JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12454

Resistance to oxidative stress shows low heritability and high common environmental variance in a wild bird

S. LOSDAT*†, F. HELFENSTEIN†‡, J. D. BLOUNT§ & H. RICHNER†

*School of Biological Sciences, Zoology Building, University of Aberdeen, Aberdeen, UK
†Evolutionary Ecology Lab, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland
‡Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland
§Centre for Ecology & Conservation, College of Life & Environmental Sciences, University of Exeter, Penryn, UK

Keywords:

additive genetic variance;
common environment;
cross-sex genetic correlation;
heritability;
oxidative stress;
Parus major.

Abstract

Oxidative stress was recently demonstrated to affect several fitness-related traits and is now well recognized to shape animal life-history evolution. However, very little is known about how much resistance to oxidative stress is determined by genetic and environmental effects and hence about its potential for evolution, especially in wild populations. In addition, our knowledge of phenotypic sexual dimorphism and cross-sex genetic correlations in resistance to oxidative stress remains extremely limited despite important evolutionary implications. In free-living great tits (*Parus major*), we quantified heritability, common environmental effect, sexual dimorphism and cross-sex genetic correlation in offspring resistance to oxidative stress by performing a split-nest cross-fostering experiment where 155 broods were split, and all siblings (n = 791) translocated and raised in two other nests. Resistance to oxidative stress was measured as both oxidative damage to lipids and erythrocyte resistance to a controlled free-radical attack. Both measurements of oxidative stress showed low additive genetic variances, high common environmental effects and phenotypic sexual dimorphism with males showing a higher resistance to oxidative stress. Cross-sex genetic correlations were not different from unity, and we found no substantial heritability in resistance to oxidative stress at adult age measured on 39 individuals that recruited the subsequent year. Our study shows that individual ability to resist to oxidative stress is primarily influenced by the common environment and has a low heritability with a consequent low potential for evolution, at least at an early stage of life.

Introduction

Oxidative stress is now well recognized as a physiological constraint affecting fitness-related traits and mediating life-history evolution in animals (Costantini, 2008; Dowling & Simmons, 2009; Monaghan *et al.*, 2009; Costantini *et al.*, 2010; Metcalfe & Alonso-Alvarez, 2010). Oxidative stress (defined as an imbalance between the formation of reactive oxygen species and the anti-oxidant response in favour of the former; Sies, 1991) is involved in cellular senescence and ageing (Finkel & Holbrook, 2000), and the ability of an individual to resist oxidative stress was found to predict its survival prospects (Bize *et al.*, 2008; Losdat *et al.*, 2013) and life expectancy (Morrow *et al.*, 2004; Alonso-Alvarez *et al.*, 2006; Saino *et al.*, 2011). Oxidative stress also has negative consequences on reproductive success (Salmon *et al.*, 2001; Alonso-Alvarez *et al.*, 2004; Wiersma *et al.*, 2004; Losdat *et al.*, 2011a; Christe *et al.*, 2012) and on male fertilizing ability through oxidative damage to sperm (Tremellen, 2008; Helfenstein *et al.*, 2010; Pike *et al.*, 2010; Almbro *et al.*, 2011).

Oxidative stress is consequently well recognized as a universal cost shaping phenotypic traits, and selection for high ability to resist oxidative stress is widely

Correspondence: Sylvain Losdat, School of Biological Sciences, Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK. Tel.: +44 (0)1224 272789; fax: +44 (0) 1224 272396; e-mail: sylvain_losdat@yahoo.fr

expected to influence the evolution of life-history traits (Costantini *et al.*, 2010; Metcalfe & Alonso-Alvarez, 2010; Isaksson *et al.*, 2011; Metcalfe & Monaghan, 2013; Costantini, 2014). Although much effort has been put in assessing the impact of oxidative stress on fitness traits, whether, and if so how, ability to resist oxidative stress itself can be inherited, and whether selection for high resistance to oxidative stress can transfer into evolution of this trait remains an important question for evolutionary ecologists and ecophysiologists (Metcalfe & Alonso-Alvarez, 2010). Specifically, our knowledge is very limited when it comes to genetic variance, heritability and common environment effects for quantitative and complex traits such as anti-oxidant capacity, ability to limit oxidative damage or structural anti-oxidant defences. Heritability of such traits could have important evolutionary consequences because individuals with high ability to resist oxidative stress could derive substantial fitness benefits through the multiple advantages (e.g. higher survival, higher ability to protect germline from oxidative damage, see above) gained by their offspring with relatively high resistance to oxidative stress. In addition, as resistance to oxidative stress is a multifactorial physiological trait (Costantini, 2014), partitioning variance in different components of oxidative stress could shed light on the determinants of an individual overall ability to resist oxidative stress.

Several laboratory studies on various taxa (e.g. mammals, worms) have identified genes involved in cell resistance to oxidative damage (Larsen, 1993; Migliaccio *et al.*, 1999) and in the control of the rate of reactive oxygen species (ROS) generation (Ito *et al.*, 2004; Tothova *et al.*, 2007), therefore providing a first line of evidence for a genetic basis of individual ability to resist oxidative stress. Cross-fostering experiments have shown that variation in the production of reactive oxygen species in painted dragon lizards *Ctenophorus pictus* (Olsson *et al.*, 2008) or variation in the level of oxidative damage in wild kestrels *Falco Tinnunculus* (Costantini & Dell'Omo, 2006) can be mostly explained by genetic factors, whereas variation in anti-oxidant levels was mainly explained by environmental conditions (Costantini & Dell'Omo, 2006; Norte *et al.*, 2009). Heritability of a measure of whole blood resistance to a ROS-induced attack was high and significant ($h^2 = 0.59$) in 8-day-old yellow-legged gulls *Larus cachinnans* (Kim *et al.*, 2010). On the contrary, a recent study on human twins reported that levels of oxidative damage were predominantly determined by environmental factors (Broedbaek *et al.*, 2011). Clearly, to what extent genetic and environmental parameters affect the variance in resistance to oxidative stress is equivocal, and a recent review by Metcalfe & Alonso-Alvarez (2010) points out the scarcity of and need for data on wild populations using quantitative genetics.

Cross-sex genetic correlations, which represent genetic correlations between the same traits expressed in males and females, may constrain the evolution of a trait in one or both sexes and are consequently assumed to play a pivotal role in the evolution of sexual dimorphism (Falconer & Mackay, 1996; Lynch & Walsh, 1998; Bonduriansky, 2007; Bonduriansky & Chenoweth, 2009; Poissant *et al.*, 2010). In fact, the independent evolution of sexes for a given trait may be genetically constrained to a degree that depends on the magnitude of the cross-sex genetic correlation for this trait (Roff, 1997; Poissant *et al.*, 2010). A meta-analysis by Poissant *et al.* (2010) revealed that cross-sex genetic correlations are generally large and positive but typically lower for fitness-related and physiological traits. This pattern is empirically supported by the low cross-sex genetic correlations observed in cell-mediated immune response and plumage colour intensity in blue tits (Drobniak *et al.*, 2010, 2013). Low cross-sex genetic correlations in resistance to oxidative stress could consequently be expected. However, to our knowledge, cross-sex genetic correlations in individual resistance to oxidative stress have not been quantified in natural conditions.

