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**Antioxidant supplementation during early development
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expression in adult ring-necked pheasants *Phasianus
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3 **Antioxidant supplementation during early development reduces parasite load but does not**
4 **affect sexual ornament expression in adult ring-necked pheasants *Phasianus colchicus***

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6 **Running headline: Early life-history trade-offs in pheasants.**

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1 *Summary*

- 2 1. The ‘parasite-mediated sexual selection’ (PMSS) hypothesis predicts that exaggerated male
3 ornamentation could provide a signal to females of a males ability to resist parasites.
4 Empirical tests of the PMSS have been largely equivocal, however, which may be because
5 most have not considered the role of early life-history effects.
- 6 2. Many sexually-selected traits are carotenoid-based. Allocation of dietary-derived carotenoids
7 to sexual ornaments may trade-off with allocation to pro-inflammatory immune response
8 and/or antioxidant functions, mediated by the oxidative status of individuals. Exposure to
9 parasites can increase oxidative stress, so under this scenario sexually-selected traits indicate
10 ability to resist oxidative stress rather than ability to resist parasites per se. Such life-history
11 trade-offs, mediated by oxidative status of individuals, are particularly acute during growth
12 and development.
- 13 3. Here we use ring-necked pheasants, *Phasianus colchicus*, a strongly sexually-selected
14 species, to test whether supplementation with dietary antioxidants (vitamin E) can mitigate
15 the effects of early exposure to parasites (the nematode, *Heterakis gallinarum*), via alteration
16 of the oxidative status of individuals, and positively affect the expression of sexual ornaments
17 at adulthood.
- 18 4. We found that vitamin E mediated the effect of early exposure to parasites on levels of
19 oxidative damage at 8 weeks of age and reduced the parasite load of individuals at adulthood
20 as predicted. However, the expression of sexual ornaments, immune function, and growth
21 were unaffected by either early vitamin E supplementation or manipulation of parasite load.
22 In contrast to the predictions of the PMSS hypothesis the intensity of sexual ornament
23 expression was not related to either parasite load or oxidative status of individuals (current or
24 long-term). Consequently there was no evidence that the expression of sexual ornaments
25 provided information on the ability of males to resist infection from parasites.

26

27 **Key words: sexual selection, oxidative damage, antioxidants, trade-offs, growth**

1 INTRODUCTION

2 Females in many animal species prefer to mate with the most elaborately ornamented males
3 (Andersson & Simmons 2006). In species in which males contribute nothing beyond their sperm
4 (Kirkpatrick & Ryan 1991; Andersson 1994) females are expected to choose mates based on
5 'indirect benefits' (Borgia 1979; Reynolds & Gross 1990); males differ in their viability and quality
6 so that mate preference confers genetic benefits to the fitness of offspring ('good genes'; Norris
7 1993; Petrie 1994; Wedell & Tregenza 1999). More specifically, Hamilton and Zuk (1982) suggested
8 that exaggerated male ornamentation could provide a signal to females of their ability to resist
9 parasite infection (the 'parasite-mediated sexual selection' or 'bright male' hypothesis). If the ability
10 to resist parasites is heritable then females could improve the fitness of their offspring by choosing
11 males with the most exaggerated ornaments (Hamilton & Poulin 1997). Experiments with controlled
12 infections show that sexual ornaments are more sensitive to parasite infection than other
13 morphological traits (Zuk, Thornhill & Lignon 1990; Houde & Torio 1992; Møller 1994). Therefore,
14 females could potentially choose males for their genetic quality (disease resistance) based on the
15 expression of their sexually selected traits (Hamilton & Zuk 1982). Tests of Hamilton and Zuk's idea
16 has produced equivocal results however (Hamilton & Poulin 1997; Møller et al. 1999; Getty 2002),
17 and one reason for this may be because the majority of studies only consider parasite infections in
18 adults and do not consider early life-history effects (Borgia et al. 2004).

19

20 Sexually selected traits are often highly sensitive to variations in the environmental conditions
21 experienced during growth and development (e.g. David et al. 2000; Ohlsson et al. 2002; McGraw et
22 al. 2005; Royle et al. 2005). Despite this very few studies have assessed how exposure to parasites
23 during life-history stages prior to adulthood affects the expression of sexually selected traits. Borgia
24 *et al.* (2004) studied adult satin bowerbirds to determine whether male display could provide an
25 indication of parasitic infections experienced during juvenile life history stages. They found that the
26 most attractive males were those that had experienced a lower parasite burden as juveniles, whilst no
27 significant relationship was found to exist between current adult parasite burden and male

1 attractiveness (Borgia et al. 2004). If sexually-selected traits reflect the long-term condition of
2 individuals and/or the ability to cope with environmental insult throughout development this is likely
3 to be more informative of genetic quality than traits that just reflect current condition, which may be
4 more transient in character.

5

6 Many sexually-selected traits expressed in birds and fish in particular are carotenoid-based.
7 Carotenoids are dietary derived, highly pigmented antioxidants that have immuno-enhancing
8 properties (Blount et al. 2003; McGraw & Ardia 2003). The intensity of the colouration of
9 carotenoid-mediated traits has been found to be negatively affected by parasite burden in many
10 species (Milinski & Bakker 1990; Zuk et al. 1990; Houde & Torio 1992; Thompson et al. 1997;
11 Brawner, Hill & Sundermann 2000; McGraw & Hill 2000; Baeta et al. 2008; Mougeot et al. 2010).
12 The intensity of parasite infection can affect carotenoid-mediated ornament expression either
13 directly, by reducing the ability of an individual to assimilate carotenoids (Hörak et al. 2004), or by
14 affecting resource allocation trade-offs between signalling and self-maintenance (Martinez-Padilla et
15 al. 2007).

16

17 The allocation of carotenoids to signalling is therefore expected to reduce the amount available for
18 allocation to immune function (Lozano 1994). Moreover, activation of the immune system in
19 response to parasite infection also results in the production of higher amounts of reactive oxygen
20 species (ROS) during the respiratory burst activity of phagocytes (Babior 1984), leading to increased
21 potential for oxidative stress. Oxidative stress results from an imbalance between the production of
22 damaging ROS and antioxidant defences (Sies 1997). Carotenoids are also antioxidants, so the
23 intensity of carotenoid-mediated sexually selected traits may therefore signal the oxidative status of
24 individuals (von Schantz et al. 1999). There is increasing evidence that oxidative stress provides a
25 potentially unifying mechanism that mediates fundamental resource allocation trade-offs underlying
26 the evolution of life-history traits in animals (e.g. Costantini 2008; Monaghan, Metcalfe & Torres
27 2009; Hall et al. 2010). Under this scenario early exposure to parasite infection can be viewed as a

1 contributory factor influencing oxidative stress, so that sexually-selected traits do not reflect
2 exposure to parasites *per se*, but the oxidative status of individuals. However, the antioxidant
3 properties of carotenoids are thought to be comparatively poor compared to non-pigmentary
4 antioxidants such as vitamin E (Costantini & Moller 2008) and it has been suggested that the
5 presence of carotenoid based signals may, instead, signal the prevalence of these more efficient, non-
6 pigmentary, antioxidants ('The carotenoid protection theory'; Hartley & Kennedy 2004). This is
7 supported by the observation that oxidation causes the structural alteration of carotenoids, rendering
8 them colourless and therefore not available for signalling (Hartley & Kennedy 2004).

