Functional Ecology

British Ecological Society

Functional Ecology 2010, **24**, 950–959 doi: 10.1111/j.1365-2435.2010.01746.x

THE ECOLOGY OF ANTIOXIDANTS & OXIDATIVE STRESS IN ANIMALS

From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology

David Costantini*[,1], Melissah Rowe[2], Michael W. Butler[2] and Kevin J. McGraw[2]

[1]*Division of Ecology and Evolutionary Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK; and* [2]*School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501, USA*

Summary

1. Initial work on oxidative stress and antioxidant defences described basic chemical and biochemical properties and processes and applied this information to issues of animal health and husbandry. Seminal experimental investigations on the damaging effects of oxidative stress and the mitigating effects of antioxidant defences were conducted primarily in domesticated organisms.

2. In recent years, ecologists have taken to studying antioxidants and oxidative stress in free-ranging organisms and have integrated principles of oxidative stress into several core evolutionary concepts, such as life-history trade-offs (e.g. survival vs. reproduction), senescence and sexual selection. This initial flurry of studies has provided major advances in our understanding of how antioxidant defences evolve and function.

3. In this overview, it is our goal to provide ecologists with an accessible summary of (i) the biochemical basis and conceptual frameworks behind oxidative stress and antioxidants, (ii) the research questions and hypotheses that are generated by incorporating antioxidants and oxidative stress into models of life-history theory, ageing, mate selection, and honest signalling, and (iii) the trends in the evidence that have emerged from initial studies in these areas.

4. Though much progress has been made on the ecological and evolutionary relevance of antioxidant and oxidative stress physiology, no consensus has emerged regarding the primacy of how oxidative stress challenges or antioxidant limits or values shape organismal life-histories. However, there are many taxonomic biases in studies to date and several ideal environmental systems that are as-of-yet untapped.

Key-words: ageing, antioxidants, free radicals, life-history, reproduction, sexual signals, sperm quality, stress physiology, vertebrates

Introduction

The integration of ecology and physiology is central for understanding proximate mechanisms underlying the evolution of life-history strategies and the way that animals respond to environmental perturbations. In the last several years, a new area of ecological research has emerged, in which levels of antioxidants (AO) and oxidative stress (OS) are quantified in organisms and used as proximate endpoints to explain a number of ecological patterns. In this article, we review this relatively young field, placing particular emphasis on the fact that there are extensive bodies of literature in other fields (e.g. medicine, nutrition) that catalysed this new subdiscipline and that must be more completely understood to advance AO-ecology research techniques and questions. Though some recent reviews exist in this area (von Schantz *et al*. 1999; Costantini 2008; Monaghan, Metcalfe & Torres 2009), we focus on topics such as the evolution of sexual ornaments and the costs of reproduction that have received comparatively less attention to date. Also, we try to regularly integrate biochemistry with ecology to describe the biochemical

*Correspondence author. E-mail: d.costantini@bio.gla.ac.uk

patterns underlying ecological phenomena and why such knowledge is important to ecological investigations. Finally, we provide historical information on reactive chemical species, OS physiology, and AO to provide an accessible compilation of this material for functional ecologists.

Historical overview of reactive species biochemistry

Around 2·45 billion years ago, during the Great Oxidation Event, atmospheric oxygen increased dramatically as a result of the adaptive radiation of photosynthetic organisms (Sessions *et al*. 2009). An important consequence of this change in atmospheric chemistry was that living organisms evolved physiological mechanisms to use oxygen to produce energy. Additionally, organisms found it necessary to protect themselves against the toxicity of oxygen and to mitigate the negative effects of oxidation. Thus, oxygen free radicals were likely an important selective pressure during the incipient stages of oxidative metabolism, and indeed throughout much of the history of life.

The damaging effects of oxygen involve a loss of cell functionality resulting from reactive species-induced oxidation of macromolecules (i.e. oxidative damage; OD). Oxygen free radicals, which are predominately, but not exclusively, derived from oxidative metabolism, are responsible for this oxidation. The modern organic chemistry of free radicals dates back to 1900, when Moses Gomberg identified the triphenylmethyl radical. After much subsequent debate, a free radical was defined as any transient (chemically unstable) species (atom, molecule, or ion; Bernthsen 1942; Herzberg 1971). Most recently, a free radical has been defined as any chemical species capable of independent existence that contains one or more unpaired electrons (Halliwell & Gutteridge 2007). Radicals can be formed through several chemical reactions (see Figs S1). In addition, there are a number of other chemicals (e.g. H_2O_2, HOCl, singlet oxygen, peroxynitrite) that, despite lacking radical properties, are important oxidizing agents in biological systems because they quickly and readily react with, and thus damage, lipids, proteins, and nucleic acids. Reactive oxygen species (ROS) is a collective term that describes all pro-oxidants derived from O_2. However, there are also pro-oxidants derived from other elements, such as nitrogen (reactive nitrogen species; RNS). In this review, we have referred to the biochemistry of 'reactive species' (RS), which is the most inclusive name for all pro-oxidants.

It was only between the 1950s and 1960s that biochemists demonstrated the existence of free radicals in living systems and suggested free radicals were responsible for cell deterioration and senescence (Commoner, Townsend & Pake 1954; Gershman *et al*. 1954; Harman 1956; McCord & Fridovich 1968, 1969). Since that time, the number of studies on chemicals with pro-oxidant activity increased dramatically and today the study of oxidative physiology has a central role in modern medicine and biology (e.g. Fridovich 1978; Beckman & Ames 1998; Halliwell & Gutteridge 2007).

Antioxidants: the other side of the coin

In the 19th century, engineers discovered that certain substances – AO – were able to prevent metal from corroding by shutting off the oxidation process. Although the reasons why AO were effective at preventing oxidation were poorly understood, scientists began applying these principles to biotechnology and found that adding AO to foods high in unsaturated fat prevented the onset of rancidity (Halliwell & Gutteridge 2007). The importance of AO in biological systems gained further support with the discovery of superoxide dismutase (SOD), the enzyme responsible for scavenging the superoxide radical (McCord & Fridovich 1968, 1969). Subsequently, a variety of AO classes have been identified, including diet-derived and endogenous chemicals (e.g. vitamins, carotenoids) and endogenously produced enzymes (e.g. SOD).

Today, even in the biomedical and biochemical literature, the term AO is still not clearly and consistently defined. Here, we define AO as molecules or structures that prevent or minimize oxidative damage. We adopt a broader view here than in traditional definitions to allow the inclusion of structural and repair mechanisms that remove oxidative damage to a target molecule. Ecologists have measured AO capacity both at the individual molecule level and as generalized AO capacity. However, because AO have other biological functions, and because levels may be regulated in response to need, interpretation of AO levels in relation to oxidative balance (i.e. homeostatic/homeodynamic equilibrium between reactive species production and the control of their potentially harmful effects) is not straightforward and should be undertaken with caution (see also Hõrak & Cohen 2010).

Oxidative stress

Cells are in a dynamic redox state when RS and AO are in balance. Typically, when RS moderately increase, the AO system responds and resets the former oxidative balance. However, an imbalance between RS production and AO response, in favour of the former, increases the rate at which OD takes place and results in OS (Sies 1991; Halliwell & Gutteridge 2007; Costantini & Verhulst 2009). In practice, ecologists use proxy measures of OS. How these measures relate to fitness and the baseline stable redox state, however, is often unclear. Nonetheless, in this review, we sometimes use the term OS in relation to a proxy measure even when the utility of the measure is weak or unknown. In the future, it will be vital to consider how indicators of increased free radical production, free radical levels, free radical damage, AO levels, or decreased resistance to damage actually relate to OS (see Hõrak & Cohen 2010).