We conducted a split-nest cross-fostering study (i.e. offspring from a given nest of origin were raised in two different foster nests) on a free-living population of great tits to quantify the amount of additive genetic and environmental variation in two measures of resistance to oxidative stress. Our experimental design allowed to separate additive genetic from common environment effects and thus to estimate narrow-sense heritability using animal models.

Because individual resistance to oxidative stress at an early age likely depends to a large extent on parental ability to provide their offspring with a diet rich in anti-oxidant compounds (Catoni *et al.*, 2008), additive genetic effects on resistance to oxidative stress may be higher later in life, as observed in other traits (e.g. Charmantier *et al.*, 2006). We therefore also estimated heritability at adult age using genetic parent vs. mid-offspring regressions with resistance to oxidative stress measured in offspring that were captured as adults the following year (recruits).

We also quantified phenotypic sexual dimorphism and cross-sex genetic correlations in both measures of individual resistance to oxidative stress.

Finally, besides heritability, nongenetic inheritance, which produces resemblance between parents and offspring through processes such as epigenetic, parental effects, ecological inheritance or cultural inheritance, is now increasingly recognized as having a strong impact on the evolutionary potential of a trait (Bonduriansky & Day, 2009; Danchin & Wagner, 2010; Danchin *et al.*, 2011). We estimated nongenetic inheritance in individual resistance to oxidative stress by comparing phenotypic resemblance between foster parents and offspring

measured i) at the offspring stage and ii) as recruiting adults.

Materials and methods

The experiment was conducted during spring 2010 in a natural population of great tits breeding in nest boxes in a forest near Bern, Switzerland (46°7′N, 7°8′E). We used plastic-covered wooden nest boxes (dimensions: 28 × 13 cm × 17 cm, entrance hole: 32 mm) that were metal-hooked to tree branches 2–4 m above ground. Nest boxes were regularly visited from the beginning of the breeding season to determine for 174 nests the start of egg laying and hatching dates. Among the 282 individual parents involved in the study (see below), 115 were of unknown origin and ringed as adults in 2010, 86 were ringed in 2009 (75 as nestlings and 11 as adults) and 39 were already ringed but not by the authors themselves (42 individual adults were not captured). As the vast majority of parents were of unknown origin, and because all individuals ringed as nestlings by SL were not experimentally treated, individual parents involved in this study were unrelated to past experiments conducted in the study area.

Cross-fostering procedure

We performed a split-nest full cross-fostering experiment, a standardized experimental design to partition variance and quantify heritability and environmental variance (Merilä, 1997; Merilä & Sheldon, 2001; Hadfield *et al.*, 2006), by matching nest triads of similar brood size (±1 nestling) and similar hatching date (all offspring in a given nest were therefore of similar age after cross-fostering). This cross-fostering procedure where all nestlings are translocated avoids the problem of having two types of nestlings: those who are moved to a foster nest and experience discontinuity between their pre- and post-hatching environments and those who stay in their original nest and enjoy matching pre- and post-hatching environment (Berthouly *et al.*, 2007). On the second day post-hatching, nestlings were ranked according to body mass and individually marked by removing dorsal tuft feathers. This design allowed to block for body mass at hatching, a correlate of hatching order, nestling competitive ability and survival (Magrath, 1990). Odd-ranked nestlings from one of the nests in the triad were then randomly assigned to one of the two other nests, and even-ranked ones were transferred to the third nest of the triad. Nestlings were further cross-fostered so that each nest of a triad contained half of the nestlings from the two other nests following the procedure described by Brinkhof *et al.* (1999). Of the 174 nests that hatched, 155 were cross-fostered and included in the analyses (14 of the 155 nests were abandoned before fledging). Nestling rank (based on body mass) before and after cross-fostering was highly correlated (mixed-effect model controlling for original and recipient nest effects; $r = 0.76$, $F_{1,537} = 2325.6$, $P < 0.001$), confirming that cross-fostering did not modify within-brood hierarchies.

Eleven days after hatching, we ringed all nestlings ($n = 791$) with uniquely numbered aluminium rings. On day 13 post-hatch, we recorded their body mass (±0.1 g), measured their tarsus and wing lengths (±0.05 mm) and took a 20-μL blood sample from the brachial vein. Blood samples were kept on ice in a cool box for 6 ± 3 h and further stored at −80 °C before analysis.

Recruits

In the subsequent breeding season (spring 2011), we captured adult breeders in the same forest aiming at capturing birds that recruited to estimate heritability of resistance to oxidative stress at adult age. Catching effort was high as 85% of the 2011 breeding individuals were captured. All individuals were captured at their nests while feeding their 13-day-old nestlings using clap-traps and were blood-sampled (as detailed above) for oxidative stress analyses.

Erythrocyte resistance to oxidative stress

We assessed nestling and adults 2011 erythrocyte resistance to oxidative stress using the KRL® (Kit Radicaux Libres®) test purchased from Brevet Spiral (Couternon, France; http://www.nutriteck.com/sunyatakrl.html) adapted to bird physiological parameters (Alonso-Alvarez *et al.*, 2004). This assay provides a quantitative measurement of the whole blood resistance to oxidative stress as it assesses the time required to haemolyze 50% of red blood cells of the sample when exposed to a controlled free-radical attack. It reflects the current availability of total antioxidant defences (enzymatic and nonenzymatic) as well as the past oxidative insults experienced by red blood cells (Esterbauer & Ramos, 1996; Brzezinska-Slebodzinska, 2001) and also indicates the rates of lipid peroxidation in the erythrocyte membrane (Zou *et al.*, 2001). Briefly, 7 μL of whole blood was diluted in 255.5 μL of KRL buffer (150 mM Na, 120 mM Cl^-, 6 mM K, 24 mM $HCO3^-$, 2 mM Ca^2, 340 mOsM, pH 7.4) immediately after sampling and stored at 4 °C before analysis and 6.2 ± 4 h after blood collection. We loaded 80 μL of KRL-diluted whole blood into wells of a 96-well microplate. We subsequently added to each well 136 μL of a 150-mM solution of 2,2-azobis(amidinopropane) hydrochloride (AAPH; a free-radical generator; 646 mg diluted in 20 mL of KRL buffer; Rojas Wahl *et al.*, 1998). The microplate was subsequently read with a microplate spectrophotometer (PowerWave XS reader, Witec Ag, Switzerland) with temperature set at 40 °C. The rate of haemolysis

was determined by the change in optical density measured at 540 nm (Bertrand *et al.*, 2006). Readings were made every 3.5 min for 80 min, and the microplate was shaken immediately before each reading to prevent cells from settling at the bottom of the wells. The repeatability of the method, assessed using samples from adult great tits 2011, was high ($r = 0.78$, $P < 0.001$, $n = 80$).