9

10 Previous studies testing the carotenoid protection theory have been conducted on adults (e.g.
11 Bertrand, Faivre & Sorci 2006; Pike et al. 2007; Perez, Lores & Velando 2008). However, resource
12 allocation trade-offs are particularly prevalent during early growth and development (e.g. Cucco et
13 al. 2006; Hall et al. 2010) and can lead to long-lasting effects. Early diet can determine the ability to
14 assimilate and metabolise antioxidants in adulthood (Kim et al. 1996; Blount et al. 2003; Koutsos et
15 al. 2003; Orledge et al. 2012) for example, and somatic growth results in the production of higher
16 levels of ROS (Stoks, De Blok & McPeck, 2006). Supplementation of vitamin E during early
17 development resulted in increased circulating vitamin E at adulthood in zebra finches (Blount et al.
18 2003a) and pheasants (Orledge et al. 2012) suggesting that the quality of the rearing diet may
19 permanently affect the ability of individuals to assimilate circulating antioxidants at adulthood
20 (Blount et al. 2003a). The availability of dietary antioxidants, and the degree of environmental insult
21 (e.g. exposure to parasite infection) may therefore alter the balance of trade-offs during growth and
22 development that affect the expression of phenotypic traits during adulthood, such as sexual
23 ornaments, through affecting the oxidative status of individuals.

24

25 We used a sexually dimorphic galliform, the ring-necked pheasant, *Phasianus colchicus* (Fig. 1) as a
26 study species to examine whether supplementation of a non-pigmentary antioxidant (vitamin E)
27 could mitigate the effects of environmental insult (exposure to parasite infection) during early

1 development on the expression of sexually selected traits at adulthood (one year old), immune
2 function, oxidative damage and growth. Male ring-necked pheasants have bright plumage,
3 conspicuous wattles, long tail feathers, spurs and ear tufts. Females are smaller than males with a
4 duller yellowish buff plumage and a long banded tail. Pheasants exhibit a harem polygyny social
5 mating system and females choose mates based on multiple sexual ornaments (Hill & Robertson
6 1988). These ornaments include facial wattles (Hillgarth 1990), the colour of which is likely to be
7 carotenoid-mediated (Czeczuga 1979), and the length of spurs on the legs (Göransson et al. 1990).
8 The bright wattle of males is expanded during sexual displays to attract females (Hill & Robertson
9 1988) and females have been shown to prefer larger males (Göransson et al. 1990), and males with
10 larger wattles (Hillgarth 1990). We used the nematode *Heterakis gallinarum*, a major parasite of wild
11 ring-necked pheasants in the UK (Draycott et al. 2000), to manipulate the health of the birds during
12 development. *H. gallinarum* release single cell eggs into the host faeces that remain in the soil before
13 reaching the infective stage. Infection occurs through ingestion of the eggs from the soil or ingestion
14 of earthworms that can act as transport hosts. The eggs develop into adults in 14 days within the
15 caeca and begin ovipositing 24 to 36 days after infection (Olsen 1974).

16
17 If early life-history effects are important in determining the expression of traits in adults then we
18 predict that early exposure to both parasites and antioxidants will have long-term effects. Specifically
19 we predict that early exposure to parasites will lead to increased susceptibility (increased parasite
20 burden) in adulthood, and that access to supplementary dietary antioxidants (vitamin E) during early
21 growth will lead to an increase in circulating levels of antioxidants when mature. Furthermore we
22 predict that if oxidative stress is an important mechanism underlying trade-offs during development
23 then males supplemented with dietary antioxidants will have more resources available to allocate to
24 sexually selected traits than unsupplemented males. In contrast, males infected with parasites will
25 have higher levels of oxidative damage, so will have to allocate more resources to self-maintenance
26 and less will be available for the expression of sexually-selected traits. Individuals supplemented

1 with vitamin E are therefore expected to have more exaggerated sexual signals than those that
2 receive a control diet or individuals infected with parasites.

3

4 **MATERIALS AND METHODS**

5 *(a) General Methods and experimental design*

6 240 ring-necked day-old pheasants of mixed genetic stock (Holme Farm Hatcheries, Wokingham)
7 were allocated randomly to one of four treatment groups (n=60 in each treatment) at the Game and
8 Wildlife Conservation Trust HQ, Hampshire. The game farm that supplied the pheasants maintains
9 breeding stock in groups of 30 hens with 3 cock pheasants (i.e. replicating the natural harem
10 polygyny mating system). As a result, males and females have multiple potential copulation partners.
11 The pheasants are not intensively farmed or artificially selected for traits such as high egg production
12 or disease resistance either, so there is no evidence that the phenotypes of the pheasants are
13 uncoupled from past natural and sexual selection pressures. Treatment diets over the first 8 weeks
14 were (1) vitamin E supplement with addition of *Heterakis* nematode parasites (P-E) (2) vitamin E
15 supplement without parasites (NP-E) (3) control diet with *Heterakis* parasites (P-C) (4) control diet
16 without parasites (NP-C). An 8 week period of dietary manipulation was chosen to include the early
17 developmental window identified by previous studies on pheasants (Ohlsson & Smith 2001; Ohlsson
18 et al. 2002). Birds supplemented in treatment groups with *Heterakis* nematodes were infected at 21
19 days of age, the optimal age for successful infection in chickens (Olsen 1974). The diet provided
20 after 8 weeks was identical for all birds. Morphometric measurements were taken initially on day one
21 then subsequently at 8, 21 and 47 weeks of age. To assay plasma concentrations of vitamin E and
22 carotenoids blood samples were taken at 8 and 47 weeks of age and, because vitamin E is fat soluble
23 and known to be an important antioxidant in the lipid-rich cell membrane (Wang & Quinn 1999),
24 oxidative stress was measured by assay of the concentration of a biomarker of lipid peroxidation,
25 malondialdehyde (MDA) at 8 and 47 weeks of age. Phytohaemagglutinin injection was used to
26 measure immune response at 21 weeks of age. Sexual signals including wattle colour, size and shape
27 and spur length were measured at 47 weeks of age. Females may use multiple cues during mate

1 choice that may reflect different aspects of male quality (Candolin 2003), so although we focused on
2 a carotenoid-mediated trait, wattle colouration, we measured multiple pheasant ornaments. Previous
3 studies have shown that the expression of ornaments is responsive to dietary quality manipulation
4 during development (Ohlsson et al. 2001) and in adulthood (one year old; Smith et al. 2007).

6 (b) Husbandry

7 General husbandry followed standard pheasant rearing practice (The Game Conservancy 2006). For
8 the first 8 weeks (commencing in early May) birds were housed in groups of 30 in indoor pens (1.8m
9 x 1.5m) under dim light conditions within a semi-intensive brooder hut system. Additional (non-
10 experimental) birds were reared and introduced to experimental pens following mortality of
11 experimental birds as necessary, in order to maintain standardised rearing densities during the first 8
12 weeks (N = 8 birds). At 2 weeks of age birds were also given daily access to outdoor pens with wire
13 floors (3m x 1.5m). At 8 weeks of age the birds were sexed and then transferred to two outdoor
14 single-sex pens (30m x 27m) with access to grass for the remainder of the experiment.

16 (c) Dietary Supplementation

17 Vitamin E is used as a descriptor a group of compounds that include both tocopherols and
18 tocotrienols. In this study we supplemented treatment groups with α -tocopherol. However, we refer
19 to the supplement using the more general description of vitamin E throughout the paper. Vitamin E
20 was supplemented to the P-E and NP-E treatment groups at a concentration of 100mg/kg of feed. The
21 basal diet of individuals in the P-C and NP-C received no vitamin E supplement (0 mg/kg of feed).
22 Birds were given treatment diets from the day after hatching (day 1) until 8 weeks of age. The
23 concentration of vitamin E supplemented was chosen to match the concentrations used in previous
24 studies on poultry that have shown effects of vitamin E on lipid peroxidation following exposure to a
25 toxin (Hoehler and Marquardt 1996), improved growth and feed utilisation (Guo et al. 2001) and
26 increased plasma vitamin E concentrations (Bartov & Frigg 1992). Supplements were added to a
27 basal diet made to specification with no added vitamin E, low levels of vitamin A (10.0mg/kg) and

