size vary with helminth load in greylag geese?

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Abstract: It has been suggested that carotenoid-derived coloration serves as an indicator of parasite levels and (or) the general health of birds. We investigated relationships among spleen size (sometimes considered an indicator of the cellular immune response capacity of birds), carotenoid level, helminth load, and leg and bill coloration of greylag geese, *Anser anser* (L., 1758), from Doñana National Park (southwest Spain). Nematode abundance was positively related to spleen size and negatively to body condition, but only in males. Coloration of bill and legs was a reliable indicator of cestode but not nematode abundance. Individuals with many cestodes had greater carotenoid stores, suggesting that helminths do not limit carotenoid absorption. Rather, it suggests either that parasitized geese were accumulating more carotenoids to control parasite-induced damage, or that carotenoid-rich diets expose geese to more intermediate hosts of cestodes. Our results support the role of integumentary carotenoid-derived coloration as an indicator of parasitism, but only for particular taxonomic groups.

Résumé : On a suggéré que la coloration dérivée des caroténoïdes peut servir d'indicateur de la charge parasitaire et(ou) de la santé globale des oiseaux. Nous avons examiné les relations entre la taille de la rate (quelquefois considérée comme un indicateur de la capacité de réaction immunitaire cellulaire des oiseaux), les concentrations de caroténoïdes, la charge d'helminthes, ainsi que la coloration des pattes et du bec chez l'oie cendrée, *Anser anser* (L., 1758), à la Parque National de Doñana (sud-ouest de l'Espagne). L'abondance des nématodes est en corrélation positive avec la taille de la rate et négative avec la condition corporelle, mais seulement chez les mâles. La coloration du bec et des pattes est un indicateur fiable de l'abondance des cestodes, mais pas de celle des nématodes. Les individus porteurs de nombreux cestodes ont des réserves plus importantes de caroténoïdes, ce qui laisse croire que les helminthes ne réduisent pas l'absorption des caroténoïdes. Au contraire, cela indique ou bien que les oies parasitées accumulent plus de caroténoïdes pour mitiger les dommages causés par les parasites, ou alors que les régimes alimentaires riches en caroténoïdes exposent les oies à un plus grand nombre d'hôtes intermédiaires de cestodes. Nos résultats appuient l'hypothèse qui veut que la coloration du tégument à partir des caroténoïdes soit un indicateur du parasitisme, mais seulement chez certains groupes taxonomiques particuliers.

[Traduit par la Rédaction]

Introduction

Some comparative analyses of the interaction between bird life histories and investment in immunity have used spleen size to estimate immune capacity (Møller et al. 1998, 2003; Morand and Poulin 2000; but see Smith and Hunt 2004). The spleen is thought to be involved in the production, maturation, and storage of lymphocytes, and in antibody synthesis and phagocytosis, although the functioning of the avian spleen is poorly understood (for a review see John 1994). The extent to which interspecific variation in spleen size reflects variation in investment in the immune system or just plastic responses to the level of infestation by parasites is not known (Smith and Hunt 2004). Spleen size changes seasonally (Silverin et al. 1999; Møller et al. 2003), but little is known about other factors related to intraspecific variation in spleen size. We are aware of only two studies analysing the relationship between spleen size and parasite load, with opposing results. Shutler et al. (1999) found little evidence for a relationship between parasitism by cestodes, trematodes, and nematodes and spleen size in snow geese, *Chen caerulescens* (L., 1758). However, Brown and Brown (2002) found a positive relationship between exposure to ectoparasites and spleen size in cliff swallows, *Petrochelidon pyrrhonota* (Vieillot, 1817).

Brightly ornamented individuals are presumed to signal their quality (health, immune capacity, knowledge regarding access to food, etc.) to potential mates or competitors (Andersson 1994). Carotenoids cannot be synthesized by birds and must be obtained from food (Fox 1976). These lipophilic molecules are responsible for most yellow, red, and orange coloration (Brush 1978). Carotenoids may also provide important health benefits because of their antioxi-

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dant and immunostimulating properties (Britton 1995; Surai et al. 2001). For these reasons, it has been suggested that there is trade-off between allocating carotenoids to fight parasites versus allocating them to carotenoid-derived, sexually selected ornamentation (Olson and Owens 1998). Endoparasites can reduce the capacity of organisms to absorb carotenoids through the digestive system (Allen 1987; McGraw and Hill 2000). In consequence, the presence of endoparasites will not only increase the demand for carotenoids for immunological functions (Møller et al. 2000), but may also affect the carotenoid-absorption rate. The possible trade-off between the health and ornamental functions of carotenoids, and the role of parasites and the immune system in determining the development of sexual ornaments, are foci of intense current research and debate (Møller et al. 2000; Lozano 2001; Brown and Brown 2002).