Oxidative damage to lipids

We measured nestling and adult 2011 plasma concentrations of malondialdehyde (MDA). MDAs are end products of lipid peroxidation and constitute a measure of *in vivo* oxidative damage (Halliwell & Chirico, 1993). Variation in MDA levels therefore reflects variation in oxidative damage and hence variation in individual ability to resist oxidative stress. MDA is formed by the β-scission of peroxidized fatty acids. We assessed plasma MDA (both protein-bound and free MDA) using HPLC with fluorescence detection, as described previously (Mougeot *et al.*, 2009) with some modifications. All chemicals were HPLC grade, and chemical solutions were prepared using ultra pure water (Milli-Q Synthesis; Millipore, Watford, UK). Sample derivitization was performed in 2 mL capacity screw-top microcentrifuge tubes. To a 5-μL aliquot of sample or standard (1,1,3, 3-tetraethoxypropane, TEP; see below) 5 μL butylated hydroxytoluene solution (0.05% w/v in 95% ethanol), 40 μL phosphoric acid solution (0.44 M) and 10 μL thiobarbituric acid (TBA) solution (42 mM) were added. Samples were capped, vortex-mixed for 5 s and then heated at 100 °C for exactly 1 h in a dry bath incubator to allow formation of MDA–TBA adducts. Samples were then cooled on ice for 5 min, before 80 μL n-butanol was added and tubes vortex-mixed for 10 s. Tubes were then centrifuged at 13,000 rpm and 4 °C for 4 min, before a 55-μL aliquot of the epiphase was collected and transferred to an HPLC vial for analysis. Samples (40 μL) were injected into a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA, USA) fitted with a 2-μm precolumn filter and a Hewlett-Packard Hypersil 5 μ ODS 100 × 4.6 mm column maintained at 37 °C. The mobile phase was methanol-buffer (40 : 60, v/v), the buffer being a 50-mM anhydrous solution of potassium monobasic phosphate at pH 6.8 (adjusted using 5 M potassium hydroxide solution), running isocratically over 3.5 min at a flow rate of 1 mL min^{-1}. Data were collected using a fluorescence detector (RF2000; Dionex) set at 515 nm (excitation) and 553 nm (emission). For calibration, a standard curve was prepared using a TEP stock solution (5 μM in 40% ethanol) serially diluted using 40% ethanol. TEP standards were assayed in triplicate and showed high repeatability ($r = 0.99$, $P < 0.0001$, $n = 12$). We also observed high repeatability of the method on a subsample of individual offspring ($r = 0.98$, $P < 0.001$, $n = 32$). SL did all analyses.

Genetic analyses

Blood samples of the nestlings and the parents were transferred into 1000 μL of absolute ethanol. Hundred and fifty-seven families were analyzed for paternity at 11 microsatellite loci (PmaC25, PmaCAn1, PmaD105, PmaD22, PmaGAn27, PmaGAn30, PmaTAGAn71, PmaTAGAn86, PmaTGAn33, PmaTGAn42 and PmaTGAn45; Saladin *et al.*, 2003). We used Cervus 3.0 software package (Kalinowski, 2007) to calculate allele frequencies, heterozygosity values, exclusion probabilities and deviation from Hardy–Weinberg equilibrium based on the genetic data of 237 adult great tits of both sexes captured in the same study area. Our population significantly deviated from Hardy–Weinberg equilibrium only at locus PmaCAn1 due to an increased presence of null alleles. Exclusion power of all loci was 0.99982 for the first parent and 0.999998 for the second parent. Nestlings were categorized as within-pair if all their loci matched those of their candidate social father. They were considered extra pair if their genotype mismatched their putative social father's genotype at two or more loci. Nestlings were sexed using the sexing primers 2917/3088 (Ellegren, 1996).

Statistical analyses

We used 'animal models' to partition variance in nestling ability to resist oxidative stress as measured by their erythrocyte resistance to oxidative stress and oxidative damage to lipids (both $\log_{10}$-transformed to achieve normally distributed residuals). 'Animal models' are linear mixed models that use relatedness among individuals to estimate additive genetic (co)variances in phenotypic traits (Wilson *et al.*, 2010). We also ran animal models to estimate heritabilities of body mass, tarsus length and wing length, for which values are well documented (see discussion), to provide a comparison with our estimates for physiological traits. We used the genetic parentage data to assign every individual to its genetic mother and father and compiled a two-generational pedigree (offspring & parents), which allowed correcting for the 9% of extra-pair offspring in our study population in 2010. We used the 'asreml' package (Butler *et al.*, 2007) implemented in R software (R Core Team, 2013) to run the animal models that used our two-generational pedigree to estimate the variance components with a restricted estimate maximum likelihood procedure (REML).

Total phenotypic variance (V_P) was partitioned into $V_A + V_C + V_R$, where V_A is the additive genetic variance, V_C is the common environment variance and V_R the residual variance. Note that as offspring were cross-fostered two days after hatching, the potential pre-cross-fostering environmental effects occurring after hatching would fall into the additive genetic variance V_A, which is therefore likely to be slightly over-estimated. Offspring identity (associated with the pedigree)

Table 1 Variance component, heritability, common environment and fixed effect estimates for both measures of resistance to oxidative stress extracted from the univariate models. Analyses included 791 nestlings originating from 155 nests of origin, which received parental care in 141 foster nests. Chi-square and *P*-values correspond to the difference between the full model and a reduced model that did not contain the variance component of interest (variance components) or likelihood-ratio tests (fixed effects). Significant values are highlighted in bold.

	Level of oxidative damage					Erythrocyte resistance to oxidative stress				
	Estimate ± SE		χ^2	$F_{d.f.}$	*P*	Estimate ± SE		χ^2	$F_{d.f.}$	*P*
Variance components										
Additive genetic V_A	0.0005 ± 0.0004	h^2 = 0.05 ± 0.04	1.48	–	0.22	0.0009 ± 0.003	h^2 = 0.02 ± 0.07	0.10	–	0.75
Common environment. V_C	**0.005 ± 0.0007**	**c^2 = 0.53 ± 0.04**	**141.96**	**–**	**<0.001**	**0.02 ± 0.007**	**c^2 = 0.15 ± 0.04**	**23.24**	**–**	**<0.001**
Residual V_R	0.004 ± 0.0004	–	–	–	–	0.087 ± 0.015	–	–	–	–
Fixed effects										
Sex				**$13.38_{1,668}$**	**<0.001**				**$7.46_{1,754}$**	**0.006**
Brood size				$2.67_{1,148}$	0.12				$3.87_{1,668}$	0.051
Hatching date				**$153.3_{1,145}$**	**<0.001**				$0.42_{1,130}$	0.52
Hatching rank				$0.82_{8,602}$	0.58				$0.99_{8,660}$	0.44

and the foster nest were fitted as random effects to estimate V_A and V_C, respectively. Sex of the nestlings, hatching date, brood size and hatching rank were included as fixed factors, and their significance assessed using a conditional Wald test with an F-approximation to its sampling distribution (Butler *et al.*, 2007). Nonsignificant fixed factors were backward eliminated from the models with a 5% significance threshold.

