doi:10.1111/j.1420-9101.2010.01942.x

Heritability of resistance to oxidative stress in early life

S.-Y. KIM, J. C. NOGUERA, J. MORALES & A. VELANDO

Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo, Spain

Keywords:

antioxidant;
brood effect;
cross-fostering;
heritability;
life-history evolution;
maternal effect;
oxidative stress;
reactive oxygen species;
yellow-legged gull.

Abstract

Oxidative stress has recently been suggested to play an important role in life-history evolution, but little is known about natural variation and heritability of this physiological trait. Here, we explore phenotypic variation in resistance to oxidative stress of cross-fostered yellow-legged gull (*Larus cachinnans*) chicks. Resistance to oxidative stress was not related to plasma antioxidants at hatching, which are mostly derived from maternal investment into eggs. Common environmental effects on phenotypic variation in resistance to oxidative stress were not significant. Heritability was relatively low and nonsignificant in hatchlings, but interestingly, the chicks of age 8 days showed high and significant heritability ($h^2 = 0.59$). Our results suggest that resistance to oxidative stress is determined mainly by the genotype as chicks grow. Further work is required to explore the genetic role of oxidative stress in life-history evolution.

Introduction

The organism's capacity to mitigate cellular damaging effects of reactive oxygen species (ROS) has been recently proposed as an important physiological trait that mediates life-history trade-offs and evolution (Alonso-Alvarez *et al.*, 2004; Dowling & Simmons, 2009; Monaghan *et al.*, 2009). ROS are highly reactive oxygen-containing molecules, mostly generated as by-products of normal metabolic processes, and have damaging effects on biomolecules through oxidative stress (Finkel & Holbrook, 2000). In both laboratory and wild animals, oxidative stress is involved in trade-offs between reproduction and self-maintenance (Alonso-Alvarez *et al.*, 2004, 2006; Bertrand *et al.*, 2006; Bize *et al.*, 2008), possibly affecting their senescence patterns (Kirkwood & Austad, 2000). Individuals more resistant to oxidative stress seem to live longer (Morrow *et al.*, 2004; Alonso-Alvarez *et al.*, 2006; Khazaeli *et al.*, 2007) or reproduce more successfully (Bize *et al.*, 2008) than those who suffer more severe oxidative costs, possibly by mitigating cellular senescence (Finkel & Holbrook, 2000).

As oxidative stress is not only related to senescence (Harman, 1957), but also to sexual reproduction (Nedelcu *et al.*, 2004), expression of secondary sexual traits (von Schantz *et al.*, 1999; Velando *et al.*, 2008) and breeding effort (Salmon *et al.*, 2001; Alonso-Alvarez *et al.*, 2004), it is expected that selection upon strong resistance to oxidative stress possibly influences life-history evolution (Dowling & Simmons, 2009). However, the evolvability of the antioxidant system will depend not only on selection but also on genetic basis of this physiological trait, i.e. narrow-sense heritability (h^2), the fraction of phenotypic variance (V_P) owing to additive effects of genes (V_A) (Falconer & Mackay, 1996). Evidence for a genetic basis of resistance to oxidative stress has been provided by studies on classic model species at the molecular level (Ishii *et al.*, 1998; Lee *et al.*, 2003; Paaby & Schmidt, 2009). Furthermore, quantitative genetic studies using *Drosophila* have suggested the importance of genetic variation in oxidative-stress-related traits for life-history evolution (Arking *et al.*, 2000; Pasyukova *et al.*, 2000; Vermeulen *et al.*, 2005; Khazaeli *et al.*, 2007). However, little is known about natural variation and heritability of this physiological trait in vertebrates.

A recent study of a wild lizard (*Ctenophorus pictus*) showed high heritability and variability among families in the superoxide levels (Olsson *et al.*, 2008). Another recent study showed low and nonsignificant heritability of resistance to oxidative stress in captive zebra finch (*Taeniopygia guttata*), although a positive genetic correlation between resistance to oxidative stress at sexual maturity and reproductive lifespan was found (Kim *et al.*,

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

2010). As far as we know, no quantitative genetic studies have explored phenotypic variation and heritability of an organism's capacity to defend against oxidative stress in a natural population.