In this paper we analyse the relationships among helminth presence, spleen size, carotenoid reserves, and the expression of carotenoid-derived coloration in greylag geese, *Anser anser* (L., 1758). Greylag geese have a pinkish orange leg and bill colour derived from carotenoids. Their bills are redder during the breeding season (A.D. Fox, cited in Negro et al. 2001), and redder in adults than in immatures (Baker 1993). First, we analysed the association of carotenoid-derived coloration, spleen size, and endoparasite load. Second, we analysed the extent to which parasitism by helminths is related to carotenoid stores.

Materials and methods

As part of a study to develop non-intrusive techniques to analyse levels of xenobiotics in birds (Green et al. 2003), 45 greylag geese were shot under license in Doñana National Park (southwest Spain) between 14 and 29 January 2003. Body mass was measured to the nearest 1 g and wing chord to the nearest 1 mm. The colour characteristics of the bill and legs were determined by means of a Minolta DR200 colorimeter (Minolta Corporation 1994), using a standard D65 light flash (see Figuerola et al. 1999). Colour was characterized by its brightness, chroma, and hue. Hue corresponds to the wavelength of maximum reflectance and is expressed in degrees of a circle starting with red (0°), continuing through yellow (90°), green (180°), and blue (270°), and completing the circle with red. Chroma (also known as intensity or saturation) corresponds to colour purity on a scale from 0 for white to 100 for pure colour. Brightness corresponds to physical light intensity on a scale from 0 for black to 100 for white.

Before colour was measured, the bill and legs were wiped with a wet paper towel to remove dirt. Colour was measured on the front, tip, and both sides of the bill (4 measurements). Tarsus coloration was measured at the interdigital palm and at the external side of each leg (3 measurements per leg). Colour variables were analysed as the average values for bill and legs.

Birds were sexed by dissection and their intestines were labeled and deep-frozen until helminthological examination. Spleen and liver mass were measured to the nearest 0.01 g and a sample of blood was taken by heart puncture. Blood was centrifuged (10 min at 2500 r/min at 601*g*) and plasma stored at –20 °C until analysis of carotenoids. Fat stores in the belly were removed manually and weighed (±0.01 g). Three juvenile (less than 1 year old) birds were excluded from the analyses to reduce age-related effects in the variables analysed, blood could not be sampled from one of the birds, and colour measurements were not available for another individual, so the final sample size was 41 (40 for analyses of coloration).

Helminthological analysis

All intestinal tracts of greylag geese were thawed at ambient temperature and the small and large intestines were removed. Each section was immediately placed in an individual container filled with tap water to prevent desiccation of the tissue. Each section was split longitudinally and the mucosa scraped using the blunt handles of dissecting scissors. All helminths found were removed by forceps or pipetting into vials containing fixative and sorted into Trematoda, Cestoda, and Nematoda. The fixative used was a mixture of 85% ethanol, 10% glacial acetic acid, and 5% formalin for Platyhelminthes and 70% ethanol for Nematoda. Helminths were processed according to the species, identified, and counted. Trematodes and cestodes were stained with Semichon's acetic carmine, dehydrated, and permanently mounted in Canada balsam. Nematodes were studied using lactophenol wet mounts in depression slides. Previous descriptions were used to identify helminth species (Levine 1980; Moravec 1982; Moravec et al. 1987; Cordero del Campillo et al. 1994; Cordero del Campillo and Rojo Vázquez 1999). Some cestodes were only identified as belonging to the family Hymenolepididae because they were not properly fixed and their scolices were lost.

Carotenoid analyses

Pigments were extracted from plasma by adding acetone to the plasma samples in a ratio of 1:1 (*v*/*v*). The mixture was centrifuged at 13 000 r/min at 16 249*g* for 10 min to precipitate the flocculant proteins (Negro and Garrido 2000). The supernatant was retained and stored at –20 °C until high-performance liquid chromatography (HPLC) analysis. Carotenoids in fat samples were extracted and purified by saponification as described by Negro et al. (2001). Liver samples were treated like the fat samples above except that they were previously extracted with acetone (9–35 g liver / 30 mL acetone) and then transferred to 50 mL of diethyl ether. The resulting pigments, from both fat and liver, were redissolved in an adequate volume of acetone and kept frozen at –20 °C until analysis by HPLC.