Univariate models were run first to estimate the variance components of nestlings' ability to resist oxidative stress. We then ran bivariate models to partition variance in resistance to oxidative stress for both sexes separately and to quantify cross-sex genetic correlations using bivariate formulations of the response variables, whereby values of resistance to oxidative stress in the two different sexes were treated as two different dependent variables. In bivariate models, residual covariances were set to 0 because each individual can be measured for only one sex.

Because running animal models on a two-generational pedigree is rather unusual, we also ran classic REML mixed-effect models using the nest of origin rather than the pedigree to estimate V_A and V_C. These models yielded qualitatively similar results, and we therefore only present here the outputs of the animal models as they account for extra-pair paternity.

Narrow-sense heritability (h^2) and common environmental effect (c^2) were computed for both dependent variables as $h^2 = V_A/V_P$, and $c^2 = V_C/V_P$. V_P was calculated as the sum of the variance components, after the variance explained by the fixed effects was accounted for. To assess the statistical significance of V_C and V_A, we used likelihood-ratio tests (LRT) to compare the final model with a reduced model that did not include the random effect tested. LRTs compare nested models based on twice the difference in log-likelihood scores that follow a χ^2 distribution with a number of degree of freedom equaling the number of parameters removed (d.f. = 1). In bivariate models, we used LRTs to test the significance of cross-sex genetic correlations by comparing the full model with one where the genetic correlation was set to 1. Genetic correlations were tested against unity because the null genetic model predicts high cross-sex genetic correlations (Lynch & Walsh, 1998; Bonduriansky, 2007; Poissant *et al.*, 2010).

Lastly, we performed parent–offspring regressions (1) between offspring at the nestling stage and their rearing parents to test for their potential resemblance (nongenetic inheritance) and (2) between recruiting offspring (the subsequent year) and their genetic and rearing parents to test whether resistance to oxidative stress at adult age is related to genetic (genetic inheritance) and/or rearing (nongenetic inheritance) parents. Offspring oxidative stress values were standardized using the residuals of GLMM models with nest of origin (for rearing parent–offspring regressions) or nest of rearing

(for genetic parent–offspring regression) fitted as a random factor, and sex as a fixed factor. Nestlings, as well as some recruiting adults, were born and raised in the same nests, and we therefore averaged their values to avoid pseudo-replication. The heritability estimates were inferred from the mid-parent–mid-offspring regression coefficients (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

Results

Resistance to oxidative stress at the nestling stage

We found a highly significant effect of sex on both traits (Table 1) with males having superior erythrocyte resistance to oxidative stress (8.05 ± 0.40 min.) and less oxidative damage (14.89 ± 0.47 nmol mL^{-1}) when compared to females (6.62 ± 0.33 min. and 16.30 ± 0.51 nmol mL^{-1}, respectively). Hatching date also had a significant negative effect on the levels of oxidative damage (Table 1). Heritability of morphological traits was substantial, 0.56 ± 0.08 for body mass, 0.18 ± 0.08 for tarsus length and 0.54 ± 0.19 for wing length.

Additive genetic variances (V_A) were not significantly different from 0, resulting in low estimates of narrow-sense heritability for both traits (Tables 1 and 2). Because V_A may also involve some pre-cross-fostering environmental effects of the nests of origin, the reported absence of significant additive genetic variance is a very conservative result. The common environment effect (V_C) explained a significant proportion of the variance for both traits with moderate and high estimates of the common environment effect c^2, except for male erythrocyte resistance to oxidative stress (Tables 1 and 2). Cross-sex genetic correlations for both measures of oxidative stress were high and did not significantly differ from unity (Table 2).

Genetic parents vs. adult offspring regressions

In the subsequent breeding season (2011), we captured 39 breeding adults that recruited from 2010 (5.1%, a low but typical recruitment rate in the great tit; Verhulst *et al.*, 1995; including in our study population; Losdat *et al.*, 2013). Genetic mid-parent-mid-offspring regressions were not significant for both measures of resistance to oxidative stress ($h^2 = -0.03 \pm 0.10$, $P = 0.74$; $h^2 = -0.09 \pm 0.09$, $P = 0.32$, respectively).

Foster parent vs. offspring regressions

Regressions of erythrocyte resistance to oxidative stress and oxidative damage of foster mid-parent vs. mid-offspring trait values measured at the nestling stage were not significant, therefore yielding no significant nongenetic inheritance for both traits (−0.05 ± 0.04, $P = 0.18$; −0.01 ± 0.03, $P = 0.61$, respectively).

Based on the 39 breeding adults captured in 2011 (ringed as nestlings in 2010), foster mid-parent vs. mid-offspring regressions yielded nonsignificant nongenetic inheritances (−0.10 ± 0.13, $P = 0.43$; −0.05 ± 0.12, $P = 0.69$, respectively).

Discussion

Our split-nest full cross-fostering experiment on a free-living vertebrate reveals that the phenotypic variance in offspring resistance to oxidative stress, as measured by erythrocyte resistance to oxidative stress and oxidative damage to lipids, was primarily explained by the common environment and had low heritability and low nongenetic inheritance (Bonduriansky & Day, 2009; Danchin *et al.*, 2011). We report remarkable sexual dimorphism with males showing superior ability to resist oxidative stress at the phenotypic level. We did not find evidence for genotype-by-sex interactions,

Table 2 Variance components, heritabilities (h^2), common environment effects (c^2) estimated for each sex separately and cross-sex genetic correlations (r_A) for both measures of resistance to oxidative stress. Values are extracted from bivariate models. Significance of each cross-sex genetic correlation was assessed by comparing the full model with a model where the genetic correlation was constrained to unity (see methods for details). Significant values are highlighted in bold.

