JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12136

Vitamins, stress and growth: the availability of antioxidants in early life influences the expression of cryptic genetic variation

S.-Y. KIM*, J. C. NOGUERA*†, A. TATO* & A. VELANDO*

*Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo, Spain
†College of Medical, Veterinary and Life Science, University of Glasgow, Glasgow, G12 8QQ, UK

Keywords:

corticosterone;
full-sibling model;
genetic variance;
heritability;
malondialdehydes (MDA);
reactive oxygen metabolites (ROMs);
vitamin supplement;
yellow-legged gull.

Abstract

Environmental inputs during early development can shape the expression of phenotypes, which has long-lasting consequences in physiology and life history of an organism. Here, we study whether experimentally manipulated availability of dietary antioxidants, vitamins C and E, influences the expression of genetic variance for antioxidant defence, endocrine signal and body mass in yellow-legged gull chicks using quantitative genetic models based on full siblings. Our experimental study in a natural population reveals that the expression of genetic variance in total antioxidant capacity in plasma increased in chicks supplemented with vitamins C and E despite the negligible effects on the average phenotype. This suggests that individuals differ in their ability to capture and transport dietary antioxidants or to respond to these extra resources, and importantly, this ability has a genetic basis. Corticosterone level in plasma and body mass were negatively correlated at the phenotypic level. Significant genetic variance of corticosterone level appeared only in control chicks nonsupplemented with vitamins, suggesting that the genetic variation of endocrine system, which transmits environmental cues to adaptively control chick development, appeared in stressful conditions (i.e. poor antioxidant availability). Therefore, environmental inputs may shape evolutionary trajectories of antioxidant capacity and endocrine system by affecting the expression of cryptic genetic variation.

Introduction

Phenotypic plasticity is the ability of a genotype to respond to different environments by changing phenotypes (Pigliucci, 2001). This ability, when exposed to changing environments or new environmental inputs, may generate novel developmental variation, for example, by expressing hidden genetic variation (West-Eberhard, 2003; Badyaev, 2009). Indeed, a growing body of evidence suggests that environmental conditions influence heritable variance of quantitative traits (reviewed in Hoffmann & Merilä, 1999; Charmantier & Garant, 2005) and covariance between different traits (reviewed in Sgrò & Hoffmann, 2004). The inconstancy of heritable variation across different environmental conditions suggests the presence of genotype by environment interaction (G × E), which is the genetic basis for phenotypic plasticity (Pigliucci, 2005). Environmental inputs can provoke previously unexpressed genetic variation or suppress otherwise expressed genetic variation in natural populations (West-Eberhard, 2003). This environmental sensitivity of quantitative genetic parameters in the wild can be assessed by testing the effects of environmental manipulations on the expression of genetic variations (e.g. Charmantier *et al.*, 2004; Dingemanse *et al.*, 2009).

Environmental conditions during early development are especially important because a large part of phenotypic programming or epigenetic changes occurs during such a brief period in an individual's life history (West-Eberhard, 2003). Therefore, early environmental conditions experienced by an organism have long-lasting consequences in its morphology, physiology, behaviour and life history through differential gene expression (West-Eberhard, 2003; Weaver *et al.*, 2004; Hager *et al.*, 2009).

Correspondence: Sin-Yeon Kim, Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, 36310 Vigo, Spain.
Tel.: +34 986 812590; fax: +34 986 812556; e-mail: yeonkim@uvigo.es

One important developmental mechanism underlying life-history evolution is trade-offs involving oxidative stress (Monaghan *et al.*, 2009; Metcalfe & Alonso-Alvarez, 2010; Kim *et al.*, 2010a). Oxidative stress occurs when the rate of production of reactive oxygen species (ROS) exceeds the capacity of antioxidant defence and repair mechanisms (Finkel & Holbrook, 2000). Rapid growth can give rise to decreased resistance to oxidative stress (Alonso-Alvarez *et al.*, 2007; Kim *et al.*, 2011) and increased oxidative damage (Nussey *et al.*, 2009; Noguera *et al.*, 2011b). Fast metabolism required for rapid growth or maintenance of a large body could directly generate oxidative stress by increasing ROS production at cellular level (Beckman & Ames, 1998; Rollo, 2002) as predicted by 'rate of living free radical theory' (Harman, 1956). Nevertheless, evidence that oxidative stress mediates life-history evolution is often weaker than acknowledged and rarely conclusively demonstrates the proposed links between mechanism and evolutionary significance, particularly for natural populations (Isaksson *et al.*, 2011). On the other hand, individuals with high metabolic rates may have greater mitochondrial uncoupling and state three respiration and increased flow of electrons, all of which can lead to lower production of ROS ('uncoupling to survive' hypothesis: Brand, 2000; Speakman *et al.*, 2004). Therefore, it may be necessary to simultaneously measure antioxidant capacity, oxidative damage and ROS production to study the structure of trade-offs involving oxidative stress.

The availability of exogenous antioxidants may shape phenotypes of organisms by directly influencing trade-offs mediated by oxidative stress and provoke programmed responses that may remain throughout the lifetime. In birds, for example, experimental increase in dietary antioxidants during development accelerated growth of chicks, although these extra resources did not reduce oxidative damage (Hall *et al.*, 2009; Noguera *et al.*, 2011b). However, the effects of increased dietary antioxidants on life-history traits or oxidative damage are not always found. Between-individual differences in the effects of dietary antioxidants on life-history traits and oxidative balance may be linked to individual differences in ability to produce endogenous antioxidants and physiological interactions between different antioxidants. Recent studies of some wild vertebrates suggest that a high proportion of phenotypic variation in antioxidant capacity can be attributed to their family of origin (Costantini & Dell'Omo, 2006; Rubolini *et al.*, 2006; Olsson *et al.*, 2008; Kim *et al.*, 2010b). However, it remains unexplored whether the gene expression for traits involved in oxidative stress could change in response to exogenous antioxidants.

In vertebrates, during development, control systems translate environmental cues perceived by sense organs into molecular (neuroendocrine) signals that influence programmed responses in physiology, gene expression and behaviour (Lessells, 2008). Most vertebrates across diverse taxa respond to exogenous stressors by up-regulating the production of glucocorticoid stress hormones (Silverin, 1998; Romero *et al.*, 2000; Evans *et al.*, 2006). Corticosterone is the main avian glucocorticoid hormone that modulates metabolic processes such as the mobilization of energy stores and the shutdown of digestive processes (Buchanan, 2000). Recent studies showed that experimentally elevated corticosterone concentration gives rise to increased oxidative stress (Lin *et al.*, 2004; Stier *et al.*, 2009; Cote *et al.*, 2010; Haussmann *et al.*, 2012) and decreased growth rate (Hayward & Wingfield, 2004; Lin *et al.*, 2006). This suggests that corticosterone may mediate trade-offs between oxidative balance and growth, thereby shaping how individual genotype responds to environmental cues (see illustration in Fig. 1). However, the mechanism by which corticosterone influences oxidative balance and growth is not entirely clear. It is necessary to simultaneously examine the relationships among the levels of corticosterone, oxidative stress and growth.