1 selenium (0.20mg/kg) (Target Feeds Ltd., Shropshire). All feed was sprayed daily using a 5 litre
2 spray pump with the following: Vitamin E supplementation (NP-E and P-E) – vitamin E was sprayed
3 in soybean oil onto the feed and stored in refrigerated vacuum pumped containers until it was given
4 to the birds. Soybean oil was selected as a medium for vitamin E supplementation because it contains
5 low levels of α -tocopherol (0.07 μ g/mg) in comparison to other food oils such as sunflower or olive
6 oils (Carpenter 1979). Equal volumes of soybean oil but without the supplemental vitamin E were
7 sprayed onto the other feeds (NP-E and P-E). Each afternoon the feed was replenished with fresh
8 refrigerated treatment feed. Following standard pheasant rearing practice four basal diets were
9 provided over the 8 week period of supplementation with medium levels of protein (starter crumb 1-
10 2 weeks: 29.8%, starter pellets 3-4 weeks: 25.5%, rearer pellets 5-6 weeks: 21.4%, grower pellets 7-8
11 weeks: 18.1%). Feed, grit and water were provided *ad libitum*. Protein levels therefore averaged
12 23.7% over the 8 week experimental period, which is mid-way between the levels used by Ohlsson *et*
13 *al.* (2001) in a previous experiment that manipulated the amount of protein available during the first
14 8 weeks of life (low protein diet = 20.5%, high protein diet = 27% protein). The overall protein
15 levels in our experiment were moderate in order to reduce the risk of high protein levels masking
16 among individual variation in quality. After 8 weeks of age all birds were fed a commercial feed with
17 a standard protein content (13%) for adult pheasants (Woodard *et al.* 1977; Sheppard *et al.* 1998).

18

19 (d) *Heterakis* infection and counts

20 *Heterakis gallinarum* eggs were embryonated by maintaining female nematodes in 0.5% formalin
21 solution at 21°C for 21 days. Eggs were then released by blending the female nematodes in saline
22 solution. Eggs were counted using a McMaster egg slide (Hawksley Ltd. Z11000) and the solution
23 was diluted with saline solution until a solution containing approximately 100 eggs per ml was
24 produced. Individuals were infected with *Heterakis gallinarum* eggs at 21 days of age. The timing of
25 infection was chosen to match the ‘optimal’ age of development for infection success (Olsen 1974).
26 A spring survey of wild hen pheasants in England found a median of 84 and range of 9-331 *H.*
27 *gallinarum* nematode worms per individual bird across 21 sites in England and Wales (Draycott *et al.*

1 2000). We also recorded similar numbers of nematodes in a sample of wild pheasants found dead on
2 the road (Orledge et al. *unpublished data*). Individual pheasant chicks were each infected with 100
3 embryonated *H. gallinarum* eggs administered directly into the throat in 1ml of saline using a pipette
4 (Tompkins et al. 2000; Sage et al. 2002). Tompkins et al. (2000) found that this dosage resulted in a
5 mean infection of 59 (\pm 14.83 SE) *H. gallinarum* worms. 1ml of saline solution without nematode
6 eggs was administered to individuals in treatment groups without infection. An infective dose of 100
7 eggs was used, as this was the largest number that could be used to avoid documented density-
8 dependent effects on *H. gallinarum* fecundity (Tompkins & Hudson 1999). The nematode *Heterakis*
9 *gallinarum* is found in the lumen of the caecum and occasionally in the small intestine. At 47 weeks
10 of age, all individuals were euthanized and dissected and the numbers of *Heterakis gallinarum* were
11 counted. Each caecum was cut open and the contents were scraped from the gut lining into a fine
12 mesh sieve (aperture 100 microns). The worms were then washed into a petri dish and counted
13 (Doster and Goater 1997).

14

15 (e) *Morphometric measurements*

16 The morphometric measurements of individuals were recorded at 0, 8, 21 and 47 weeks of age. Body
17 mass was measured using a variety of Pesola® spring balances (30g, 60g, 100g, 300g, 600g, 1000g,
18 2500g). Tarsus length and head to bill length were measured using a sliding calliper (\pm 0.01mm) and
19 wing length was recorded using a wing rule (\pm 0.1mm). Spur length was measured at 21 and 47
20 weeks using dial calliper measurements of the tarsus width just above the spur and by subtracting
21 this from a measurement of the tarsus width and spur length (Ohlsson et al. 2001).

22

23 (f) *Measurement of plasma antioxidants and oxidative stress*

24 Blood samples were taken at 8 weeks (at the end of the supplementation period) and at 47 weeks of
25 age. Whole blood (up to 0.3ml) was collected from the brachial vein under Home Office licence in
26 5/8" 26 gauge Microlance™ needles (Fisher Scientific UK Ltd.) and BD Plastipak™ 1ml syringes
27 (Fisher Scientific UK Ltd.) flushed with heparin (Sigma-Aldrich Inc.) and microhaematocrit EDTA-

1 coated capillary tubes (Bilbate Ltd.). Syringe samples were transferred to 1.5ml EDTA-coated micro
2 tubes (Sarstedt) and stored in a dark cool bag. The samples were centrifuged and plasma was
3 removed and stored at -20°C within 1 hour of collection. The samples were then transferred to a -
4 80°C freezer within 5 days before biochemical analysis.

5

6 α -Tocopherol was measured within a month using high-performance liquid chromatography
7 (HPLC). Plasma (50 μ l) was mixed with 5% sodium chloride (50 μ l) and ethanol (100 μ l). The mixture
8 was vortexed for 20s. Hexane (600 μ l) was added to the solution and vortexed for 20s and centrifuged
9 for 4min (13.8 x g). The hexane layer was removed and the absorbance measured at 450nm using a
10 spectrophotometer (Nicolet Evolution 500) to determine total carotenoid concentration using 2500 as
11 an average extinction coefficient for all carotenoids. The hexane (400 μ l) was dried down and
12 samples redissolved in methanol (150 μ l), centrifuged for 4 minutes, then injected (50 μ l) into a
13 Dionex HPLC system (Dionex Corporation, California, USA) fitted with a 3 μ C₁₈ reverse-phase
14 column (15 cm x 4.6 mm) (Spherisorb S30DS2; Phase separations, Clwyd, UK) and using a mobile
15 phase of methanol:distilled water (97:3) at a flow rate of 1.1ml min⁻¹. Fluorescence detection was
16 carried out at 295 nm (excitation) and 330 nm (emission). Known concentrations of α -tocopherol
17 (Sigma-Aldrich T36634) dissolved in methanol were used for calibration.

18

19 To measure plasma concentrations of malondialdehyde (MDA), 20 μ l butylated hydroxytoluene
20 (BHT) (0.05% w/v in 95% ethanol), 160 μ l of phosphoric acid (0.44M) solution and 20 μ l of 2-
21 thiobarbituric acid (TBA) (42mM) was added to either 20 μ l of plasma or 1,1,3,3-tetraethoxypropane
22 (TEP) which was used for calibration (see below). The mixture was vortexed for 10s and heated in a
23 dry bath incubator for 1hour at 100°C. Samples were then cooled on ice for 5 minutes. 80 μ l of *n*-
24 butanol (HPLC grade) was added and the mixture was vortexed for 20s and centrifuged for 3 minutes
25 at 4°C (13.8 x g) and 20 μ l of the butanol phase containing MDA-TBA adduct was injected into a
26 Dionex HPLC system fitted with a Hewlett-Packard Hypersil 5 μ m ODS 100 x 4.6 mm column and a
27 5 μ ODS guard column maintained at 37°C. The mobile phase was 50mM potassium monobasic

1 phosphate (pH 6.8 adjusted using 5M potassium hydroxide) mixed with methanol (HPLC grade)
2 running isocratically at 60:40 (v/v), at a flow rate of 1ml min⁻¹. Fluorescence detection was
3 performed at 515 nm (excitation) and 553 nm (emission). For calibration a standard curve was
4 prepared using a TEP stock solution (5 mM in 40 % ethanol) serially diluted using 40 % ethanol.