In our study, we explore additive genetic and common environmental effects on total phenotypic variation in resistance to oxidative stress in yellow-legged gull (*Larus cachinnans* Pallas) chicks in a wild population. Resistance to oxidative stress, measured as the time needed for haemolysis of red blood cells under free-radical attack, represents the overall efficiency of an organism's antioxidant machineries for cellular defence against ROS damage and is commonly used in ecological studies (e.g. Alonso-Alvarez *et al.*, 2004, 2006; Bertrand *et al.*, 2006; Bize *et al.*, 2008). Blood resistance to oxidative stress is negatively related to growth rate in gull chicks, suggesting that fast metabolism has oxidative costs and damaging effects of ROS might mediate a trade-off between growth and physiological conditions (our unpublished work). Here, we disentangle genetic vs. environmental variation in resistance of red blood cells to a controlled free-radical attack in chicks by cross-fostering eggs during the early incubation period. However, an offspring's phenotype can be influenced by maternal investment (Wade, 1999; Wilson *et al.*, 2005), and it is difficult to disentangle the prenatal maternal effects from genetic variance by cross-fostering. In birds, maternally derived antioxidants such as vitamin E and the carotenoids play an important defending role against ROS damage (Rubolini *et al.*, 2006; Surai, 2007). Therefore, we also examine whether maternal investment influences susceptibility of chicks to oxidative stress by examining maternally derived antioxidants in the plasma of hatchlings (e.g. uric acid, vitamin C, polyphenols and proteins; see also Erel, 2004).

Methods

Field procedure and cross-fostering

We carried out this study from May to June 2009 in a colony of yellow-legged gulls in the Parque Nacional das Illas Atlánticas, Sálvora Island, Galicia, Spain (42°28′N, 09°00′W). We surveyed the study areas once daily during egg laying and marked nests with numbered bamboo sticks. We visited each nest until clutch completion and marked eggs individually on the day of laying with a permanent marker. A total of 72 nests with a clutch of three eggs (modal clutch size; > 85% of breeding pairs lay a clutch of three eggs in the study population; our unpublished data) were used for this study. To examine additive genetic and common environmental effects on trait variation among individuals, 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-days interval). This design resulted in all three eggs from the same original nest being incubated (then the hatchlings being raised) in three different recipient nests other than the original nest, but conserving the original order within the clutch. The length and breadth of each egg were measured to the nearest 0.01 mm using electronic callipers, and the volume of each egg was calculated as $0.52 \times \text{length} \times \text{breadth}^2$ (Hoyt, 1979).

We checked each nest daily beginning 2 days before the estimated hatching date until all the chicks were hatched. A total of 191 chicks hatched among the 72 study nests. We marked all hatchlings using leg flags made with coloured Velcro on the day of hatching (day 0) for identification. Chicks were weighed, measured and blood sampled at day 0 and day 8. Body condition of each chick at day 0 was calculated as the residual from a linear regression of body mass against tarsus length (day 0: $r = 0.376$, $P < 0.001$; see Schulte-Hostedde *et al.*, 2005). Body mass growth (mass at day 8 and mass at day 0) and skeletal size growth (tarsus length at day 8-tarsus length at day 0) were significantly related in the study chicks ($r = 0.698$, $P < 0.001$). Therefore, relative mass growth calculated as the residual from a linear regression of body mass growth against skeletal size growth was used as an index of chick growth during the first 8 days of life.

Measurements of blood resistance to oxidative stress and plasma antioxidants

Resistance to oxidative stress was assessed as the time needed to haemolyse 50% of red blood cells exposed to controlled free-radical attack (see also Alonso-Alvarez *et al.*, 2006) for all hatchlings ($n = 191$) and 115 chicks recaptured at age 8 days. Around at age 8 days, efficiency of resistance to oxidative stress may be important because basal metabolism increases exponentially in gull chicks (Drent *et al.*, 1992), and increasing metabolic activity causes oxidative-stress-related damage to the organism (Rollo, 2002; Alonso-Alvarez *et al.*, 2007). To assess blood resistance to oxidative stress and plasma antioxidants, we collected blood from each chick using a sterile needle and heparinised capillary tubes. Twenty microlitres of whole blood was mixed with 730 μL of saline buffer (158 mM Na^+, 144 mM Cl^-, 6 mM K^+, 24 mM HCO_3^-, 2 mM Ca^{2+}, 340 mOsm, pH 7.4) immediately after blood sampling. The sample mixed with the saline buffer and the rest of blood were stored at 4 °C before analysis of resistance to oxidative stress and centrifugation, respectively, that occurred within 10 h. Chicks were observed 1–2 days after blood sampling (at day 2 and day 9) in our other studies, but no ill effect caused by blood sampling (e.g. inflammation and mortality) was found in the chicks (unpublished data).