In 2004, Dotan *et al*. suggested that there can be different types of OS, depending on what macromolecular component is oxidatively damaged. Furthermore, because an oxidative

insult can damage certain molecules (e.g. DNA) but not others (e.g. proteins, lipids), Dotan, Lichtenberg & Pinchuk (2004) suggested that the level of peroxidation to certain molecules cannot be used as a measure of universal OS. We do not yet have the data necessary to conclude whether differences in peroxidation by molecular type are ecologically relevant; while it may be that simply measuring lipid peroxidation provides a general picture regarding resource-based trade-offs, it is also plausible that different fitness-related phenomena (e.g. physiological dysfunction, disease, senescence) may be differentially affected by OD of different molecules or tissues.

Mechanisms of OD differ by cellular target, and include lipid peroxidation, protein oxidation, and nucleic acid oxidation (see Figs S2–S4). For more comprehensive biochemical descriptions of lipid peroxidation and damage to proteins and nucleic acids, we suggest the following reviews (Porter, Caldwell & Mills 1995; Cadet *et al*. 2003; Chakravarti & Chakravarti 2007; Halliwell & Gutteridge 2007; Laguerre, Lecomte & Villeneuve 2007; Houben *et al*. 2008). However, it is important to note that, although RS have been traditionally viewed as toxic products of metabolism, RS are also essential components of cell signalling and regulation (i.e. redox messengers; Thannickal & Fanburg 2000; Dröge 2002), and ecologists should be careful to avoid the oversimplification of pronouncing all RS as 'bad'. This is particularly relevant in light of evidence that the exposure of an organism to a low dose of a chemical agent or environmental stressor that is damaging at higher doses induces an adaptive beneficial effect on the organism itself (hormesis, Mattson & Calabrese 2010).

Complex interactions among RS, AO and OS

There exist a number of challenges to quantifying and tracking interactions between AO and OS within an organism (see Hõrak & Cohen 2010). For example, food intake (and associated AO supplies) can be challenging to track for almost any wild animal. Moreover, the synergistic nature of AO contributes to the difficulties of quantifying organismal redox balance. Additionally, levels of AO and OS differ among tissues (Powers *et al*. 2004), some of which can only be sampled post-mortem. Dietary AO are particularly susceptible to mobilization and redistribution among tissues. However, their contribution to protection against OS is still unclear in many animal models, including humans, because some of them (e.g. flavonoids) exert a myriad of biological functions (Halliwell & Gutteridge 2007). In the future, it will be important to evaluate the contribution and relative strengths of each AO type to protect against OD *in vivo* (e.g. Costantini & Møller 2009; Costantini & Verhulst 2009). In doing so, it will be important to consider that exogenous AO can interact with endogenous AO, potentially resulting in a decrease in overall AO protection. For example, high dietary doses of vitamin C are ineffective at prolonging lifespan in mice because any positive benefits derived as an AO are offset by compensatory reductions in endogenous protection mechanisms, leading to no net reduction in accumulated OD (Selman *et al*. 2006). Interactions between different AO may also arise during specific life-history stages and may modulate allocation trade-offs of AO (e.g. allocation of AO to eggs in birds; Costantini 2010). Finally, it is also important to remember that AO levels by themselves are not enough to make inferences regarding OS (Costantini & Verhulst 2009), as an animal exposed to less OS may simply circulate or accumulate lower levels of AO, and care should be taken when interpreting results from studies that measure AO protection but not OD.

Ecological and evolutionary issues of OS

ENVIRONMENT

Environmental conditions across a range of habitat types are associated with OS threats. For example, marine environments vary widely in thermal characteristics, partial pressure of oxygen, degree of pollution and light environment, all of which can influence the degree of oxidative insult experienced by an individual, and many marine organisms adjust their AO systems in response to this environmental variability to maintain a stable redox state (reviewed in Lesser 2006; Buttemer, Adele & Costantini 2010). Furthermore, within species, population densities can vary with habitat and, at least in freshwater clams (*Sphaerium* sp), some of this variation has been tied to OD; clams were most abundant and showed lower levels of nucleic acid damage in relatively more hypoxic environments (Joyner-Matos *et al*. 2007).

Further environmentally driven variation in an organism's oxidative exposure and AO system can be a product of season (snails, *Helix aspersa*; Ramos-Vasconcelos, Cardoso & Hermes-Lima 2005), thermotolerance (grasses; Banowetz *et al*. 2007), osmotic stress (reeds; Zhao *et al*. 2008), atrophy (burrowing frogs, *Cyclorana alboguttata*; Hudson *et al*. 2006), nutrient starvation (*Mycobacterium avium*; Gumber *et al*. 2009) and seed dormancy (Oracz *et al*. 2009), sometimes with RS playing an essential signalling role (Bailly, El-Maarouf-Bouteau & Corbineau 2008). These few examples demonstrate the wide variety of ecological contexts in which RS affect organismal behaviour, physiology, distribution and fitness, and highlight the breadth of research areas that remain to be explored.

ORGANISMAL SELF-MAINTENANCE AND SURVIVAL BEHAVIOURS

In sports medicine and laboratory studies of exercise, the role of locomotor activity in promoting RS production (Davies *et al*. 1982), and of regular exercise in nullifying these effects (reviewed in Powers *et al*. 2004), has received considerable attention (Alessio 1993; Di Meo & Venditti 2001; Gomez-Cabrera, Domenech & Viña 2008). Although physical performance has been linked to aspects of organismal fitness, specifically as a proxy for escape from predators (e.g. flight performance; Veasey, Metcalfe & Houston 1998), the effect of RS and role of AO in maintaining the redox balance during activity remains unclear. Nonetheless, a diversity of ecologi-

cally relevant phenomena conducive to this type of research exists, and clearly warrants further investigation. For example, the expression of a number of physically demanding behaviours (e.g. migration, hunting, predator escape, sexual displays) may be evolutionary shaped by OS.

Changes in tissue oxygen levels can directly affect rates of OD, a phenomenon most commonly studied in rodents, rabbits, dogs and pigs during ischaemia-reperfusion as a model for treating injuries in humans (e.g. Aksoy *et al.* 2004; Hirayama *et al.* 2005; Ovechkin *et al.* 2007). However, similar non-pathological changes (e.g. ischaemia) can be found in natural contexts, such as reanimation following estivation, torpor, or hibernation, recovery from extracellular freezing, or temporally variable anoxia conditions commonly found in both invertebrates and vertebrates (Storey 1996; Willmore & Storey 1997; Bickler & Buck 2007; Issartel *et al.* 2009; Orr *et al.* 2009). Many of these organisms elevate AO levels during the oxygen-limited state to limit OD upon return to normoxia (Storey 1996; Hermes-Lima, Storey & Storey 1998).

There are also a variety of survival behaviours that are related to OS. For example, degree of torpor decreases with an increased polyunsaturated fatty acid (PUFA) content in the food hoard of chipmunks (*Tamias striatus*; Munro, Thomas & Humphries 2005), possibly due to the susceptibility of PUFAs to oxidation. Perceived predation risk also seems to have a direct influence on OS, as predator presence can increase oxygen consumption and decrease levels of AO enzymes (Slos & Stoks 2008). Finally, Bize *et al.* (2008) found that male alpine swifts (*Apus melba*) with red blood cells that were more resistant to an *in vitro* free radical attack tended to have greater inter-annual survival rates, providing one of the first clear links between oxidative balance and survival in a wild animal.

