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REVIEW AND SYNTHESIS

Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation

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Abstract

The concept of trade-offs is central to our understanding of life-history evolution. The underlying mechanisms, however, have been little studied. Oxidative stress results from a mismatch between the production of damaging reactive oxygen species (ROS) and the organism's capacity to mitigate their damaging effects. Managing oxidative stress is likely to be a major determinant of life histories, as virtually all activities generate ROS. There is a recent burgeoning of interest in how oxidative stress is related to different components of animal performance. The emphasis to date has been on immediate or short-term effects, but there is an increasing realization that oxidative stress will influence life histories over longer time scales. The concept of oxidative stress is currently used somewhat loosely by many ecologists, and the erroneous assumption often made that dietary antioxidants are necessarily the major line of defence against ROS-induced damage. We summarize current knowledge on how oxidative stress occurs and the different methods for measuring it, and highlight where ecologists can be too simplistic in their approach. We critically review the potential role of oxidative stress in mediating life-history trade-offs, and present a framework for formulating appropriate hypotheses and guiding experimental design. We indicate throughout potentially fruitful areas for further research.

Keywords

Antioxidants, carotenoid, free radical, life-history strategies, oxidative stress, reactive oxygen species, reproduction, senescence.

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INTRODUCTION

Life-history theory has provided a powerful conceptual framework for investigating the evolution of life-history strategies. Central to this is the concept of life-history trade-offs. Such trade-offs are presumed to have a physiological basis (Zera & Harshman 2001), but we actually know rather little about the mechanisms that determine their nature and outcome, or indeed constrain the evolution of particular life-history strategies. Trade-offs are most usually thought of in terms of resource allocation. That is, where the allocation of limiting resources to one trait has negative consequences for other traits requiring the same resource (Zera & Harshman 2001). However, trade-offs can also be produced as a consequence of the performance of one activity generating negative consequences for other traits. A prime candidate mechanism that might drive this second type of trade-off, and in which there has recently been a great deal of interest amongst evolutionary ecologists, is oxidative stress. All organisms need to fuel the biological processes on which they depend. Aerobic species have evolved the capacity to use oxygen for the efficient release of energy. They also need to prevent oxidation of their body components by reactive substances produced in the process, which is what happens during oxidative stress. The need for energy efficiency therefore needs to be balanced against potential by-product toxicity. This is the basis for the suggestion that the management of oxidative stress might mediate life-history trade-offs.

The extent of uncontrolled oxidative processes in animals, and their consequences for life-history traits, is still poorly understood (Balaban *et al.* 2005). There is no

simple relationship between energy generation and oxidative damage, as all organisms have evolved mechanisms to prevent damage by means of sophisticated and multifaceted antioxidant defence systems. Nonetheless, oxidative damage does occur, and that such damage might accumulate with age is the basis of the well-known free-radical hypothesis of ageing (Gershman *et al.* 1954; Harman 1956; Beckman & Ames 1998; Finkel & Holbrook 2000). The fitness consequences of such damage accumulation crucially depend on the extent to which changes in longevity or late-life performance have negative effects on lifetime reproductive output. Following the suggestion by von Schantz *et al.* (1999) that sexually selected male traits might advertise oxidative stress levels, and that this might be linked to genetic variation in fitness, there has been a burgeoning of interest in how oxidative stress influences many other components of life histories, such as growth, ability to combat disease, sexual signalling, diet and foraging behaviour. Understanding the role of oxidative stress in this context is a multidisciplinary topic that requires the integration of knowledge from different sources. The concept of oxidative stress (and how it can be detected) is currently used somewhat loosely by ecologists (for instance, the erroneous assumption is often made that levels of dietary antioxidants can indicate the likely level of oxidative stress, with a consequent over-emphasis on this component of the antioxidant defence system; Catani *et al.* 2008), and a more comprehensive view is therefore needed.

In this mini-review, we critically review the current state of knowledge of the potential role of oxidative stress in mediating life-history trade-offs. We outline what is meant by oxidative stress, antioxidant defences and oxidative damage, and discuss why it is important to choose an appropriate method for measuring each of these components. We present a cohesive framework that can be used to formulate appropriate hypotheses and guide experimental design, and highlight where ecologists may be in danger of being too simplistic in their approach to the underlying biochemistry. We also indicate the areas where knowledge is currently lacking and suggest where future research should be directed.

THE OXIDATIVE THREAT

The threat of uncontrolled oxidation of biomolecules largely comes from the so-called reactive oxygen species (ROS). Although ROS from exogenous sources (e.g. from ultra violet radiation, ozone or pollutants) can attack biomolecules, in most circumstances the greatest threat seems to come from endogenous ROS production (Balaban *et al.* 2005). The term ROS is used to cover both the free radical and non-radical oxidants. Free radicals are atoms or compounds containing one or more unpaired electrons in their outer orbits. Amongst the most potent and important in a biological context are superoxide, hydroxyl and nitric oxide (Finkel & Holbrook 2000). These radicals are highly reactive and unstable, existing for only micro- or nanoseconds before triggering chain reactions in which reactivity is passed along to even more damaging compounds. The main non-radicals are hydrogen peroxide, hypochlorous acid and singlet oxygen. These can persist for longer, minutes in the case of hydrogen peroxide, and also cause oxidative damage to biomolecules (Surai 2002). Reactive nitrogen species (RNS) also occur, causing analogous nitrosative effects (Surai 2002). While less well studied, RNS are generally thought not to be so important as ROS in the context of damage generation and so will not be considered further here.

It is important to realize that not all endogenous ROS production has negative consequences. Around 10% of ROS in animal cells is produced in a controlled and compartmentalized manner, usually by specific enzymes such as NADPH oxidases and NO synthases. These ROS are channelled to serve many important functions, for example in cell signalling and cell transformation, regulation of smooth muscle relaxation, regulation of blood flow and in immune defence (Dröge 2002). In the latter case, pathogen encapsulation by the macrophages is followed by an intense production of ROS in the membrane surrounding the vacuole, which stimulates protease production and thereby destroys the invader (Dröge 2002; Finkel 2003).

The remaining 90% of ROS are generated as a by-product of normal metabolic processes (Balaban *et al.* 2005). In the cells of higher organisms, energy is produced in the form of ATP, generated in the mitochondria via the electron transport chain (ETC). Over 80% of all the oxygen used by the cells is consumed in the ETC, which is a highly efficient power house. However, *in vitro* studies suggest that 1–2% of the oxygen molecules used are converted into superoxide anions. Although the figure *in vivo* is likely to be much less, possibly more like 0.2% (Balaban *et al.* 2005), it is still a very significant amount. Furthermore, even more damaging ROS can then be formed if uncontrolled downstream reactions are not prevented.

OXIDATIVE STRESS

Oxidative stress occurs when the enzymatic and non-enzymatic antioxidants cannot fully neutralize the ROS that are produced, so that unquenched ROS remain for long enough to cause further reactions. As a consequence of this imbalance, a so-called oxidative event then occurs in which biomolecules are oxidized by ROS, the extent of the damage being dependent on their susceptibility. The basic homeostatic situation can change either as a consequence of increased ROS production, or by reduced defences. Figure 1

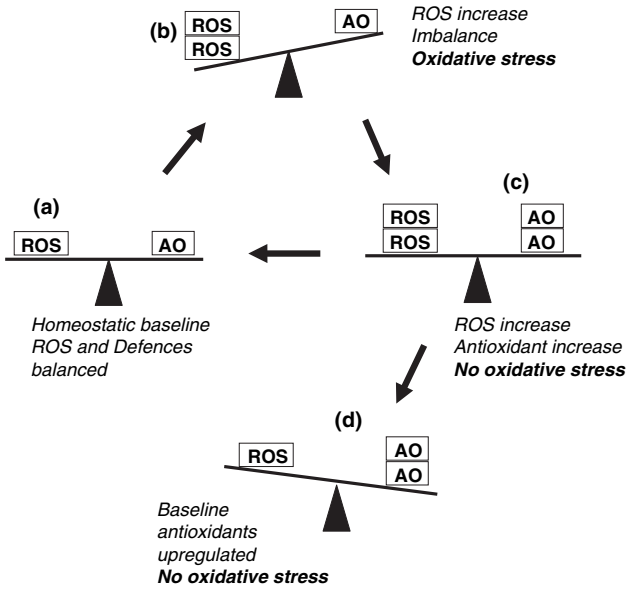

Figure 1 A simplified illustration of the relationships between reactive oxygen species (ROS), antioxidant defence (including repair) systems and oxidative stress. (a) In the homeostatic baseline condition, both ROS and antioxidant (AO) levels are low, with defences adequate to balance ROS production so that there is no oxidative stress. (b) An increase in ROS production may initially exceed the capacity of the antioxidant system, leading to a period of oxidative stress. (c) If the increase in ROS is small, it can be matched by increased deployment of antioxidants, preventing further oxidative stress. If the elevation of ROS is only temporary, there will then be a return to (a), the homeostatic position. (d) Exposure to more prolonged elevation of ROS can induce the organism to permanently increase its baseline antioxidant levels, making it better able to cope with future oxidative events. Note that measurement of only ROS or only antioxidants can give a misleading picture: high levels of the former can suggest oxidative stress (as in b) whereas this is not necessarily the case (see c); similarly, a given level of antioxidant defences does not indicate whether oxidative stress is (b) or is not (a) occurring. Note also that an elevation of antioxidant defences does not necessarily prevent the occurrence of oxidative damage, as the effectiveness of this will depend on the extent to which it cancels out the increase in ROS.

illustrates this in a simplified form. High levels of ROS do not necessarily result in oxidative stress if this can be balanced by upregulation of defences; nor does it follow that individuals having relatively high levels of antioxidants are necessarily in a better redox state than those with lower levels, as this will depend on the level of ROS that these defences have to deal with. Therefore, oxidative stress cannot be inferred simply by measuring just one side of the delicate balance that generally exists between ROS generation and damage limitation by the antioxidant system. In practice, we infer oxidative stress from measuring either damage or the presence of increased levels of ROS that suggest quenching is inadequate. In terms of the consequences for the organism, it is the outcome of oxidative stress that matters, i.e. the level of impaired function as a consequence of oxidative damage.