A Jasco PU-2089 Plus instrument equipped with a quaternary pump (Jasco Analítica Spain, S.L., Madrid) was used for carotenoid analyses, with a reverse-phase C_{18} column (Phenomenex Synergi 4 µ) and a precolumn of the same material with a particle size of 5 µm. Samples were prefiltered using an OEM nylon filter, 0.45 µm × 4 mm) and later injected using a Rheodyne 7725i valve equipped with a 20-µL loop (Rheodyne, Rohnent Park, California, USA). The eluent system was that described in Mínguez-Mosquera and Hornero-Méndez (1993), except that the flow rate was 1 mL·min^{-1}. Data were acquired between 195 and 650 nm with a multiwavelength detector (MD-2010 Plus, Jasco Analítica Spain, S.L.).

Reference carotenoids were obtained from fresh green plants in J. Garrido's laboratory, according to Mínguez-Mosquera (1997). Known dilutions of reference lutein and β-carotene were injected into the HPLC instrument to build a calibration curve at 450 nm. The concentration of individual carotenoids was calculated from HPLC areas recorded at 450 nm. The total carotenoid concentration used in the analyses was obtained by adding together the lutein and β-carotene values of each individual.

Statistical analyses

Spleen sizes and abundances of Nematoda or Cestoda were log-transformed to fit a normal distribution. Body condition of geese (defined here as size-corrected body mass) was analysed by regarding body mass as the dependent variable, while wing length was entered in the model as a covariate to control for differences in structural size (García-Berthou 2001). Carotenoid concentrations in liver and fat were expressed in absolute terms (by multiplying the concentration per gram of tissue by the mass of the liver and fat of each individual), to control for individual differences in the size of liver and fat stores, given the important negative relationship between carotenoid concentration and size of fat stores (see Negro et al. 2001). Log-transformed carotenoid concentrations in liver, fat, and blood were analysed with a principal component analysis. Two components yielded eigenvalues larger than 1 and were retained as interpretable (Kaisser–Guttman criterion; Guttman 1954). The first principal component (PC1) explained 42.4% of the variance and represents an estimate of the carotenoid concentration in liver and blood (loadings were 0.70250 for liver, 0.12694 for fat, and 0.70027 for blood). The second principal component (PC2) summarized 33.2% of the variance and is mainly related to carotenoids in fat (loadings were –0.07190 for liver, 0.99159 for fat, and –0.10761 for blood).

Sex and its two-way interactions with the other independent variables were also included in the initial models. For model selection we used a stepwise backwards removal procedure by eliminating the less explanatory variable and recalculating the model until all the variables in the model were significant at $\alpha = 0.05$ and retaining the main effects of significant two-way interactions. Our models could have suffered from over-parameterization, given the number of factors included in the initial models in relation to the number of observations. To reduce the biases that this may have introduced, model selection was also based on the Akaike Information Criterion (AIC). Models with the lowest AIC values are preferred and represent an equilibrium between increased model fit and increases in the number of explanatory variables (Burnham and Anderson 1998). Differences in AIC of less than 2 represent models with equal support from the data, and in this case we selected the model with fewer parameters (Burnham and Anderson 1998). Final models were always supported by AIC and backwards selection based on the *P* value. In our analyses we considered 6 different dependent variables at the risk of reporting as statistically significant, relationships that were due to chance. We have not applied a Bonferroni or any similar correction, for several reasons. First, our small sample size results in low statistical power (capacity to detect real correlations), and any correction for multiple tests will further reduce the power. Second, reducing the number of analyses would lead to a loss of detail in our results without any significant change in the output of the presented tests (for further discussion see Moran 2003). And third, a significant increase in sample size is not desirable for ethical reasons.

Results

Endoparasite community

Nine helminth species were found (prevalence is shown in parentheses): two trematodes, *Echinostoma* sp. aff. *revolutum* Rudolphi, 1809 (2.4%) and *Notocotylus attenuatus* (Rudolphi, 1809) (2.4%); three cestodes, *Drepanidotaenia lanceolata* (Bloch, 1782) (9.5%), *Tschertkovilepis setigera* (Froelich, 1789) (14.3%), and unidentified hymenolepidids (7.1%); and four nematodes, *Amidostomum anseris* (Zeder, 1800) (2.4%), *Heterakis dispar* (Schrank, 1790) (9.5%), *Baruscapilaria anseris* (Madsen, 1945) (88.1%), and *Trichostrongylus tenuis* (Eberth) (95.2%). All the geese sampled had between one and five different species, with 2.3 ± 0.1 species/individual (mean ± SE) and 27.8 ± 3.3 helminths/individual.