Another component of survival is immunocompetence. The study of ecoimmunology has proliferated in recent years, and a large number of studies have examined relationships between AO (particularly carotenoids) and immunity (e.g. Blount *et al.* 2003; Costantini & Dell'Omo 2006; Hõrak *et al.* 2007). As this field has been reviewed elsewhere in an evolutionary context (Dowling & Simmons 2009), we do not explore the relationship between RS and immune function in depth here. Briefly, however, during an innate immune response, phagocytic cells use an oxidative burst (RS production) to destroy pathogens. While these molecules effectively neutralize bacterial invaders, they are non-specific and may also destroy self-tissue. Therefore, individuals need to be able to negate the potential immunopathological effects of an immune response to maintain the fitness-related benefit of the immune response (Costantini & Møller 2009; Sorci & Faivre 2009).

SENESCENCE

Senescence is a process in which organisms undergo physiological degeneration with age (Rose 1991). OS has been linked to evolutionary theories of senescence since Harman (1956) first proposed the free radical theory of ageing, which suggests that cumulative OD on cell constituents caused by free radicals produced during aerobic respiration results in ageing and ultimately death. Since this time, the free radical theory has been refined and a number of hypothetical iterations have been presented, including the mitochondrial theory of ageing (Harman 1972) and the OS hypothesis of ageing (Yu & Yang 1996; Sohal, Mockett & Orr 2002). In 2003, Hulbert further developed the hypothesis and proposed the membrane pacemaker theory of ageing, suggesting that it is variation in membrane fatty acid composition, and the consequent differential vulnerability to lipid peroxidation, which determines lifespan: cells richer in PUFA should be more susceptible to OD and will therefore be more prone to cell senescence (Hulbert 2003, 2005; Hulbert *et al.* 2007). There is considerable support for the free radical theory of ageing in a wide range of taxa. However, much of this evidence is correlative and based on a limited number of laboratory taxa; thus definitive proof that alterations in OS play a role in longevity remains elusive (Wickens 2001; Sohal, Mockett & Orr 2002). Several recent, comprehensive reviews discuss the evidence for the broad free radical theory of ageing in considerable detail (see Beckman & Ames 1998; Ashok & Ali 1999; Wickens 2001; Sohal, Mockett & Orr 2002), and it is not our intention to review such evidence here; instead we present a brief overview of the relationship between lifespan and metabolic rate and discuss the role of OS in this relationship.

Given that RS production is partly a function of metabolic rate (i.e. a higher metabolic rate should lead to greater RS production), the free radical theory of ageing is often thought of synonymously with the rate of living theory: species with higher basal metabolic rates (BMR) are suggested to have shorter maximum lifespan potential (MLSP) as a result of increased RS production (Beckman & Ames 1998; Dowling & Simmons 2009). However, this integrated theory is somewhat erroneous. While there is some empirical support for a relationship between MLSP and BMR (e.g. birds, Cohen *et al.* 2008; humans, Ruggiero *et al.* 2008), many studies show no relationship between mortality and BMR (e.g. microchiropteran bats, Filho *et al.* 2007; colubrid snakes, Robert, Brunet-Rossinni & Bronikowski 2007; zebra finches *Taeniopygia guttata*, Moe *et al.* 2009). In addition, several statistical and methodological issues appear to compromise the validity of much of the data supporting this hypothesis; though, at least in one study of small mammals, when these issues are eliminated and a more robust measure of daily energy expenditure is employed (e.g. elimination rates of stable isotopes as opposed to BMR), high energy expenditure remains associated with shorter lifespan (Speakman *et al.* 2002). Finally, BMR does not appear to be a good predictor of lifespan within a species. For example, experimental manipulation of energy expenditure via cold exposure does not shorten lifespan in field voles (*Microtus agrestis*; Selman *et al.* 2008). Moreover, in mice, lifespan is not affected by an increase in energy expenditure induced by either cold exposure (Vaanholt *et al.* 2009) or aerobic exercise (Vaanholt *et al.* 2010).

One reason for these mixed results appears to be an uncoupling between metabolism and OS. Specifically, the transition of mitochondria from state 4 (resting) to state 3 (respiratory active, producing ATP) is not accompanied by a proportionate increase of free radical production (Loschen, Flohé & Chance 1971). Consequently, OD cannot be massive during phases of increased metabolic rate because the mitochondrial free radical leak strongly decreases over the states 4 to 3 transition (Herrero & Barja 1997). This uncoupling between metabolism and OS appears to be influenced by uncoupling proteins (Criscuolo *et al*. 2005) and likely represents an important biochemical adaptation to OS. Importantly, this uncoupling can result in a positive relationship between metabolic rate and lifespan ('uncoupling to survive hypothesis' Brand 2000; Speakman *et al*. 2002). However, we suggest that more studies on the link between metabolic rate and RS production/OD (especially among wild animals and understudied taxonomic groups) are necessary to understand if this uncoupling is a general rule or occurs only under certain circumstances. Finally, we recommend further empirical studies of the link between BMR (or daily energy expenditure) and lifespan, but suggest that such studies should incorporate a wider range of taxa and might be best addressed in long-lived organisms or organisms that withstand environmental extremes (e.g. bristlecone pine, *Pinus longaeva*, MLSP >4500 years, Lanner & Connor 2001; periodical cicadas, *Magicicada*; Iceland clam, *Arctica islandica*, MLSP >400 years, Buttemer, Adele & Costantini 2010; waved albatross, *Diomedea irrorata*; estivating snails or anoxia-tolerant turtles, see Storey 1996).

REPRODUCTION

OS may have its most dramatic effect on animal fitness by directly impairing reproduction (Harshman & Zera 2007). Age-specific accumulation of OS has been shown to damage oocytes and embryos of mammal mothers later in life (Tarín 1996), but in many taxa, OS can also have age-independent reproductive detriments. For example, mating increases susceptibility to OS in virgin female *Drosophila melanogaster* (Rush *et al*. 2007). In domesticated zebra finches, two reproductive phenomena – a greater number of breeding bouts per lifetime (Alonso-Alvarez *et al*. 2006) and raising more offspring per breeding bout (Alonso-Alvarez *et al*. 2004; Wiersma *et al*. 2004) – reduce AO defences. Some nutrients like carotenoids, however, seem to be able to modulate reproduction and trade-offs with other life-history aspects like OS resistance (e.g. birds, Biard, Surai & Møller 2005; Bertrand *et al*. 2006; mollusks, Petes, Menge & Harris 2008). Maintaining a positive seasonal carotenoid balance also predicts reproductive success in wild barn swallows (*Hirundo rustica*, Safran *et al*. 2010). In contrast to emphases on AO, only a handful of studies have investigated OS and female reproduction in wild animals (see de Almedia *et al*. 2007 for a review in marine bivalves). In a wild, long-lived avian species (alpine swifts), number of eggs laid per attempt (clutch size) was positively correlated with resistance of red blood cells to an *in vitro* free radical attack (Bize *et al*. 2008). However, in a wild, long-lived mammal (soay sheep, *Ovis aries*), lipid peroxidation was unrelated to recent and past reproductive effort (Nussey *et al*. 2009).