		Level of oxidative damage				Erythrocyte resistance to oxidative stress			
Variance components	Sex	Estimate ± SE		χ^2	P	Estimate ± SE		χ^2	P
Additive genetic V_A	F	0.00 ± –*	$h^2 = 0.00 \pm$ –*	0	1	0.0082 ± 0.015	$h^2 = 0.07 \pm 0.13$	0.60	0.44
	M	0.0032 ± 0.003	$h^2 = 0.09 \pm 0.09$	1.03	0.31	0.0272 ± 0.0197	$h^2 = 0.21 \pm 0.15$	0.57	0.45
Additive genetic correlation		$r_A = 0.99 \pm$ –*		0	1	$r_A = 0.41 \pm 0.81$	–	0.39	0.53
Common environment V_C	**F**	**0.021 ± 0.004**	**$c^2 = 0.56 \pm 0.05$**	**8.22**	**0.004**	**0.02 ± 0.007**	**$c^2 = 0.18 \pm 0.06$**	**14.4**	**<0.001**
	M	**0.019 ± 0.003**	**$c^2 = 0.54 \pm 0.05$**	**20.45**	**<0.001**	0.013 ± 0.008	$c^2 = 0.10 \pm 0.06$	2.42	0.12
Residual V_R	F	0.018 ± 0.0017	–	–	–	0.087 ± 0.015	–	–	–
	M	0.012 ± 0.0028	–	–	–	0.093 ± 0.018	–	–	–

*Parameter estimate was bound to 0 or 1; hence, no standard error was estimated.

which may partly reflect the low additive genetic variance in resistance to oxidative stress.

We report low heritabilities for both erythrocyte resistance to oxidative stress and levels of oxidative damage. Traits closely related to fitness tend to have lower heritabilities than traits less associated to fitness because the former capture more environmental variance due to their dependence on more components (e.g. sexual selection) resulting in higher nongenetic variance (Fisher, 1930; Gustafsson, 1986; Price & Schluter, 1991; Wilson, 2008), as shown in long-term studies on wild vertebrates (Kruuk *et al.*, 2000; Merilä & Sheldon, 2000; McCleery *et al.*, 2004). The low heritabilities observed here may therefore reflect the close link between resistance to oxidative stress and fitness (reviewed in Costantini *et al.*, 2010; Metcalfe & Alonso-Alvarez, 2010). Indeed, links between resistance to oxidative stress as measured in this study and fitness-related traits have been identified in our study system (Losdat *et al.*, 2011a, 2013). Importantly, heritabilities of morphological traits estimated here are typical values (i.e. moderate to high) for these traits, as reported for many species (e.g. Alatalo & Lundberg, 1986; Gustafsson, 1986; Mousseau & Roff, 1987; Noordwijk *et al.*, 1988; Gebhardt-Henrich & van Noordwijk, 1991; Kruuk *et al.*, 2000; Jensen *et al.*, 2003; Visscher *et al.*, 2008). Hence, the low heritabilities of resistance to oxidative stress we report do not reflect a general pattern of low heritabilities in our study system.

Both measures of resistance to oxidative stress did also not show significant additive genetic variance. This is unexpected because fitness-related traits usually harbour substantial additive genetic variance, potentially because they capture variation and accumulate mutations from many loci (Price & Schluter, 1991; Merilä & Sheldon, 1999). Additive genetic variance has been shown to be age-dependent in some species (Charmantier *et al.*, 2006), and this is to be expected in species where individuals highly depend on their environment such as passerine birds at an early age that fully rely on their parents. One could therefore expect a higher additive genetic variance in resistance to oxidative stress when measured at the adult stage, when individuals become independent. Here, measurements performed on individual recruits did not reveal greater resemblance at adulthood than at the fledging stage between genetic parents and their offspring. However, measurements at adulthood in our study comprised only 39 individuals (i.e. 5% of the individual sampled as offspring), and therefore, potential age dependence of additive genetic variance in resistance to oxidative stress remains to be tested on a more representative sample size.

Despite low additive genetic variances and heritabilities, resistance to oxidative stress may still exhibit some evolutionary potential if it showed significant nongenetic inheritance as produced by mechanisms such as parental effects, epigenetic or ecological inheritance (Bonduriansky & Day, 2009; Danchin *et al.*, 2011). Although nongenetic inheritance can be expected at least through parental effects (maternal effects: deposition of anti-oxidant in the yolk; Mousseau & Fox, 1998; Blount *et al.*, 2002; Berthouly *et al.*, 2008; paternal effects: territory quality reflecting availability of anti-oxidant rich food; Catoni *et al.*, 2008; van de Crommenacker *et al.*, 2011), parent–offspring regressions both at nestling and at adult stages indicated no resemblance between foster parents and their offspring in terms of erythrocyte resistance to oxidative stress and oxidative damage.

In contrast, our results reveal a large environmental effect on erythrocyte resistance to oxidative stress and levels of oxidative damage. This is in line with previous studies reporting a large environmental effect on resistance to oxidative stress, including measures of enzymatic and nonenzymatic anti-oxidants, in different species (Costantini & Dell'Omo, 2006; Geens *et al.*, 2009; Norte *et al.*, 2009). Such environmental effects have important evolutionary implications because they potentially allow parents to strengthen their offspring resistance to oxidative stress through parental care, with potential benefits to offspring fitness-related traits (Alonso-Alvarez *et al.*, 2006). A significant environmental effect may be expected if some components of the anti-oxidant system (e.g. dietary anti-oxidants) are linked to food sources and environmental conditions (Catoni *et al.*, 2008). The anti-oxidant system involves several lines of defence (reviewed in Monaghan *et al.*, 2009; Pamplona & Costantini, 2011), for instance (i) reducing levels of uncontrolled reactive species in the cell, (ii) cellular anti-oxidant enzyme groups counteracting the effects of the superoxide anion, (iii) chain-breaking anti-oxidant compounds (endogenously produced and dietary) and (iv) structural defences of tissues. The common environment shared by offspring has the potential to influence all levels of the anti-oxidant system. First, the food resources nestlings get from their rearing parents constitute their only source of dietary anti-oxidants, and only source of energy for the synthesis of endogenous anti-oxidants. Thus, the amount and quality of food provided by the parents will likely influence the nestling ability to neutralize reactive species and adjust their structural defences (e.g. membrane lipid composition through ratio of polyunsaturated vs. monounsaturated and saturated fatty acids) and consequently their susceptibility to oxidative stress. Second, the intensity of sibling competition depends on common environment factors such as parental investment and parent–offspring conflict (Parker *et al.*, 2002; Hinde *et al.*, 2010), brood size (Neuenschwander *et al.*, 2003) or sex ratio in the brood (Bonisoli-Alquati *et al.*, 2011). Sibling competition may entail costs in terms of oxidative stress and damage (Hall *et al.*, 2010; Noguera *et al.*, 2010; Boncoraglio *et al.*, 2012), and variation in levels of sibling competition is expected to produce variance in

resistance to oxidative stress among broods. Our results, however, did not support this hypothesis as brood size and hatching rank had no effect on both measures of oxidative stress.