In this study, we examine the effects of dietary antioxidant supply during early development on the sources of individual variations in growth, corticosterone and antioxidant capacity. Here, we used a field experiment, combining cross-fostering design and vitamin supplement (vitamins C and E), to examine the differences in genetic and phenotypic variations of physiological parameters and growth in yellow-legged gull *Larus michahellis* chicks that experience different antioxidant availability in the early life. Vitamin C (ascorbic acid, hydrophilic antioxidants) and vitamin E (tocopherols, lipophilic antioxidants) are two of the most important dietary antioxidants synthesized mainly by plants (vitamin C, also by some animals) to protect themselves against oxidative stress. Vitamin C is antioxidant, but it is also involved in recycling oxidized

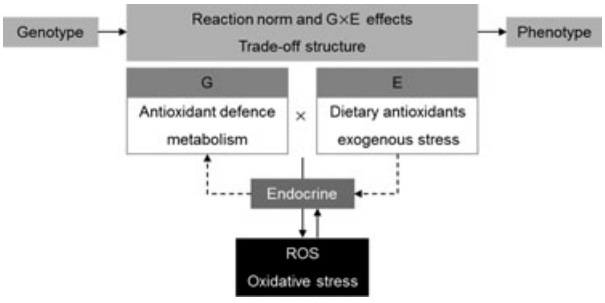

Fig. 1 A simplified illustration of relationships between intrinsic and extrinsic antioxidant defences, endocrine and oxidative stress. This assumes that the phenotypes involving oxidative balance are plastic to environmental conditions and that the plasticity has genetic basis. Endocrine signals may mediate trade-offs between oxidative balance and growth, thereby shaping how individual genotype responds to environmental cues.

vitamin E (Halpner *et al.*, 1998; Woods *et al.*, 2001). The intake of these dietary antioxidants affects life-history traits such as fertility and growth in a variety of vertebrates including fish, birds and mammals (reviewed in Catoni *et al.*, 2008). This experiment enabled us to examine how the availability of exogenous antioxidants shapes individual phenotypes of antioxidant capacity, corticosterone and growth (see illustration in Fig. 1).

If the phenotypes involving oxidative balance are plastic to environmental conditions (i.e. exogenous sources of antioxidants) and the plasticity has genetic basis, the expression of genetic variation will differ between chicks supplemented with dietary antioxidants and control chicks. Indeed, the main finding presented here is that despite negligible effects of vitamin supplement on the average phenotype, the expression of genetic and/or phenotypic variances in body mass, total antioxidants and corticosterone level was altered by the experimental treatment.

Materials and methods

General procedures and experimental design

We performed the field experiment from April to June 2010 at a colony of yellow-legged gulls in the Parque Nacional das Illas Atlánticas, Sálvora Island, Galicia, Spain (N42°28′ W09°00′). In the study population, modal clutch size is three, and the semi-precocial chicks hatch asynchronously, with the third chick hatching normally 1–3 days after the first two (Kim *et al.*, 2010b). We surveyed the study area once daily during the egg laying and marked nests with numbered bamboo sticks. We visited each nest every day until clutch completion to mark eggs and register laying dates.

We used 108 nests with a clutch of three eggs for our experiment. To disentangle genetic and environmental (co)variances of chicks, all three eggs were swapped 1 day after clutch completion. A set of cross-fostering was performed within a group of four randomly selected nests in which the second and third eggs were laid on the same days (at 2- or 3-days interval). This cross-fostering design resulted in all three eggs from the same original nest being incubated, then the hatchings being raised in three different foster nests other than the original nest, but conserving the expected chick order. Each cross-foster group of four nests was randomly assigned to either nonsupplement (hereafter NS, $n = 52$ nests) or vitamin supplement experimental group (hereafter VS, $n = 56$ nests). Therefore, all chicks from the same nest of origin and foster nest receive the same treatment. Note that due to the clutch size constraint (three eggs), full siblings from the same nest of origin could not be assigned to both experimental treatments in this study designed for quantitative genetic analyses. The cross-fostering design and experimental assignment are illustrated in Supporting Information (Fig. S1).

We checked each nest daily beginning 2 days before the estimated hatching date. Before hatching, we installed a fence enclosure around each nest with plastic garden mesh measured 5 m × 0.25 m to keep chicks in their territory. All hatchlings were marked using numbered leg flags made with Velcro on the day of hatching (day 0, $n = 280$). Chicks assigned to NS group received daily only water, and those of VS group, a suspension of vitamins C and E mixed in water, beginning on the day of hatching (day 0) until age 11 days. Water-soluble vitamin C and water-miscible form of vitamin E, commercially available diet supplement for domestic animals (Lohmann Animal Health GmbH & Co. KG, Cuxhaven, Germany), were used. We moderated the level of dosage to prevent hypervitaminosis because it is unknown to which extent the dosage is safe in wild birds. We calculated the supplement dosage of vitamins C and E based on the estimated natural intake from main food items in the study population, fish and marine molluscs (Dias *et al.*, 2003). From a suspension of 10 mg vitamin E and 6 mg vitamin C mixed in 100 mL water, each chick daily received 0.6 mL on days 0–3, 1 mL on days 4–7 and 2 mL on days 8–11 via oral administration with a syringe. These daily doses contain approximately 20% the estimated natural daily intake of vitamins C and E (i.e. 500 μg VE and 300 μg VC per 100 g body mass; see also Hall *et al.*, 2009). Control group received the same amount of water but without vitamins C and E.

We weighed chicks with spring balances and measured the tarsus length with callipers on days 0, 2, 7 and 12. Blood for sex identification and measurement of total antioxidant capacity, oxidative damage and hormone concentration was sampled from the brachial vein of each chick on days 0, 7 and 12, using a sterile needle and heparinized capillary tubes. We sampled blood from each chick immediately after capture (within 1 min) and before biometry measurement and experimental administration because the plasma corticosterone level of birds increases in response to handling, typically starting several minutes after capture (Cockrem & Silverin, 2002). The blood samples were stored at 4 °C before centrifugation that occurred within 10 h. Immediately after centrifugation, plasma fractions were stored at −80 °C until analyses of antioxidants and oxidative damage. Red blood cells from day 0 were mixed with alcohol and stored at room temperature until molecular sexing. Chick sex was identified from blood cell DNA by detecting two CHD genes (*CHD1W* and *CHD1Z*), using a pair of primers (2550F and 2718R; Fridolfsson & Ellegren, 1999).

The study was carried out under permissions by the Parque Nacional das Illas Atlánticas and Xunta de Galicia, and all the field procedures that we performed complied with the current laws of Spain. The

experimental protocol was approved by the Committee on the Ethics of Animal Welfare of the University of Vigo.

Measuring total antioxidants, lipid peroxidation and reactive oxygen metabolites

The concentration of total antioxidants was measured in the plasma sampled for each chick using the method described by Erel (2004). In brief, coloured 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation [ABTS] was added to each plasma sample in a 96-well microplate. The ABTS is decolourized by antioxidants according to their concentrations and antioxidant capacities. This change in colour was measured as a change in absorbance at 660 nm (Synergy™ 2 Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT, USA), and the assays were calibrated with Trolox (Erel, 2004). Duplicate sample analyses showed that the antioxidant assays were repeatable throughout all ages of sampling (day 0: $r = 0.930$, $F_{281,281} = 27.634$, $P < 0.001$; day 7: $r = 0.815$, $F_{214,203} = 9.307$, $P < 0.001$; day 12: $r = 0.708$, $F_{114,104} = 5.333$, $P < 0.001$). Main sources of antioxidants that contribute to this assay include hydrophilic antioxidants, such as -SH group of proteins, uric acid and vitamin C, and also some hydrophobic antioxidants such as vitamin E (Erel, 2004). Although uric acid may have a dominant influence on the total antioxidant capacity of plasma in birds (Cohen *et al.*, 2007; Costantini, 2011), our previous analysis did not reveal a strong relationship between the level of uric acid and total antioxidants in gull plasma samples ($r = 0.048$, $n = 54$, $P = 0.728$; unpublished data). Within-individual repeatability of each physiological measurement was estimated by following Lessells & Boag (1987).