Some aspects of parental care (e.g. nestling food provisioning in birds, Helfenstein *et al*. 2008; egg fanning in fish, Pike *et al*. 2007) may also be linked to parental AO status. Given the high growth and metabolic rates in developing young, it is not surprising that OS strongly influences offspring; for example, Costantini *et al*. (2006) found that nestling Eurasian kestrels (*Falco tinnunculus*) from larger broods experience higher level of OS. Pre-natal OS can even induce neural damage and cognitive function in young rats (Song *et al*. 2009). There is also significant interest in how OS impacts fertility in human females (Agarwal, Gupta & Sharma 2005). OS can affect early (e.g. oocyte maturation) and late (e.g. pre-eclampsia, pre-term labour) stages of the female reproductive cycle, and various dietary interventions (e.g. vitamin supplementation) have been implemented before and during pregnancy. In most cases, however, either pre-embryonic or post-hatch/-birth OS is studied in isolation; we encourage more comprehensive studies across life-history stages to better understand how OS is transmitted from mother to offspring and which sources are more significant for long-term offspring survival and reproduction. Additionally, investigation of the role of OS in transgenerational effects that are not the result of genetic origin or developmental environment may help us understand if these effects 'program' offspring for the future environment or if they simply function as a constraint on phenotypic plasticity (Jablonka & Raz 2009). Finally, we recommend expanding tests of these AO/OS ideas to new taxa, including organisms that are semelparous, very long-lived (and thus infrequently breeding), and that live under extreme environmental conditions (e.g. crowded, inbred, thermal extremes), to permit robust examination of how various reproductive stages and tactics are influenced by oxidative balance.

SEXUAL ORNAMENTATION

Many animals put substantial energy into developing and maintaining secondary sexual traits (e.g. colours, songs, antlers), whose expression may be shaped by AO and OS in a number of ways (Sheldon & Verhulst 1996; von Schantz *et al*. 1999). Carotenoid-based sexual traits have commanded particular attention in this area because carotenoid molecules function both as colorants (McGraw 2006) and immunostimulants (e.g. Blount *et al*. 2003), with potential for AO activity via RS scavenging (Krinsky 2001; but see Costantini & Møller 2008). Consequently, individuals may face an allocation trade-off between investment in ornamentation vs. immunocompetence (Lozano 1994; Olson & Owens 1998), a hypothesis for which there is some empirical support (e.g. Faivre *et al*. 2003; Alonso-Alvarez *et al*. 2004; Clotfelter, Ardia & McGraw 2007).

OS has also been suggested to mediate the trade-off between health and other forms of ornamentation. Specifi-

cally, ornaments, from fleshy combs and wattles to feathers and spurs, are proposed to be sensitive to OS, such that production of RS resulting from immune system activation leads to reduced ornamentation (von Schantz *et al*. 1999). However, studies examining whether immune activation induces OS, and those examining the relationship between OS and ornamentation, provide mixed support for this hypothesis (Costantini & Dell'Omo 2006; Hõrak *et al*. 2006; Torres & Veldano 2007; Galván & Alonso-Alvarez 2008; Isaksson & Andersson 2008).

SPERM PERFORMANCE

Sperm cells appear to be particularly vulnerable to attack by RS due to their high PUFA content and metabolic activity (Sikka 2001; Surai *et al*. 2001). In humans and a limited number of domesticated animals, RS attack has been shown to induce a lipid peroxidation chain event that can lead to decreased motility and viability, and an inability to fuse with the oocyte (Fujihara & Howarth 1978; Wishart 1984; Aitken, Clarkson & Fishel 1989; deLamirande & Gagnon 1992; Baumber *et al*. 2000; Bilodeau *et al*. 2002). Importantly, RS may also damage nuclear and mitochondrial DNA of sperm, with negative consequences for fertilizing capacity and post-fertilization embryo survival (Aitken & Krausz 2001). Sperm are also subject to RS attack in the testes, resulting in impaired steroidogenesis, a reduced capacity to differentiate normal spermatozoa, and, ultimately, a reduction in sperm fertilizing ability (Wu *et al*. 1973; Aitken & Roman 2008). Thus, OS can have a significant impact on male fitness, and may influence a number of evolutionary processes including the evolution of polyandry and mating strategies, sexual conflict, and sperm competition dynamics (Siva-Jothy 2000; Dean, Bonsall & Pizzari 2007; Pizzari *et al*. 2008; Dowling & Simmons 2009).

Both sperm and seminal plasma possess AO systems that protect sperm from RS-induced OS. Specifically, SOD, glutathione peroxidase, and catalase appear to be the major semen AO in a range of taxa, with additional protection provided by vitamins C and E, ubiquinols, and glutathione (Sikka 2001; Surai *et al*. 2001; Weirich, Collins & Williams 2002). Dietary carotenoid supplementation is associated with improved sperm quality (presumably via prevention of RS-induced damage) in humans (Gupta & Kumar 2002) and fishes (Ahmadi *et al*. 2006; Pike *et al*. 2010). Furthermore, carotenoids are present in the ejaculates of birds (Rowe & McGraw 2008) and insects (Heller, Fleischmann & Lutz-Röder 2000), suggesting the AO role of carotenoids in semen deserves further research. Future investigations should also focus on the relative contribution of each AO type to total AO capacity of semen, and examine how these metrics covary with sperm performance.

The impact of OS may be particularly important in species with high levels of sperm competition. In these species, males with a greater capacity to defend sperm against OS should gain a greater share of paternity. Consequently, sexual selection may target cellular mechanisms underlying sperm function aimed at avoiding OS. In a recent comparative study of *Mus* species, the level of sperm competition was associated with rapid changes in protamine 2 genes, which, in turn, appear to be associated with the efficiency of DNA compaction (Martin-Coello *et al*. 2009). In sperm nuclei, protamine-induced DNA compaction protects DNA from double-strand breaks by reducing the ability of RS to access DNA (Suzuki *et al*. 2009), suggesting that the rapid changes observed in *Mus* species may reflect selection for increased resistance to RS-induced sperm DNA damage. Similarly, protective mechanisms aimed at preventing OD to sperm structures may have evolved in response to sperm competition, though these ideas remain largely unexplored.

AO defences aimed at minimizing OD to sperm should also be particularly important for species with prolonged sperm storage. Stored sperm (i.e. aged sperm) show reduced fertilizing success (Tarín, Pérez-Albalá & Cano 2000; Wagner, Helfenstein & Danchin 2004), which can impact female reproductive success, generate sexual conflict and influence behaviour (e.g. sperm ejection, re-mating strategies; Siva-Jothy 2000; Dean, Bonsall & Pizzari 2007). To date, studies of AO in the sperm storage organs of females are limited to a few species of agricultural importance (i.e. poultry, Brèque, Surai & Brillard 2003; bees, Collins, Williams & Evans 2004). However, a wide range of taxa show extended periods of sperm storage (e.g. bats, up to 198 days; reptiles, up to 2555 days; Birkhead & Møller 1993), and future studies utilizing these species should provide substantial insight into the biological significance of OS-related sperm deterioration in wild organisms. Perhaps the most exciting potential study systems can be found among the insects: in some species, females maintain viable populations of stored sperm for periods as long as thirty years (e.g. narrow-headed ant *Formica exsecta*, Pamilo 1991). Male extragonadal sperm stores may also experience RS-induced deterioration with important consequences for fitness (Tarín, Pérez-Albalá & Cano 2000) and male behaviour (e.g. sperm wastage; Pizzari *et al*. 2008). Finally, OS and AO may modulate the relationship between male sexual ornamentation and sperm quality and allow females to choose reproductive partners with high functional fertility (Blount, Møller & Houston 2001; Velando, Torres & Alonso-Alvarez 2008); for example more colourful males appear better able to protect their sperm from OS in both birds (Helfenstein *et al*. 2010) and fishes (Pike *et al*. 2010).