Our study demonstrates clear phenotypic sexual dimorphism with males having a higher resistance to oxidative stress than females for both our measurements. This is in line with a recent study on decorated crickets that reported the same pattern of sexual dimorphism with more oxidative damage found in females (Archer *et al.*, 2013). This important result suggests that selection for a higher ability to resist oxidative stress is stronger on males. One potential explanation could stem from the impact of oxidative stress on traits under sexual selection. For example, oxidative stress can affect sperm performance (Aitken, 1999; Aitken & Baker, 2006; Helfenstein *et al.*, 2010; Losdat *et al.*, 2011b; Costantini, 2014) and sexually selected colour signals (Garratt & Brooks, 2012) with negative consequences for paternity (reviewed in Pizzari & Parker, 2009) and mating (Andersson & Simmons, 2006) successes, respectively. Males with higher ability to resist oxidative stress could consequently gain substantial fitness benefits through higher mating and/or paternity success, especially in genetically polygamous reproductive systems with intense competition for access to social (and extra pair) mates and substantial sperm competition.

We reported high cross-sex genetic correlations, therefore suggesting a strong genetic constraint on the independent evolution of male vs. female resistance to oxidative stress. In fact, cross-sex genetic correlations in resistance to oxidative stress did not differ from unity which is unexpected in light of the generally low cross-sex genetic correlations observed in physiological traits (Poissant *et al.*, 2010). However, our data provided limited power to estimate genetic correlations because of the low additive genetic variance in both measures of resistance to oxidative stress. Additional studies are required to clarify whether the high cross-sex genetic correlations in ability to resist oxidative stress reported here is a general pattern across species and systems.

Based on a substantial number of free-living individuals and families, we surprisingly found in a wild vertebrate no evidence for substantial heritability or nongenetic inheritance in two biomarkers of resistance to oxidative stress, which were shown to be biologically and ecologically meaningful (e.g. Alonso-Alvarez *et al.*, 2004; Bize *et al.*, 2008). Our study shows that resistance to oxidative stress has, at least early in life, a very low potential for evolution, because any selective advantage is not transmitted to the new generation. Our study cannot, however, entirely exclude the possibility that additive genetic variance increases with age (as we could estimate it for only 39 individual recruits) and therefore that resistance to oxidative stress has some potential for evolution expressed later in life. Importantly, an absence of additive genetic variance does not preclude the existence of other inheritance processes in relation to this trait. In fact, because resistance to oxidative stress shows strong links to fitness and provided that individuals with a higher ability to resist oxidative stress may confer direct benefits to their sexual partner (e.g. fertility, embryo viability; Tremellen, 2008; Velando *et al.*, 2008), the ability of an individual to resist oxidative stress has the potential to drive the evolution of mate choice based on this trait (Andersson & Simmons, 2006). Furthermore, although there appears to be only low evolutionary potential in resistance to oxidative stress *per se,* the strategies individuals develop to adaptively allocate their anti-oxidant resources to competing functions (e.g. reproduction vs. survival) may be heritable. Despite having assessed resistance to oxidative stress using two reliable and sensitive biomarkers, one should not forget the extremely complex nature of the oxidative stress processes, and the nonsignificance of genetic inheritance of the markers used here may not preclude genetic inheritance of other aspects of resistance to oxidative stress (e.g. production of reactive species).

Future research should aim at examining heritability of oxidative stress measured at adulthood and heritability of anti-oxidant allocation strategies, combining long-term monitoring with experimental manipulations inducing individual adjustment in investment into competing life-history traits and physiological functions.

Acknowledgments

This work was conducted under licence of the Ethical Committee of the Agricultural Office of the Canton Bern. Ringing permits were provided by the Swiss Federal Agency for Environment, Forests and Landscapes. The authors thank Julien Martin and Matthew Wolak for helpful advice and David Costantini and two anonymous reviewers for constructive comments on previous versions of the manuscript. The study was financially supported by the Swiss National Science Foundation. SL was supported by a Swiss NSF and a Marie Curie IEF Post-Doctoral Fellowships. JDB was supported by a Royal Society Research Fellowship. The authors have declared no conflict of interest.

References

Aitken, R.J. 1999. The amoroso lecture. The human spermatozoon – a cell in crisis? *J. Reprod. Fertil.* **115**: 1–7.

Aitken, R.J. & Baker, M.A. 2006. Oxidative stress, sperm survival and fertility control. *Mol. Cell. Endocrinol.* **250**: 66–69.

Alatalo, R.V. & Lundberg, A. 1986. Heritability and selection on Tarsus length in the Pied Flycatcher (*Ficedula hypoleuca*). *Evolution* **40**: 574–583.

Almbro, M., Dowling, D.K. & Simmons, L.W. 2011. Effects of vitamin E and beta-carotene on sperm competitiveness. *Ecol. Lett.* **14**: 891–895.

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B. & Sorci, G. 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecol. Lett.* **7**: 363–368.

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O. *et al.* 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution* **60**: 1913–1924.

Andersson, M. & Simmons, L.W. 2006. Sexual selection and mate choice. *Trends Ecol. Evol.* **21**: 296–302.

Archer, C.R., Sakaluk, S.K., Selman, C., Royle, N.J. & Hunt, J. 2013. Oxidative stress and the evolution of sex differences in life span and ageing in the decorated cricket, *Gryllodes sigillatus*. *Evolution* **67**: 620–634.

Berthouly, A., Helfenstein, F. & Richner, H. 2007. Cellular immune response, stress resistance and competitiveness in nestling great tits in relation to maternally transmitted carotenoids. *Funct. Ecol.* **21**: 335–343.

Berthouly, A., Cassier, A. & Richner, H. 2008. Carotenoid-induced maternal effects interact with ectoparasite burden and brood size to shape the trade-off between growth and immunity in nestling great tits. *Funct. Ecol.* **22**: 854–863.

Bertrand, S., Alonso-Alvarez, C., Devevey, G., Faivre, B., Prost, J. & Sorci, G. 2006. Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches. *Oecologia* **147**: 576–584.

Bize, P., Devevey, G., Monaghan, P., Doligez, B. & Christe, P. 2008. Fecondity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology* **89**: 2584–2593.

Blount, J.D., Surai, P.F., Houston, D.C. & Møller, A.P. 2002. Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. *Funct. Ecol.* **16**: 445–453.

Boncoraglio, G., Caprioli, M. & Saino, N. 2012. Solicitation displays reliably reflect oxidative damage in barn swallow nestlings. *Behav. Ecol. Sociobiol.* **66**: 539–546.

Bonduriansky, R. 2007. The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition-dependence. In: *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism* (D.J. Fairbairn, W.U. Blanckenhorn & T. Szekely, eds), pp. 176–184. Oxford University Press, Oxford, UK.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280–288.