Lipid peroxidation in plasma (i.e. oxidative damage in lipids) was assessed in triplicate by quantifying malondialdehyde (MDA) using high-performance liquid chromatography. We followed the methods described by Karatas *et al.* (2002), but modified the volume of sample (10 μL) and reagents (see also Noguera *et al.*, 2011a). Briefly, a plasma sample (10 μL) was mixed with 50 μL to 0.1 M $HClO_4$. Then, 140 μL of ultra-pure water was added, and the mixture was centrifuged at 1677 *g* for 1 min. The addition of acid was necessary to purify the sample and release the MDA bound to the amino groups of proteins and other amino compounds. An aliquot of 100 μL of the supernatant was taken and stored in cold for the analysis. Purified samples were injected into a HPLC system (JASCO Comparison Proven, model 1500) fitted with a Security Guard precolumn (particle size 5 μm) and a C18 reverse phase analytical column (Sphere Clone type ODS(2), Phenomenex, Torrance, CA). The absorbance of the eluent was monitored at 254 nm, and 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St. Louis, MO) was used as a external standard (calibration curves, $R^2 = 0.999$). Lipid peroxidation was expressed as microgram of MDA per mL of plasma (Noguera *et al.*, 2011b). The lipid peroxidation assays were repeatable at both age 7 days ($r = 0.970$, $F_{219,439} = 199.659$, $P < 0.001$) and age 12 days ($r = 0.988$, $F_{113,225} = 486.049$, $P < 0.001$).

The level of reactive oxygen metabolites (ROMs) in plasma was also assessed in duplicate using the method described in Brambilla *et al.* (2001). ROMs, including hydroperoxides, are generated by the oxidation of organic molecules such as lipids, amino acids and nucleotides. Thus, the level of ROMs is a general indicator of cellular oxidative stress. ROMs also have the potential to act as oxidants themselves, thereby amplifying damage throughout the body. Briefly, ROMs present in plasma (5 μL) were reacted with *N,N*-diethyl-p-phenylenediamine to produce a coloured complex that can be measured spectrophotometrically at 495 nm (Synergy™ 2 Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc.). This measure was expressed as mmol H_2O_2 equivalent/L (repeatability: day 7: $r = 0.776$, $F_{215,218} = 7.944$, $P < 0.001$; day 12: $r = 0.674$, $F_{113,114} = 5.144$, $P < 0.001$). This assay of ROMs mainly measures the reaction of alkoxy and peroxy radicals derived from the hydroperoxides with *N,N*-diethyl-*para*-phenylenediamine (Alberti *et al.*, 2000).

Measuring corticosterone level

We measured corticosterone concentration in plasma sampled at age 7 days using a commercially available enzyme-linked immunosorbent assay (ELISA Kit EIA-4164 from DRG Diagnostics, Marburg, Germany), based on the principle of competitive binding. The samples were analysed in duplicates according to the manufacturer's instructions. In brief, plasma samples previously diluted in ultrapure water were incubated with a corticosterone-horseradish peroxidase conjugate for 60 min in a microtiter plate. Afterwards, microtiter plate was washed three times and allowed to react with a substrate solution leading to a blue–green complex. The change in absorbance at 450 nm (Synergy™ 2 Multi-Mode Microplate Reader, Bio-Tek Instruments, Inc.) of the blue–green complex was reverse proportional to the concentration of corticosterone. Duplicate sample analyses showed that the assays were significantly repeatable ($r = 0.752$, $F_{194,192} = 7.053$, $P < 0.001$).

General statistics

The effects of vitamin supplement experiment were analysed using a linear mixed effect model (LMM) or generalized linear mixed model (GLMM) using PROC MIXED and PROC GLIMMIX in SAS (SAS Institute, 1999). Identities of original and foster nests were included as random effects nested within the cross-foster group (i.e. a group of four nests in which

cross-fostering was performed, see Fig. S1) to account for the nonindependence of chicks from the same original and foster broods. Cross-foster group was also included as a random effect. Body mass, total antioxidant capacity, lipid peroxidation (i.e. MDA) and ROMs were analysed using repeated-measure LMMs that include data obtained from chicks at ages 7 and 12 days. Corticosterone concentration was analysed using a standard LMM because this was measured only once in each chick at age 7 days. Chick survival was analysed using a GLMM with a binomial error distribution.

Initially, a mixed model with all explanatory variables and two-way interactions of interest were fitted. Then, nonsignificant interactions and main terms were dropped sequentially to simplify the model until we obtained a minimum adequate model that included only terms significant at the $P < 0.05$ level. The structure of fixed effects in models for examining the effects of vitamin supplement was as follows: body mass = treatment + age + order + sex + hatch date + hatching mass + age × treatment + order × treatment + sex × treatment; antioxidants = treatment + age + order + sex + hatch date + antioxidants (day 0) + MDA + age × treatment + order × treatment + sex × treatment + MDA × treatment + MDA × age; MDA or ROMs = treatment + age + order + sex + hatch date + antioxidants (day 0) + age × treatment + order × treatment + sex × treatment; corticosterone = treatment + order + sex + hatch date + order × treatment + sex × treatment; chick survival = treatment + order + sex + hatch date + order × treatment + sex × treatment. The structure of models examining the effects of corticosterone level measured at age 7 days on body mass and oxidative status at 12 days was as follows: corticosterone treatment + order + sex + hatch date (+hatching mass) + corticosterone × treatment + order × treatment + sex × treatment.

Quantitative genetic analyses

We estimated genetic and environmental (co)variances in quantitative traits using a cross-fostering design and robust quantitative genetic analysis (see also Kim *et al.*, 2010b, 2011) using ASReml v3 (VSN International, Hemel Hempstead, UK; Gilmour *et al.*, 2008). Genetic variance component and two common environmental components ($V_{Origin(CF)}$, $V_{Foster(CF)}$ and V_{CF}, respectively) of total phenotypic variance (V_P) for each trait were estimated in a restricted maximum-likelihood (REML) univariate linear mixed effect model, with original and foster nests (nested within the cross-foster group) and cross-foster group included as random effects.

Our preliminary analyses suggested that the levels of MDA and ROMs in plasma did not have a heritable variance (MDA: $V_{Origin(CF)} < 10^{-7}$, $\chi^2_1 = 0$, $P = 1$; ROMs: $V_{Origin(CF)} < 10^{-6}$, $\chi^2_1 = 0$, $P = 1$). Therefore, subsequent quantitative genetic analyses were performed for only body mass and total antioxidants and corticosterone in plasma at age 7 days. Note that body mass and total antioxidants at age 12 days, although measured, were not used for quantitative genetic analyses because few genetic or foster siblings were sampled (only 42.9% of hatched chicks were alive and sampled at age 12 days). Significant fixed effects in the previous analyses explained above were included in the quantitative genetic models. Additive genetic variance (V_A) was calculated as twice the variance component of the original nest ($V_{Origin(CF)}$) because full siblings share on average 50% of their genes. Note that extra-pair paternity is rare in gulls (Bukacinska *et al.*, 1998; Gilbert *et al.*, 1998). Total phenotypic variance was calculated as the sum of the original nest, foster nest, cross-foster group and residual variance components ($V_P = V_{Origin(CF)} + V_{Foster(CF)} + V_{CF} + V_R$). Heritability and common environmental effects were calculated as $h^2 = V_A/V_P$, $f^2 = V_{Foster(CF)}/V_P$ and $cf^2 = V_{CF}/V_P$, respectively, for each trait. The statistical significance of each variance component was tested using likelihood ratio test (LRT) that compares models based on -two times the difference in REML log-likelihood scores distributed as chi-square where the degree of freedom equalled the number of variance terms removed.