OXIDATIVE DAMAGE

Key biological molecules, notably DNA, proteins, lipids, can all be adversely affected by ROS. Furthermore, the reaction of ROS with these macromolecules generates additional ROS, setting in train a cascade of damage if left unchecked. It is estimated that ROS are responsible for *c.* 10 000 DNA base modifications per cell per day (Ames *et al.* 1991). Oxidation or methylation of bases is thought to have the most serious phenotypic consequences (Falnes *et al.* 2007). Mitochondrial DNA appears to be particularly vulnerable, in part due to its proximity to the site of most uncontrolled ROS generation, and because of the low level of repair that occurs (Finkel & Holbrook 2000; Balaban *et al.* 2005; Falnes *et al.* 2007). Telomeres, the caps at the chromosome end that are critical for genome stability, are also vulnerable to attack from ROS, and the accelerated reduction in telomere length that results from oxidative stress can hasten cell senescence (Richter & von Zglinicki 2007). Oxidation of proteins produces reversible disulphide bridges, changes their formation and eventually impairs their function. The magnitude of the damage will depend in part on the location of the proteins relative to the site of ROS generation, and their composition and structure (Dröge 2002). Some amino acids, notably tryptophan, tyrosine, histidine and cysteine, are much more susceptible to oxidation than others, and ROS can also alter the secondary and tertiary structure of proteins (Dröge 2002; Surai 2002).

Damage to lipids is also of great significance, as this can have major consequences for membrane structure and function in particular. Membrane composition, which is very important to the membrane function and possibly to metabolic rate (Hulbert *et al.* 2007; but see Valencak & Ruf 2007), influences susceptibility to oxidative damage. Polyunsaturated fatty acids (PUFA) are much less resistant to peroxidation than monounsaturated or saturated fatty acid acids, and so variation in the proportion of PUFA in membranes can influence the rate of oxidative damage (Hulbert *et al.* 2007). Furthermore, oxidative damage to lipids has widespread effects, as lipid peroxidation triggers a complex chain reaction involving a range of reactive intermediates that can then also cause protein and DNA damage (Hulbert *et al.* 2007).

THE ANTIOXIDANT SYSTEM

Given the constant attack by potentially destructive, endogenously generated ROS, it is not surprising that

sophisticated defence mechanisms to prevent damage and impaired function as a consequence of oxidation have evolved. These, which collectively are termed the antioxidant system (Surai 2002), can be summarized under five main headings:

(1) The first line of defence is minimizing uncontrolled ROS release within the cell. ROS generation from the mitochondria can vary for several reasons (Balaban *et al.* 2005). For example, the composition of the mitochondrial membranes and their state (which can vary across species and tissues, and with age) can affect ROS production (Hulbert *et al.* 2007). Increased uncoupling of oxygen consumption and ATP generation, which results in the production of heat, reduces mitochondrial ROS production; the metabolic substrate is also important because the use of fatty acids as a metabolic substrate seems to increase uncoupling (Brand 2000; Hulbert *et al.* 2007).

(2) Three main antioxidant enzyme groups located in the cell counteract the effects of the superoxide anion and its damaging derivatives. Superoxide dismutase acts on the superoxide anion to produce hydrogen peroxide and singlet oxygen. Both of these products are themselves potentially damaging ROS. However, metal-binding proteins prevent transition metal (mainly iron and copper) ions from facilitating the production of the highly reactive hydroxyl radical from hydrogen peroxide, while two further enzymes (catalase, located in particular cellular subcompartments, and the more widely distributed glutathione peroxidase) convert the hydrogen peroxide to water. Singlet oxygen is quenched by other antioxidants, notably vitamin E (see the next section). The recently discovered peroxiredoxin enzymes also play an important ROS scavenging role in the mitochondria (Balaban *et al.* 2005).

(3) The next level of defence involves the chain-breaking antioxidant compounds, which neutralize ROS without passing on reactivity. These are a mixture of endogenously produced antioxidants and those obtained through the diet. The endogenous compounds include the intra-cell thioredoxin systems, ubiquinones and glutathione. The last is probably the most active antioxidant in biological systems (Surai 2002), and is especially effective at neutralizing the destructive hydroxyl radicals against which there is no enzymatic neutralization. Other endogenously produced antioxidants include the water-soluble vitamin C (synthesized by most vertebrates with a few notable exceptions, including humans, fruit bats and guinea pigs) and uric acid, though production of the latter can generate further radicals (Dröge 2002). Many other antioxidants are obtained from the diet, including the well-known plant-derived compounds such as vitamin E (the collective name for the tocopherols and tocotrienols) and the carotenoids (Catani *et al.* 2008). These are generally fat soluble, and vitamin E is the main antioxidant in membranes, mopping up singlet oxygen and breaking the chain reaction of lipid peroxidation. Interaction amongst these various antioxidants is very important, and thus levels of any one group are not necessarily good indicators of the overall level of defence. For example, in reacting with ROS, vitamin E is itself turned into a radical; this is then reduced by other antioxidants, notably the carotenoids, allowing the vitamin E molecule to again be used as an antioxidant; low levels of vitamin E can thus still provide high antioxidant protection if this recycling by other antioxidants is efficient, and such recycling is an extremely important part of the antioxidant system (Surai 2002). A further important consideration is that the production or deployment of the various antioxidants can be altered in response to increased ROS production. The endogenous production of antioxidants can be increased, and stored dietary antioxidants can be mobilized (Surai 2002). In addition, mild exposure to oxidative events can result in a more permanent upregulation of defence levels, producing what is termed a hormetic effect, i.e. where exposure to relatively mild stress improves the efficacy of the protective mechanisms (Rattan 2008).

(4) Another line of defence against ROS damage is structural, such that the most sensitive or important structures are more resistant to ROS attack, as a consequence of their amino acid or fatty acid composition for example. This may play an important role in determining differences between tissues, individuals or species in the amount of damage that particular levels of oxidative stress produce. Furthermore, the vulnerability of tissues to ROS attack may increase with age as a consequence of changes in their composition (Hulbert *et al.* 2007).

(5) Despite the above defences, some oxidative damage still occurs. The final line of defence against the damaging effects of oxidants is the removal or repair of damaged molecules. Repair of DNA is very important to cell function, and several complex damage recognition and repair pathways exist (Kastan & Bartek 2004). Similarly, damaged proteins and lipids are either repaired, or destroyed and replaced, but these processes are less well understood (Surai 2002; Halliwell & Gutteridge 2007; Hulbert *et al.* 2007).

MEASUREMENT OF OXIDATIVE STRESS

It is possible to measure all four components of oxidative stress (free radical production, antioxidant defences, oxidative damage and repair mechanisms), but not equally easily. However, many ecological studies have only quantified a single component (usually an indicator of antioxidant capacity, as this is relatively easy) on the erroneous assumption that by itself this can provide information on levels of oxidative stress. Moreover, it is important to know exactly what each assay is measuring, as the most

appropriate technique will depend on the question being investigated and the kind of sample available. The battery of methods available to assess any of these components of oxidative stress is enormous and comprehensive reviews have recently been published (Guetens *et al.* 2002; Del Rio *et al.* 2005; Mateos & Bravo 2007; Somogyi *et al.* 2007). Here, we briefly review a sample of methods that we think are likely to be useful for field ecologists and evolutionary biologists interested in measuring oxidative stress (Table 1). We have favoured methods utilizing plasma and other body fluids because these are less invasive and do not require terminal sampling; however, some of these methods are equally applicable to other tissues such as muscle and the nervous system, which could be particularly useful in the studies of senescence.

Free radicals

The direct analysis of ROS in biological material is difficult because of their intrinsic reactivity and short half-lives. The only technique that allows direct observation of free radicals is electron spin resonance (ESR; Halliwell & Gutteridge 2007). However, this technique is only able to detect the less-reactive radical species. To overcome this limitation, ESR is commonly used in combination with the technique of spin trapping, in which a trap molecule is allowed to react with a radical to produce a more stable and measurable product. Besides ESR, alternative methods based on the detection of ROS reaction products with a variety of spectroscopic probes (i.e. substances that change their properties of light absorption or emission, or magnetic characteristics upon reaction with ROS) are available (reviewed by Bartosz 2006). However, the use of spectroscopic probes for the detection and quantification of ROS production has some inherent limitations and sources of artefacts, such as low stability of some probes and products formed that may be additionally metabolized in cellular systems, undesired probe reactions in the system studied, production of ROS by the probes themselves and perturbation of the systems studied by the probes (Bartosz 2006; Halliwell & Gutteridge 2007). Because of these limitations, methods currently available for the direct measurement of ROS are of limited applicability in field studies in ecology. Therefore, it is more common to measure not the ROS themselves, but the damage that they cause; there is some justification for this as ultimately it is the damage caused by ROS that matters rather than the total amount of ROS produced.