Each blood sample was added to two wells (for duplicated analysis, 80 μL per well) of a 96-well microplate, then 136 μL of a 150 mM solution of 2,2′-azobis-(aminodinopropane)hydrochloride [AAPH] was

subsequently added to each well. The microplate was incubated at 40 °C, and the haemolysis of red blood cells was assessed with a microplate reader device, which measures the decrease of optical density at a wavelength of 540 nm, every 10 min during 3–4 h until all samples reach their minimum values (see also Alonso-Alvarez *et al.*, 2006).

To determine maternal investment into antioxidant defence, the concentration of total antioxidants was measured (see Erel, 2004) using the plasma sampled for each chick on the day of hatching. All plasma antioxidants (extracellular) at hatching have a maternal origin (Surai, 2007). Plasma and cellular fractions were separated by centrifuging a capillary tube filled with whole blood sample. Immediately after centrifugation, plasma fractions were stored at −200 °C until antioxidant analyses. 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. All the process was applied to an automated analyser, and the assay was calibrated with Trolox (see Erel, 2004 for details).

Quantitative genetic analyses

The phenotypic variation caused by the common environment experienced by siblings may bias estimates of additive genetic variances (Kruuk, 2004). We avoided such bias by cross-fostering all siblings to be raised in different recipient nests and including both original and recipient nests as random effects in quantitative genetic analyses (see also Hadfield *et al.*, 2007; Nilsson *et al.*, 2009). Genetic and common environmental components (V_{Origin} and $V_{Recipient}$) of total phenotypic variances (V_P) for resistance to oxidative stress at day 0 and day 8 were estimated using REML univariate mixed-effect models, with original and recipient nests fitted as random effects. Additive genetic variances (V_A) were calculated as twice the variance component of original nest (V_{Origin}), because full siblings share 50% of their genes (Lynch & Walsh, 1998). Note that extra-pair paternity is very rare in gulls (Bukacinska *et al.*, 1998; Gilbert *et al.*, 1998). Environmental variances (V_E) were equal to the recipient nest component ($V_{Recipient}$ in univariate models), and phenotypic (co)variances were calculated as the sum of the original nest, recipient nest and residual variance components ($V_P = V_{Origin} + V_{Recipient} + V_R$). Narrow-sense heritability (h^2) and common environmental effect, i.e. recipient nest effect, (c^2) were estimated as $h^2 = V_A/V_P$ and $c^2 = V_E/V_P$ for each trait. We also provide the coefficient of additive genetic variance CV_A (Houle, 1992), in which the additive genetic variance is scaled by the trait mean ($\overline{X}$): $CV_A = 100 \times \sqrt{V_A}/\overline{X}$ to enable comparison with other traits and populations.

As heritability can be over-estimated by egg maternal effects (see Introduction), factors possibly related to maternal effects on blood resistance to oxidative stress were included as fixed effects in the initial mixed-effect models and then only significant factors were retained in the selected models. Hatching date, chick order (egg laying order), plasma antioxidants (day 0), egg volume and hatchling condition were fitted as fixed effects in the initial model for resistance to oxidative stress at hatching. For resistance to oxidative stress at the age of 8 days, hatching date, chick order, egg volume, resistance to oxidative stress at day 0, plasma antioxidants (day 0) and chick growth were fitted in the initial model.

The statistical significance of each random effect was assessed using likelihood ratio tests that compare models based on -2 times the difference in REML log-likelihood scores distributed as χ^2 where the number of degrees of freedom equalled the number of variance terms removed. The significance of each variance component function (h^2 and c^2) was assessed using a *t*-test. ASReml v2 (VSN International; Gilmour *et al.*, 2006) was used to fit full-sibling univariate models and calculate variance component functions and standard errors (see Lynch & Walsh, 1998).