To compare $V_{Origin(CF)}$, $V_{Foster(CF)}$ and V_{CF} of the same trait between the two experimental groups (i.e. NS and VS), first, we performed separated univariate model analysis for each treatment. Then, we tested explicitly whether the size of each variance component differs significantly between the two experimental groups using a bivariate mixed model fitted to the data from both NS and VS for each trait (see also Husby *et al.*, 2010). The same fixed effects as in the univariate analysis were included in the bivariate analysis. In the model, all covariances, including residual covariance, were constrained to zero. The comparison between NS and VS was carried out by constraining each variance component in the two groups to be equal, then using a LRT to compare the likelihood of this model with the unconstrained model.

Additionally, phenotypic, genetic and environmental correlations (r_P, r_G, r_F and r_{CF}) among body mass, total antioxidants capacity and corticosterone level in chicks at age 7 days were estimated using a REML multivariate mixed-effect model fitted to each experimental group. Genetic and environmental covariances (Cov_G, Cov_F and Cov_{CF}) among the three traits were estimated by including original and foster nests (nested within the cross-foster group) and cross-foster group as random effects. Cov_G was calculated as twice the original nest covariance, and Cov_F and Cov_{CF} were equal to the foster nest and cross-foster group covariances. The significance of each covariance was assessed using LRT that compares the full model and a reduced model by fixing covariance parameter of the random effect to zero.

Results

Effects of vitamin supplement on body mass and survival

Overall, the mean body mass was 120.7 (±32.2 SD) g at age 7 days (n = 221 chicks) and 206.1 (±57.4) g at age 12 days (n = 120). Repeated-measure analysis of body mass of chicks at age 7 and 12 days revealed that the third chicks (i.e. chicks from the third eggs in three eggs clutch) supplemented with vitamins C and E (VS) were heavier than those supplemented only with water (NS), but this pattern of treatment effect did not appear in the first and second chicks, while the significant effects of chick age on the day of weighing, chick order in the brood, hatching date and body mass at hatching were taken into account (Fig. 2; repeated-measure LMM: age: $F_{1,125}$ = 303.94, P < 0.001; order: $F_{2,178}$ = 4.57, P = 0.012, treatment: $F_{1,108}$ = 0.01, P = 0.938; hatch date: $F_{1,161}$ = 12.13, P < 0.001; hatching mass: $F_{1,200}$ = 9.03, P = 0.003; treatment × order: $F_{2,146}$ = 3.31, P = 0.039). Chick sex and other interaction terms, treatment × age and treatment × sex, were nonsignificant (P > 0.237).

A total of 59 chicks survived to day 12 among 117 hatchlings from VS broods, and 60 chicks survived among 115 hatchlings from NS broods. These numbers exclude the chicks missed from sampling at day 12 and not found dead. Probability of survival to day 12 did not differ between VS and NS chicks (P = 0.979); only chick order and hatching date were significant in the model of chick survival (GLMM with a binomial error distribution: order: $F_{2,51}$ = 3.39, P = 0.042; hatching date: $F_{1,51}$ = 18.50, P < 0.001). The third chicks (from the third eggs) were less likely to survive than the first and second chicks (mean survival probability: first: 0.62; second: 0.61; third: 0.26); the probability of chick survival decreased with hatching date.

Effects of vitamin supplement on oxidative stress and corticosterone

Vitamin supplement did not affect any of the physiological traits related to oxidative stress (i.e. total antioxidants, MDA and ROMs in plasma) and the corticosterone level in study chicks.

None of the fixed factors, covariates and interactions (i.e. age, order, sex, treatment, hatch date, total antioxidants at hatching, treatment × age, treatment × order, treatment × sex) explained the level of lipid peroxidation measured as MDA concentration and the ROMs level in plasma sampled from chicks at ages 7 and 12 days (MDA: P > 0.094; ROMs: P > 0.107). The overall mean of total antioxidant capacity was 1.395 (±1.029) mM Trolox equivalents. In the analysis of total antioxidants in plasma, additionally the effect of MDA was examined. There was a significant interaction between MDA and chick age (age: $F_{1,179}$ = 0.04, P = 0.836; MDA: $F_{1,172}$ = 76.37, P < 0.001; age ×MDA: $F_{1,183}$ = 4.65, P = 0.032). In general, increased level of MDA concentration was associated with raised total antioxidants in plasma, and the positive relationship was stronger at age 12 days than at age 7 days (Fig. 3). All other factors, covariates and interactions were nonsignificant (P > 0.079). Some samples, particularly those sampled at age 7 days, showed extremely low levels of total antioxidants (Fig. 3). Excluding those 72 samples, which showed consistently low values in duplicate sample analyses, did not change the results (age: $F_{1,159}$ = 0.46, P = 0.497; MDA: $F_{1,147}$ = 75.72, P < 0.001; age × MDA: $F_{1,158}$ = 6.87, P = 0.010).

The level of corticosterone in plasma sampled at age 7 days differed significantly between the two sexes ($F_{1,164}$ = 19.35, P < 0.001; mean ± SE: males: 90.84 ± 5.14 nM, n = 100; females: 127.83 ± 7.51 nM, n = 90). All other fixed effects, vitamin treatment, chick order and hatching date, and all interactions of interest (i.e. treatment × order, treatment × sex) were nonsignificant (P > 0.077).

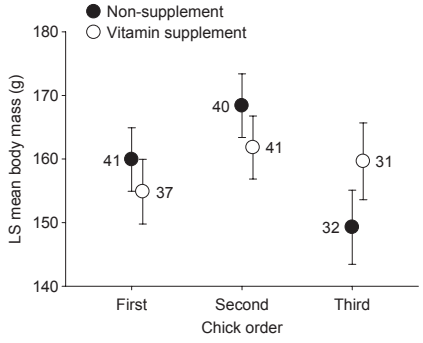

Fig. 2 Body mass according to chick order (laying order) in vitamin supplement and nonsupplement broods. Least square means ± SE from LME, including hatching date, hatching mass and age on the day of weighing to account for their effects, are presented. Number of individuals is presented in each error bar.

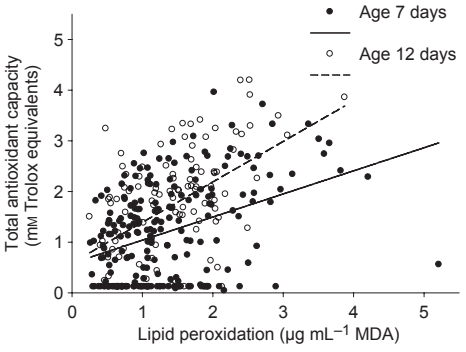

Fig. 3 Relationship between lipid peroxidation (MDA concentration) and total antioxidants in plasma. The fitted lines are linear regressions at ages 7 and 12 days.

Effects of corticosterone on body mass and oxidative stress

Body mass of chicks at age 12 days was significantly correlated with corticosterone level in plasma sampled at age 7 days (LMM: $F_{1,101} = 9.40$, $P = 0.003$). Chicks that showed lower level of corticosterone in plasma at day 7 were heavier 5 days later (Fig. 4a). A significant term, hatching date, was retained in the model ($F_{1,52.8} = 4.89$, $P = 0.031$), but vitamin treatment, sex, order, hatching mass, treatment × order, treatment × sex and treatment × corticosterone were nonsignificant ($P > 0.184$). Excluding three possible outliers (corticosterone level >294.2 nM; all are female chicks) did not alter these results (corticosterone: $F_{1,94.6} = 4.74$, $P = 0.032$; hatching date: $F_{1,61.5} = 5.23$, $P = 0.026$).