Antioxidant defences

The concentration of individual enzymatic and non-enzymatic antioxidants can be measured in different tissues and fluids (e.g. Selman *et al.* 2002b). These measurements can be appropriate for some experimental studies. However, the antioxidant capacity is not a simple additive function of the concentration of individual antioxidants in a sample because of the synergistic and antagonistic interactions among antioxidants as mentioned earlier. Thus, data on measurements of individual antioxidant concentrations are generally more difficult to interpret than estimations of overall antioxidant capacity of a sample against a radical attack (Cohen *et al.* 2007; Somogyi *et al.* 2007).

There is a wide range of methods that aim to estimate the total antioxidant capacity (TAC) in the plasma and other body fluids. This usually involves estimating the ability of the sample to quench a free oxygen/nitrogen radical, thus providing an integrated parameter of measurable antioxidants (Cohen *et al.* 2007; Somogyi *et al.* 2007). These methods can be classified into assays that apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals, and those that measure the capacity of an antioxidant to reduce (and so change the colour of) an oxidant, with the degree of colour change correlating with the antioxidant capacity of the sample (reviews in Halliwell & Gutteridge 2007; Somogyi *et al.* 2007). However, although these methods provide an estimate of TAC, some important caveats intrinsic to the methods should be considered. (1) When interpreting results, it needs to be born in mind that assays estimating TAC in plasma or serum only reflect the antioxidant capacity of circulating antioxidants with respect to a specific free radical, and the response may vary depending on the free radical challenge used. (2) Tissue antioxidant protection is mostly enzymatic and it is not known how well the circulating and the enzymatic components of the antioxidant defence system correlate. (3) The correlations between measurements of TAC *in vitro* and *in vivo* are not known, thus estimations of TAC *in vitro* may not have direct implications for estimations of TAC *in vivo* (Somogyi *et al.* 2007).

Oxidative damage

Free radicals and other reactive species can cause oxidative damage to lipids, proteins and DNA. A global indicator of oxidative damage is not yet available, and thus the assessment of the oxidative status of an organism may require a combination of various methods to measure different oxidative damage biomarkers (Mateos & Bravo 2007).

Lipids are one of the major targets of oxidative stress. As mentioned earlier, lipid peroxidation gives rise to a number of secondary, highly damaging products. The two most used as biomarkers of lipid peroxidation are F2-isoprostanes and malondialdehyde (MDA). Both are important secondary decomposition products of PUFA (Halliwell & Gutteridge

Table 1 Methods to estimate reactive oxygen species, antioxidant defences, oxidative damage and repair mechanisms

Method	Measures	Sample	Storage of samples*	Principle of test and comments	Ref.
Reactive oxygen species (ROS)					
Electron spin resonance (ESR) with spin trapping	Reactive intermediates of free radicals	Blood, tissues		Adds a trap molecule that reacts with ROS to form more stable radicals that can be measure by ESR Requires an expensive ESR spectrometer	1,2
Flow cytometry	Singlet oxygen, superoxide, H_2O_2, peroxynitrite, hydroxyl radicals	Blood, different tissues	No	Used in combination with probes that fluoresce when oxidized by specific ROS; much more sensitive than spectroscopic probes	1,3,4
Antioxidant defences					
Indirect assay with spectrophotometry	Superoxide dismutase (SOD) activity	Red blood cells	No	SOD in the sample competes with cytochrome c for superoxide; activity estimated as changes in absorbance at 550 nm	2
Indirect assay with spectrophotometry	Catalase or Se-dependent glutathione peroxidase activity	Different tissues (e.g. liver, muscle)	Yes	Activity determined by the disappearance of free radical. Samples need to be frozen at −70°C immediately after dissection. Requires terminal sampling or biopsy	5
Kirial Laboratories (KRL) test	Antioxidant defence in red blood cells	Blood	No	Time needed to haemolyse 50% of whole red blood cells when under free radical attack. Measures mainly membrane resistance to oxidants, therefore very linked to vitamin E. Requires fresh, unfrozen blood	6
OXY-adsorbent test	Antioxidant capacity	Plasma, yolk	Yes	Colorimetric assessment of capacity of non-enzymatic antioxidants to prevent oxidation by hypochlorous acid	7,8
Trolox equivalent antioxidant capacity	Antioxidant capacity	Plasma, serum	Yes	Colorimetric assessment of capacity to quench and decolorize chromogenic-free radical	9
Total antioxidant activity	Total non-enzymatic antioxidant capacity	Plasma	Yes	Colorimetric assessment of capacity to quench and decolorize ABTS+ radical; measurement is compared to an internal standard (Trolox, a vitamin E analogue)	2,10
Oxidative damage					
Damage to lipids					
HPLC or GC	Isoprostanes	Plasma, urine, tissues, seminal fluid, breath condensate	Yes	Measurements of isoprostanes in plasma and urine are a 'whole body' measurement, but prone to artefactual lipid peroxidation during storage (reduced by immediate freezing, storage at −80 °C, and addition of antioxidants and enzyme inhibitors)	2,11
TBARS (thiobarbituric acid reacting substance) assay	Malondialdehyde (MDA)	Plasma, erythrocytes, egg, different tissues (liver, heart)	Yes	MDA determined spectrophotometrically after reaction with thiobarbituric acid (TBA). Simple and inexpensive, but potentially inaccurate since TBA reacts with other compounds; oxidation during preparation can also occur. Modifications can reduce problems	2,12,13

Table 1 (*Continued*)

Method	Measures	Sample	Storage of samples*	Principle of test and comments	Ref.
HPLC with ultraviolet-visible or fluorescence detection	MDA	Plasma, serum or breath condensate	Yes	MDA–TBA adduct is identified by HPLC after a deproteinization step. Reaction conditions involve incubation at high temperature, thus potential oxidation during sample preparation. Using diaminonaphthalene instead of TBA overcomes some biases, which are less in breath samples	2,12,14,15
HPLC	MDA	Plasma	Yes	Protein-free plasma is directly injected onto an HPLC and the very quick elution of MDA allows rapid analyses. Method is precise and robust, and amount of plasma needed is small	16
GC	Ethane, pentane	Breath condensate	Yes	Ethane and pentane are stable by-products of lipid peroxidation exhaled in the breath. The method has been used to assess "whole body" lipid peroxidation	2,11
Damage to proteins					
Carbonyl assay	Protein carbonyl groups	Tissue or plasma	Yes	Products of protein oxidation contain carbonyl groups, measured by spectrophotometry, HPLC–UV, ELISA or electrophoresis/Western blot after reaction with 2,4-dinitrophenylhydrazine. Carbonyls are good biomarkers because of their relatively early formation during oxidative stress and high stability	11
HPLC	Gamma-glutamic semialdehyde and aminoadipic semialdehyde	Plasma		Measures major contributors to total protein carbonyls by HPLC after derivatization with fluoresceinamine, or by fluorimetric HPLC after derivatization with a fluorescent reagent	11
Damage to DNA					
GC–MS	A wide range of modified bases in a single DNA sample	Different tissues	Yes	Can simultaneously identify and quantify modified nucleotide bases. Risk of overestimation of damage due to artefact oxidation of DNA during sample processing. Requires highly specialized personnel and expensive instrumentation, and few of the internal standards are commercially available	11,17,18
HPLC with electrochemical detection (HPLC–ECD), HPLC with mass spectrometry or capillary electrophoresis	8-Oxog, 8-OHdG and 8-OH-G concentration	Cells, tissues, urine, various blood fractions	Yes	Measures the oxidized basis 8-oxoG and the nucleoside forms, 8-OH-Gua and 8-OH-G, in extracted and digested DNA. Only looks for damage to one nucleotide (guanine), though technique is being extended to other bases. Capillary electrophoresis allows greater separation efficiency than HPLC methods	2,11,17–19

Table 1 (*Continued*)

Method	Measures	Sample	Storage of samples*	Principle of test and comments	Ref.
Comet assay single-cell electrophoresis	DNA strand breakage	Lymphocytes and different types of tissue	No	Extent of movement of DNA from lysed cells (visualized using fluorescent DNA stain) on electrophoresis gel depends on strand breaks or supercoiling changes. Also possible to detect oxidized bases. As strand breakage also occurs during DNA repair, breakage cannot be attributed solely to oxidative DNA damage, but inclusion of lesion-specific enzymes may overcome this limitation	11,17,20,21
Repair					
Spectrophotometry	Proteasome activity	Plasma and different tissues	Yes	Measures degradations of fluoropeptides (chymotrypsin-like and trypsin-like) by spectrophotometry	22
Comet assay	OGG1 (8-oxoguanine DNA glycosylase) activity	Lymphocyte extracts	No	Measures activity of OGG1, the enzyme in mammalian cells that removes 8-oxoGua, a specific indicator of oxidative damage to DNA. Assay creates 8-oxoGua oxidation then measures repair enzyme activity in extract of lysed cells	21

*Whether or not samples can be stored; 'No' indicates samples need to be analysed shortly after collection (< 2 days).