5

6 (g) *Wattle colour measurement and quantification*

7 Wattle reflectance data were collected using a USB2000 UV-Visible spectrophotometer and
8 OOIBase32 Software (Ocean Optics Inc., Dunedin, FL) (Mougeot et al. 2005). The
9 spectrophotometer was fitted with a 90° probe pointer to ensure perpendicular contact with the wattle
10 surface and to exclude ambient light (Mougeot et al. 2005). Reflected radiance was measured across
11 a spectral range of 260-680nm at 0.3nm resolution relative to a WS-1 (Ocean Optics Inc.) white
12 standard. The probe was held against the wattle and the spectra allowed to stabilize before capture
13 (Keyser & Hill 1999). Three spectra were collected for the left wattle and 3 for the right wattle. The
14 brightness of the wattle has been identified as being important in female mate choice (Keyser and
15 Hill, 1999), so we calculated brightness as it is likely to be perceived by female pheasants, using the
16 method detailed in Endler and Mielke (2005). In Galliforms, brightness is likely to be perceived by
17 the double cones which show broader spectral tuning and a greater absolute sensitivity suggesting
18 that they are of greater importance for luminance than for colour vision (Vorobyev et al. 1998;
19 Osorio et al. 1999). Because no data on photoreceptor spectral sensitivity have been collected for
20 ring-necked pheasants we used data for the closely-related species, the blue peafowl (*Pavo cristatus*)
21 (Hart 2002). The pheasants' double cone has a peak sensitivity at 567 nm, and is associated with a
22 carotenoid-coloured oil droplet (Hart 2002). Effective double cone sensitivity functions were
23 modelled using the visual pigment template of Govardovskii et al. (2000) and incorporating the
24 transmittance spectra of the combined ocular media for peafowl (Hart 2002), and estimated oil
25 droplet transmission spectra calculated using the equations of Hart & Vorobyev (2005) and data from
26 Hart (2002). The birds were reared outdoors, so a standard daylight-simulating illumination spectrum
27 (D65) was used in the model (Wyszecki & Stiles 1982).

1

2 *(h) Wattle Size and Shape parameters*

3 An image of the male wattle at 46 weeks of age was taken with the head held on the same plane as a
4 fixed scale. Image J software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda,
5 Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2009) was used to calibrate the scale of the image
6 and a polygon was drawn around the wattle to calculate area. The outlines of the wattles for all
7 individuals were included in a common elliptic fourier analysis (EFA) (Rohlf 1992) using Morpheus
8 *et al.* software (D. E. Slice, *Morpheus et al.: Software for Morphometric Research. Revision 01-31-*
9 *00* Department of Ecology and Evolution, State University of New York). The EFA decomposed the
10 curved edges of the polygon into a sum of 15 harmonically related ellipses (to produce 60 Fourier
11 coefficients). Normalisation allowed for variation in the size, position and the rotation of images
12 taken of each wattle. The Fourier coefficients were then used as variables in principal component
13 analyses. The number of principal components that described over 95% of the wattle shape variation
14 was used for analyses (South & Arnqvist 2009).

15

16 *(i) Immune response*

17 Immune response was measured in all birds at 21 weeks of age. Phytohaemagglutinin (PHA) a lectin
18 from the red kidney bean (*Phaseolus vulgaris*) is used as a standard measurement of pro-
19 inflammatory immune response in avian studies (Smits et al. 1999, Vinkler et al. 2010). An area of
20 feathers (approx. 1cm²) from the patagium of both wings for each bird was plucked and sterilised
21 with ethanol. The wing web diameters were then measured using callipers (0.01mm). In the right
22 patagium 0.2mg of phytohaemagglutinin (PHA) (Sigma-Aldrich Inc.) in 0.1ml of sterilised
23 phosphate buffer solution (PBS) (Sigma-Aldrich Inc.) was injected subcutaneously using 5/8” 26
24 gauge Microlance™ needles (Fisher Scientific UK Ltd.) and BD Plastipak™1ml needles (Fisher
25 Scientific UK Ltd.). 0.1ml of sterilised PBS was injected into the left wing patagium. The thickness
26 of the wing patagium of each wing was measured directly before injection using callipers (0.01mm).
27 24 hours (± 10 minutes) after the injection the thickness of the patagium of the wings was measured.

1 The original thickness measurement was subtracted from this measurement to identify the pro-
2 inflammatory response to PHA 24 hours after exposure.

3

4 *(j) Statistical analyses*

5 Normality checks were carried out in SPSS (SPSS Inc., Chicago IL) and data was log-transformed
6 where necessary. Nine individuals died before 47 weeks, approximately equally distributed across
7 the treatment groups. Only measurements taken from individuals that survived to 47 weeks of age
8 were used in analyses (P-E N=59, NP-E N=57, P-C N=57, NP-C N=58). Principal components were
9 produced using the coefficients calculated by an elliptic fourier analysis of wattle shape data. These
10 principal components were used in a multivariate analysis of covariance (MANCOVA) as dependent
11 variables with parasite and vitamin E treatments as fixed effects to determine the effects of
12 treatments on wattle shape. Other response variables were analysed using general linear mixed
13 models (GLMMs) with hatch date (batch) as a random effect. Parasite treatment and vitamin E
14 treatment were included as 2 factors each with 2 levels in a 2 x 2 factorial design in all models. The
15 date on which the HPLC assay was run for each sample was also included as a covariate to control
16 for inter-assay variation, but was dropped from all models during simplification. Growth was
17 analysed using morphometric measurements for males and females at 0, 8, 21 and 47 weeks of age
18 with repeated measures GLMMs. Plasma concentration of either vitamin E or carotenoids were used
19 as the dependent variables in repeated measures GLMMs that included age (for males) as an
20 additional fixed effect to those listed above and bird ID as an additional random effect to determine
21 the effects of the treatments on circulating levels of antioxidants. The effect of the treatments on
22 oxidative damage was examined using a repeated measures GLMM with plasma MDA concentration
23 as the response variable and including sex and age as fixed effects. Similar GLMMs (including sex
24 as a fixed effect, but not repeated measures) were used to examine treatment effects on immunity
25 (PHA measurement as the dependent), parasite burden, and, for males, the expression of secondary
26 sexual traits (spur length, wattle colouration, wattle size and wattle shape). GLMMs were completed
27 in R version 2.9.2 (© R Development Core Team 2009). General linear mixed models were tested

1 using the *lme* function. All interactions were included in the maximal model. For model
2 simplification we removed the highest order interactions, followed by lower order terms in turn from
3 the maximal model using maximum likelihood tests (Likelihood ratios – LR; Crawley 2007) to
4 identify the minimum adequate model (MAM). For post hoc tests involving treatment groups
5 GLMMs in which the focal treatment groups were paired were compared to the original GLMM (i.e.
6 with unpaired treatments) using ANOVA model comparison.

7

8 RESULTS

9 (a) Parasitic Burden at 47 weeks of age

10 The number of *Heterakis* worms in the guts of individual pheasants was measured in both males and
11 females at 47 weeks of age (N = 231 individuals). The MAM of a GLMM with parasite burden at
12 adulthood as the dependent variable included significant main effects of sex (LR = 12.87, $p < 0.001$),
13 vitamin E treatment (LR = 7.99, $p < 0.01$) and parasite treatment (LR = 13.34, $p < 0.001$) and a vitamin
14 E treatment * parasite treatment interaction (LR = 6.45, $p = 0.03$; see Table 1a for parameter estimates
15 for the MAM). All other interactions were dropped from the model during simplification (all
16 $p > 0.20$). Individuals infected with parasites and given a control diet had more parasites at 47 weeks
17 of age than individuals from other treatment groups (Fig. 2). Birds that were infected with parasites
18 but did not receive vitamin E had a higher number of parasites at 47 weeks than those birds that did
19 not receive either vitamin E or parasites in early life. Individuals that received a diet with
20 supplementary vitamin E during development had a lower parasite burden at 47 weeks of age,
21 whereas individuals that were infected with parasites during early life had a higher parasite burden at
22 47 weeks of age than those individuals that did not receive the parasite treatment (Fig. 2). Males had
23 a significantly higher mean parasitic burden than females (Table 1a).