Parasite abundance and host condition

Cestode and nematode abundances and carotenoid concentrations in liver and blood (PC1) were negatively related to body condition, while no significant relationship with spleen size was found (Table 1). A significant interaction between body condition and nematode abundance was found: the relationship was significant for males ($F_{[1,18]} = 8.60$, $P = 0.009$) but not for females ($F_{[1,13]} = 0.10$, $P = 0.75$; Fig. 1).

Spleen size and endoparasites

Spleen size was positively related to nematode abundance ($F_{[1,40]} = 5.42$, $P = 0.03$) but unrelated to cestode abundance, carotenoid concentration (PC1 or PC2), sex, or the interaction of the other factors with sex ($P > 0.14$).

Integumentary coloration as an indicator of endoparasite burden, carotenoid level, or spleen size

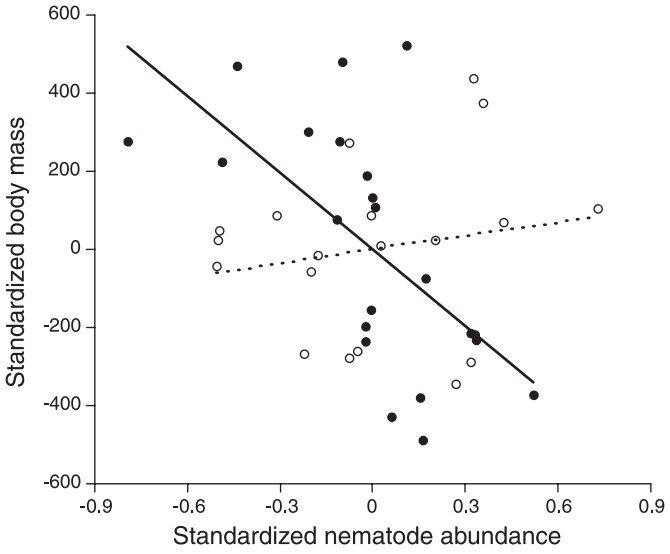
Brightness was negatively related to carotenoid concentrations in liver and blood (PC1) but positively with carotenoid concentration in fat (PC2). Although spleen size was not significantly related to brightness, a significant interaction between sex and spleen size occurred (Table 2), indicating a negative relationship between spleen size and brightness that was only significant in males ($F_{[1,17]} = 8.20$, $P = 0.01$; females: $F_{[1,13]} = 0.26$, $P = 0.64$; Fig. 2). Chroma was positively related only to cestode abundance ($F_{[1,39]} = 10.15$, $P = 0.0003$), while all the other factors were not significant ($P > 0.73$). Hue was also positively related to cestode abundance ($F_{[1,36]} = 11.81$, $P = 0.002$) and was significantly related to carotenoid concentration in fat (PC2) ($F_{[1,36]} = 6.77$, $P = 0.01$; $P > 0.09$ for all the other factors), indicating that the relationship with hue was only significant when carotenoid concentrations in fat ($F_{[1,38]} = 6.94$, $P = 0.01$) but not in liver ($F_{[1,38]} = 1.21$, $P = 0.28$) or blood ($F_{[1,37]} = 1.42$, $P = 0.24$) were considered.

Table 1. Results of multiple regression analysing variability in body mass in greylag geese (*Anser anser*).

	Slope (mean ± SE)	*F*	*P*
Sex		$2.49_{[1,34]}$	0.12
Wing length	13.17±2.27	$33.66_{[1,34]}$	<0.0001
Nematode abundance	–274.51±124.73	$4.77_{[1,34]}$	0.04
Cestode abundance	–486.45±193.98	$6.29_{[1,34]}$	0.02
Spleen size		$0.57_{[1,33]}$	0.46
Carotenoid concn. (PC1)	–85.87±39.34	$4.76_{[1,34]}$	0.04
Carotenoid concn. (PC2)		$0.45_{[1,33]}$	0.51
Sex × wing length		$0.16_{[1,33]}$	0.69
Sex × nematode abundance	313.20±123.60	$6.42_{[1,34]}$	0.02
Sex × cestode abundance		$0.00_{[1,33]}$	0.97
Sex × spleen size		$0.28_{[2,32]}$	0.76
Sex × carotenoid concn. (PC1)		$0.24_{[1,33]}$	0.63
Sex × carotenoid concn. (PC2)		$0.23_{[2,32]}$	0.79

Note: Model selection followed a backwards removal procedure. Only variables significant at the 0.05 level and the main factors of significant interactions were retained in the model. For variables not included in the final model the significance when added to that model is given. Slopes are given for significant variables.