1: Bartosz (2006); 2: Halliwell & Gutteridge (2007); 3: Olsson *et al.* (2008); 4: Soh (2006); 5: Selman *et al.* (2000); 6: Alonso-Alvarez *et al.* (2006); 7: Rubolini *et al.* (2006); 8: Costantini & Dell'Omo (2006); 9: Cohen *et al.* (2007); 10: Re *et al.* (1999); 11: Mateos & Bravo (2007); 12: Del Rio *et al.* (2005); 13: Freitas *et al.* (2007); 14: Steghens *et al.* (2001); 15: Larstad *et al.* (2002); 16: Karatas *et al.* (2002); 17: Guetens *et al.* (2002); 18: Dizdaroglu *et al.* (2002); 19: Cadet *et al.* 2003; 20: Selman *et al.* (2006); 21: Collins (2007); 22: Selman *et al.* (2002a).

2007; Mateos & Bravo 2007). A major potential drawback for the use of MDA is the fact that it may be present in ingested food and can be absorbed through the gastrointestinal tract, thus altering background MDA levels *in vivo*. While isoprostanes may also be present in food, they do not seem to be absorbed through the gastrointestinal tract in sufficient quantities to affect plasma or urinary levels (Halliwell & Gutteridge 2007). Several spectrophotometric and chromatographic methods have been developed for the measurement of MDA and isoprostanes (Del Rio *et al.* 2005; Mateos & Bravo 2007). Results from methods measuring lipid peroxidation by the thiobarbituric acid assay (TBA assay) should be interpreted carefully. In this assay, measurements of MDA are based on its derivatization with TBA. However, the specificity of the test based on this reaction is low, as TBA may react with several other compounds apart from MDA. Furthermore, a considerable proportion of the MDA determined in the assay may be formed by decomposition of lipid peroxides during the acid heating stage of the assay, which generates further oxidation of the sample matrix with obvious overestimation of the results (Halliwell & Gutteridge 2007). To minimize matrix oxidation, most modified TBA assays involve the precipitation of protein prior to the TBA reaction as a pretreatment of plasma samples.

Proteins oxidation occurs as a result of either direct attack by ROS or indirectly through peroxidation of lipids that further degrade and attack proteins (Halliwell & Gutteridge 2007; Mateos & Bravo 2007; Mirzaei & Regnier 2008). As a result of this oxidation, carbonyls are introduced into proteins either by direct oxidation of amino acids or indirectly by attachment of a carbonyl-containing moiety (Mateos & Bravo 2007; Mirzaei & Regnier 2008). Carbonyl formation often alters protein conformation, which enhances non-specific protein–protein interactions that compromise cell viability and impair protein turnover (Mirzaei & Regnier 2008). Thus, oxidative damage to proteins leads to a loss of functional and structural efficiency, with increased levels of protein carbonyls, one of the most used biomarker of oxidative damage to proteins (Halliwell & Gutteridge 2007; Mateos & Bravo 2007). Some of the methods for the detection and quantification of protein carbonyl groups include spectrophotometry, enzyme-linked immunosorbent assay (ELISA) and electrophoresis followed by Western blot (Halliwell & Gutteridge 2007; Mateos & Bravo 2007; Table 1).

Oxidative damage to DNA can result in the modification of sugars and bases, deoxyribose damage, strand breakage and DNA–protein cross-links (Dizdaroglu *et al.* 2002; Halliwell & Gutteridge 2007; Mateos & Bravo 2007). The most commonly used biomarkers of DNA damage through modified bases are the concentrations of nucleosides 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-hydroxyguanine (8-OH-G). These products are the oxidized derivates of guanine, the nucleotide most prone to oxidation (Mateos & Bravo 2007). Both of these biomarkers show high specificity, can be relatively abundant in DNA and measure a biologically important mutagenic lesion (Guetens *et al.* 2002; Halliwell & Gutteridge 2007; Mateos & Bravo 2007). High-performance liquid chromatography with electrochemical detection (HPLC–ECD) is the most common means of determining the levels of 8-OHdG or 8-OH-G. However, it should be borne in mind that the levels of 8-OHdG are a marker of DNA damage by reactive species; this will depend not only on the intensity of the ROS attack, but also on the cell environment. ROS attack upon guanine generates other products, and the ratio of these products to 8-OHdG will depend on the redox state of the cell and the presence of transition metal ions, thus the same amount of attack on DNA by reactive species could generate different levels of 8-OHdG (Halliwell & Gutteridge 2007). In addition, because exposure to pro-oxidant agents can lead to different types of oxidative DNA damage, it is intrinsically unreliable to measure a single product as an index of oxidative DNA damage (Guetens *et al.* 2002; Halliwell & Gutteridge 2007). A better approach is to measure multiple modified bases in DNA at the same time, as when a gas chromatography-mass spectrometry assay is used (Table 1). Other methods to assess oxidative DNA damage such as HPLC with mass spectrometry, antibody-based immunoassays, the ARP assay in combination with an ELISA-like assay and the comet assay (single-cell electrophoresis method), are also available (Kow & Dare 2000; Guetens *et al.* 2002; Cadet *et al.* 2003; Mateos & Bravo 2007 – also see Table 1).

A general caveat for assays aimed at measuring modified DNA bases is that these methods do not tell us whether the damage occurs in active genes, inactive genes, telomeres or 'junk' DNA. To evaluate the biological importance of oxidative DNA damage we need to know the location as well as the level of damage (Halliwell & Gutteridge 2007). More recently, molecular techniques have been used to determine, for example, the effect of dietary antioxidants on the expression of several specific gene sequences related to oxidative stress (Selman *et al.* 2006). The combined use of techniques of analytical chemistry and molecular genetics is likely to be very productive.

Repair mechanisms

Repair of damaged DNA, lipids and proteins is essential to cell function and ultimately the fitness of the organism. Several methods are available to estimate repair capacity or repair enzyme activity (Fracasso *et al.* 2006; Collins 2007; Glei *et al.* 2008), and these may be feasible for field ecologists (Table 1). However, to our knowledge, no studies

by field ecologists have included the assessment of these repair mechanisms; measurements of this component of oxidative stress could be particularly interesting in studies of life-history trade-offs and senescence.

OXIDATIVE STRESS AND LIFE-HISTORY TRADE-OFFS

How might oxidative stress influence life-history trade-offs? The outcomes of a trade-off involving oxidative stress can be manifest over different time scales. For instance, there may be an immediate trade-off involving resources dedicated to combating ROS and its effects, as these may have alternative roles within the body (this is especially true for the dietary antioxidants – see later). Alternatively, there may be a longer term trade-off, involving an indirect pathway, as many activities incur a 'hidden' cost in that they generate ROS. This ROS production could increase the rate at which unrepaired cellular damage accumulates, leading to accelerated senescence, unless there is an increased counter investment in the antioxidant system. However, any increase in investment in the antioxidant system can only come at a cost to investment elsewhere. Such knock on effects need not involve changes in senescence rates and thereby longevity, as other life-history components (e.g. growth, immune function, reproduction) could be compromised instead, affecting fitness in different ways. Figure 2 shows the possible consequences of these different responses to an increase in ROS production. Note that if the instantaneous rate of mortality increases as a consequence of, say, reduced ability to fight disease or resist predators, then lifespan will decrease without there being any change in the rate of senescence [an outcome noted, for example, when three-spined sticklebacks *Gasterosteus aculeatus* were subjected to repeated phases of rapid growth (Inness & Metcalfe 2008), which are likely to have caused elevated ROS production]. Figure 2 also illustrates that animals can show no change in lifespan when subjected to higher levels of ROS, if the cost of the extra defences and/or repair is paid in terms of reduced reproductive potential. However, this potential loss of fitness is rarely measured in laboratory studies as the animals are usually not allowed to breed.

The optimal life history could therefore depend on the balance between resources devoted to combating oxidative stress vs. other requirements – and investment in the former divided between reducing ROS production, quenching those ROS that are produced and repairing any oxidative damage (McNamara & Buchanan 2005; Yearsley *et al.* 2005). Variation in the importance of these parameters (and hence the optimal solution to the trade-offs) may explain why the sexes do not always respond equally to a perturbation in either ROS or antioxidant resources (Alonso-Alvarez *et al.* 2004; Wiersma *et al.* 2004; Magwere *et al.* 2006b; Pike *et al.* 2007a). We will first consider ecological factors that create variation in ROS production and oxidative damage, before going on to discuss the extent to which animals might signal their resistance to oxidative stress in order to obtain mating advantages.

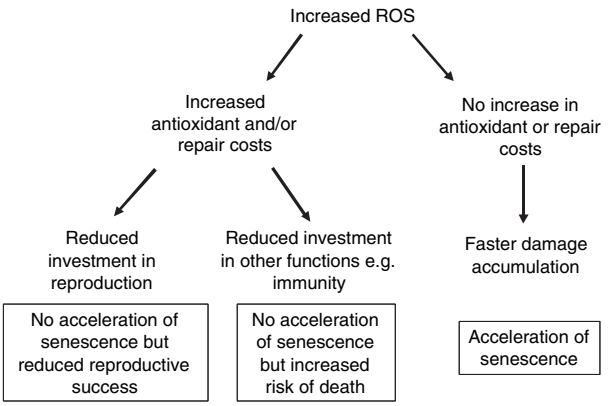

Figure 2 A simplified flow chart of the possible consequences of increased reactive oxygen species (ROS) for life histories, depending on the organism's response. Consequences for fitness are shown in the boxes at the bottom. In response to the increased ROS levels, which could occur for many reasons, animals can increase their investment in antioxidant defences. Assuming that this is costly, this will have consequences for other traits. The extent to which such increased investment occurs will affect the rate of damage accumulation. The optimal level of investment in antioxidant defence will depend on the fitness consequences of reducing oxidative damage, which in turn will depend on other factors such as extrinsic mortality risk. If the chances of dying from other causes unrelated to damage accumulation are high, diverting resources away from important fitness traits such as reproduction or immune defence will not be favoured by selection. Therefore outcomes are likely to vary between species and environments.