24

25 (b) Concentrations of plasma antioxidants

26 The concentration of α -tocopherol (vitamin E) decreased from a mean across groups of 87.66 $\mu\text{g/ml}$
27 at 8 weeks to 2.59 $\mu\text{g/ml}$ by 47 weeks of age in male pheasants (N = 115 individuals and 218

1 observations) The MAM of a repeated measures GLMM with bird ID and hatch date as random
2 effects and plasma vitamin E concentration as the response variable included main effects of vitamin
3 E supplementation group (LR = 75.00, $p < 0.001$) and age (LR = 204.91, $p < 0.001$), and a significant
4 interaction between age and vitamin E supplementation (LR = 115.19, $p < 0.001$; see Table 1b for
5 parameter estimates). The greatest decrease in plasma vitamin E concentration occurred in those
6 birds that received vitamin E in their diet up to 8 weeks of age (Table 1b, Fig. 3a, b).

7

8 In analyses separated by age (N = 115), males in groups that were supplemented with vitamin E had
9 higher concentrations of plasma vitamin E at 8 weeks of age than males given a control diet (Vitamin
10 E treatment, LR = 98.36, $p < 0.001$). Plasma concentrations of vitamin E in males that received a diet
11 supplemented with vitamin E in early life remained higher at 47 weeks than birds given a diet
12 without the vitamin E supplement (Vitamin E treatment, LR = 45.63, $p < 0.001$). Infection with
13 parasites did not affect the concentration of vitamin E in the plasma at 8 (parasite treatment, LR =
14 2.42, $p = 0.11$; vitamin E * parasite, LR = 2.28, $p = 0.14$) or 47 weeks of age (parasite treatment, LR =
15 0.88, $p = 0.35$; vitamin E * parasite, LR = 0.10, $p = 0.76$), and males did not differ from females in the
16 concentrations of vitamin E circulating in the plasma at 8 weeks of age (Sex, LR = 0.85, $p = 0.47$; N =
17 231 individuals;). There were no effects of vitamin E supplementation (LR = 0.93, $p = 0.69$), parasite
18 treatment (LR = 1.37, $p = 0.33$), age (LR = 0.42, $p = 0.85$) or any significant interactions between these
19 variables on the concentrations of carotenoids circulating in plasma (all interactions were $p > 0.06$;
20 The MAM included just the model intercept; Fig. 3c, d).

21

22 (c) Oxidative Stress

23 The concentration of MDA in plasma did not differ between males and females (LR = 0.11, $p = 0.74$),
24 or parasite treatment (LR = 1.36, $p = 0.26$) but decreased with age (from an overall mean of 6.61
25 $\mu\text{g/ml}$ at 8 weeks to a mean of 1.61 $\mu\text{g/ml}$ at 47 weeks of age; LR = 252.12, $p < 0.001$; Fig. 3e, f). The
26 MAM included significant interactions between vitamin E treatment and age (LR = 9.47, $p = 0.002$),
27 parasite treatment and age (LR = 4.18, $p = 0.041$) and vitamin E treatment and parasite treatment (LR

1 = 5.70, $p=0.017$) respectively ($N = 231$ individuals and 462 observations; Table 1c). GLMMs
2 separated by age for males showed that birds given a control diet and infected with parasites had a
3 higher concentration of plasma MDA at 8 weeks of age (Parasite treatment * vitamin E treatment:
4 $LR = 3.92$, $p=0.03$; vitamin E treatment, $LR = 9.39$, $p<0.01$; parasite treatment, $LR = 2.85$, $p=0.09$;
5 Fig. 3e, f). However, by 47 weeks there were no differences in plasma MDA concentrations between
6 individuals given the parasite treatment or the vitamin E treatment (GLMM for birds at 47 weeks:
7 vitamin E treatment* parasite treatment: $LR = 2.42$, $p=0.12$; vitamin E treatment: $LR= 1.72$, $p=0.17$;
8 parasite treatment: $LR = 1.38$, $p=0.24$; Fig. 3e, f).

9

10 *(d) Morphometric measurements*

11 There were no initial differences in the size of chicks allocated to different vitamin E or parasite
12 infection treatments (GLMM, $N = 231$ individuals: treatment group, $LR = 6.22$, $p=0.10$; sex, $LR =$
13 0.44 , $p=0.51$; treatment * sex, $LR = 2.83$, $p=0.42$). Repeated measures GLMMs with mass, tarsus
14 length, wing length or head-bill length as response variables ($N = 231$ individuals and 693
15 observations) and sex, age and treatment group as explanatory variables showed that males were
16 larger and faster growing than females (mass, $LR = 91.87$, $p<0.001$; head-bill length, $LR = 87.19$,
17 $p<0.001$; tarsus, $LR = 124.15$, $p<0.001$, wing length, $LR = 12.18$, $p=0.04$), but that there were no
18 significant differences in growth among treatments, either for vitamin E supplementation (mass, LR
19 $= 0.03$, $p=0.98$; head-bill length, $LR = 0.27$, $p=0.89$; tarsus, $LR = 0.28$, $p=0.84$; wing length, $LR =$
20 0.81 , $p=0.67$) or in relation to parasite treatment (mass, $LR = 1.47$, $p=0.55$; head-bill length, $LR =$
21 2.45 , $p=0.43$; tarsus, $LR = 0.25$, $p=0.87$; wing length, $LR = 2.01$, $p=0.11$). There were also no
22 significant interaction terms in any of the respective MAMs (all interactions $p>0.29$; parameter
23 estimates for the MAMs are given in Table 2)).

24

25 *(e) Immune function*

26 The MAM of a model including immune response at adulthood as the dependent variable and
27 vitamin E treatment, parasite treatment and sex with hatch date as a random effect included only the

1 intercept (N = 231 individuals). Immune response did not vary in relation to either sex (LR = 0.54, p
2 = 0.46), parasite treatment (LR = 0.83, p=0.36), or vitamin E treatment (LR = 0.20, p=0.65). All
3 interactions were also dropped from the model during simplification (all p>0.38).

4

5 (f) *Secondary Sexual Signals*

6 The expression of sexual signals in males (N = 115 individuals) was not affected by parasite load
7 (parasite treatment: wattle size LR = 2.10, p=0.15, spur length: LR = 2.62, p=0.11, wattle brightness:
8 LR = 0.59, p=0.44) or the supplementation of vitamin E (vitamin E treatment: wattle size LR = 2.23,
9 p=0.14, spur length: LR = 0.29, p=0.59, wattle brightness: LR = 0.18, p=0.67). A MANCOVA of the
10 5 principal components that collectively described 95% of the shape variation calculated by EFA
11 analysis indicated that there was also no difference in the shape of the wattles of males in relation to
12 parasite treatment ($F = 0.34$, $df = 1,110$, $p=0.54$) or vitamin E treatment ($F = 1.25$, $df = 1,110$,
13 $p=0.23$). There were no significant interaction terms in any of these models (all p>0.09).

14

15

16 **DISCUSSION**

17 The results show that, contrary to expectations, the expression of sexually-selected traits in adulthood
18 was unaffected by the experimental manipulation of parasite load or antioxidant (vitamin E)
19 availability during the first 8 weeks of development. However, adult males had greater numbers of
20 parasites than females in their guts at 47 weeks of age regardless of which treatment they had
21 received during development. In addition the experimental treatments did not have any effect on the
22 growth or immune response of individual ring-necked pheasants of either sex, but early exposure to
23 parasites and vitamin E did, as predicted, have some long-term effects. Individuals exposed to
24 *Heterakis* nematode worms at 21 days of age had higher numbers of the parasite at adulthood (47
25 weeks) than individuals that were not infected with *Heterakis*, unless they also received
26 supplementary vitamin E during early growth. Early exposure to parasites without supplementary
27 vitamin E was also associated with elevated levels of oxidative damage at 8 weeks of age. In

1 contrast, the reduced oxidative stress (lower levels of damage during early growth and higher
2 circulating levels of vitamin E throughout development) and lower numbers of intestinal parasites at
3 adulthood (47 weeks) of individuals that received supplementary vitamin E during the first 8 weeks
4 of growth may have positive downstream effects on fitness prospects, even if sexually-selected traits
5 were unaffected.