Fig. 1. Relationship between body mass (standardized by regression of the other variables in the final model in Table 1, excluding nematode abundance) and nematode abundance (also standardized by regression of the other independent variables in the models in Table 1) for male (●) and female (○) greylag geese (*Anser anser*). The solid line is the regression line for males and the dotted line is the regression line for females.



Endoparasite burden and carotenoid concentration

Cestode abundance was positively related to carotenoid concentrations in liver and blood ($F_{[1,39]} = 11.28$, $P = 0.002$), while none of the other factors was retained in the model ($P > 0.51$). No relationship between parasite levels and carotenoid concentration in fat was found ($P > 0.51$ for all factors).

Discussion

Our results support the hypothesis that carotenoid-derived colours are affected by parasite levels in birds, but suggest that the relationship is specific to particular parasite groups. Parasites vary greatly in their effects upon hosts, and these effects can also be modulated by stressful environmental conditions (Sanz et al. 2002; Hanssen et al. 2003). Effects on carotenoid levels and coloration were related to the abundance of cestodes but not nematodes.

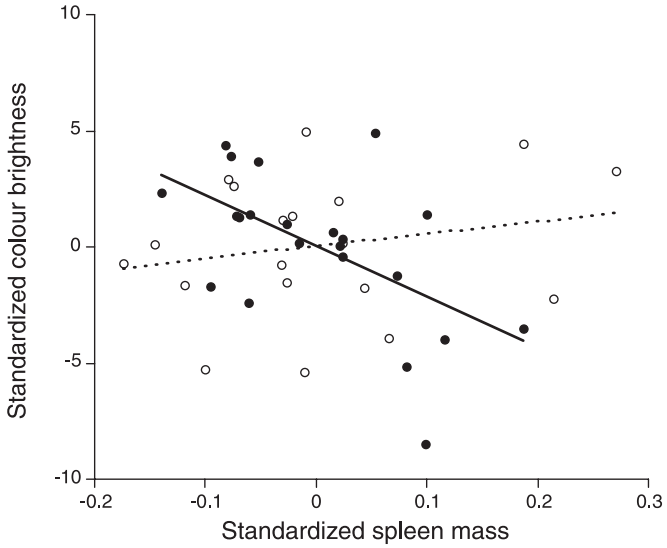
The abundances of both parasite groups were negatively related to host body condition, supporting the hypothesis that these endoparasites had a negative effect on birds or that birds in poor body condition are unable to control their infections. The most surprising results of our study are the negative relationship between carotenoid concentration and body condition and the positive relationship between cestode abundance and carotenoid concentration. There are several non-mutually exclusive hypotheses that can explain these relationships. First, carotenoids may be more abundant because they are being concentrated in the tissues, especially the liver, to control physiological stress, so we are in fact measuring not carotenoid availability but rather the carotenoid requirements of the organisms. Helminth infection and other stressors induce catabolism of proteins in muscle and synthesis of acute-phase proteins in liver, and other metabolic changes (Colditz 2003) that can increase the demand for carotenoids in the liver. This interpretation conflicts with the view which is sometimes espoused that carotenoids are limiting in the environment (Endler 1980; see the discussion in Lozano 2001). Although this may be true for some species with carotenoid-poor diets (see Tella et al. 2004), the vegetarian diet of greylag geese is very rich in carotenoids, so the geese are not likely to be limited in their access to these substances. A second possible explanation is that individuals feeding on more carotenoid-rich diets may be more exposed to cestodes. Geese in Doñana National Park feed mainly on grasses, tubers, and aerial parts of *Scirpus* spp. (Amat et al. 1991), although individuals with well-differentiated diets seem to coexist during the winter (some birds feed in *Scirpus* marshes, while others regularly use rice fields). In this case the relationship between carotenoids, cestodes, and body condition could be a spurious result reflecting the dif-

Table 2. Results of multiple regression analysing variability in colour brightness.