Corticosterone level in plasma at age 7 days was not correlated with MDA concentration and total antioxidants in plasma sampled 5 days later ($P = 0.404$ and $P = 0.170$, respectively). However, chicks with higher level of corticosterone showed increased ROMs level in plasma at age 12 days ($F_{1,99.4} = 23.29$, $P < 0.001$; Fig. 4b). All other effects and interactions were nonsignificant ($P > 0.160$). An additional analysis excluding three possible outliers also showed significant effect of corticosterone ($F_{1,97.4} = 9.60$, $P = 0.003$).

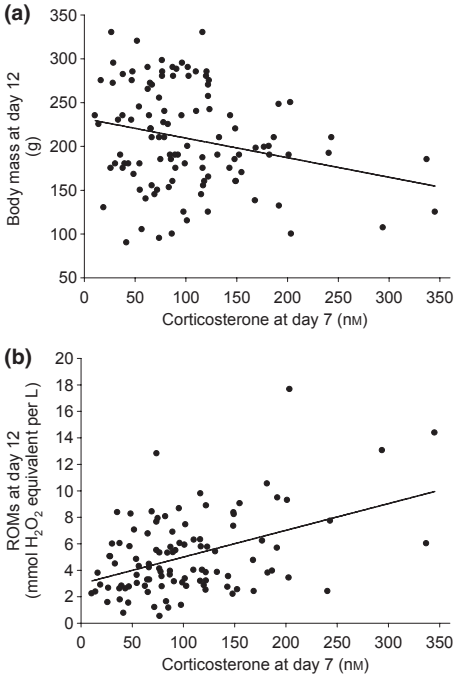

Fig. 4 Effects of corticosterone level in plasma sampled at age 7 days. (a) Body mass and (b) ROMs level in plasma measured at age 12 days. The fitted lines are linear regressions.

Quantitative genetics of growth, antioxidants and corticosterone

All quantitative genetic models converged successfully. The between-individual variation (V_P) in body mass showed nonsignificant genetic component and significant common environmental component, estimated as original nest and foster nest variances, respectively, in both NS and VS broods (see Table 1 for estimates and tests). Cross-foster group variance was nonsignificant in both NS and VS broods. Both heritability and foster nest effect of body mass were comparable between VS chicks and control chicks (NS: $h^2 = 0.290 \pm 0.211$ SE, $f^2 = 0.308 \pm 0.123$, $cf^2 = 0.099 \pm 0.121$; VS: $h^2 = 0.306 \pm 0.239$, $f^2 = 0.313 \pm 0.119$, $cf^2 = 0$), although V_P increased in VS broods. The additional analysis using a bivariate model showed that the differences in $V_{Origin(CF)}$, $V_{Foster(CF)}$ and V_{CF} between the two experimental groups were statistically nonsignificant (Table 1).

Results from the univariate models fitted to all chicks that survived to age 7 days showed that nest of origin explained a high and significant proportion of phenotypic variance for total antioxidants in plasma in VS chicks ($h^2 = 0.586 \pm 0.259$, $f^2 = 0$, $cf^2 = 0$) whereas $V_{Origin(CF)}$ was null in NS chicks (Table 1; $h^2 = 0$, $f^2 = 0.036 \pm 0.124$, $cf^2 = 0.015 \pm 0.062$). The difference in $V_{Origin(CF)}$ between NS and VS broods was statistically significant (Table 1).

Results from the univariate model of corticosterone concentration in plasma showed that the phenotypic variance in this trait had a significant genetic component ($V_{Origin(CF)}$), but a nonsignificant variance by common environment components ($V_{Foster(CF)}$ and V_{CF}) in NS broods (Table 1; $h^2 = 0.573 \pm 0.284$, $f^2 = 0.136 \pm 0.109$, $cf^2 = 0.076 \pm 0.116$). All variance components decreased and were nonsignificant in VS chicks (Table 1; $h^2 = 0.190 \pm 0.309$, $f^2 = 0$, $cf^2 = 0$). However, the bivariate analysis did not provide evidence for a statistical difference between NS and VS chicks in all variance components (Table 1). Neither NS nor VS broods showed significant genetic covariance between body mass, total antioxidants and corticosterone level (Table 2).

Discussion

Dietary supplement of vitamins C and E did not influence the average body mass (except for the third chicks) and the levels of total antioxidants, lipid peroxidation (MDA), ROMs and corticosterone in plasma of growing gull chicks. However, an exciting finding in the present study is that, despite these negligible effects on the average phenotypes, the expression of genetic and/or phenotypic variances in body mass, total antioxidants and corticosterone level was altered by the experimental treatment. This change in genetic variance of each trait was not attributed to that of the

Table 1 Quantitative genetic results from univariate mixed-effect models of body mass, total antioxidants and corticosterone level in chicks at age 7 days; comparisons between nonsupplement (NS) and vitamin supplement (VS) broods from bivariate mixed-effect models.

		NS			VS			Comparison NS vs. VS	
Trait	Variance	Estimate	χ^2_1	*P*	Estimate	χ^2_1	*P*	χ^2_1	*P*
Body mass		(*n* = 112)			(*n* = 109)				
	$V_{Origin(CF)}$	110.88 ± 82.992	1.806	0.179	160.93 ± 130.20	1.582	0.208	2.826	0.093
	$V_{Foster(CF)}$	235.43 ± 104.42	8.286	0.004	329.15 ± 147.56	6.774	0.009	0.280	0.597
	V_{CF}	75.44 ± 97.68	0.840	0.359	$<10^{-4}$	0	1	0.250	0.617
	V_P	763.84 ± 125.87			1050.50 ± 156.45				
Antioxidants		(*n* = 107)			(*n* = 108)				
	$V_{Origin(CF)}$	$<10^{-7}$	0	1	0.277 ± 0.140	4.014	0.045	19.732	<0.001
	$V_{Foster(CF)}$	0.023 ± 0.078	0.096	0.757	$<10^{-7}$	0	1	3.456	0.063
	V_{CF}	0.010 ± 0.039	0.070	0.791	$<10^{-6}$	0	1	0.384	0.535
	V_P	0.631 ± 0.087			0.945 ± 0.138				
Corticosterone		(*n* = 100)			(*n* = 90)				
	$V_{Origin(CF)}$	1108.2 ± 601.7	4.198	0.040	356.5 ± 588.6	0.388	0.533	1.664	0.197
	$V_{Foster(CF)}$	526.8 ± 429.3	2.038	0.153	$<10^{-2}$	0	1	2.960	0.085
	V_{CF}	292.4 ± 463.7	0.500	0.480	$<10^{-3}$	0	1	0.382	0.537
	V_P	3866.7 ± 627.5			3759.5 ± 570.0				

Fixed effects: body mass = chick order + hatch date + hatching mass; antioxidants = MDA; corticosterone = sex.

Table 2 Genetic, environmental and phenotypic covariances (±SE) and correlations among body mass and total antioxidants and corticosterone level in NS and VS chicks at age 7 days from multivariate mixed-effect models.