Results

Chick order, plasma antioxidants (day 0), hatchling condition did not influence resistance to oxidative stress at day 0 ($P \geq 0.18$). Resistance to oxidative stress of chicks at hatching was negatively correlated to hatching date and egg volume, and their significant effects (ANOVA: hatching date: $F_{1,188} = 19.47$, $P < 0.001$; egg volume: $F_{1,188} = 5.21$, $P = 0.02$) were included as fixed effects in the REML univariate mixed-effect model. Both heritability ($h^2 = 0.16 \pm 0.16$) and common environmental effect ($c^2 = 0.06 \pm 0.08$) were low and nonsignificant for resistance to oxidative stress on the day of hatching (Table 1).

Resistance to oxidative stress of chicks at age of 8 days was negatively correlated to hatching date (ANOVA: $F_{1,112} = 7.11$, $P = 0.01$) and chick growth ($F_{1,112} = 5.12$, $P = 0.03$). Nonsignificant fixed effects, chick order, egg volume, resistance to oxidative stress (day 0) and plasma antioxidants (day 0) ($P \geq 0.38$), were dropped from the final model. Resistance to oxidative stress at day 8 showed significant heritability ($h^2 = 0.59 \pm 0.25$, $P = 0.01$), but the rearing environment of the chicks did not influence the variation in resistance to oxidative stress ($c^2 = 0.11 \pm 0.11$, $P = 0.18$).

Discussion

Our quantitative genetic analyses using cross-fostered gull chicks showed that variation in blood resistance to oxidative stress was not affected by common rearing environment. However, chicks at age 8 days showed

Table 1 Quantitative genetics for resistance to oxidative stress at hatching (day 0) and age of 8 days. REML univariate mixed-effect models.

	Resistance at day 0 (min)			Resistance at day 8 (min)		
	Estimate		*P*	Estimate		*P*
n	191			115		
V_{Origin} ± SE	4.49 ± 4.59	$\chi^2_1 = 1.06$	0.30	17.33 ± 8.49	$\chi^2_1 = 4.77$	0.03
$V_{Recipient}$ ± SE	3.55 ± 4.44	$\chi^2_1 = 0.71$	0.40	6.22 ± 6.88	$\chi^2_1 = 0.85$	0.36
V_P ± SE	55.06 ± 5.75			58.42 ± 8.27		
h^2 ± SE	0.16 ± 0.16	$t_{188} = 1.00$	0.16	0.59 ± 0.25	$t_{113} = 2.34$	0.01
c^2 ± SE	0.06 ± 0.08	$t_{188} = 0.80$	0.21	0.11 ± 0.11	$t_{113} = 0.93$	0.18
Trait mean ± SD	28.06 ± 7.95			34.42 ± 7.89		
CV_A	10.68			17.10		

The variance components reported are original nest (V_{Origin}), recipient nest ($V_{Recipient}$) and total phenotypic variance (V_P). Heritability (h^2) and common environmental effect (c^2) were calculated from these variance components.

high resemblance to original siblings in resistance to oxidative stress.

The defence system to prevent oxidative damage (Surai, 2007) includes various mechanisms such as (i) cellular antioxidant enzyme groups that counteract the effects of the superoxide anion and its damaging derivatives, (ii) the chain-breaking antioxidant compounds (endogenously produced antioxidants and dietary antioxidants) that neutralize ROS and (iii) structural defence of tissues through their amino acid or fatty acid composition (reviewed in Monaghan *et al.*, 2009). Our measurement of the haemolysis rate of red blood cells exposed to a controlled free-radical attack not only represents total antioxidant defences (enzymatic and nonenzymatic antioxidants) in the blood but also indicates the rate of lipid peroxidation in the erythrocyte membrane that would affect the cell capacity to resist free-radical aggressions (e.g. Zou *et al.*, 2001). Thus, blood resistance to oxidative stress is influenced by both exogenous and endogenous components. Among these, only dietary antioxidants are mainly influenced by parental provisioning, and variations in the other defence systems against oxidative stress possibly depend on genetic, maternal and unexplained environmental influences. Molecular genetic studies based on laboratory animals have shown that resistance to a ROS-induced haemolysis has a genetic basis (Zou *et al.*, 2001; Ito *et al.*, 2004; Armutcu *et al.*, 2005; Tothova *et al.*, 2007; see also Warren & Rossi, 2009).