	Slope (mean ± SE)	F	P
Sex	–1.17±0.43	$7.38_{[1,34]}$	0.01
Nematode abundance		$3.00_{[1,33]}$	0.09
Cestode abundance		$0.10_{[1,33]}$	0.75
Spleen size	–7.74±4.70	$2.71_{[1,34]}$	0.11
Carotenoid concn. (PC1)	–1.30±0.40	$10.74_{[1,34]}$	0.002
Carotenoid concn. (PC2)	0.99±0.44	$5.22_{[1,34]}$	0.03
Sex × nematode abundance		$1.57_{[2,32]}$	0.22
Sex × cestode abundance		$0.99_{[2,32]}$	0.38
Sex × spleen size	13.52±4.60	$8.63_{[1,34]}$	0.006
Sex × carotenoid concn. (PC1)		$1.49_{[1,33]}$	0.23
Sex × carotenoid concn. (PC2)		$1.19_{[1,33]}$	0.28

Note: Model selection followed a backwards removal procedure. Only variables significant at the 0.05 level and the main factors of significant interactions were retained in the final model. For variables not in the final model the significance when added to that model is given. Slopes are given for significant variables.

Fig. 2. Relation between colour brightness (standardized by regression of the other variables in the final model in Table 2, excluding spleen size) and spleen size (also standardized by regression of the other independent variables in the model in Table 2) for male (●) and female (○) greylag geese. The solid line is the regression line for males and the dotted line is the regression line for females.



ferences in diet and exposure to parasites of birds exploiting different habitats.

A third explanation is that geese are long-lived birds and the relationship between cestode abundance and carotenoid concentration simply reflects age-related increases in cestode (but not nematode) numbers and carotenoid stores. However, we excluded birds less than 2 years of age from our analyses. Although some differences in cestode prevalence and abundance have been reported between juvenile and adult birds in other species (e.g., Glass et al. 2002), these effects are by no means general, and age-related effects probably do not extend for more than the first few months of life (see e.g., Wallace and Pence 1986; Haukos and Neaville 2003).

Finally, some studies have suggested that high levels of carotenoids can have deleterious effects on host condition (Olson and Owens 1998; Hartley and Kennedy 2004). However, no toxic levels are currently recognised for birds, and the levels found in our study do not seem high enough, so we can refute this hypothesis. Further experimental work is necessary to test the relevance of the two first hypotheses. In any case we failed to find support for the hypothesis that helminths reduce the levels of carotenoids in their hosts; in fact, the results indicate the opposite.

Carotenoid concentration was related to two of the three components of coloration analysed. While abundance of carotenoids in liver and blood was related to brightness, hue was related to carotenoid levels in fat but not in liver or blood. Consequently, bill and leg integumentary colours in greylag geese reflect the carotenoid levels in individuals. In the case of the spleen, nematode but not cestode abundance was related to larger organ size. Based on previous intra- and inter-specific analyses, similar conclusions have been reached, namely that individuals or species that are more exposed to nematodes have a larger spleen, while no such relationship is apparent with cestodes or other helminth groups (John 1995; Shutler et al. 1999). Interestingly, Sagerup et al. (2000) reported a lack of association between intensity of parasitism by helminths and levels of organochlorines in the host. However, a positive relationship was found between intensity of nematode infection and organochlorine levels. The authors considered that this supported a negative impact of PCBs on the avian immune system, and on the ability to resist nematodes, but apparently not cestodes. Although the spleen can be involved in the immune response to cestode larvae, it is less important for the control of adult cestodes (John 1995). Greylag geese are the final hosts of all the cestode species found, and become infected by consuming the intermediate hosts in which the larvae have developed, and this may explain the lack of a relationship between cestode abundance and spleen size.

The negative relationship between nematode abundance and body condition and between spleen size and colour brightness were significant for males but not for females. These patterns have also been reported in some previous studies on the susceptibility of birds to parasites (e.g., Atkinson et al.

1995; Bosch et al. 2000). Males have higher blood levels of testosterone, a hormone presumed to have an immunosuppressive effect (Owens and Wilson 1999). Testosterone supplementation can reduce spleen size in mice (Harder et al. 1992). We consider that this factor may explain the sex-dependent nature of the relationships between nematode abundance and body condition and between spleen size and colour brightness

In conclusion, coloration was related to the presence of some endoparasite groups, but was not a good indicator of parasitism levels. We also found support for the hypothesis that some parasite groups are associated with greater spleen development. An overview of the few studies published up to now suggest positive relationships of spleen size with nematode abundance (Shutler et al. 1999; this study) and ectoparasite abundance (Brown and Brown 2002) but no relationship with cestode or trematode abundance (Shutler et al. 1999; this study). Finally, carotenoid concentration is related to cestode abundance and spleen size, but in a way that is more consistent with mobilization to cope with higher stress levels than with depletion of carotenoid stores as a result of increased stress or reduced absorption rates caused by the presence of helminths. Further experimental work is necessary to unravel the nature of the relationships among spleen size, carotenoid concentration, and endoparasite-induced stress.