The production of RS in semen may also provide a range of beneficial effects for sperm function, including the ability to achieve and sustain hyperactivation and the promotion of capacitation (deLamirande & Gagnon 1993; Zini, deLamirande & Gagnon 1995). Although these initial studies were performed *in vitro* with human sperm and the mechanisms underlying these effects remain unclear, these reports demonstrate that RS are not always detrimental to the fertilization process. Therefore, understanding the balance between the beneficial and harmful effects of RS and the balance between RS production and AO defences is integral to understanding sperm function. Future ecological studies can benefit by

avoiding the oversimplification of RS as purely detrimental and investigating these other potential roles of RS in species with different mating strategies, modes of fertilization (internal vs. external fertilizers), or fertilization environments (marine vs. terrestrial spawners). Finally, as the majority of studies concerning OS in semen have been performed *in vitro* and in the fields of human infertility and poultry semen cryopreservation, future research across a wider range of taxa (as suggested above) may provide a clearer picture of the influence of OS on gametic performance and individual fitness.

Conclusions

Even at this early stage of investigation, it is evident that AO availability and OS can be ecologically relevant selection pressures linked to organismal survival and reproduction. However, the complexity of mechanisms underlying the physiology of RS and AO, combined with the dominance of lab studies and paucity of studies on free-ranging organisms, makes it difficult to draw broad ecological and evolutionary conclusions at this time. It is also becoming clear that different taxa may respond to the same environmental perturbations in very different ways. Therefore, it is important to take into account the characteristics of the species under study and to be conservative in claiming the ability of any species to function as a model for understanding the great diversity of comparative OS physiology.

We believe that a useful way to think about the emergence and impact of the field of AO and OS ecology is to draw from another physiological subdiscipline, specifically the interface between endocrinology and ecology (e.g. Ketterson *et al.* 1996). Similar to hormones, AO can be sensitive to numerous stressors, highly labile within and among organisms over space and time, and challenging to manipulate. However, the insights gained by taking endocrinological approaches to phenotypic development, ornament expression, territory defence, and a host of other life history questions have proven invaluable to the study of ecology. By investigating the roles of RS, AO, and OS with the same rigour that ecologists have used in evaluating hormonal causes and consequences, we will be able to ask new questions concerning life-history traits at the individual, population, and community levels. Additionally, the ubiquity of similar AO defences in widely dissimilar organisms (cf. many hormone systems), allows biologists to ask broad evolutionary and ecological questions, such as those surrounding survival-reproduction trade-offs. Evolutionary endocrinologists have recently investigated such a question and found robust evidence of selection on hormones as a function of annual survival, within-pair siring success and extra-pair mating success (McGlothlin *et al.* 2010). If functional ecologists develop methodologies to manipulate and measure AO or OS levels, we may also be able to more concretely investigate causal mechanisms regarding constraints on life-history strategies, expression of sexual ornaments, and rates of both individual and sperm senescence. In conclusion, future research that investigates longer-term, integrated measures of OD, AO, and OS across a wide range of taxa from a variety of ecological niches will help to elucidate the extent to which these factors are important selective pressures under wild conditions.

Acknowledgements

During manuscript preparation, DC was supported by a postdoctoral NERC research fellowship (NE/G013888/1). KJM, MR, and MWB were supported by the National Science Foundation (IOS-0746364).

References

Agarwal, A., Gupta, S. & Sharma, R.K. (2005) Role of oxidative stress on female reproduction. *Reproductive Biology and Endocrinology*, **3**, 1477–1498.

Ahmadi, M.R., Bazyar, A.A., Safi, S., Ytrestøyl, T. & Bjerkeng, B. (2006) Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology*, **22**, 388–394.

Aitken, R.J., Clarkson, J.S. & Fishel, S. (1989) Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biology of Reproduction*, **40**, 183–197.

Aitken, R.J. & Krausz, C. (2001) Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, **122**, 497–506.

Aitken, R.J. & Roman, S.D. (2008) Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity*, **1**, 15–24.

Aksoy, Y., Yapanoglu, T., Aksoy, H. & Kadir Yildirim, A. (2004) The effect of dehydroepiandrosterone on renal ischemia-reperfusion-induced oxidative stress in rabbits. *Urological Research*, **32**, 93–96.

Alessio, H.M. (1993) Exercise-induced oxidative stress. *Medicine and Science in Sports and Exercise*, **25**, 218–224.

de Almedia, E.A., Biany, A.F.C., Loureiro, A.P.M., Martinez, G.R., Miyamoto, S., Onuki, J. *et al.* (2007) Oxidative stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation and DNA damage. *Comparative Biochemistry and Physiology A*, **146**, 588–600.

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B. & Sorci, G. (2004) An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *The American Naturalist*, **164**, 651–659.

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.

Ashok, B.T. & Ali, R. (1999) The aging paradox: free radical theory of aging. *Experimental Gerontology*, **34**, 293–303.

Bailly, C., El-Maarouf-Bouteau, H. & Corbineau, F. (2008) From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies*, **331**, 806–814.

Banowetz, G.M., Azevedo, M.D., El-Nashaar, H.M., Martin, R.C. & Stout, R.G. (2007) Temperature-induced increase in cellular chelating potential associated with reduced thermotolerance. *Journal of Thermal Biology*, **32**, 12–19.

Baumber, J., Ball, B.A., Gravance, C.G., Medina, V. & Davies-Morel, M.C.G. (2000) The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. *Journal of Andrology*, **21**, 895–902.

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

Bernthsen, A. (1942). *A Textbook of Organic Chemistry*. Blackie and Sons, London and Glasgow.

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.

Biard, C., Surai, P.F. & Møller, A.P. (2005) Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia*, **144**, 32–44.

Bickler, P.E. & Buck, L.T. (2007) Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. *Annual Review of Physiology*, **69**, 145–170.

Bilodeau, J.-F., Blanchette, S., Cormier, N. & Sirard, M.-A. (2002) Reactive oxygen species-mediated loss of bovine sperm motility in egg yolk Tris extender: protection by pyruvate, metal chelators and bovine liver or oviductal fluid catalase. *Theriogenology*, **57**, 1105–1122.