	NS				VS			
Traits	Cov_P	r_P	χ^2_1	*P*	Cov_P	r_P	χ^2_1	*P*
Mass–antioxidants	3.533 ± 2.181	0.167 ± 0.100	4.392	0.036	5.087 ± 3.294	0.158 ± 0.099	2.986	0.084
Mass–corticosterone	−444.3 ± 191.6	−0.265 ± 0.105	11.700	<0.001	−599.5 ± 226.3	−0.302 ± 0.100	8.166	0.004
Antioxidants–corticosterone	0.515 ± 4.706	0.011 ± 0.098	1.278	0.258	5.251 ± 7.195	0.083 ± 0.113	0.598	0.439
	Cov_G	r_G	χ^2_1	*P*	Cov_G	r_G	χ^2_1	*P*
Mass–antioxidants	n.e.	n.e.	–	–	1.755 ± 6.817	0.102 ± 0.384	0.062	0.803
Mass–corticosterone	164.3 ± 315.33	0.209 ± 0.435	0.160	0.689	−559.8 ± 438.9	−0.782 ± 0.517	1.738	0.187
Antioxidants–corticosterone	n.e.	n.e.	–	–	9.533 ± 14.564	0.296 ± 0.456	0.418	0.518
	Cov_F	r_F	χ^2_1	*P*	Cov_F	r_F	χ^2_1	*P*
Mass–antioxidants	−0.714 ± 2.166	−0.224 ± 0.751	0.138	0.710	n.e.	n.e.	–	–
Mass–corticosterone	−253.8 ± 185.3	−0.559 ± 0.288	0.004	0.950	n.e.	n.e.	–	–
Antioxidants–corticosterone	−0.975 ± 4.321	−0.185 ± 0.845	0.052	0.820	n.e.	n.e.	–	–
	Cov_{CF}	r_{CF}	χ^2_1	*P*	Cov_{CF}	r_{CF}	χ^2_1	*P*
Mass–antioxidants	0.006 ± 1.143	0.100 ± 18.92	0.094	0.759	n.e.	n.e.	–	–
Mass–corticosterone	−1.017 ± 116.5	−0.221 ± 24.12	0.196	0.658	n.e.	n.e.	–	–
Antioxidants–corticosterone	−0.004 ± 2.584	−0.028 ± 20.70	0.872	0.350	n.e.	n.e.	–	–

n.e.: nonestimable due to one or more 0 variance.
Fixed effects: Mass–Antioxidants–Corticosterone = chick order + sex + hatch date + hatching mass + MDA.

other traits because there was no significant genetic covariance among these traits according to our analyses using multivariate models.

At the phenotypic level, our results showed that the third chicks (but not the first and second chicks) supplemented with vitamins C and E grew faster than the control chicks during an early stage of life. In *Larus* gull species, the third chicks (from eggs laid in the third position) are generally the smallest in the brood at hatching and receive a lower level of maternally derived antioxidants such as vitamin E and carotenoids (Royle *et al.*, 2001). The difference in maternal alloca-

tion among eggs within a clutch might give rise to variation in the effect of extra antioxidants on growth in gull chicks, whereas such effect appeared irrespective of hatch rank in nestling red-winged blackbirds (Hall *et al.*, 2009). Therefore, the third chicks, which received vitamin supplement, accelerated growth possibly by allocating extra antioxidants to defend against ROS or by enhancing intensity of begging to their foster parents (Hall *et al.*, 2009; Noguera *et al.*, 2010). However, this benefit of vitamin supplement in growth did not change early survival rate of the third chicks.

Recent studies of antioxidant supplement to avian young have revealed that chicks allocate extra antioxidants in increasing begging intensity, growing faster and larger and improving immune competence (de Ayala *et al.*, 2006; Hall *et al.*, 2009; Noguera *et al.*, 2010; Orledge *et al.*, 2012). However, antioxidant supplement often has no direct influence in improving oxidative balance or reducing oxidative damage in the wild (e.g. Hall *et al.*, 2009; Larcombe *et al.*, 2010; Noguera *et al.*, 2011b; but see studies of poultries, Surai, 2002) as is the case also in the present study. Dietary antioxidants are probably a scarce and limiting resource for wild birds. Thus, developing birds may allocate extra antioxidants preferentially to biological functions related to fitness such as growth, size at maturation, sibling competition for parental care, immunity and resistance to pathogens, thereby influencing trade-offs involving oxidative balance rather than oxidative balance itself.

Our result of the positive correlation between antioxidant capacity and lipid peroxidation in plasma is also interesting. This indicates that the greater antioxidant capacity cannot be understood as a proxy of reduced oxidative damage as studies of oxidative stress often do. Rather, the positive correlation between antioxidant capacity and lipid peroxidation measured at the same age suggests that individuals with a higher level of oxidative damage invested more in the antioxidant system to subsequently recover oxidative balance (Monaghan *et al.*, 2009) or that the increase in oxidative stress triggered the up-regulation of antioxidant defence (Scandalios, 2005).

Our correlative results suggest that a high level of corticosterone in plasma during early growth was associated with suppressed body mass gain and increased production of ROMs in chicks regardless of vitamin supplement as illustrated in Fig. 1. In vertebrates, the release of glucocorticoids after exposure to a stressor (acute stress response) promotes the reallocation of resources from energy-consuming systems (immunity, growth, etc.) to short-term survival (Wingfield & Sapolsky, 2003). Baseline (low) glucocorticoid levels are nonetheless required for normal metabolism (Wingfield & Sapolsky, 2003). A previous study showed that experimentally elevated corticosterone reduced body growth and resistance to oxidative stress in barn owl nestlings (Stier *et al.*, 2009), and a recent meta-analysis suggests that administration of glucocorticoids has a significant effect on oxidative stress (Costantini *et al.*, 2011). Nevertheless, it has been unknown whether nonmanipulated level of corticosterone has the same effect. Another possibility is that as a response (or a result) to stressful situations, corticosterone and ROMs levels were increased independently, and they interactively suppressed growth of chicks.

The most important result in this study is that phenotypic variance in total antioxidants in plasma increased in the chicks supplemented with vitamins mainly due to increased additive genetic variance. Common environmental effects (from shared environment within foster nest and cross-foster group) on phenotypic variation in total antioxidants were negligible in the control chicks (5.1% of phenotypic variance) and disappeared in the vitamin-supplemented chicks. The low similarity between foster sibs that shared the same rearing environments suggests that the role of dietary antioxidants from parental provisioning was minor in plasma antioxidants. The total phenotypic variance of antioxidant capacity increased in the vitamin-supplemented chicks while the average phenotype was maintained. This indicates that increased availability of dietary antioxidants does not influence oxidative balance in all individuals at the same level, with some individuals lowering antioxidant capacity in response to the supplement, whereas some others increasing antioxidant capacity possibly by absorbing the exogenous sources of antioxidants. In humans, administration of vitamins E and C reduces the normal response to increased metabolism that promotes the production of endogenous antioxidants (such as superoxide dismutases 1 and 2 and glutathione peroxidase) to defend against ROS (Ristow *et al.*, 2009). Thus, in the present study, it is possible that the vitamin supplement reduces the expression of endogenous antioxidants in some experimental chicks.

The change in genetic variance for total antioxidant capacity suggests that individuals differ in the ability to capture, absorb and transport dietary antioxidants or to produce endogenous antioxidants, and importantly, this ability has a genetic basis. Our experimental study demonstrates that the level of total antioxidant capacity in plasma is under genetic control (for evidence in human, see Wang *et al.*, 2001) and that this genetic effect is probably cryptic at some extent. Availability of exogenous antioxidants in environments may influence the expression of cryptic genetic variance for antioxidant capacity.

Significant genetic variance of corticosterone level in the control chicks did not appear in the vitamin-supplemented chicks, although the difference in genetic variance between the treatment groups was nonsignificant, and the total phenotypic variance was maintained at the similar level. Some experimental studies also have shown heritability of corticosterone response in captive birds (Evans *et al.*, 2006 and references therein). The production of corticosterone is an adaptive response to

stressors in favour of activities concerned with short-term survival (Wingfield, 2002). Vitamin supplement possibly suppressed gene expression for producing corticosterone, which controls metabolic processes, thereby reducing genetic variance for this trait. Under circumstances that more dietary antioxidants are available, the importance of corticosterone as a modulator of metabolism may be reduced in growing chicks.