ECOLOGICAL FACTORS INFLUENCING OXIDATIVE STRESS

The levels of oxidative stress incurred by organisms are not constant but vary with developmental stage, environmental conditions and levels of activity. Developmental stage is relevant for two reasons. First, oxidative damage may be positively related to growth rate, due to the higher rate of ROS production that can accompany greater cellular activity. For example, transgenic mice that grow fast due to overexpression of growth hormone produce more ROS and have a greater incidence of lipid peroxidation, which may explain their shorter lifespan (Rollo *et al.* 1996). Oxidative stress could therefore be a cause of some of the well-documented long-term deleterious effects of rapid growth, including reduced longevity (Metcalfe & Monaghan 2003).

Second, the different components of the antioxidant system may not all be equally functional throughout ontogeny (Fontagne *et al.* 2008). Differences in nutritional conditions early in life might therefore have long-term consequences for antioxidant systems, although such effects are not well understood. For example, in one of our own studies (Blount *et al.* 2003a), we found that an experimentally imposed episode of low-quality food early in the development of zebra finches *Taeniopygia guttata* resulted in the birds having much lower levels of circulating dietary antioxidants in adulthood. However, no measurements were made of ROS production or oxidative damage in either chicks or adults, and only lipophilic dietary components of the antioxidant defences were quantified (i.e. carotenoids, vitamins A and E). To be confident that these adults had impaired antioxidant defences, we really need to know either that the level of ROS was high relative to the birds' antioxidant capacity (as in Fig. 1b) or that the experimental group experienced higher levels of oxidative damage. Similarly, elevated growth rates have been recorded as causing both a reduction (Alonso-Alvarez *et al.* 2007b) and an increase (De Block & Stoks 2008) in antioxidant defences; these opposite trends were nonetheless both interpreted as indicating greater oxidative damage, although again neither study included an independent measurement of damage levels. Note also that while rapid growth in early development may lead to a higher subsequent resting metabolic rate in adulthood (Criscuolo *et al.* 2008), an elevated metabolic rate cannot by itself be assumed to indicate greater ROS production; a greater oxygen consumption may be at least partly due to an increased uncoupling of the mitochondria, which actually *reduces* ROS production (Brand 2000; Speakman *et al.* 2004).

Nutritional conditions can also potentially affect levels of oxidative stress, as animals depend on acquiring some of their antioxidant defences from their diet. Therefore, it is possible that a diet lacking key antioxidants such as vitamins A and E or carotenoids could lead to impaired antioxidant defences (Catani *et al.* 2008). On the other hand, low availability of some components of the antioxidant system may be compensated for by increased mobilization or synthesis of other components, and the multifaceted nature of the antioxidant system may have evolved in order that such compensation can readily occur. Furthermore, although there have been many studies investigating the effect of dietary supplementation of these antioxidants, hardly any have assessed whether or not they are limiting in the natural diet, or whether animals alter their diet in order to increase their intake of a specific antioxidant (Catani *et al.* 2008). Moreover, supplementation experiments may show no effect on overall antioxidant defences because the animals respond by reducing their expression of endogenous antioxidants (Selman *et al.* 2006). Not surprisingly, therefore, antioxidant supplementation of the diet generally has little effect on lifespan (see later). Nonetheless, it is likely that the functioning of the antioxidant system is responsive to diet at some level, as the general nutritional state affects the ability to process antioxidant molecules such as carotenoids (McGraw *et al.* 2005).

The rate of ROS production is not constant over time but varies with the activity of the organism. One of the most intriguing links between behaviour and oxidative stress relates to the greater muscular activity that can accompany activities such as migration or reproduction. In general terms, an increase in overall physical activity can cause greater ROS generation in the mitochondria due to a greater production of ATP (Leeuwenburgh & Heinecke 2001). This can lead to measurable oxidative damage, at least in the short term (Leaf *et al.* 1999; Leeuwenburgh & Heinecke 2001; Costantini *et al.* 2008; see also Fig. 1b). However, physical activity is also known to be beneficial to health, even to the extent of increasing lifespan (Leaf *et al.* 1999; Navarro *et al.* 2004). How can the paradox of muscular activity both being detrimental and beneficial to health be resolved? The solution appears to lie in the regularity of the muscular work: while acute and unaccustomed exercise generates more ROS than can be dealt with by the animal's antioxidant defences (Leeuwenburgh & Heinecke 2001), regular exercise does not (Leaf *et al.* 1999; Selman *et al.* 2002b). The lack of oxidative damage in regularly exercising animals appears to be due to a combination of factors: ROS production may be decreased, and antioxidant defences may be enhanced [e.g. due to the hormetic effect mentioned earlier (Leeuwenburgh & Heinecke 2001; Navarro *et al.* 2004; and see Fig. 1c), although this is not always the case (Selman *et al.* 2002b; Vaanholt *et al.* 2008)]. The measurable incidence of oxidative damage to macromolecules such as DNA may also be lower because they are repaired faster in exercised animals, due to an upregulation of repair enzymes induced again as a hormetic response to mild stress (Nakamoto *et al.* 2007).

How can such findings on the effects of exercise (mostly derived from studies of humans or laboratory rodents) be extrapolated to the situation experienced by animals in their natural environment? It is likely that wild animals are generally accustomed to muscular work and so will not experience the deleterious effects of sudden exercise: short-tailed field voles *Microtus agrestis* showed little change in antioxidant enzyme activities and no elevation in oxidative damage even when voluntarily running an average of almost 6 km day^{-1} (Selman *et al.* 2002b). However, this may not be the case with animals that engage in intermittent activity, for instance migratory species that are sedentary outside of the migration period. This situation was simulated in a study of racing pigeons,

which show reduced antioxidant defences and elevated levels of oxidative damage after longer flights (Costantini *et al.* 2008), and there is some evidence that oxidative stress in migrating wild birds is inversely related to their body condition at the time (Costantini *et al.* 2007a). Moreover, all of the work on the effects of exercise quoted above refers to endothermic vertebrates, and it is quite possible that the findings do not apply to other taxa. Evidence that this is so comes from a study demonstrating that increased flight activity led to a *decrease* in the lifespan of *Drosophila*, due to a change in membrane fatty acids that made them more prone to lipid peroxidation (Magwere *et al.* 2006a). It is therefore difficult to predict whether increased activity will lead to an increase in oxidative damage in taxa other than those already studied.

OXIDATIVE STRESS AND REPRODUCTION

Reproduction can be one of the most physiologically demanding periods of an animal's life (Speakman 2008), but there is surprisingly little direct evidence that it increases oxidative stress. For instance, several studies have found that an increase in reproductive effort in zebra finches is associated with a decrease in either concentrations of specific antioxidants (Wiersma *et al.* 2004) or the overall antioxidant capacity of the blood (Alonso-Alvarez *et al.* 2004, 2006; Bertrand *et al.* 2006a). However, this does not show that they actually incurred more oxidative damage, as there were no parallel measurements of ROS production or oxidative damage. Similarly, although experimental manipulations of reproductive rate in *Drosophila* [whether by provision of extra nutrients (Wang *et al.* 2001), hormonal manipulations (Salmon *et al.* 2001) or provision of mates (Rush *et al.* 2007)] demonstrated that increased reproductive rate led to greater vulnerability to oxidative stress, the tests employed extreme oxidative stressors: flies were subjected to doses of powerful chemical oxidants that killed them all (irrespective of treatment group) within a few days. These assays shed little light on how reproduction affects responses to natural levels of oxidants, nor do they reveal whether greater reproductive effort actually causes more oxidative stress. There is clearly a need for more research in this area.

While reproduction might generate oxidative stress, oxidative stress might conversely reduce reproductive potential. In alpine swifts *Apus melba*, a female's resistance to oxidative stress has been found to be related to both the size and hatching success of her clutch (Bize *et al.* 2008), although this may reflect differences in female quality. There are few experimental data on reproductive effects, but there is correlational evidence that male mammals both produce more ROS in their testicular tissues and have lower antioxidant defences as they get older, leading to age-related increases in the rate of oxidative damage and consequent reductions in steroid production (reviewed by Martin & Grotewiel 2006). Oocyte quality in females also declines with age, possibly due to oxidative stress (Martin & Grotewiel 2006).

OXIDATIVE STRESS, SENESCENCE AND LIFESPAN

Pearl (1928) suggested that the variation in lifespan that is observed among species is due to parallel variation in metabolic rate (the 'rate of living hypothesis'): a high rate of metabolism might cause a faster rate of cellular damage and so an earlier death. This later developed into the 'rate of living/free radical' hypothesis of ageing, which suggested that high metabolic rates generate high levels of ROS, leading to greater oxidative stress and hence faster cellular and organismal senescence and a shorter lifespan (Gershman *et al.* 1954; Harman 1956). This concept has been applied to within- and well as between-species comparisons. The original hypothesis (Pearl 1928; Gershman *et al.* 1954; Harman 1956) was based on a misconception that a high rate of metabolism is necessarily linked to a high rate of ROS production. In fact the opposite may be the case, due, for example, to mitochondrial uncoupling or other mechanisms (Brand 2000; Bonawitz *et al.* 2007). This helps explain why those mice in a cohort that had the highest metabolic expenditures for their body mass tended to have the longest lifespans, rather than the shortest as would be predicted by the 'rate of living' hypothesis (Speakman *et al.* 2004).