Birkhead, T.R. & Møller, A.P. (1993) Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. *Biological Journal of the Linnean Society*, **50**, 295–311.
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., Møller, A.P. & Houston, D.C. (2001) Antioxidants, showy males and sperm quality. *Ecology Letters*, **4**, 393–396.
Blount, J.D., Metcalfe, N.B., Birkhead, T.R. & Surai, P.F. (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science*, **300**, 125–127.
Brand, M.D. (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Experimental Gerontology*, **35**, 811–820.
Brèque, C., Surai, P. & Brillard, J.-P. (2003) Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. *Molecular Reproduction and Development*, **66**, 314–323.
Buttemer, W.A., Adele, D. & Costantini, D. (2010) From bivalves to birds: oxidative stress and longevity. *Functional Ecology*, **24**, 971–983.
Cadet, J., Douki, T., Gasparutto, D. & Ravanat, J.-L. (2003) Oxidative damage to DNA: formation, measurement and biochemical features. *Mutation Research*, **531**, 5–23.
Chakravarti, B. & Chakravarti, D.N. (2007) Oxidative modification of proteins: age-related changes. *Gerontology*, **53**, 128–139.
Clotfelter, E.D., Ardia, D.R. & McGraw, K.J. (2007) Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. *Behavioral Ecology*, **18**, 1139–1145.
Cohen, A.A., McGraw, K.J., Wiersma, P., Williams, J.B., Robinson, W.D., Robinson, T.R., Brawn, J.D. & Ricklefs, R.E. (2008) Interspecific associations between circulating antioxidant levels and life-history variation in birds. *American Naturalist*, **172**, 178–193.
Collins, A.M., Williams, V. & Evans, J.D. (2004) Sperm storage and antioxidative enzyme expression in the honey bee, Apis mellifera. *Insect Molecular Biology*, **13**, 141–146.
Commoner, B., Townsend, J. & Pake, G.E. (1954) Free radicals in biological materials. *Nature*, **174**, 689–691.
Costantini, D. (2008) Oxidative stress in ecology and evolution: lessons from avian studies. *Ecology Letters*, **11**, 1238–1251.
Costantini, D. (2010). Complex trade-offs in the pigeon (*Columba livia*): egg antioxidant capacity and female serum oxidative status in relation to diet quality. *Journal of Comparative Physiology*, **180**, 731–739.
Costantini, D. & Dell'Omo, G. (2006) Effects of T-cell-mediated immune response on avian oxidative stress. *Comparative Biochemistry and Physiology, Part A*, **145**, 137–142.
Costantini, D. & Møller, A.P. (2008) Carotenoids are minor antioxidants for birds. *Functional Ecology*, **22**, 367–370.
Costantini, D. & Møller, A.P. (2009) Does immune response cause oxidative stress in birds? A meta-analysis. *Comparative Biochemistry and Physiology, Part A*, **153**, 339–344.
Costantini, D. & Verhulst, S. (2009) Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology*, **23**, 506–509.
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*). *Journal of Comparative Physiology B*, **176**, 329–337.
Criscuolo, F., del Mar Gonzalez-Barroso, M., Bouillaud, F., Ricquier, D., Miroux, B. & Sorci, G. (2005) Mitochondrial uncoupling proteins: new perspectives for evolutionary ecologists. *The American Naturalist*, **166**, 686–699.
Davies, K.J.A., Quintanilhat, A.T., Brooks, G.A. & Packert, L. (1982) Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications*, **107**, 1198–1205.
Dean, R., Bonsall, M.B. & Pizzari, T. (2007) Aging and sexual conflict. *Science*, **316**, 383–384.
Di Meo, S. & Venditti, P. (2001) Mitochondria in exercise-induced oxidative stress. *Biological Signals and Receptors*, **10**, 125–140.
Dotan, Y., Lichtenberg, D. & Pinchuk, I. (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Progress in Lipid Research*, **43**, 200–227.
Dowling, D.K. & Simmons, L.W. (2009) Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of the Royal Society B*, **276**, 1737–1745.
Dröge, W. (2002) Free radicals in the physiological control of cell function. *Physiological Reviews*, **82**, 47–95.
Faivre, B., Préault, M., Salvadori, F., Théry, M., Gaillard, M. & Cézilly, F. (2003) Bill colour and immunocompetence in the European blackbird. *Animal Behaviour*, **65**, 1125–1131.
Filho, D.W., Althoff, S.L., Dafré, A.L. & Boveris, A. (2007) Antioxidant defenses, longevity and ecophysiology of South American bats. *Comparative Biochemistry and Physiology, Part C*, **146**, 214–220.
Fridovich, I. (1978) The biology of oxygen radicals. *Science*, **201**, 875–880.
Fujihara, N. & Howarth, B.. (1978) Lipid peroxidation in fowl spermatozoa. *Poultry Science*, **57**, 1766–1768.
Galván, I. & Alonso-Alvarez, C. (2008) An intracellular antioxidant determines the expression of a melanin-based signal in a bird. *PLoS One*, **3**, e33335.
Gershman, R., Gilbert, D.L., Nye, F., Dwyer, P. & Fenw, M.V. (1954) Oxygen poisoning and X-irradiation: a mechanism in common. *Science*, **119**, 623–626.
Gomez-Cabrera, M.-C., Domenech, E. & Viña, J. (2008) Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radical Biology and. Medicine*, **44**, 126–131.
Gumber, S., Taylor, D.L., Marsh, I.B. & Whittington, R.J. (2009) Growth pattern and partial proteome of Mycobacterium avium subsp. paratuberculosis during the stress response to hypoxia and nutrient starvation. *Veterinary Microbiology*, **133**, 344–357.
Gupta, N.P. & Kumar, R. (2002) Lycopene therapy in idiopathic male infertility – a preliminary report. *International Urology and Nephrology*, **34**, 369–372.
Halliwell, B.H. & Gutteridge, J.M.C. (2007). *Free Radicals in Biology and Medicine*. 4th edn. Oxford University Press, Oxford.
Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology A*, **11**, 298–300.
Harman, D. (1972) The biologic clock: the mitochondria? *Journal of the American Geriatrics Society*, **20**, 145–147.
Harshman, L.G. & Zera, A.J. (2007) The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution*, **22**, 80–86.
Helfenstein, F., Berthouly, A., Tanner, M., Karadas, F. & Richner, H. (2008) Nestling begging intensity and parental effort in relation to prelaying carotenoid availability. *Behavioral Ecology*, **19**, 108–115.
Helfenstein, F., Losdat, S., Møller, A.P., Blount, J.D. & Richner, H. (2010) Sperm of colourful males are better protected against oxidative stress. *Ecology Letters*, **13**, 213–222.
Heller, K.-G., Fleischmann, P. & Lutz-Röder, A. (2000) Carotenoids in the spermatophores of bushcrickets (Orthoptera: Ephippigerinae). *Proceedings of the Royal Society London B*, **267**, 1905–1908.
Hermes-Lima, M., Storey, J.M. & Storey, K.B. (1998) Antioxidant defences and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. *Comparative Biochemistry and Physiology Part B*, **120**, 437–448.
Herrero, A. & Barja, G. (1997) ADP-regulation of mitochondrial free radical production is different with complex I- or complex II-linked substrates: implications for the exercise paradox and brain hypermetabolism. *Journal of Bioenergetics and Biomembranes*, **29**, 241–249.
Herzberg, G. (1971) *The Spectra and Structures of Simple Free Radicals*. Cornell University Press, Ithaca. Dover Phoenix Editions.
Hirayama, A., Nagase, S., Ueda, A., Oteki, T., Takada, K., Obara, M., Inoue, M., Yoh, K., Hirayama, K. & Koyama, A. (2005) In vivo imaging of oxidative stress in ischemia-reperfusion renal injury using electron paramagnetic resonance. *American Journal of Physiology Renal Physiology*, **288**, F597–F603.
Hõrak, P. & Cohen, A. (2010) How to measure oxidative stress in an ecological context: methodological and statistical issues. *Functional Ecology*, **24**, 960–970.
Hõrak, P., Zilmer, M., Saks, L., Ots, I., Karu, U. & Zilmer, K. (2006) Antioxidant protection, carotenoids and the costs of immune challenge in greenfinches. *The Journal of Experimental Biology*, **209**, 4329–4338.
Hõrak, P., Saks, L., Zilmer, M., Karu, U. & Zilmer, K. (2007) Do dietary antioxidants alleviate the cost of immune activation? An experiment with greenfinches *The American Naturalist*, **170**, 625–635.
Houben, J.M.J., Moonen, H.J.J., van Schooten, F.J. & Hageman, G.J. (2008) Telomere length assessment: biomarker of chronic oxidative stress? *Free Radical Biology and Medicine*, **44**, 235–246.
Hudson, N.J., Lehnert, S.A., Ingham, A.B., Symonds, B., Franklin, C.E. & Harper, G.S. (2006) Lessons from an estivating frog: sparing muscle protein despite starvation and disuse. *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, **290**, R836–R843.
Hulbert, A.J. (2003) Life, death and membrane bilayers. *Journal of Experimental Biology*, **206**, 2303–2311.
Hulbert, A.J. (2005) On the importance of fatty acid composition of membranes for aging. *Journal of Theoretical Biology*, **234**, 277–288.