6

7 Sexual traits can show higher condition dependence in response to environmental stress during early
8 development than morphological traits (e.g. Hunt & Simmons, 1997, David et al. 2000). The
9 negative effects of nutritional stress during early development on sexual signals have mostly been
10 documented for vocal sexual signals (song e.g. Buchanan et al 2003; Spencer et al. 2003) but little is
11 known about the connection between development and evolution of sexual ornaments in response to
12 an early environmental insult such as parasite infection. Borgia et al. (2004) proposed that if females
13 have evolved to gain the greatest “good genes” benefits from mate selection that they should choose
14 male display traits that include information from life history stages when parasites are most harmful.
15 The results of the Borgia et al. (2004) study with satin bowerbirds indicated that immunocompetence
16 handicap studies should consider the effects of exposure to infection in non-reproductive, not just
17 reproductive, age classes. In contrast with the results of previous experiments (Borgia et al. 2004;
18 Spencer et al. 2005) the expression of sexually selected traits in ring-necked pheasants in the current
19 study were largely unaffected by exposure to parasites (*H. gallinarum*) during development.

20

21 Furthermore, we also found that the intensity of male sexual signals did not correspond with current
22 *H. gallinarum* burden. The results of the current study therefore do not support the ‘parasite-
23 mediated sexual selection’ theory (Hamilton & Zuk, 1982) which proposes that females choose
24 bright males because elaborate displays are effective indicators of heritable male-parasite resistance
25 traits. None of the multiple ornaments measured, whether carotenoid-mediated (wattle colour) or not
26 (spur length, wattle size or body size) were related to parasite load. Previous studies have provided
27 evidence that carotenoid-mediated sexual traits can be affected by parasitic infection. Male house

1 finches infected with *Mycoplasma gallicepticum*, show reduced carotenoid plumage colour without
2 direct disruption of carotenoid absorption or transportation (Hill et al. 2004). Experimental reduction
3 of infection levels has been shown to reduce carotenoid based signalling in red grouse combs
4 (nematode; Martinez-Padilla et al. 2007) and in great tits (hemoparasite; Horak et al. 2001). Møller *et*
5 *al.* (1999) suggested that inconsistent results in tests of the ‘parasite-mediated sexual signal’ theory
6 may result from the use of relatively harmless parasites in studies. Previous studies on pheasants
7 have provided some support for parasite-mediated effects on sexual display. Hillgarth (1990), for
8 example, found a correlation between female mate-choice, coccidian numbers and male display rate.
9 Our experiment used *H. gallinarum*, a common nematode in wild pheasants which may be less
10 pathogenic than some other parasites. We found no negative effects of *H. gallinarum* infection on
11 body mass or growth, consistent with other studies (Tompkins et al. 1999; Draycott et al. 2000;
12 Tompkins et al. 2001; Woodburn et al. 2002). However, Tompkins et al. (2001) found that pheasants
13 infected with *H. gallinarum* following infection with 100 embryonated eggs, the same dosage used in
14 this study, produced a lower mass of caecal droppings, and suggested that reduced caecal activity
15 may result in reduced nutrient absorption and therefore reduce the fecundity and survival of
16 pheasants in the wild if food is limiting (see also Holmes, 1995; Coop & Holmes, 1996). In the
17 current study birds infected with parasites that were not also provided with supplementary
18 antioxidants had higher levels of oxidative damage at 8 weeks of age and higher parasite loads at
19 adulthood, which indicates that there may be significant costs of early exposure to *H. gallinarum*.

20

21 Activation of the immune system in response to parasite infection results in the production of higher
22 amounts of reactive oxygen species during the respiratory burst activity of phagocytes (Babior 1984).
23 Individuals may also experience higher levels of oxidative damage if parasitism impairs the uptake
24 of antioxidants from the diet. As a result it was predicted that individuals infected with *H. gallinarum*
25 would experience a higher degree of oxidative damage. Supplementation with vitamin E however,
26 mitigated the oxidative effects of early exposure to parasites, as P-E birds had significantly lower
27 levels of oxidative damage than infected birds given a control diet, and had similar levels of MDA to

1 uninfected individuals at 8 weeks of age. In addition, our results complement the results of previous
2 studies showing that vitamin E can reduce nematode infection. Vitamin E deficiency has been shown
3 to impair resistance to secondary nematode infection 30 days after inoculation in adult mice (Smith
4 et al. 2005). Reduced vitamin E concentrations may affect the ability of a host to respond to
5 nematode infection of the gastro-intestinal tract due to increases in oxidative stress and alterations to
6 both signal transduction and transcription factor activation (Smith et al. 2005). Supplementation with
7 vitamin E during the first 8 weeks in our experiment also resulted in increased levels of circulating
8 vitamin E (i.e. elevated antioxidant defences) at adulthood. However, there were no differences in
9 oxidative stress at 47 weeks of age despite significantly higher numbers of parasites in the P-C
10 group. As a result there was also no evidence that sexually-selected traits reflected the long-term
11 oxidative status of individuals.

12

13 Despite monitoring individuals for a year post-hatch treatment effects on sexual signal expression
14 were not detected, in contrast to a previous study on pheasants that manipulated protein content of
15 early diet and found treatment effects on the expression of sexually-selected traits on one-year old
16 adults (Ohlsson et al. 2002). However, it is possible that measurement of the sexual ornaments of
17 males at one-year of age failed to identify the longer term effects of supplementation. Hillgarth
18 (1990) found no female preferences for male morphological traits in captive birds during a study on
19 one year old ring-necked pheasants. Spur length is reportedly the most important predictor of harem
20 size in ring-necked pheasants (Göransson et al. 1990), but spur length at one year of age has been
21 found to have less influence on female mate choice than the spur length of older males (Grahn & von
22 Schantz 1994). In addition, the effects of higher circulating vitamin E at 47 weeks found in birds
23 supplemented with vitamin E during development on the oxidative status of individuals beyond the
24 first year of life are unknown.

25

26 Previous supplementation experiments during post-natal development involving vitamin E only (in
27 barn swallows; de Ayala et al. 2006) and a cocktail of antioxidants including vitamin E (in red-

1 winged blackbirds; Hall et al. 2010) have shown that additional antioxidant resources are
2 preferentially allocated to growth. Related work on pheasants showed that supplementation of a
3 combination of carotenoids and vitamin E, but not vitamin E by itself, resulted in preferential
4 allocation of resources to achieving a large body size rather than to sexually-selected traits (Orledge
5 et al. 2012). This is likely to be because in ring-necked pheasants attaining a larger body size has
6 beneficial downstream effects. Smith et al. (2007) found that pheasants in better body condition,
7 measured as residual mass, showed increased wattle colour when carotenoid supplemented as first
8 year adult males. By maintaining a better body condition it is likely that birds will be able to
9 capitalise on environmental fluctuations in carotenoid availability to allocate resources to sexual
10 signalling as adults (Smith et al. 2007). Göransson et al. (1990) and Grahn et al. (1993) also found
11 that increased body mass is correlated with dominance in pheasant male-male interactions. However,
12 in the current study extra antioxidant resources were preferentially allocated to self-maintenance
13 (reducing parasite load and oxidative damage) instead of growth or reproduction (i.e. sexually-
14 selected traits). Consequently it may be that selection favours allocation of resources to self-
15 maintenance in parasitized birds related to increased survival prospects during the first year of life.
16 Individuals ingest a cocktail of natural antioxidants and a number of studies have identified
17 synergistic interactions of dietary antioxidants when supplemented in combination (Pike et al. 2007;
18 Catoni et al. 2008; Perez et al. 2008; Orledge et al. 2012). Thus it may be that selection favours the
19 allocation of resources to self-maintenance in parasitized birds, which is related to increasing
20 survival prospects during the first year of life, or that unless vitamin E is supplemented in
21 conjunction with carotenoids it is effectively unavailable for preferential allocation towards growth
22 (Orledge et al. 2012).