Bonduriansky, R. & Day, T. 2009. Nongenetic inheritance and its evolutionary implications. *Annu. Rev. Ecol. Syst.* **40**: 103–125.

Bonisoli-Alquati, A., Boncoraglio, G., Caprioli, M. & Saino, N. 2011. Birth order, individual sex and sex of competitors determine the outcome of conflict among siblings over parental care. *Proc. R Soc. B-Biol. Sci.* **278**: 1273–1279.

Brinkhof, M.W.G., Heeb, P., Kölliker, M. & Richner, H. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc. R Soc. B-Biol. Sci.* **266**: 2315–2322.

Broedbaek, K., Ribel-Madsen, R., Henriksen, T., Weimann, A., Petersen, M., Andersen, J.T. *et al.* 2011. Genetic and environmental influences on oxidative damage assessed in elderly Danish twins. *Free Radical Biol. Med.* **50**: 1488–1491.

Brzezinska-Slebodzinska, E. 2001. Erythrocyte osmotic fragility test as the measure of defence against free radicals in rabbits of different age. *Acta Vet. Hung.* **49**: 413–441.

Butler, D., Cullis, B.R., Gilmour, A.R. & Gogel, B.J. 2007. *ASReml-S Reference Manual*. Department of Primary Industries and Fisheries, Brisbane.

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

Charmantier, A., Perrins, C., McCleery, R.H. & Sheldon, B.C. 2006. Age-dependent genetic variance in a life-history trait in the mute swan. *Proc. R Soc. B-Biol. Sci.* **273**: 225–232.

Christe, P., Glaizot, O., Strepparava, N., Devevey, G. & Fumagalli, L. 2012. Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proc. R Soc. B-Biol. Sci.* **279**: 1142–1149.

Costantini, D. 2008. Oxidative stress in ecology and evolution: lessons from avian studies. *Ecol. Lett.* **11**: 1238–1251.

Costantini, D. 2014. *Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology*. Springer, Berlin, Heidelberg.

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

Costantini, D., Rowe, M., Butler, M.W. & McGraw, K.J. 2010. From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. *Funct. Ecol.* **24**: 950–959.

van de Crommenacker, J., Komdeur, J., Burke, T. & Richardson, D.S. 2011. Spatio-temporal variation in territory quality and oxidative status: a natural experiment in the Seychelles warbler (*Acrocephalus sechellensis*). *J. Anim. Ecol.* **80**: 668–680.

Danchin, E. & Wagner, R.H. 2010. Inclusive heritability: combining genetic and non-genetic information to study animal behavior and culture. *Oikos* **119**: 210–218.

Danchin, E., Charmantier, A., Champagne, F.A., Mesoudi, A., Pujol, B. & Blanchet, S. 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nat. Rev. Genet.* **12**: 475–486.

Dowling, D.K. & Simmons, L.W. 2009. Reactive oxygen species as universal constraints in life-history evolution. *Proc. R Soc. B-Biol. Sci.* **276**: 1737–1745.

Drobniak, S.M., Wiejaczka, D., Arct, A., Dubiec, A., Gustafsson, L. & Cichon, M. 2010. Sex-specific heritability of cell-mediated immune response in the blue tit nestlings (*Cyanistes caeruleus*). *J. Evol. Biol.* **23**: 1286–1292.

Drobniak, S.M., Wiejaczka, D., Arct, A., Dubiec, A., Gustafsson, L. & Cichoń, M. 2013. Low cross-sex genetic correlation in carotenoid-based plumage traits in the blue tit nestlings (*Cyanistes caeruleus*). *PLoS One* **8**: e69786.

Ellegren, H. 1996. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proc. R Soc. B-Biol. Sci.* **263**: 1635–1641.

Esterbauer, H. & Ramos, P. 1996. Chemistry and pathophysiology of oxidation of LDL. *Rev. Physiol. Biochem. Pharmacol.* **127**: 31–64.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*. Longman, London.

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

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Dover, New York.

Garratt, M. & Brooks, R.C. 2012. Oxidative stress and condition-dependent sexual signals: more than just seeing red. *Proc. R Soc. B-Biol. Sci.* **279**: 3121–3130.

Gebhardt-Henrich, S.G. & van Noordwijk, A.J. 1991. Nestling growth in the Great Tit I. Heritability estimates under different environmental conditions. *J. Evol. Biol.* **4**: 341–362.

Geens, A., Dauwe, T. & Eens, M. 2009. Does anthropogenic metal pollution affect carotenoid colouration, antioxidative capacity and physiological condition of great tits (*Parus major*)? *Comp. Biochem. Physiol. Part C Toxicol. Pharmcol.* **150**: 155–163.

Gustafsson, L. 1986. Lifetime reproductive success and heritability: empirical support for fisher's fundamental theorem. *Am. Nat.* **128**: 761–764.

Hadfield, J.D., Burgess, M.D., Lord, A., Phillimore, A.B., Clegg, S.M. & Owens, I.P.F. 2006. Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proc. R Soc. B-Biol. Sci.* **273**: 1347–1353.

Hall, M.E., Blount, J.D., Forbes, S. & Royle, N.J. 2010. Does oxidative stress mediate the trade-off between growth and self-maintenance in structured families? *Funct. Ecol.* **24**: 365–373.

Halliwell, B. & Chirico, S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *Am. J. Clin. Nut.* **57**: 715S–724S.

Helfenstein, F., Losdat, S., Møller, A.P., Blount, J.D. & Richner, H. 2010. Sperm of colourful males are better protected against oxidative stress. *Ecol. Lett.* **13**: 213–222.

Hinde, C.A., Johnstone, R.A. & Kilner, R.M. 2010. Parent-offspring conflict and coadaptation. *Science* **327**: 1373–1376.

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

Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I. *et al.* 2004. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* **431**: 997–1002.

Jensen, H., Sæther, B.E., Ringsby, T.H., Tufto, J., Griffith, S.C. & Ellegren, H. 2003. Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (*Passer domesticus*). *J. Evol. Biol.* **16**: 1296–1307.

Kalinowski, S.T. 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**: 1099.

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

Kruuk, L.E.B., Clutton-Brock, T.H., Slate, J., Pemberton, J.M., Brotherstone, S. & Guinness, F.E. 2000. Heritability of fitness in a wild mammal population. *Proc. Natl. Acad. Sci. USA* **97**: 698–703.

Larsen, P.L. 1993. Aging and resistance to oxidative damage in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **90**: 8905–8909.

Losdat, S., Helfenstein, H., Gaude, B. & Richner, H. 2011a. Reproductive effort transiently reduces antioxidant capacity in a wild bird. *Behav. Ecol.* **22**: 1218–1226.

Losdat, S., Richner, H., Blount, J.D. & Helfenstein, F. 2011b. Immune activation reduces sperm quality in the great tit. *PLoS One* **6**: e22221.

