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Review

Reactive oxygen species as universal constraints in life-history evolution

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Evolutionary theory is firmly grounded on the existence of trade-offs between life-history traits, and recent interest has centred on the physiological mechanisms underlying such trade-offs. Several branches of evolutionary biology, particularly those focusing on ageing, immunological and sexual selection theory, have implicated reactive oxygen species (ROS) as profound evolutionary players. ROS are a highly reactive group of oxygen-containing molecules, generated as common by-products of vital oxidative enzyme complexes. Both animals and plants appear to intentionally harness ROS for use as molecular messengers to fulfil a wide range of essential biological processes. However, at high levels, ROS are known to exert very damaging effects through oxidative stress. For these reasons, ROS have been suggested to be important mediators of the cost of reproduction, and of trade-offs between metabolic rate and lifespan, and between immunity, sexual ornamentation and sperm quality. In this review, we integrate the above suggestions into one life-history framework, and review the evidence in support of the contention that ROS production will constitute a primary and universal constraint in life-history evolution.

Keywords: reactive oxygen species; life-history trade-offs; evolutionary ecology; ageing; sexual selection

1. INTRODUCTION

A fundamental principle of life-history theory is that the evolution of fitness-related traits will be constrained by the existence of trade-offs between them (Stearns 1992). That is, a beneficial change in the expression of one trait will often incur a negative change in the expression of another. Such trade-offs are ubiquitous in nature, and their existence has traditionally been explained in the context of resource limitation, with energy as the typical currency (Stearns 1992; Roff & Fairbairn 2007). As an example in the simplest form, an individual may have a limited energy budget with which to allocate resources towards either growth or reproduction. Although such a framework has been enormously helpful in the development of evolutionary theory, it does neglect the reality that the proximate mechanisms that underlie life-history trade-offs are, in many cases, unknown and might not necessarily be based around a currency involving energy or resource allocation (Råberg *et al.* 1998; Zera & Harshman 2001; Zuk & Stoehr 2002; Roff & Fairbairn 2007).

In this regard, significant advances have been made in recent times when it comes to our understanding of the physiological mechanisms underlying such trade-offs. For example, many studies have examined the role that hormonal regulation may play in the evolution of trade-offs (Zera & Harshman 2001; Harshman & Zera 2006; Martin *et al.* 2008). The goal of this review is to highlight the potentially far-reaching effects that a group of highly reactive oxygen-containing molecules, collectively termed reactive oxygen species (ROS), might have on our understanding of life-history theory. The past decade has seen an explosive surge in research into the biology of ROS across the medical and biochemical sciences (Sanz *et al.* 2006), extending into the evolutionary and ecological sciences (Apel & Hirt 2004; Constantini 2008). In this contribution, we discuss the effects of ROS on a diverse range of life-history traits in animals, summarizing the key empirical and conceptual advances. By doing so, we integrate these concepts, together with the new ideas on post-copulatory sexual selection, into a unified life-history framework, containing ROS production as a centrepiece. We contend that ROS production can provide the general cost that mediates a multitude of functional trade-offs commonly observed among fitness-related traits.

2. ROS—WHAT ARE THEY?

ROS are free radicals produced as by-products of oxidation–reduction (REDOX) reactions. At low levels, they are known to act as important signalling molecules. For instance, in plants, ROS are used to facilitate an array of essential biological processes (Apel & Hirt 2004; Foyer & Noctor 2005; Gechev *et al.* 2006), including immune defence (Apel & Hirt 2004), growth and development (Gapper & Dolan 2006; Gechev *et al.* 2006), seed germination (Schopfer *et al.* 2001) and the alleviation of seed dormancy (Oracz *et al.* 2007), programmed cell death (Jabs 1998; Dangl & Jones 2001; Van Breusegem & Dat 2006) and stress acclimation (McAinsh *et al.* 1996; Gechev *et al.* 2006). Yet, the effects of ROS are dose dependent (Gechev *et al.* 2006; Quan *et al.* 2008), and at high levels these highly reactive molecules will exert oxidative stress on the cell, and invoke profound changes in gene expression

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(Apel & Hirt 2004). If left unchecked, oxidative stress will result in cumulative oxidative damage to DNA, RNA and proteins within the cell (Harman 1956; Beckman & Ames 1998).