23

24 Males had significantly larger numbers of adult *H. gallinarum* at adulthood than females. Previous
25 studies have also shown that males are more likely to be infected with parasites and have a higher
26 load than females (Zuk & McKean 1996). Folstad and Karter (1992) have argued that
27 immunosuppressive effects of high testosterone levels that contribute to bright displays may cause

1 males to have more rather than fewer parasites. Despite evidence that vitamin E has immuno-
2 enhancing capacities we found no evidence for improved immune response to PHA injection at 21
3 weeks of age in individuals that had been supplemented with vitamin E during development. In
4 addition, we found no effect of parasite load on the degree of immune response. In this study, we
5 measured the pro-inflammatory immune response following PHA injection at 21 weeks of age,
6 which is likely to incorporate broad elements of both innate and acquired immunity, so we were
7 unable to measure more specific immune responses. In this case it may have been that humoral
8 immunity was affected by the treatments, and/or there were treatment effects at 47 weeks, but these
9 were not measured. It is also possible that the nematode *H. gallinarum* was not pathogenic enough to
10 affect the pro-inflammatory immune response, although the reduced numbers of nematodes in the
11 guts of birds supplemented with vitamin E indicates that the costs of parasite infection at the given
12 dose was sufficient to lead to treatment differences in parasite loads at 47 weeks.

13

14 In conclusion, we found that supplementation of additional vitamin E during development reduced
15 the parasite load of adults and the oxidative stress associated with maintaining a higher parasite load.
16 However, we did not find that the availability of extra antioxidant resources during development
17 resulted in increased allocation to sexual signals if infected with nematode parasites, or that the
18 degree of ornamentation in pheasants reflected either the parasite load of *H. gallinarum* or the
19 oxidative status of males. It is possible that the parasite used in our study did not produce a
20 sufficiently strong pathological response to lead to detectable differences in the allocation of
21 resources to sexually-selected traits. However, given that *H. gallinarum* is a common intestinal
22 parasite of pheasants and was administered in doses within the natural range found in wild birds, if
23 the dose was not sufficient to stimulate a strong enough response that is visibly expressed in a sexual
24 signal of quality it raises questions about how generally informative such a signal can be to females
25 if it is only expressed when males have experienced very high parasite loads. In such circumstances
26 signals may effectively become redundant. It is also possible that the effects of parasite manipulation
27 and supplementation of vitamin E in relation to the quality of the general nutritional environment

1 were too weak to detect treatment effects on sexually-selected traits in males that were not fully
2 developed (i.e. 1st year as opposed to 2nd year birds). However, the long-term effects of early
3 exposure to parasites and vitamin E on parasite load and circulating levels of vitamin E at adulthood
4 indicate that there are likely to be downstream fitness effects of the treatments that are not evident at
5 47 weeks, when the expression of sexually-selected traits is largely uninformative of the environment
6 experienced during the first 8 weeks of life in pheasants.

7

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17

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1

Table 1. Parameter estimates of explanatory terms in Minimum Adequate Models for parasite load and plasma concentrations of vitamin E and the lipid peroxidation product MDA, respectively. See main text for further model details.

Explanatory term	Parameter estimate	SE of estimate	DF	t-value	p-value
a) Parasite burden of males and females at 47 weeks of age					
Intercept	24.57	3.82	225	6.44	<0.0001
Vitamin E treatment	-5.95	4.59	225	-1.30	0.196
Parasite treatment	10.71	4.60	225	2.33	0.021
Sex	-11.45	3.18	225	-3.60	<0.001
Vitamin E * Parasite	-3.38	6.38	225	-0.53	0.031
b) Plasma vitamin E concentration ($\mu\text{g/ml}$) of males					
Intercept	48.50	5.21	113	9.32	<0.0001
Vitamin E treatment	107.47	6.20	112	17.32	<0.0001
Age	-1.00	0.13	113	-7.53	<0.0001
Vitamin E * Age	-2.26	0.18	113	-12.26	<0.0001
c) Plasma MDA concentration ($\mu\text{g/ml}$) of males and females					
Intercept	8.13	0.47	454	17.29	<0.0001
Vitamin E treatment	-0.84	0.58	454	-1.45	0.148
Parasite treatment	2.18	0.58	454	3.75	0.0002
Age	-0.15	0.01	454	-11.16	<0.0001
Vitamin E * Parasite	-2.04	0.58	454	-3.54	0.0004
Vitamin E * Age	0.05	0.01	454	3.07	0.002
Parasite * Age	-0.03	0.01	454	-2.03	0.043

2

3

Table 2. Parameter estimates of explanatory terms in Minimum Adequate Models for growth of morphological response variables. See main text for further model details.

Explanatory term	Parameter estimate	SE of estimate	DF	t-value	p-value
a) Mass (g)					
Intercept	624.54	16.76	461	37.27	<0.0001
Sex	-161.59	14.72	228	-10.98	<0.0001
Age	16.12	0.45	461	35.53	<0.0001
b) Head-bill length (mm)					
Intercept	62.77	0.27	461	228.51	<0.0001
Sex	-3.09	0.29	228	-10.71	<0.0001
Age	0.22	0.01	461	33.30	<0.0001
c) Tarsus length (mm)					
Intercept	76.85	0.33	461	235.43	<0.0001
Sex	-7.09	0.42	228	-16.99	<0.0001
Age	0.15	0.01	461	28.00	<0.0001
d) Wing length (mm)					
Intercept	18.88	0.13	461	148.79	<0.0001
Sex	-1.23	0.12	228	-10.22	<0.0001
Age	0.12	0.01	461	32.17	<0.0001

4

5

1 Figure legends

2

3 **Fig. 1:** A male ring necked pheasant [*Phasianus colchicus*] showing sexually selected ornament, the
4 facial wattle. Photo credit N.J. Royle.

5

6 **Fig. 2:** Levels of parasitic burden (*H.gallinarum*) at 47 weeks of age in relation to sex and treatment
7 group. Means are shown with 95% confidence intervals. Sample sizes are provided for each mean.

8

9 **Fig. 3:** Plasma α -tocopherol (a and b) carotenoid (c and d) and MDA (e and f) concentrations ($\mu\text{g/ml}$)
10 in relation to treatment and age at (a, c and e) 8 and (b, d and f) 47 weeks of age. Means are shown
11 with 95% confidence intervals. Note that scales differ considerably between 8 and 47 weeks of age.