Losdat, S., Helfenstein, F., Blount, J.D., Marri, V., Maronde, L. & Richner, H. 2013. Nestling erythrocyte resistance to oxidative stress predicts fledging success but not local recruitment in a wild bird. *Biol. Lett.* **9**: 20120888.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland.

Magrath, R.D. 1990. Hatching asynchrony in altricial birds. *Biol. Rev.* **65**: 587–622.

McCleery, R.H., Pettifor, R.A., Armbruster, P., Meyer, K., Sheldon, B.C. & Perrins, C.M. 2004. Components of variance underlying fitness in a natural population of the great tit *Parus major*. *Am. Nat.* **164**: E62–E72.

Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* **51**: 526–536.

Merilä, J. & Sheldon, B.C. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity* **83**: 103–109.

Merilä, J. & Sheldon, B.C. 2000. Lifetime reproductive success and heritability in nature. *Am. Nat.* **155**: 301–310.

Merilä, J. & Sheldon, B.C. 2001. Avian quantitative genetics. In: *Current Ornithology*, Vol. 16 (V. Nolan & C. F. Thompson, eds), pp. 179–255. Kluwer Academic/Plenum Publishers, New York.

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.

Metcalfe, N.B. & Monaghan, P. 2013. Does reproduction cause oxidative stress? An open question *Trends Ecol. Evol.* **28**: 347–350.

Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P.P. *et al.* 1999. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* **402**: 309–313.

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.

Morrow, G., Samson, M., Michaud, S. & Tanguay, R.M. 2004. Overexpression of the small mitochondrial Hsp22 extends *Drosophila* life span and increases resistance to oxidative stress. *FASEB J.* **18**: 598–599.

Mougeot, F., Martìnez-Padilla, J., Webster, L.M.I., Blount, J.D., Pérez-Rodrìguez, L. & Piertney, S.B. 2009. Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. *Proc. R Soc. B-Biol. Sci.* **276**: 1093–1100.

Mousseau, T.A. & Fox, C.W. 1998. *Maternal Effects as Adaptations*. Oxford University Press, New York.

Mousseau, T.A. & Roff, D.A. 1987. Natural selection and the heritability of fitness components. *Heredity* **59**: 181–197.

Neuenschwander, S., Brinkhof, M., Kolliker, M. & Richner, H. 2003. Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behav. Ecol.* **14**: 457–462.

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

Noordwijk, A.J.V., Balen, J.H.V. & Scharloo, W. 1988. Heritability of body size in a natural population of the great tit (*Parus major*) and its relation to age and environmental conditions during growth. *Genetics Res.* **51**: 149–162.

Norte, A.C., Sheldon, B.C., Sousa, J.P. & Ramos, J.A. 2009. Environmental and genetic variation in body condition and blood profile of great tit *Parus major* nestlings. *J. Avian Biol.* **40**: 157–165.

Olsson, M., Wilson, M., Isaksson, C., Uller, T. & Mott, B. 2008. Carotenoid intake does not mediate a relationship between reactive oxygen species and bright colouration: experimental test in a lizard. *J. Exp. Biol.* **211**: 1257–1261.

Pamplona, R. & Costantini, D. 2011. Molecular and structural antioxidant defenses against oxidative stress in animals. *Am. J. Physiol. – Reg. Int. Comp. Physiol.* **301**: R843–R863.

Parker, G.A., Royle, N.J. & Hartley, I.R. 2002. Intrafamilial conflict and parental investment: a synthesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **357**: 295–307.

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. 2010. Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biol. Lett.* **6**: 191–193.

Pizzari, T. & Parker, G.A. 2009. Sperm competition and sperm phenotype. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds), pp. 207–245. Academic Press, San Diego.

Poissant, J., Wilson, A.J. & Coltman, D.W. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution* **64**: 97–107.

Price, T. & Schluter, D. 1991. On the low heritability of life-history traits. *Evolution* **45**: 853–861.

R Core Team 2013. *R: A Language and Environment for Statistical Computing*. Austria, Vienna.

Roff, D.A. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, New York.

Rojas Wahl, R.U., Liansheng, Z., Madison, S.A., DePinto, R.L. & Shay, B.J. 1998. Mechanistic studies on the decomposition of water soluble azo-radical-initiators. *J. Chem. Soc., Perkin Trans. 2* **9**: 2009–2018.

Saino, N., Caprioli, M., Romano, M., Boncoraglio, G., Rubolini, D., Ambrosini, R. *et al.* 2011. Antioxidant defenses predict long-term survival in a passerine bird. *PLoS One* **6**: e19593.

Saladin, V., Bonfils, D., Binz, T. & Richner, H. 2003. Isolation and characterization of 16 microsatellite loci in the great tit *Parus major*. *Mol. Ecol. Notes* **3**: 520–522.

Salmon, A.B., Marx, D.B. & Harshman, L.G. 2001. A cost of reproduction in *Drosophila melanogaster*: stress susceptibility. *Evolution* **55**: 1600–1608.

Sies, H. 1991. *Oxidative Stress: Oxidants and Antioxidants*. London Academic Press, London.

Tothova, Z., Kollipara, R., Huntly, B.J., Lee, B.H., Castrillon, D.H., Cullen, D.E. *et al.* 2007. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* **128**: 325–339.

Tremellen, K. 2008. Oxidative stress and male infertility – a clinical perspective. *Hum. Reprod. Update* **14**: 243–258.

Velando, A., Torres, R. & Alonso-Alvarez, C. 2008. Avoiding bad genes: oxidatively damaged DNA in germ line and mate choice. *BioEssays* **30**: 1212–1219.

Verhulst, S., van Balen, J.H. & Tinbergen, J.M. 1995. Seasonal decline in reproductive success of the great tit: variation in time or quality? *Ecology* **76**: 2392–2403.

Visscher, P.M., Hill, W.G. & Wray, N.R. 2008. Heritability in the genomics era – concepts and misconceptions. *Nat. Rev. Genet.* **9**: 255–266.

Wiersma, P., Selman, C., Speakman, J.R. & Verhulst, S. 2004. Birds sacrifice oxidative protection for reproduction. *Proc. R Soc. B-Biol. Sci.* **271**: 360–363.

Wilson, A.J. 2008. Why h^2 does not always equal VA/VP? *J. Evol. Biol.* **21**: 647–650.

Wilson, A.J., Réale, D., Clements, M.N., Morrissey, M.M., Postma, E., Walling, C.A. *et al.* 2010. An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**: 13–26.

Zou, C.-G., Agar, N.S. & Jones, G.L. 2001. Oxidative insult to human red blood cells induced by free radical initiator AAPH and its inhibition by a commercial antioxidant mixture. *Life Sci.* **69**: 75–86.

Received 27 February 2013; revised 30 June 2014; accepted 30 June 2014