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References

Allen, P.C. 1987. Physiological responses of chicken gut tissue to coccidial infection: comparative effects of *Eimeria acervulina* and *Eimeria mitis* on mucosal mass, carotenoid content, and brush border enzyme activity. Poult. Sci. **66**: 1306–1315.

Amat, J.A., García-Criado, B., and García-Ciudad, A. 1991. Food, feeding behaviour and nutritional ecology of wintering Greylag Geese *Anser anser.* Ardea, **79**: 271–282.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, N.J.

Atkinson, C.T., Woods, K.L., Dusek, R.J., Sileo, L.S., and Iko, W.M. 1995. Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). Parasitology, **111**: S59–S69.

Baker, K. 1993. Identification guide to European non-passerines. British Trust for Ornithology, Thetford.

Bosch, M., Torres, J., and Figuerola, J. 2000. A helminth community in breeding Yellow-legged Gulls (*Larus cachinnans*): pattern of association and its effect on host fitness. Can. J. Zool. **78**: 777–786.

Britton, G. 1995. Structure and properties of carotenoids in relation to function. FASEB J. **9**: 1551–1558.

Brown, C.R., and Brown, M.B. 2002. Spleen volume varies with colony size and parasite load in a colonial bird. Proc. R. Soc. Lond. B Biol. Sci. **269**: 1367–1373.

Brush, A.H. 1978. Avian pigmentation. *In* Chemical zoology. Vol. X. *Edited by* A.H. Brush. Academic Press, New York. pp. 141–161.

Burnham, K.P., and Anderson, D.R. 1998. Model selection and inference: a practical information–theoretic approach. Springer-Verlag, New York.

Colditz, I.G. 2003. Metabolic effects of host defence responses during gastrointestinal parasitism in sheep. Aust. J. Exp. Agric. **43**: 1437–1443.

Cordero del Campillo, M., and Rojo-Vázquez, F.A. 1999. Parasitología veterinaria. McGraw–Hill Interamericana, Madrid, Spain.

Cordero del Campillo M., Castañon-Ordoñez, L., and Reguero-Feo, A. 1994. Indice–catálogo de zooparásitos ibéricos. Secretariado de Publicaciones, Universidad de León, León, Spain.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution, **34**: 76–91.

Figuerola, J., Senar, J.C., and Pascual, J. 1999. The use of a colorimeter in field studies of blue tit *Parus caeruleus* coloration. Ardea, **87**: 269–275.

Fox, D.L. 1976. Animal biochromes and structural colours. University of California Press, Berkeley.

García-Berthou, E. 2001. On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. J. Anim. Ecol. **70**: 708–711.

Glass, J.W., Fedynich, A.M., Small, M.F., and Benn, S.J. 2002. Helminth community structure in an expanding white-winged dove (*Zenaida asiatica asiatica*) population. J. Wildl. Dis. **38**: 68–74.

Green, A.J., Mateo, R., Lefranc, H., Figuerola, J., and Taggart, M. 2003. El estudio del plumbismo en Doñana y otros humedales andaluces. Final report, Junta de Andalucía, Sevilla, Spain.

Guttman, L. 1954. Some necessary conditions for common factor analysis. Psychometrika, **19**: 149–161.

Hanssen, S.A., Folstad, I., Erikstad, K.E., and Oksanen, A. 2003. Costs of parasites in common eiders: effects of antiparasite treatment. Oikos, **100**: 105–111.

Harder, A., Wunderlich, F., and Marinovski, P. 1992. Effects of testosterone on *Heterakis spumosa* infections in mice. Parasitology, **105**: 335–342.

Hartley, R.C., and Kennedy, M.W. 2004. Are carotenoids a red herring in sexual display? Trends Ecol. Evol. **19**: 353–354.

Haukos, D.A., and Neaville, J. 2003. Spatial and temporal changes in prevalence of a cloacal cestode in wintering waterfowl along the gulf coast of Texas. J. Wildl. Dis. **39**: 152–160.

John, J.L. 1994. The avian spleen: a neglected organ. Q. Rev. Biol. **69**: 327–351.

John, J.L. 1995. Parasites and the avian spleen: helminths. Biol. J. Linn. Soc. **54**: 87–106.