In birds, females allocate maternal antioxidants and hormones into eggs, and such maternal effects may inflate genetic effects estimated in quantitative genetic models based on full siblings (Eising *et al.*, 2001; Surai, 2002; Rubolini *et al.*, 2006). Our previous study suggested that antioxidant capacity during the growth may be independent to that at hatching, and maternally derived antioxidants in eggs probably have rather weak influences on the similarity in oxidative conditions among genetic siblings during the post-natal growth (Kim *et al.*, 2010b). However, we cannot exclude the possibility of maternal effects through hormones on the levels of antioxidant capacity and corticosterone.

In conclusion, this study suggests that the availability of exogenous antioxidants in environment influences the expression of genetic variations in important physiological functions (i.e. total antioxidants and corticosterone levels in plasma) that influence metabolic processes during early growth. Although there was no evidence that such environment-dependent genetic variances also influence the average phenotypes, it is possible that the gene expression in early life affects shaping later phenotypes (Spencer *et al.*, 2009). Under natural conditions, it is likely that the consumption of exogenous antioxidants increases as the total amount of food fed by parent birds increases. Therefore, in such circumstances enhancing individual capacity for capture and absorption of exogenous antioxidants and suppressing the production of corticosterone should help growing chicks to prepare for the maintenance of oxidative balance during fast growth. Our results suggest that environmental inputs may shape evolutionary trajectories of antioxidant capacity because this trait could evolve only in antioxidants-rich environments where the cryptic genetic variance is expressed. The inconstancy of heritable variance between the two experimental groups, containing randomly selected families, suggests the presence of G×E in response to exogenous antioxidants availability. Furthermore, the endocrine system mediates the reaction norm by transmitting environmental cues to adaptively control gene expression (Lessells, 2008). The future studies should quantify the endogenous and exogenous sources of antioxidants that contribute the changes in genetic and phenotypic variances to confirm the presence of G×E and investigate the mechanisms. Whether the cryptic genetic variation in antioxidant capacity, released by certain environmental conditions, contributes to phenotypic evolution should be explored (McGuigan & Sgrò, 2009).

Acknowledgments

We are most grateful to two anonymous reviewers for constructive comments. We thank C. Pérez, A. Lucas and C. Lazcano for invaluable help during the fieldwork, I. López-Rull and C. Alonso-Alvarez for advice in the hormone analysis and Á. Naya Alba for providing vitamin supplements for animals. Fieldwork in Sálvora Island depended on the generous support and friendship of Pepin, Cristina, Marta, Marcos, Pablo, Jacinto, Andrés and Gemma of the Parque Nacional, and los fareros, Pepe and Julio. Finance was provided by the Spanish Ministerio de Ciencia e Innovación (CGL2009-10883-C02-01 and CGL2012-40229-C02-02). S.-Y.K. is supported by the Isidro Parga Pondal fellowship (Xunta de Galicia) and J.C. N. by the FPI grants (MICINN) and the Marie Curie Intra European Fellowship within the 7th European Framework Program (PIEF-GA-2011-301093).

References

Alberti, A., Bolognini, L., Macciantelli, D. & Caratelli, M. 2000. The radical cation of *N, N*-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. *Res. Chem. Intermed.* **26**: 253–267.

Alonso-Alvarez, C., Bertrand, S., Faivre, B. & Sorci, G. 2007. Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Funct. Ecol.* **21**: 873–879.

de Ayala, R.M., Martinelli, R. & Saino, N. 2006. Vitamin E supplementation enhances growth and condition of nestling barn swallows (*Hirundo rustica*). *Behav. Ecol. Sociobiol.* **60**: 619–630.

Badyaev, A.V. 2009. Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Phil. Trans. R. Soc. B* **364**: 1125–1141.

Beckman, K.B. & Ames, B.N. 1998. The free radical theory of aging matures. *Physiol. Rev.* **78**: 547–581.

Brambilla, G., Fiori, M. & Archetti, L.I. 2001. Evaluation of the oxidative stress in growing pigs by microplate assays. *J. Vet. Med. Ser. A* **48**: 33–38.

Brand, M.D. 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Exp. Gerontol.* **35**: 811–820.

Buchanan, K.L. 2000. Stress and the evolution of condition-dependent signals. *Trends Ecol. Evol.* **15**: 156–160.

Bukacinska, M.D., Bukacinska, J.T., Epplen, J.T., Sauer, K.P. & Lubjuhn, T. 1998. Low frequency of extra-pair paternity in common gulls (*Larus canus*) as revealed by DNA fingerprinting. *J. Ornithol.* **139**: 413–420.

Catoni, C., Peters, A. & Schaefer, M. 2008. Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. *Anim. Behav.* **76**: 1107–1119.

Charmantier, A. & Garant, D. 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proc. R. Soc. B* **272**: 1415–1425.

Charmantier, A., Kruuk, L.E.B. & Lambrechts, M.M. 2004. Parasitism reduces the potential for evolution in a wild bird population. *Evolution* **58**: 203–206.

Cockrem, J.F. & Silverin, B. 2002. Variation within and between birds in corticosterone responses of great tits (*Parus major*). *Gen. Comp. Endocrinol.* **125**: 197–206.

Cohen, A., Klasing, K. & Ricklefs, R. 2007. Measuring circulating antioxidants in wild birds. *Comp. Biochem. Physiol. B* **147**: 110–121.

Costantini, D. 2011. On the measurement of circulating antioxidant capacity and the nightmare of uric acid. *Methods Ecol. Evol.* **2**: 321–325.

Costantini, D. & Dell'Omo, G. 2006. Environmental and genetic components of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J. Comp. Physiol. B.* **176**: 575–579.

Costantini, D., Marasco, V. & Moller, A.P. 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J. Comp. Physiol. B.* **181**: 447–456.

Cote, J., Meylan, S., Clobert, J. & Voituron, Y. 2010. Carotenoid-based coloration, oxidative stress and corticosterone in common lizards. *J. Exp. Biol.* **213**: 2116–2124.

Dias, M.G., Sánchez, M.V., Bártolo, H. & Oliveira, L. 2003. Vitamin content of fish and fish products consumed in Portugal. *Electron. J. Environ. Agric. Food Chem.* **2**: 510–513.

Dingemanse, N.J., Van der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D.A., *et al.* 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proc. R. Soc. B* **276**: 1285–1293.

Eising, C.M., Eikenaar, C., Schwabl, H. & Groothuis, T.G.G. 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. *Proc. R. Soc. B* **268**: 839–846.

Erel, O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.* **37**: 277–285.

Evans, M.R., Roberts, M.L., Buchanan, K.L. & Goldsmith, A.R. 2006. Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *J. Evol. Biol.* **19**: 343–352.

Finkel, T. & Holbrook, N.J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* **408**: 239–247.

Fridolfsson, A.K. & Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* **30**: 116–121.

Gilbert, L., Burke, T. & Krupa, A. 1998. No evidence for extrapair paternity in the western gull. *Mol. Ecol.* **7**: 1549–1552.

Gilmour, A.R., Gogel, B.J., Cullis, B.R. & Thompson, R. 2008. *ASReml User Guide, Release 3.0*. VSN International Ltd., Hemel Hempstead.

Hager, R., Cheverud, J.M. & Wolf, J.B. 2009. Change in maternal environment induced by cross-fostering alters genetic and epigenetic effects on complex traits in mice. *Proc. R. Soc. B.* **276**: 2949–2954.