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

Isaksson, C. & Andersson, S. (2008) Oxidative stress does not influence carotenoid mobilization and plumage pigmentation. *Proceedings of the Royal Society B*, **275**, 309–314.

Issartel, J., Hervant, F., de Fraipont, M., Clobert, J. & Voituron, Y. (2009) High anoxia tolerance in the subterranean salamander *Proteus anguinus* without oxidative stress nor activation of antioxidant defences during reoxygenation. *Journal of Comparative Physiology B*, **179**, 543–551.

Jablonka, E. & Raz, G. (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, **84**, 131–176.

Joyner-Matos, J., Chapman, L.J., Downs, C.A., Hofer, T., Leeuwenburgh, C. & Julian, D. (2007) Stress response of a freshwater clam along an abiotic gradient: too much oxygen may limit distribution. *Functional Ecology*, **21**, 344–355.

Ketterson, E.D., Nolan, V. Jr, Cawthorn, M.J., Parker, P.G. & Zeigenfus, C. (1996) Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis*, **138**, 70–86.

Krinsky, N.I. (2001) Carotenoids as antioxidants. *Nutrition*, **17**, 815–817.

Laguerre, M., Lecomte, J. & Villeneuve, P. (2007) Evaluation of the ability of antioxidants to counteract lipid oxidation: existing methods, new trends and challenges. *Progress in Lipid Research*, **46**, 244–282.

deLamirande, E. & Gagnon, C. (1992) Reactive oxygen species and human spermatozoa. I. Effect on the motility of intact spermatozoa and on sperm axonemes. *Journal of Andrology*, **13**, 368–378.

deLamirande, E. & Gagnon, C. (1993) Human sperm hyperactivation and capacitation as parts of an oxidative process. *Free Radical Biology & Medicine*, **14**, 157–166.

Lanner, R.M. & Connor, K.F. (2001) Does bristlecone pine senesce? *Experimental Gerontology*, **36**, 675–685.

Lesser, M.P. (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Reviews of Physiology*, **68**, 253–278.

Loschen, G., Flohé, L. & Chance, B. (1971) Respiratory chain linked H_2O_2 production in pigeon heart mitochondria. *FEBS Letters*, **18**, 261–264.

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

Martin-Coello, J., Dopazo, H., Arbiza, L., Ausió, J., Roldan, E.R.S. & Gomendio, M. (2009) Sexual selection drives weak positive selection in protamine genes and high promoter divergence, enhancing sperm competitiveness. *Proceedings of the Royal Society London B*, **276**, 2427–2436.

Mattson, M.P. & Calabrese, E.J. (2010). *Hormesis: A Revolution in Biology, Toxicology and Medicine*. Springer, New York and London.

McCord, J.M. & Fridovich, I. (1968) The reduction of cytochrome c by milk xanthine oxidase. *Journal of Biological Chemistry*, **243**, 5753–5760.

McCord, J.M. & Fridovich, I. (1969) Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, **244**, 6049–6055.

McGlothlin, J.W., Whittaker, D.J., Schrock, S.E., Gerlach, N.M., Jawor, J.M., Snajdr, E.A. & Ketterson, E.D. (2010) Natural selection on testosterone production in a wild songbird population. *American Naturalist*, **175**, 687–701.

McGraw, K.J. (2006). Mechanisms of carotenoid-based coloration. *Bird Coloration: Mechanisms and Measurements* (eds G.E. Hill & K.J. McGraw), pp. 177–242. Harvard University Press, Cambridge,

Moe, B., Rønning, B., Verhulst, S. & Bech, C. (2009) Metabolic ageing in individual zebra finches. *Biology Letters*, **5**, 86–89.

Monaghan, P., Metcalfe, N.B. & Torres, R. (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurement and interpretation. *Ecology Letters*, **12**, 75–92.

Munro, D., Thomas, D.W. & Humphries, M.M. (2005) Torpor patterns of hibernating eastern chipmunks *Tamias striatus* vary in response to the size and fatty acid composition of food hoards. *Journal of Animal Ecology*, **74**, 692–700.

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

Olson, V.A. & Owens, I.P.F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution*, **13**, 510–514.

Oracz, K., El-Maarouf-Bouteau, H., Kranner, I., Bogatek, R., Corbineau, F. & Bailly, C. (2009) The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. *Plant Physiology*, **150**, 494–505.

Orr, A.L., Lohse, L.A., Drew, K.L. & Hermes-Lima, M. (2009) Physiological oxidative stress after arousal from hibernation in Arctic ground squirrel. *Comparative Biochemistry and Physiology, Part A*, **153**, 213–221.

Ovechkin, A.V., Lominadze, D., Sedoris, K.C., Robinson, T.W., Tyagi, S.C. & Roberts, A.M. (2007) Lung ischemia–reperfusion injury: implications of oxidative stress and platelet–arteriolar wall interactions. *Archives of Physiology and Biochemistry*, **113**, 1–12.

Pamilo, P. (1991) Life span of queens in the ant *Formica exsecta*. *Insectes Sociaux*, **38**, 111–119.

Petes, L.E., Menge, B.A. & Harris, A.L. (2008) Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs*, **78**, 387–402.

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

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. (2010) Dietary carotenoid availability, sexual signaling and functional fertility in sticklebacks. *Biology Letters*, **6**, 191–193.

Pizzari, T., Dean, R., Pacey, A., Moore, H. & Bonsall, M.B. (2008) The evolutionary ecology of pre- and post-meiotic sperm senescence. *Trends in Ecology and Evolution*, **23**, 131–140.

Porter, N.A., Caldwell, S.E. & Mills, K.A. (1995) Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, **30**, 277–290.

Powers, S.K., DeRusseau, K.C., Quindry, J. & Hamilton, K.L. (2004) Dietary antioxidants and exercise. *Journal of Sports Sciences*, **22**, 81–94.

Ramos-Vasconcelos, G.R., Cardoso, L.A. & Hermes-Lima, M. (2005) Seasonal modulation of free radical metabolism in estivating land snails *Helix aspersa*. *Comparative Biochemistry and Physiology, Part C*, **140**, 165–174.

Robert, K.A., Brunet-Rossinni, A. & Bronikowski, A.M. (2007) Testing the 'free radical theory of aging' hypothesis: physiological differences in long-lived and short-lived colubrid snakes. *Aging Cell*, **6**, 395–404.

Rose, M.R.. (1991). *Evolutionary Biology of Aging*. Oxford University Press, New York.