Our results show that resistance to oxidative stress at hatching was not related to plasma antioxidants, which are mostly derived from maternal investment into eggs (Surai, 2007). However, we acknowledge that plasma antioxidants in hatchlings are only a part of maternal antioxidants allocated into eggs and other potential maternal effects could have influenced embryonic development (Price, 1998) and thereby blood resistance to oxidative stress at hatching. Hatchlings from larger eggs were less resistant to oxidative stress, suggesting a complex influence of maternal effects on resistance to oxidative stress at hatching. Hatchlings from large eggs are generally bigger and heavier than those from small eggs (Bolton, 1991), and embryonic growth rates in skeletal size and mass are positively correlated between egg mass and volume (Ricklefs & Starck, 1998). Therefore, the accelerated embryonic growth might trade off with physiological conditions at hatching (Metcalfe & Monaghan, 2001), including resistance to oxidative stress. Similarly, chicks growing relatively fast in mass, compared to their skeletal growth, across the first 8 days of life had decreased resistance to oxidative stress. The later a chick hatched in the season, the less resistant its red blood cells to oxidative stress at both hatching and age of 8 days. As individuals breeding in suboptimal time in season are generally poor quality breeders in gulls (e.g. Sydeman *et al.*, 1991; Brouwer *et al.*, 1995), poor maternal investment in yolk composition, including vitamin E and the carotenoids (Rubolini *et al.*, 2006; Surai, 2007), may have resulted in the negative relationship between hatching date and resistance to oxidative stress of offspring.

Common environmental effects on phenotypic variation in resistance to oxidative stress are expected to increase with age during the nestling periods because chicks obtain dietary antioxidants derived from parental provisioning (Catani *et al.*, 2008). However, common environmental effects on variation in resistance to oxidative stress, estimated as the similarity between post-sibs that shared the same rearing environments, were not significant in chicks from both day 0 and day 8, although c^2 increased slightly from day 0 ($c^2 = 0.06$) to day 8 ($c^2 = 0.11$). These results may suggest that the role of dietary antioxidants from parental provisioning is minor in blood resistance to oxidative stress or that differences among broods in parental favouritism (Lessells, 2002) or sibling competition (Costantini *et al.*, 2006) could influence estimates of common environmental variances. Alternatively, relatively low c^2 in day 8 than expected could arise as a consequence of the increase in importance of additive genetic effects.

Heritability in resistance to oxidative stress was relatively low and nonsignificant in hatchlings, but interestingly, the chicks of age 8 days showed high and significant heritability ($h^2 = 0.59$). The increase of

heritability with age cannot be attributed to a decrease in the total phenotypic variance as it was comparable between day 0 and day 8. Some quantitative genetic studies of laboratory and wild populations have shown that heritability in morphology and life-history traits can vary with age possibly as a result of age-dependent environmental and maternal effects (e.g. Réale *et al.*, 1999; Albuquerque & Meyer, 2001; Charmantier *et al.*, 2006). In hatchlings, additive genetic variance in resistance to oxidative stress can be over- or under-estimated because of the possible interferences by unexplained early maternal effects that vary between or within genetic broods, respectively. Particularly in the latter case, differential strategies in maternal allocation of egg antioxidants can make it difficult to estimate similarity between siblings. Total yolk antioxidant capacity decreases with laying order in the yellow-legged gull *Larus michahellis* (Rubolini *et al.*, 2006). However, females may be able to adjust antioxidant allocation within clutches to influence offspring quality variation within broods, controlling over sibling competition (Blount *et al.*, 2002; Royle *et al.*, 2003). The significant estimate of genetic variance at day 8 could be partly inflated by unexplained maternal effects on egg composition that are difficult to disentangle by cross-fostering. However, maternal investment in offspring conditions can play a major role early in life; whereas later on, offspring phenotype is determined mainly by the genotype and/or the environment (Charmantier *et al.*, 2006). In our study, resistance to oxidative stress and plasma antioxidants at hatching (proxy of maternal investment for the defence system against oxidative stress) had no influence on resistance to oxidative stress at day 8, suggesting that indeed influence of maternally derived antioxidants on the heritability estimate at this age should be little.