Levine, N.D. 1980. Nematode parasites of domestic animals and of man. Burgess Publishing Co., Minneapolis, Minn.

Lozano, G.A. 2001. Carotenoids, immunity, and sexual selection: comparing apples with oranges? Am. Nat. **158**: 200–203.

McGraw, K.J., and Hill, G.E. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc. R. Soc. Lond. B Biol. Sci. **267**: 1525–1531.

Mínguez-Mosquera, M.I. 1997. Clorofilas y carotenoides en tecnología de los alimentos. Universidad de Sevilla, Sevilla, Spain.

Mínguez-Mosquera, M.I., and Hornero-Méndez, D. 1993. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika and oleoresin by reversed-phase HPLC. J. Agric. Food Chem. **41**: 1616–1620.

Minolta Corporation. 1994. Precise color communication: color control from feeling to instrumentation. Minolta Corp., Ramsey, N.J.

Moran, M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos, **100**: 403–405.

Morand, S., and Poulin, R. 2000. Nematode parasite species richness and the evolution of spleen size in birds. Can. J. Zool. **78**: 1356–1360.

Moravec, F. 1982. Proposal of a new systematic arrangement of nematodes of the family Capillariidae. Folia Parasitol. **29**: 119–132

Moravec, F., Prokopic, J., and Shlikas, A.V. 1987. The biology of nematodes of the family Capillariidae Neveu-Lemaire, 1936. Folia Parasitol. **34**: 39–56.

Møller, A.P., Christe, P., Erritzøe, J., and Mavarez, J. 1998. Condition, disease and immune defence. Oikos, **83**: 301–306.

Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N., and Surai, P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poult. Biol. Rev. **11**: 137–159.

Møller, A.P., Erritzøe, J., and Saino, N. 2003. Seasonal changes in immune response and parasite impact on hosts. Am. Nat. **161**: 657–671.

Negro, J.J., and Garrido, J. 2000. Astaxanthin is the major carotenoid in tissue of white storks (*Ciconia ciconia*) feeding on introduced crayfish (*Procambarus clarkii*). Comp. Biochem. Physiol. B, **126**: 347–352.

Negro, J.J., Figuerola, J., Garrido, J., and Green, A.J. 2001. Fat stores in birds: an overlooked sink for carotenoid pigments? Funct. Ecol. **15**: 297–303.

Olson, V.A., and Owens, I.P.F. 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol. Evol. **13**: 510–514.

Owens, I.P.F., and Wilson, F. 1999. Immunocompetence: a neglected life history trait or conspicuous red herring? Trends Ecol. Evol. **14**: 170–172.

Sagerup, K., Henriksen, E.O., Skorping, A., Skaares, J.U., and Gabrielsen, G.W. 2000. Intensity of parasitic nematodes increases with organochlorine levels in the glaucous gull. J. Appl. Ecol. **37**: 532–539.

Sanz, J.J., Moreno, J., Arriero, E., and Merino, S. 2002. Reproductive effort and blood parasites of breeding pied flycatchers: the need to control for interannual variation and initial health state. Oikos, **96**: 299–306.

Shutler, D., Alisauskas, R.T., and McLaughlin, J.D. 1999. Mass dynamics of the spleen and other organs in geese: measures of immune relationships to helminths? Can. J. Zool. **77**: 351–359.

Silverin, B., Fänge, R., Viebke, P.-A., and Westin, J. 1999. Seasonal changes in mass and histology of the spleen in willow tits *Parus montanus*. J. Avian Biol. **30**: 255–262.

Smith, K.G., and Hunt, J.L. 2004. On the use of spleen mass as a measure of avian immune system strength. Oecologia (Berl.), **138**: 28–31.

Surai, P.F., Speake, B.K., and Sparks, N.H.C. 2001. Carotenoids in avian nutrition and embryonic development. 1. Absorption availability and levels in plasma and egg yolk. J. Poult. Sci. **38**: 1–27.

Tella, J.L., Figuerola, J., Negro, J.J., Blanco, G., Rodríguez-Estrella, R., Forero, M.G., et al. 2004. Ecological, morphological and phylogenetic correlates of interspecific variation in plasma carotenoid concentration in birds. J. Evol. Biol. **17**: 156–164.

Wallace, B.M., and Pence, D.B. 1986. Population dynamics of the helminth community from migrating blue-winged teal: loss of helminths without replacement on the wintering grounds. Can. J. Zool. **64**: 1765–1773.