Nonetheless, the idea that oxidative stress may cause cellular senescence has proved extremely influential, prompting intense research into the relationships between oxidative stress, senescence (both cellular and at the level of the organism) and lifespan (Beckman & Ames 1998; Monaghan *et al.* 2008; Ricklefs 2008). One focus of this research has been on the link between oxidative stress and telomere length. Oxidative stress causes telomeres to shorten (Richter & von Zglinicki 2007), and it has recently been shown that a range of environmental stressors [such as psychological stress (Epel *et al.* 2004) or elevated reproductive effort (Kotrschal *et al.* 2007)] can cause changes in telomere lengths. The implication is that these environmental stressors can cause oxidative stress, which if not counteracted causes telomere shortening; this then triggers cellular senescence, which may accelerate organismal senescence. While many of the links in this chain of events are as yet untested (Monaghan & Haussmann 2006), they do reveal a plausible long-term trade-off between oxidative stress and future life histories, as a reduction in investment in antioxidant defences or repair early in life can lead to more rapid cellular senescence later.

On a more general level, there is much correlative evidence that oxidative damage does indeed increase with age in a variety of species, lending indirect support to the free radical hypothesis of ageing (Martin & Grotewiel 2006). However, investigation into the causes of this age-related accumulation of damage shows that the situation is far from simple. Cellular damage accumulates as animals get older, due to increases in both the production of ROS (due to altered mitochondrial function) and susceptibility to ROS (e.g. as a result of increases in membrane PUFA; Hulbert *et al.* 2007), reductions in the rate of repair and reductions in the rate of degradation of excised damaged molecules by the proteosome, but (contrary to expectations) there is little consistent evidence for a decline in antioxidant defences (Martin & Grotewiel 2006). Perhaps as a result, supplementation with extra antioxidants does not necessarily influence lifespan or the rate of senescence (Magwere *et al.* 2006b). While provision of antioxidant enzyme mimics increased the lifespan of *Caenorhabditis elegans* (Melov *et al.* 2000), in *Drosophila* this only had an effect if the flies were otherwise deficient in these enzymes (Magwere *et al.* 2006b). Long-term provision of extra dietary antioxidants to mice may sometimes increase lifespan, but this does not seem to be due to any reduction in oxidative damage (Selman *et al.* 2006, 2008).

An alternative approach is to use selection experiments to elucidate the links between antioxidant systems and lifespan. Selection for increased longevity in *Drosophila* is associated with an upregulation of enzymes involved in the antioxidant defence system (Arking *et al.* 2000b), although the same extension of lifespan can be achieved by genetic selection on different antioxidant defence genes, indicating the complexity of this trait (Arking *et al.* 2000a). However, the general finding from experiments that select directly for overexpression of components of the antioxidant system is that this does not lead to increased longevity (Hulbert *et al.* 2007). This finding is supported by comparisons between species, which show that most variation in lifespan is not driven by differences in antioxidant defences, but in ROS production and susceptibility of proteins and lipid to damage. Indeed, long-lived species tend to have *lower* levels of antioxidant defences, but only because of a disproportionate reduction in rates of ROS production and susceptibility to oxidative stress (Barja 2002). This is likely to be in part attributable to interspecific differences in the PUFA content of membranes, which could explain the variation in lifespan between mammals and birds, and between mammals of contrasting lifespan (Hulbert *et al.* 2007); differences in the types of membrane unsaturated fatty acids can explain why naked mole rats *Heterocephalus glaber* live over seven times longer than other rodents of similar body size (Hulbert *et al.* 2006).

SEXUAL SIGNALLING OF OXIDATIVE STRESS AND THE ROLE OF CAROTENOIDS

An interesting feature of the antioxidant system is the multiple functions of several of the circulating antioxidant molecules. One of the most self-evident of their alternative roles (and the one that sparked much of the initial interest of behavioural ecologists) is as pigments used to produce animal coloration, often in sexual ornamentation. The most studied of these are the carotenoids, although many other substances (e.g. melanins, porphyrins and flavonoids) also have this dual role (McGraw 2005). Carotenoids have diverse properties, including acting as antioxidants, re-cycling other antioxidants, immunostimulants and as pigments producing many animal and plant colours. These multiple roles, and the fact that animals cannot synthesize carotenoids *de novo* and so must obtain them from the diet, led to the (largely untested) assumption that carotenoids must be limiting in animals and therefore the focus of a resource allocation trade-off. It was hypothesized that carotenoid-based sexual ornamentation might be signalling an ability to combat parasites (Lozano 1994) and/or oxidative stress (von Schantz *et al.* 1999). These ideas prompted many investigations that have examined possible correlations between circulating levels of carotenoids, coloration, immune function and/or oxidative stress (reviewed by Blount 2004), or have examined the effect of increasing the dietary intake of carotenoids on these other traits (e.g. Blount *et al.* 2003b; Grether *et al.* 2004).

However, the interpretation of such studies remains controversial for several reasons. First, it is difficult to measure the natural intake of carotenoids in wild animals (and so to test directly whether or not they are actually limiting in the diet). Second, their power as antioxidants is relatively limited (Hartley & Kennedy 2004), although, as mentioned above, they are important indirectly in recycling vitamin E once it has been oxidized (Surai 2002). Third, there may only be a weak or non-existent relationship between carotenoid levels and overall antioxidant capacity of the blood, presumably because there are other antioxidants present that are either more effective or are at higher concentrations (Costantini *et al.* 2007b; Costantini & Møller 2008). This has led to a re-evaluation of the basic assumption of a simple trade-off between the antioxidant and ornamentation roles of carotenoids. A more sophisticated hypothesis is that the intensity of carotenoid-based ornamentation is an indication of the overall level of antioxidant protection, either because it shows the extent to which carotenoids can be 'spared' from their antioxidant role or because the other antioxidant defences are protecting those carotenoids incorporated in the coloured tissues from being oxidized (Hartley & Kennedy 2004). This latter 'protection' hypothesis suggests that the

carotenoids incorporated into colourful ornaments can still indicate an animal's level of oxidative stress even if they have no significant antioxidant capability themselves, so long as they change colour when oxidized (leading to bleaching of the ornament; Hartley & Kennedy 2004). Recent studies that demonstrate a reduction in the intensity of coloration when the level of oxidative stress is increased (Torres & Velando 2007), or an increase in carotenoid coloration when animals are supplemented with other (colourless) antioxidants (Bertrand *et al.* 2006b; Pike *et al.* 2007b; Pérez *et al.* 2008), support the concept that carotenoids signal overall antioxidant status. However, it is difficult to tell whether the carotenoids are themselves acting as antioxidants (i.e. whether the change in coloration is due to their being switched to/from their role as antioxidants, or due to a change in the rate of pigment bleaching as a consequence of the general increase in oxidative stress in the body).

Either way, sexual ornaments based on pigments such as carotenoids have the potential to signal the oxidative status (and capacity to avoid oxidative stress) of the individual, and hence its likely rate of senescence. The colour of such ornaments can potentially change rapidly in intensity, depending on the state of the animal (Faivre *et al.* 2003; Velando *et al.* 2006), but can also show longer-term trends, consistent with organismal senescence (Pike *et al.* 2007a; Torres & Velando 2007). Indeed, it has been shown experimentally that older male blue-footed boobies *Sula nebouxii* show the greatest reduction in foot colouration when oxidative stress is increased, and that females alter their level of reproductive investment in accordance with the colour intensity of their partner's feet (Velando *et al.* 2006). These effects are not restricted to long-lived species: experiments on an annual population of three-spined sticklebacks *G. aculeatus* have shown that carotenoid availability in the diet affects both levels of oxidative damage and lifespan in breeding males (but not females), and females prefer males that will be longer lived (Pike *et al.* 2007a), presumably because they are better able to work intensively at ventilating the nest (Pike *et al.* 2007c). The expression of many sexual signals is also dependent on levels of androgens such as testosterone. While the honesty of such signals has been assumed to be maintained by the suppressive effect of androgens on the immune system, the link between androgens and immunocompetence is far from clear (Roberts *et al.* 2004). A recently proposed alternative explanation for the prevalence of androgen-based sexual signals is that they are (also) advertising oxidative status, as they suppress antioxidant defences (Alonso-Alvarez *et al.* 2007a). In these interpretations, both carotenoid- and androgen-based signals are based on handicaps, but in different senses: a carotenoid-based ornament handicaps the owner because either (depending on the exact role of carotenoids) the carotenoids forming the ornament cannot be used for fighting ROS or must be protected from ROS. In contrast, an androgen-based signal is a handicap because higher levels of androgens are thought to generate greater levels of ROS (Alonso-Alvarez *et al.* 2007a). The relationship between the two types of signal is clearly complex, although they appear to be physiologically connected (Blas *et al.* 2006). However, while all individuals (regardless of antioxidant status) may be able to produce a strong sexual signal of either type for a short period (Blount *et al.* 2003a) due to putting a greater proportion of resources into the signal (Pike *et al.* 2007a), only those with good resistance to oxidative stress can maintain this over time. However, the production of sexual signals may themselves generate additional oxidative stress, which could explain negative relationships between signal production and lifespan (Hunt *et al.* 2004).