Hall, M.E., Blount, J.D., Forbes, S. & Royle, N.J. 2009. Does oxidative stress mediate the trade-off between growth and self-maintenance in structured families? *Funct. Ecol.* **24**: 365–373.

Halpner, A.D., Handelman, G.J., Harris, J.M., Belmont, C.A. & Blumberg, J.B. 1998. Protection by vitamin C of loss of vitamin E in cultured rat hepatocytes. *Arch. Biochem. Biophys.* **359**: 305–309.

Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**: 298–300.

Haussmann, M.F., Longenecker, A.S., Marchetto, N.M., Juliano, S.A. & Bowden, R.M. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proc. R. Soc. B.* **279**: 1447–1456.

Hayward, L.S. & Wingfield, J.C. 2004. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen. Comp. Endocrinol.* **135**: 365–371.

Hoffmann, A.A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* **14**: 96–101.

Husby, A., Nussey, D.H., Visser, M.E., Wilson, A.J., Sheldon, B.C. & Kruuk, L.E.B. 2010. Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. *Evolution* **64**: 2221–2237.

SAS Institute1999. *SAS/STAT, Version 8*. SAS Institute, Cary, NC.

Isaksson, C., Sheldon, B.C. & Uller, T. 2011. The challenges of integrating oxidative stress into life-history biology. *Bioscience* **61**: 194–202.

Karatas, F., Karatepe, M. & Baysar, A. 2002. Determination of free malondialdehyde in human serum by high performance liquid chromatography. *Anal. Biochem.* **311**: 76–79.

Kim, S.-Y., Velando, A., Sorci, G. & Alonso-Alvarez, C. 2010a. Genetic correlation between resistance to oxidative stress and reproductive life span in a bird species. *Evolution* **64**: 852–857.

Kim, S.-Y., Noguera, J.C., Morales, J. & Velando, A. 2010b. Heritability of resistance to oxidative stress in early life. *J. Evol. Biol.* **23**: 769–775.

Kim, S.-Y., Noguera, J.C., Morales, J. & Velando, A. 2011. Quantitative genetic evidence for trade-off between growth and resistance to oxidative stress in a wild bird. *Evol. Ecol.* **25**: 461–472.

Larcombe, S.D., Mullen, W., Alexander, L. & Arnold, K.E. 2010. Dietary antioxidants, lipid peroxidation and plumage colouration in nestling blue tits *Cyanistes caeruleus*. *Naturwissenschaften* **97**: 903–913.

Lessells, C.M. 2008. Neuroendocrine control of life histories: what do we need to know to understand the evolution of phenotypic plasticity? *Phil. Trans. R. Soc. B* **363**: 1589–1598.

Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116–121.

Lin, H., Decuypere, E. & Buyse, J. 2004. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 1. Chronic exposure. *Comp. Biochem. Physiol. B* **139**: 737–744.

Lin, H., Sui, S.J., Jiao, H.C., Buyse, J. & Decuypere, E. 2006. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. *Comp. Biochem. Physiol. A* **143**: 400–405.

McGuigan, K. & Sgrò, C.M. 2009. Evolutionary consequences of cryptic genetic variation. *Trends Ecol. Evol.* **24**: 305–311.

Metcalfe, N.B. & Alonso-Alvarez, C. 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* **24**: 984–996.

Monaghan, P., Metcalfe, N.B. & Torres, R. 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**: 75–92.

Noguera, J.C., Morales, J., Pérez, C. & Velando, A. 2010. On the oxidative cost of begging: antioxidants enhance vocalizations in gull chicks. *Behav. Ecol.* **21**: 479–484.

Noguera, J.C., Alonso-Álvarez, C., Kim, S.Y., Morales, J. & Velando, A. 2011a. Yolk-testosterone reduces levels of

oxidative damages during postnatal development. *Biol. Lett.* **7**: 93–95.

Noguera, J.C., Lores, M., Alonso-Álvarez, C. & Velando, A. 2011b. Thrifty development: early-life diet restriction reduces oxidative damage during later growth. *Funct. Ecol.* **25**: 1144–1153.

Nussey, D.H., Pemberton, J.M., Pilkington, J.G. & Blount, J.D. 2009. Life history correlates of oxidative damage in a free-living mammal population. *Funct. Ecol.* **23**: 809–817.

Olsson, M., Wilson, M., Uller, T., Mott, B., Isaksson, C., Healey, M., *et al.* 2008. Free radicals run in lizard families. *Biol. Lett.* **4**: 186–188.

Orledge, J.M., Blount, J.D., Hoodless, A.N., Pike, T.W. & Royle, N.J. 2012. Synergistic effects of supplementation of dietary antioxidants during growth on adult phenotype in ring-necked pheasants, *Phasianus colchicus*. *Funct. Ecol.* **26**: 254–264.

Pigliucci, M. 2001 *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore, MD.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now?. *Trends. Ecol. Evol.* **20**: 481–486.

Ristow, M., Zarse, K., Oberbach, A., Klöting, N., Birringer, M., Kiehntopf, M. *et al.* 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl Acad. Sci. USA* **106**: 8665–8670.

Rollo, C.D. 2002. Growth negatively impacts the life span of mammals. *Evol. Dev.* **4**: 55–61.

Romero, L.M., Reed, J.M. & Wingfield, J.C. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. *Gen. Comp. Endocrinol.* **118**: 113–122.

Royle, N.J., Surai, P.F. & Hartley, I.R. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? *Behav. Ecol.* **12**: 381–385.

Rubolini, D., Romano, M., Alquati, A.B. & Saino, N. 2006. Early maternal, genetic and environmental components of antioxidant protection, morphology and immunity of yellow-legged gull (*Larus michahellis*) chicks. *J. Evol. Biol.* **19**: 1571–1584.

Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.* **38**: 995–1014.

Sgrò, C.M. & Hoffmann, A.A. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**: 241–248.

Silverin, B. 1998. Behavioural and hormonal responses of pied flycatchers to environmental stressors. *Anim. Behav.* **55**: 1411–1420.

Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., *et al.* 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* **3**: 87–95.

Spencer, K.A., Evans, N.P. & Monaghan, P. 2009. Post-natal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology* **150**: 1931–1934.

Stier, K.S., Almasi, B., Gasparini, J., Piault, R., Roulin, A. & Jenni, L. 2009. Effects of corticosterone on innate and humoral immune functions and oxidative stress in barn owl nestlings. *J. Exp. Biol.* **212**: 2085–2091.

Surai, P.F. 2002 *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham University Press, Nottingham.

Wang, X.L., Rainwater, D.L., VandeBerg, J.F., Mitchell, B.D. & Mahaney, M.C. 2001. Genetic contributions to plasma total antioxidant activity. *Arterioscl. Throm. Vas. Biol.* **21**: 1190–1195.

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C.D., Sharma, S., Seckl, R.S., *et al.* 2004. Epigenetic programming by maternal behavior. *Nature Neurosci.* **7**: 847–854.

West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press, New York, NY.

Wingfield, J.C. 2002. Ecophysiological studies of hormone-behavior relations in birds. In: *Hormones, Brain and Behavior*, vol. **2** (D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fairbach, R.T. Rubin, eds), pp. 587–647. Academic Press, San Diego, CA.

Wingfield, J.C. & Sapolsky, R.M. 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* **15**: 711–724.

Woods, J.R. Jr, Plessinger, M.A. & Miller, R.K. 2001. Vitamins C and E: missing links in preventing preterm premature rupture of membranes? *Am. J. Obstet. Gynecol.* **185**: 5–10.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 The cross-fostering design and experimental assignment.

Data deposited at Dryad: doi:10.5061/dryad.4v178

Received 11 October 2012; revised 23 January 2013; accepted 23 January 2013