Rowe, M. & McGraw, K.J. (2008) Carotenoids in the seminal fluid of wild birds: interspecific variation in fairy-wrens. *The Condor*, **110**, 694–700.

Ruggiero, C., Metter, E.J., Melenovsky, V., Cherubini, A., Najjar, S.S., Ble, A., Senin, U., Longo, D.L. & Ferrucci, L. (2008) High basal metabolic rate is a risk factor for mortality: the Baltimore longitudinal study of aging. *Journal of Gerontology A Biological Sciences*, **63**, 698–706.

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.

Safran, R.J., McGraw, K.J., Wilkins, M.R., Hubbard, J. & Marling, J.. (2010). Positive carotenoid balance over the breeding season predicts reproductive performance in a wild bird. *PLoS ONE*, **5**, e9420.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B*, **266**, 1–12.

Selman, C., McLaren, J.S., Meyer, C., Duncan, J.S., Redman, P., Collins, A.R., Duthie, G.G. & Speakman, J.R. (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. *Mechanisms of Ageing and Development*, **127**, 897–904.

Selman, C., McLaren, J.S., Collins, A.R., Duthie, G.G. & Speakman, J.R. (2008) The impact of experimentally elevated energy expenditure on oxidative stress and lifespan in the short-tailed field vole *Microtus agrestis*. *Proceedings of the Royal Society of London B*, **275**, 1907–1916.

Sessions, A.L., Doughty, D.M., Welander, P.V., Summons, R.E. & Newman, D.K. (2009) The continuing puzzle of the Great Oxidation Event. *Current Biology*, **19**, 567–574.

Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, **11**, 317–321.

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

Sikka, S.C. (2001) Relative impact of oxidative stress on male reproductive function. *Current Medical Chemistry*, **8**, 851–862.

Siva-Jothy, M.T. (2000) The young sperm gambit. *Ecology Letters*, **3**, 172–174.

Slos, S. & Stoks, R. (2008) Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology*, **22**, 637–642.

Sohal, R.S., Mockett, R.J. & Orr, W.C. (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radical Biology & Medicine*, **33**, 575–586.

Song, L., Zheng, J., Li, H., Jia, N., Suo, Z., Cai, Q., Bai, Z., Cheng, D. & Zhu, Z. (2009) Prenatal stress causes oxidative damage to mitochondrial DNA in hippocampus of offspring rats. *Neurochemical Research*, **34**, 739–745.
Sorci, G. & Faivre, B. (2009) Inflammation and oxidative stress in vertebrate host-parasite systems. *Philosophical Transactions of the Royal Society B*, **364**, 71–83.
Speakman, J.R., Selman, C., McLaren, J.S. & Harper, E.J. (2002) Living fast, dying when? The link between aging and energetics *Journal of Nutrition*, **132**, 1583S–1597S.
Storey, K.B. (1996) Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research*, **29**, 1715–1733.
Surai, P.F., Fujihara, N., Speake, B.K., Brillard, J.-P., Wishart, G.J. & Sparks, N.H.C. (2001) Polyunsaturated fatty acids, lipid peroxidation and antioxidant protection in avian semen. *Asian-Australasian Journal of Animal Science*, **14**, 1024–1050.
Suzuki, M., Crozatier, C., Yoshikawa, K., Mori, T. & Yoshikawa, Y. (2009) Protamine-induced DNA compaction but not aggregation shows effective radioprotection against double-strand breaks. *Chemical Physics Letters*, **480**, 113–117.
Tarín, J.J. (1996) Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. *Molecular Human Reproduction*, **2**, 717–724.
Tarín, J.J., Pérez-Albalá, S. & Cano, A. (2000) Consequences on offspring of abnormal function in ageing gametes. *Human Reproduction Update*, **6**, 532–549.
Thannickal, V.J. & Fanburg, B.L. (2000) Reactive oxygen species in cell signaling. *American Journal of Physiology*, **279**, L1005–L1028.
Torres, R. & Veldano, A. (2007) Male reproductive senescence: the price of immune induced oxidative damage on sexual attractiveness in the blue-footed booby. *Journal of Animal Ecology*, **76**, 1161–1168.
Vaanholt, L.M., Daan, S., Schubert, K.A. & Visser, G.H. (2009) Metabolism and aging: effects of cold exposure on metabolic rate, body composition, and longevity in mice. *Physiological and Biochemical Zoology*, **82**, 314–324.
Vaanholt, L.M., Daan, S., Garland T., Jr & Visser, G.H. (2010) Exercising for life? Energy metabolism, body composition, and longevity in mice exercising at different intensities. *Physiological and Biochemical Zoology*, **83**, 239–251.
Veasey, J.S., Metcalfe, N.B. & Houston, D.C. (1998) A reassessment of the effect of body mass upon flight speed and predation risk in birds. *Animal Behaviour*, **56**, 883–889.
Velando, A., Torres, R. & Alonso-Alvarez, C. (2008) Avoiding bad genes: oxidatively damaged DNA in germ line and mate choice. *Bioessays*, **30**, 1212–1219.
Wagner, R.H., Helfenstein, F. & Danchin, E. (2004) Female choice of young sperm in a genetically monogamous bird. *Proceedings: Biological Sciences*, **271**, S134–S137.
Weirich, G.F., Collins, A.M. & Williams, V.P. (2002) Antioxidant enzymes in the honey bee, *Apis mellifera*. *Apidologie*, **33**, 3–14.
Wickens, A.P. (2001) Ageing and the free radical theory. *Respiration Physiology*, **128**, 379–391.
Wiersma, P., Selman, C., Speakman, J.R. & Verhulst, S. (2004) Birds sacrifice oxidative protection for reproduction. *Proceedings of the Royal Society London B (Suppl.)*, **271**, S360–S363.
Willmore, W.G. & Storey, K.B. (1997) Antioxidant systems and anoxia tolerance in a freshwater turtle *Trachemys scripta elegans*. *Molecular and Cellular Biochemistry*, **170**, 177–185.
Wishart, G.J. (1984) Effects of lipid peroxide formation in fowl semen on sperm motility, ATP content and fertilizing ability. *Journal of Reproduction and Fertility*, **71**, 113–118.
Wu, S.H., Oldfield, J.E., Whanger, P.D. & Weswig, P.H. (1973) Effect of selenium, vitamin E, and antioxidants on testicular function in rats. *Biology of Reproduction*, **8**, 625–629.
Yu, B.P. & Yang, R. (1996) Critical evaluation of the free radical theory of aging. A proposal for the oxidative stress hypothesis. *Annals of the New York Academy of Sciences*, **786**, 1–11.
Zhao, L., He, J., Wang, X. & Zhang, L. (2008) Nitric oxide protects against polyethylene glycol-induced oxidative damage in two ecotypes of reed suspension cultures. *Journal of Plant Physiology*, **165**, 182–191.
Zini, A., deLamirande, E. & Gagnon, C. (1995) Low levels of nitric oxide promote human sperm capacitation *in vitro*. *Journal of Andrology*, **16**, 424–431.

Received 20 February 2010; accepted 7 June 2010
Handling Editor: Peeter Hõrak

Supporting information

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

Figure S1. Free radicals can be formed by losing or by gaining a single electron from a nonradical species.

Figure S2. Example of mechanisms of lipid peroxidation.

Figure S3. Example of oxidation of the methionine residues of proteins, which are the most susceptible to oxidation by RS.

Figure S4. Examples of interactions between DNA base products and RS (•OH = hydroxyl; ONOO− = peroxynitrite).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.