In conclusion, our results show natural variation and heritability of resistance to a ROS-induced haemolysis in gull chicks. Importantly, our results suggest that genetic effects on variation in resistance to oxidative stress increase as gull chicks grow. As early resistance to oxidative stress is genetically linked to reproductive life-history traits, oxidative-stress-related genes may constrain reproductive senescence (Kim *et al.*, 2010). Our study suggests that the resistance to oxidative stress may play an important role in life-history evolution (Dowling & Simmons, 2009; Monaghan *et al.*, 2009). Further work is required to confirm the evolutionary role of oxidative stress by exploring genetic correlations between resistance to oxidative stress and life-history traits.

Acknowledgments

We thank A. Tato for analysing plasma antioxidants, C. Pérez for invaluable help during the fieldwork, M. Lores for advice in preparing for the saline buffer, and J. Dominguez and L. Sampedro for logistic helps. Fieldwork in Sálvora Island depended on the generous support and friendship of M. Caneda, M. Costas, P. Fernandez, P. Rivadulla, J. Torrado, P. Valverde and P. Vázquez of the Parque Nacional, and los fareros, P. Pertejo and J. Vilches. Finance was provided by the Spanish Ministerio de Ciencia e Innovación (CGL2009-10883-C02-01). J.M. was supported by Juan de la Cierva fellowship from MICINN.

References

Albuquerque, L.G. & Meyer, K. 2001. Estimates of covariance functions for growth from birth to 630 days of age in Nelore cattle. *J. Anim. Sci.* **79**: 2776–2789.

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. & Sorci, G. 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution* **60**: 1913–1924.

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.

Arking, R., Burde, V., Graves, K., Hari, R., Soliman, S., Saraiya, A., Sathrasala, K. & Buck, S. 2000. Identical longevity phenotypes are characterized by different patterns of gene expression and oxidative damage. *Exp. Gerontol.* **35**: 353–373.

Armutcu, F., Coskun, O., Gurel, A., Sahin, S., Kanter, M., Cihan, A., Numanoglu, K.V. & Altinyazar, C. 2005. Vitamin E protects against acetone-induced oxidative stress in rat red blood cells. *Cell Biol. Toxicol.* **21**: 53–60.

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. Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology* **89**: 2584–2593.

Blount, J.D., Surai, P.F., Nager, R.G., Houston, D.C., Møller, A.P., Trewby, M.L. & Kennedy, M.W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proc. R. Soc. Lond. B* **269**: 29–36.

Bolton, M. 1991. Determinants of chick survival in the lesser black-backed gull: relative contributions of egg size and parental quality. *J. Anim. Ecol.* **60**: 949–960.

Brouwer, A., Spaans, A.L. & De Wit, A.A.N. 1995. Survival of herring gull *Larus argentatus* chicks: an experimental analysis of the need for early breeding. *Ibis* **137**: 272–278.

Bukacinska, M., Bukacinski, D., 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.

Catani, C., Peters, A. & Schaefer, H.M. 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* **273**: 225–232.

Costantini, D., Casagrande, S., De Filippis, S., Brambilla, G., Fanfani, A., Tagliavini, J. & Dell'Omo, G. 2006. Correlates of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J. Comp. Physiol. B* **176**: 329–337.

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

Drent, R.H., Klaassen, M. & Zwaan, B. 1992. Predictive growth budgets in terns and gulls. *Ardea* **80**: 5–17.

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.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*, 4th edn. Longman, Essex.

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

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

Gilmour, A.R., Gogel, B.J., Cullis, B.R. & Thompson, R. 2006. *ASReml User Guide. Release 2.0.* VSN International Ltd, Hemel Hempstead, UK.

Hadfield, J.D., Nutall, A., Osorio, D. & Owens, I.P.F. 2007. Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. *J. Evol. Biol.* **20**: 549–557.

Harman, D. 1957. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **2**: 298–300.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.

Hoyt, D.F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **92**: 270–279.

Ishii, N., Fujii, M., Hartman, P.S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanase, S., Ayusawa, D. & Suzuki, K. 1998. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature* **394**: 694–697.

Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., Ikeda, Y., Mak, T.W. & Suda, T. 2004. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* **431**: 997–1002.

Khazaeli, A.A., Nuzhdin, S.V. & Curtsinger, J.W. 2007. Genetic variation for life span, resistance to paraquat, and spontaneous activity in unselected populations of *Drosophila melanogaster*: implications for transgenic rescue of life span. *Mech. Ageing Dev.* **128**: 486–493.