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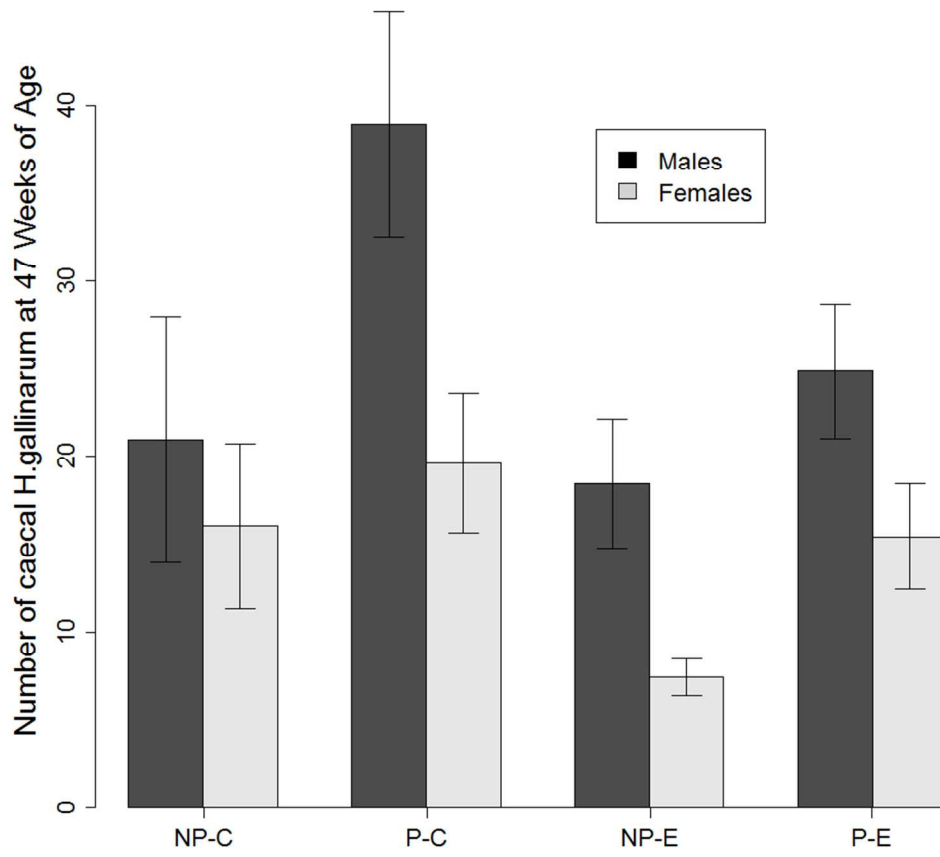
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Review



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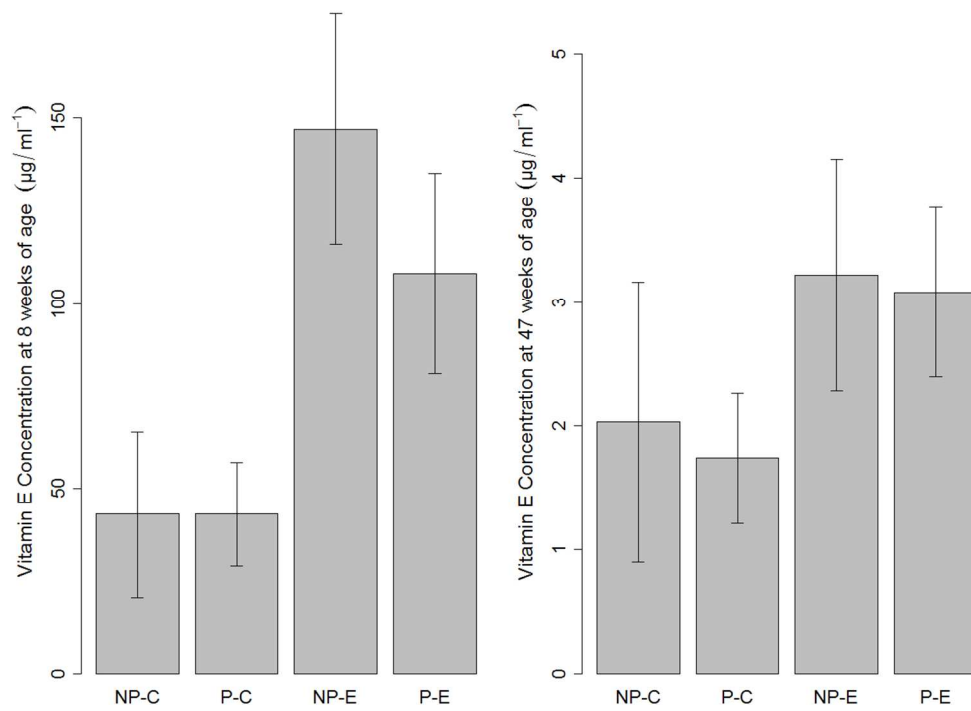


Figure 3 a & b
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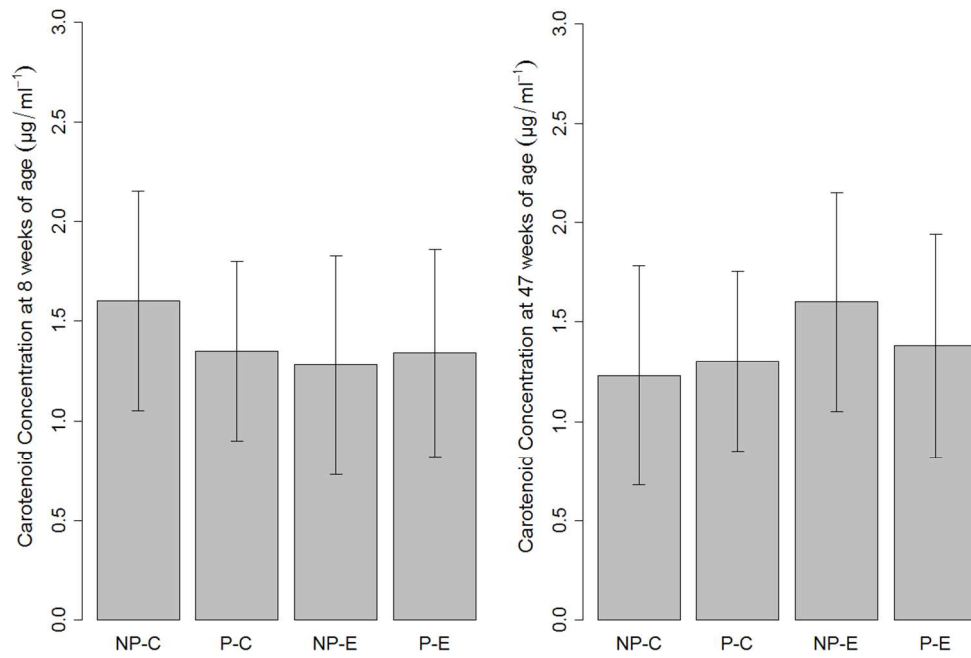
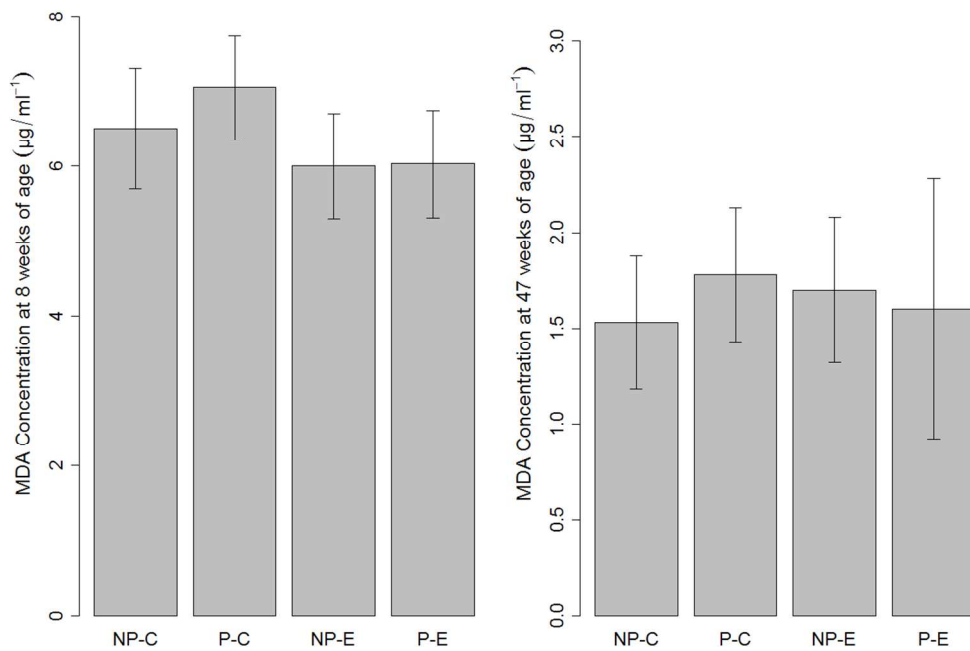


Figure 3c & d
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Review



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