FUTURE DIRECTIONS

Combining the study of function and mechanism is an increasing focus of ecology, and one that is likely to provide important insights into the evolution and flexibility of life-history strategies. Oxidative stress is a potentially very important mediator of life-history trade-offs, with wide reaching consequences for investment patterns and senescence rates. Measuring oxidative stress is complex, and requires a combination of different measurements, and care needs to be taken in the interpretation of the results. There is much, however, that we do not know. How costly are antioxidant defences? This is very difficult to study, as bombarding an animal with exogenous antioxidants, or inducing it to exercise to a level that it has no evolved capacity to cope with, tells us little. Realistic increases are required in experimental work. Hormetic upregulation of antioxidant defences, as induced by moderate frequent exercise, is particularly interesting in this context, and we know little of the effect of this on traits other than lifespan. If there are no costs associated with such upregulation, then why are antioxidant defences not always at a maximum? Both diet and level of food intake will influence oxidative stress; dietary intake will influence the availability of exogenous antioxidants for example, and reduced caloric intake is known to be associated with reduced oxidative damage, though how this comes about is currently unclear (Masoro 2006). Nonetheless, links between food availability and longevity are likely to be of great interest to ecologists.

An important point to be borne in mind in the experimental study of oxidative stress and life histories is the time scale over which we might see an effect. While some effects could be immediate, others that are mediated through accumulated damage might not be come evident until much

later in life. Several studies of oxidative stress have focussed on lifespan, as this seems the fitness trait most likely to be affected in the long term. However, care needs to be taken to distinguish between effects that operate on life expectancy by increasing the risk of death as opposed to altering the rate of senescence: only the latter effect is related to ageing. Furthermore, as illustrated in Figure 2, other traits might be compromised instead of lifespan. This is mostly likely in species with low extrinsic mortality rates, where lifespan is a more important contributor to fitness than the outcome of a particular reproductive event. More research on the effects on immune function and reproductive output are needed. That the antioxidant system is likely to have co-evolved with other traits is a further interesting aspect that has received little attention in comparative studies. Finally, an area that we have touched on little is variability in the extent to which animals encounter exogenously generated ROS, either natural (e.g. UV radiation, ozone exposure) or of anthropogenic origin (e.g. pollutants). The consequences this has for their antioxidant defences and life-history strategies might well be illuminating.

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REFERENCES

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.

Alonso-Alvarez, C., Bertrand, S., Faivre, B., Chastel, O. & Sorci, G. (2007a). Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proc. R. Soc. Lond. B Biol. Sci.*, 274, 819–825.

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

Ames, B.N., Shingenaga, M.K. & Park, E.M. (1991). DNA damage by endogenous oxidants as a cause of ageing and cancer. In: *Oxidation Damage and Repair: Chemical, Medical and Biological Aspects* (ed. Davies, K.J.A.). Pergamon press, New York, pp. 181–187.

Arking, R., Burde, V., Graves, K., Hari, R., Feldman, E., Zeevi, A. *et al.* (2000a). Identical longevity phenotypes are characterized by different patterns of gene expression and oxidative damage. *Exp. Gerontol.*, 35, 353–373.

Arking, R., Burde, V., Graves, K., Hari, R., Feldman, E., Zeevi, A. *et al.* (2000b). Forward and reverse selection for longevity in *Drosophila* is characterized by alteration of antioxidant gene expression and oxidative damage patterns. *Exp. Gerontol.*, 35, 167–185.

Balaban, R.S., Nemoto, S. & Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell*, 120, 483–495.

Barja, G. (2002). Rate of generation of oxidative stress-related damage and animal longevity. *Free Radic. Biol. Med.*, 33, 1167–1172.

Bartosz, G. (2006). Use of spectroscopic probes for detection of reactive oxygen species. *Clin. Chim. Acta*, 368, 53–76.

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

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

Bertrand, S., Faivre, B. & Sorci, G. (2006b). Do carotenoid-based sexual traits signal the availability of non-pigmentary antioxidants? *J. Exp. Biol.*, 209, 4414–4419.

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.

Blas, J., Perez-Rodriguez, L., Bortolotti, G.R., Vinuela, J. & Marchant, T.A. (2006). Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signaling. *Proc. Natl Acad. Sci. USA*, 103, 18633–18637.

Blount, J.D. (2004). Carotenoids and life-history evolution in animals. *Arch. Biochem. Biophys.*, 430, 10–15.

Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F., Devevey, G.L. & Monaghan, P. (2003a). Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proc. R. Soc. Lond. B Biol. Sci.*, 270, 1691–1696.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R. & Surai, P.F. (2003b). Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science*, 300, 125–127.

Bonawitz, N.D., Chatenay-Lapointe, M., Pan, Y. & Shadel, G.S. (2007). Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metab.*, 5, 265–277.

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

Cadet, J., Douki, T., Gasparutto, D. & Ravanat, J.L. (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res.*, 531, 5–23.

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.

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

Collins, A.R. (2007). Investigating oxidative DNA damage and its repair using the comet assay. *Mutat. Res.*, in press (doi: 10.1016/j.mrrev.2007.10.002).

Costantini, D. & Dell'Omo, G. (2006). Effects of T-cell-mediated immune response on avian oxidative stress. *Comp. Biochem. Physiol. A*, 145, 137–142.

Costantini, D. & Møller, A.P. (2008). Carotenoids are minor antioxidants for birds. *Funct. Ecol.*, 22, 367–370.

Costantini, D., Cardinale, M. & Carere, C. (2007a). Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site. *Comp. Biochem. Physiol. C*, 144, 363–371.

Costantini, D., Fanfani, A. & Dell'Omo, G. (2007b). Carotenoid availability does not limit the capability of nestling kestrels (*Falco tinnunculus*) to cope with oxidative stress. *J. Exp. Biol.*, 210, 1238–1244.

Costantini, D., Dell'Ariccia, G. & Lipp, H.P. (2008). Long flights and age affect oxidative status of homing pigeons (*Columba livia*). *J. Exp. Biol.*, 211, 377–381.

Criscuolo, F., Monaghan, P., Nasir, L. & Metcalfe, N.B. (2008). Early development and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proc. R. Soc. Lond. B Biol. Sci.*, 275, 1565–1570.

De Block, M. & Stoks, R. (2008). Compensatory growth and oxidative stress in a damselfly. *Proc. R. Soc. Lond. B Biol. Sci.*, 275, 781–785.

Del Rio, D., Stewart, A.J. & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab. Cardiovasc. Dis.*, 15, 316–328.

Dizdaroglu, M., Jaruga, P., Birincioglu, M. & Rodriguez, H. (2002). Free radical-induced damage to DNA: mechanisms and measurement. *Free Radic. Biol. Med.*, 32, 1102–1115.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.*, 82, 47–95.

Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D. *et al.* (2004). Accelerated telomere shortening in response to life stress. *Proc. Natl Acad. Sci. USA*, 101, 17312–17315.

Faivre, B., Grégoire, A., Préault, M., Cézilly, F. & Sorci, G. (2003). Immune activation rapidly mirrored in a secondary sexual trait. *Science*, 300, 103.

Falnes, P.Ø., Klungland, A. & Alseth, I. (2007). Repair of methyl lesions in DNA and RNA by oxidative demethylation. *Neuroscience*, 145, 1222–1232.

Finkel, T. (2003). Oxidant signals and oxidative stress. *Curr. Opin. Cell Biol.*, 15, 247–254.

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

Fontagne, S., Lataillade, E., Breque, J. & Kaushik, S. (2008). Lipid peroxidative stress and antioxidant defence status during ontogeny of rainbow trout (*Oncorhynchas mykiss*). *Br. J. Nutr.*, 100, 102–111.

Fracasso, M.E., Doria, D., Franceschetti, P., Perbellini, L. & Romeo, L. (2006). DNA damage and repair capacity by comet assay in lymphocytes of white-collar active smokers and passive smokers (non- and ex-smokers) at workplace. *Toxicol. Lett.*, 167, 131–141.

Freitas, D.R.J., Rosa, R.M., Moraes, J., Campos, E., Logullo, C., Vaz, I.D. *et al.* (2007). Relationship between glutathione S-transferase, catalase, oxygen consumption, lipid peroxidation and oxidative stress in eggs and larvae of *Boophilus microplus* (Acarina : Ixodidae). *Comp. Biochem. Physiol. A*, 146, 688–694.

Gershman, R., Gilbert, D.L., Nye, S.W., Dwyer, P. & Fenn, W.O. (1954). Oxygen poisoning and X-irradiation – a mechanism in common. *Science*, 119, 623–626.

Glei, M., Hovhannisyan, G. & Pool-Zobel, B.L. (2008). Use of Comet-FISH in the study of DNA damage and repair. *Mutat. Res.* (doi: 10.1016/j.mrrev.2008.01.006).

Grether, G.F., Kasahara, S., Kolluru, G.R. & Cooper, E.L. (2004). Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proc. R. Soc. Lond. B Biol. Sci.*, 271, 45–49.

Guetens, G., De Boeck, G., Highley, M., van Oosterom, A.T. & de Bruijn, E.A. (2002). Oxidative DNA damage: biological significance and methods of analysis. *Crit. Rev. Clin. Lab. Sci.*, 39, 331–457.

Halliwell, B. & Gutteridge, J. (2007). *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford UK.

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

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

Hulbert, A.J., Faulks, S.C. & Buffenstein, R. (2006). Oxidation-resistant membrane phospholipids can explain longevity differences among the longest-living rodents and similarly-sized mice. *J. Gerontol. A Biol. Sci. Med. Sci.*, 61, 1009–1018.

Hulbert, A.J., Pamplona, R., Buffenstein, R. & Buttemer, W.A. (2007). Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol. Rev.*, 87, 1175–1213.

Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussiere, L.F. (2004). High-quality male field crickets invest heavily in sexual display but die young. *Nature*, 432, 1024–1027.

Inness, C.L.W. & Metcalfe, N.B. (2008). The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. *Proc. R. Soc. Lond. B Biol. Sci.*, 275, 1703–1708.

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

Kastan, M.B. & Bartek, J. (2004). Cell-cycle checkpoints and cancer. *Nature*, 432, 316–323.

Kotrschal, A., Ilmonen, P. & Penn, D.J. (2007). Stress impacts telomere dynamics. *Biol. Lett.*, 3, 128–130.

Kow, Y.W. & Dare, A. (2000). Detection of abasic sites and oxidative DNA base damage using an ELISA-like assay. *Methods*, 22, 164–169.

Larstad, M., Ljungkvist, G., Olin, A.C. & Toren, K. (2002). Determination of malondialdehyde in breath condensate by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 766, 107–114.

Leaf, D.A., Kleinman, M.T., Hamilton, M. & Deitrick, R.W. (1999). The exercise-induced oxidative stress paradox: the effects of physical exercise training. *Am. J. Med. Sci.*, 317, 295–300.

Leeuwenburgh, C. & Heinecke, J.W. (2001). Oxidative stress and antioxidants in exercise. *Curr. Med. Chem.*, 8, 829–838.

Lozano, G.A. (1994). Carotenoids, parasites, and sexual selection. *Oikos*, 70, 309–311.

Magwere, T., Pamplona, R., Miwa, S., Martinez-Diaz, P., Portero-Otin, M., Brand, M.D. *et al.* (2006a). Flight activity, mortality rates, and lipoxidative damage in *Drosophila*. *J. Gerontol. A Biol. Sci. Med. Sci.*, 61, 136–145.

Magwere, T., West, M., Riyahi, K., Murphy, M.P., Smith, R.A.J. & Partridge, L. (2006b). The effects of exogenous antioxidants on lifespan and oxidative stress resistance in *Drosophila melanogaster*. *Mech. Ageing Dev.*, 127, 356–370.

Martin, I. & Grotewiel, M.S. (2006). Oxidative damage and age-related functional declines. *Mech. Ageing Dev.*, 127, 411–423.

Masoro, E.J. (2006). Dietary restriction-induced life extension: a broadly based biological phenomenon. *Biogerontology*, 7, 153–155.

Mateos, R. & Bravo, L. (2007). Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *J. Sep. Sci.*, 30, 175–191.

McGraw, K.J. (2005). The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Anim. Behav.*, 69, 757–764.

McGraw, K.J., Hill, G.E. & Parker, R.S. (2005). The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. *Anim. Behav.*, 69, 653–660.

McNamara, J.M. & Buchanan, K.L. (2005). Stress, resource allocation, and mortality. *Behav. Ecol.*, 16, 1008–1017.

Melov, S., Ravenscroft, J., Malik, S., Gill, M.S., Walker, D.W., Clayton, P.E. *et al.* (2000). Extension of life-span with superoxide dismutase/catalase mimetics. *Science*, 289, 1567–1569.

Metcalfe, N.B. & Monaghan, P. (2003). Growth versus lifespan: perspectives from evolutionary ecology. *Exp. Gerontol.*, 38, 935–940.

Mirzaei, H. & Regnier, F. (2008). Protein:protein aggregation induced by protein oxidation. *J. Chromatogr. B*, 873, 8–14.

Monaghan, P. & Haussmann, M.F. (2006). Do telomere dynamics link lifestyle and lifespan? *Trends Ecol. Evol.*, 21, 47–53.

Monaghan, P., Charmantier, A., Nussey, D.H. & Ricklefs, R.E. (2008). The evolutionary ecology of senescence. *Funct. Ecol.*, 22, 371–378.

Nakamoto, H., Kaneko, T., Tahara, S., Hayashi, E., Naito, H., Radak, Z. *et al.* (2007). Regular exercise reduces 8-oxodG in the nuclear and mitochondrial DNA and modulates the DNA repair activity in the liver of old rats. *Exp. Gerontol.*, 42, 287–295.

Navarro, A., Gomez, C., Lopez-Cepero, J.M. & Boveris, A. (2004). Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Am. J. Physiol.*, 286, R505–R511.

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

Pearl, P. (1928). *The Rate of Living*. University of London, London.

Pérez, C., Lores, M. & Velando, A. (2008). Availability of non-pigmentary antioxidant affects red coloration in gulls. *Behav. Ecol.* 19, 967–973.

Pike, T.W., Blount, J.D., Bjerkeng, B., Lindström, J. & Metcalfe, N.B. (2007a). Carotenoids, oxidative stress and female mating preference for longer-lived males. *Proc. R. Soc. Lond. B Biol. Sci.*, 274, 1591–1596.

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. (2007b). Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornament. *Biol. Lett.*, 3, 353–356.

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. (2007c). Dietary carotenoid availability influences a male's ability to provide parental care. *Behav. Ecol.*, 18, 1100–1105.

Rattan, S.I.S. (2008). Hormesis in ageing. *Ageing Res. Rev.*, 7, 63–78.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, 26, 1231–1237.

Richter, T. & von Zglinicki, T. (2007). A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp. Gerontol.*, 42, 1039–1042.

Ricklefs, R.E. (2008). The evolution of senescence from a comparative perspective. *Funct. Ecol.*, 22, 379–392.

Roberts, M.L., Buchanan, K.L. & Evans, M.R. (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.*, 68, 227–239.

Rollo, C.D., Carlson, J. & Sawada, M. (1996). Accelerated aging of giant transgenic mice is associated with elevated free radical processes. *Can. J. Zool.*, 74, 606–620.

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

Rush, B., Sandver, S., Bruer, J., Roche, R., Wells, M. & Giebultowicz, J. (2007). Mating increases starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster* females. *Aging Cell*, 6, 723–726.

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

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

Selman, C., McLaren, J.S., Himanka, M.J. & Speakman, J.R. (2000). Effect of long-term cold exposure on antioxidant enzyme activities in a small mammal. *Free Radic. Biol. Med.*, 28, 1279–1285.

Selman, C., Grune, T., Stolzing, A., Jakstadt, M., McLaren, J.S. & Speakman, J.R. (2002a). The consequences of acute cold exposure on protein oxidation and proteasome activity in short-tailed field voles, *Microtus agrestis*. *Free Radic. Biol. Med.*, 33, 259–265.

Selman, C., McLaren, J.S., Collins, A.R., Duthie, G.G. & Speakman, J.R. (2002b). Antioxidant enzyme activities, lipid peroxidation, and DNA oxidative damage: the effects of short-term voluntary wheel running. *Arch. Biochem. Biophys.*, 401, 255–261.

Selman, C., McLaren, J.S., Meyer, C., Duncan, J.S., Redman, P., Collins, A.R. *et al.* (2006). Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mech. Ageing Dev.*, 127, 897–904.

Selman, C., McLaren, J.S., Mayer, C., Duncan, J.S., Collins, A.R., Duthie, G.G. *et al.* (2008). Lifelong alpha-tocopherol supplementation increases the median life span of C57BL/6 mice in the cold but has only minor effects on oxidative damage. *Rejuvenation Res.*, 11, 83–95.

Soh, N. (2006). Recent advances in fluorescent probes for the detection of reactive oxygen species. *Anal. Bioanal. Chem.*, 386, 532–543.

Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z. & Nagy, G. (2007). Antioxidant measurements. *Physiol. Meas.*, 28, R41–R55.

Speakman, J.R. (2008). The physiological costs of reproduction in small mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 363, 375–398.

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

Steghens, J.P., van Kappel, A.L., Denis, I. & Collombel, C. (2001). Diaminonaphthalene, a new highly specific reagent for HPLC-UV measurement of total and free malondialdehyde in human plasma or serum. *Free Radic. Biol. Med.*, 31, 242–249.

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

Torres, R. & Velando, A. (2007). Male reproductive senescence: the price of immune-induced oxidative damage on sexual attractiveness in the blue-footed booby. *J. Anim. Ecol.*, 76, 1161–1168.

Vaanholt, L.M., Speakman, J.R., Garland, T., Lobley, G.E. & Visser, G.H. (2008). Protein synthesis and antioxidant capacity in aging mice: effects of long-term voluntary exercise. *Physiol. Biochem. Zool.*, 81, 148–157.

Valencak, T.G. & Ruf, T. (2007). N-3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. *Aging Cell*, 6, 15–25.

Velando, A., Beamonte-Barrientos, R. & Torres, R. (2006). Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia*, 149, 535–542.

Wang, Y., Salmon, A.B. & Harshman, L.G. (2001). A cost of reproduction: oxidative stress susceptibility is associated with increased egg production in *Drosophila melanogaster*. *Exp. Gerontol.*, 36, 1349–1359.

Wiersma, P., Selman, C., Speakman, J.R. & Verhulst, S. (2004). Birds sacrifice oxidative protection for reproduction. *Proc. R. Soc. Lond. B Biol. Sci.*, 271, S360–S363.

Yearsley, J.M., Kyriazakis, A., Gordon, I.J., Johnston, S.L., Speakman, J.R., Tolkamp, B.J. *et al.* (2005). A life history model of somatic damage associated with resource acquisition: damage protection or prevention? *J. Theor. Biol.*, 235, 305–317.

Zera, A.J. & Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Ann. Rev. Ecol. Syst.*, 32, 95–126.

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