Kim, S.-Y., Velando, A., Sorci, G. & Alonso-Alvarez, C. 2010. Genetic correlation between resistance to oxidative stress and reproductive lifespan in a bird species. *Evolution*, in press, doi:10.1111/j.1558-5646.2009.00862.x.

Kirkwood, T.B.L. & Austad, S.N. 2000. Why do we age? *Nature* **408**: 233–238.

Kruuk, L.E.B. 2004. Estimating genetic parameters in natural populations using the 'animal model'. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**: 873–890.

Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J. & Ruvkun, G. 2003. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**: 40–48.

Lessells, C.M. 2002. Parentally biased favouritism: why should parents specialize in caring for different offspring? *Philos. Trans. R. Soc. Lond. B* **357**: 381–403.

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

Metcalfe, N.B. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**: 254–260.

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.

Nedelcu, A.M., Marcu, O. & Michod, R.E. 2004. Sex as a response to oxidative stress: a two-fold increase in cellular reactive oxygen species activates sex genes. *Proc. R. Soc. Lond. B* **271**: 1591–1596.

Nilsson, J.-Å., Kesson, M.Å. & Nilsson, J.F. 2009. Heritability of resting metabolic rate in a wild population of blue tits. *J. Evol. Biol.* **22**: 1867–1874.

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

Paaby, A.B. & Schmidt, P.S. 2009. Dissecting the genetics of longevity in *Drosophila melanogaster*. *Fly* **6**: 29–38.

Pasyukova, E.G., Vieira, C. & Mackay, T.F.C. 2000. Deficiency mapping of quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Genetics* **156**: 1129–1146.

Price, T. 1998. Maternal and paternal effects in birds: effects on offspring fitness. In: *Maternal Effects as Adaptations* (T.A. Mousseau & C.W. Fox, eds), pp. 202–226. Oxford University Press, New York.

Réale, D., Festa-Bianchet, M. & Jorgenson, J.T. 1999. Heritability of body mass varies with age and season in wild bighorn sheep. *Heredity* **83**: 526–532.

Ricklefs, R.E. & Starck, J.M. 1998. Embryonic growth and development. In: *Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum* (J.M. Starck & R.E. Ricklefs, eds), pp. 31–58. Oxford University Press, Oxford.

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

Royle, N.J., Surai, P.F. & Hartley, I.R. 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. *Funct. Ecol.* **17**: 472–481.

Rubolini, D., Romano, M., Bonisoli Alquati, A. & 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.

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

von Schantz, T.V., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good genes oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* **266**: 1–12.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. & Hickling, G.J. 2005. Restitution of mass-size residuals: validating body condition indices. *Ecology* **86**: 155–163.

Surai, P.F. 2007. *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham University Press, Nottingham.

Sydeman, W.J., Penniman, J.F., Penniman, T.M., Pyle, P. & Ainley, D.G. 1991. Breeding performance in the western gull: effects of parental age, timing of breeding and year in relation to food availability. *J. Anim. Ecol.* **60**: 135–149.

Tothova, Z., Kollipara, R., Huntly, B.J., Lee, B.H., Castrillon, D.H., Cullen, D.E., McDowell, E.P., Lazo-Kallanian, S.,

Williams, I.R., Sears, C., Armstrong, S.A., Passegue, E., DePinho, R.A. & Gilliland, D.G. 2007. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* **128**: 325–339.

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

Vermeulen, C.J., van de Zande, L. & Bijlsma, R. 2005. Resistance to oxidative stress induced by paraquat correlates well with both decreased and increased lifespan in *Drosophila melanogaster*. *Biogerontology* **6**: 387–395.

Wade, M.J. 1999. The evolutionary genetics of maternal effects. In: *Maternal Effects as Adaptations* (T.A. Mousseau & C.W. Fox, eds), pp. 5–21. Oxford University Press, Oxford.

Warren, L.A. & Rossi, D.J. 2009. Stem cells and aging in the hematopoietic system. *Mech. Ageing Dev.* **130**: 46–53.

Wilson, A.J., Coltman, D.W., Pemberton, J.M., Overall, A.D.J., Byrne, K.A. & Kruuk, L.E.B. 2005. Maternal genetic effects set the potential for evolution in a free-living vertebrate population. *J. Evol. Biol.* **18**: 405–414.

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 14 October 2009; accepted 6 January 2010