The mitochondria are a primary site for ROS production (Barja 2007). Most of our energy production is generated via oxidative phosphorylation (OXPHOS), which entails five enzyme complexes (Blier *et al.* 2001). Complexes I–IV (the electron transport chain) are involved in transporting electrons through a series of proteins via REDOX reactions, with the final destination being an oxygen molecule. Under normal circumstances, this oxygen is then converted to water in complex IV, and the energy stored in the proton gradient is used to drive ATP production in complex V. However, during this process, a small percentage of the oxygen consumed by the mitochondria at complex IV is converted to one of several ROS (such as superoxide anions, hydrogen peroxide or hydroxyl radicals), rather than water. Thus, somewhat paradoxically, the most fundamental of processes in enabling eukaryote life (OXPHOS) is also one of the chief culprits of ROS production.

ROS are also produced via other REDOX reactions, such as those involved in defence against pathogens (e.g. NADPH oxidase). In these enzyme complexes, ROS are generated rapidly as part of an oxidative burst and then harnessed to fulfil a range of functions, such as to kill incoming microbes (Apel & Hirt 2004). However, ROS are non-targeting molecules and therefore this oxidative burst may also result in self-harm (autoimmunity) if surplus ROS are not efficiently dealt with (Sadd & Siva-Jothy 2006). To counter these risks, eukaryotic organisms are equipped with an arsenal of enzymatic and non-enzymatic mechanisms, which tightly controls unwanted ROS accumulation (Nappi & Ottaviani 2000; Apel & Hirt 2004). For instance, one of the primary enzymatic ROS scavenging mechanisms involves dismutating superoxide to hydrogen peroxide (H_2O_2), via the enzyme superoxide dismutase. The H_2O_2 is subsequently detoxified via other enzymatic reactions. Non-enzymatic mechanisms involve the use of antioxidants, such as cellular redox buffers ascorbate and glutathione, tocopherol, flavonoids, alkaloids and carotenoids (Apel & Hirt 2004).

We note that ROS are not the only reactive molecules generated as by-products of enzymatic reactions. In this regard, much research has been conducted recently into the effects of reactive nitrogen species (RNS), produced by a group of enzymes called nitric oxide (NO) synthases. RNS are free radicals that are similar to ROS in that they are not only necessary in various biological processes, but also exert harmful effects owing to their reactivity (Drew & Leeuwenburgh 2002). For simplicity, the focus of our review is fixed on ROS.

3. ROS AND THE EVOLUTION OF AGEING

Ageing is the physiological deterioration of an organism, leading to decreased reproductive performance and an increased chance of death with age (Rose 1991; Charlesworth 1994). It is more than half a century since Harman first proposed the *Free Radical Theory of Ageing* (Harman 1956), providing the conceptual link between ROS production and the process of ageing. The theory acknowledges that oxidative enzyme complexes are a ubiquitous feature of living organisms, and states that the unwanted ROS produced as a result of such reactions will exert irreversible deleterious effects on molecules within the soma (e.g. DNA, RNA, proteins, lipids), leading to ageing. This theory is well supported empirically as a major mechanistic process underlying ageing (Beckman & Ames 1998; Landis *et al.* 2004; Chen *et al.* 2007), and was later refined into the *Mitochondrial Theory of Ageing* (Harman 1972), acknowledging that the mitochondria are a main source of ROS production (through OXPHOS). Under this theory, it is predicted that mitochondrial DNA (mtDNA) will be a primary target of free radical attack, because it lies within the mitochondrion, which is a major site of ROS production. As mtDNA degrades via oxidative stress, it will result in disruptions to OXPHOS function, thus augmenting ROS production and resulting in a downward spiral of further damage to mtDNA and ROS production, causing ageing (Harman 1972; Balaban *et al.* 2005; Chen *et al.* 2007).

The *Free Radical Theory of Ageing* can be readily integrated into what is probably the most prominent, but contentious, concept in ageing, the *Rate-of-Living Theory of Ageing* (Pearl 1928), which aims to link the rate of ageing to metabolic rates (Beckman & Ames 1998; Brys *et al.* 2007). The resulting integrated theory hinges on a 'live fast–die young' metabolic trade-off, mediated by the production and damaging effects of ROS. That is, individuals, or species, with faster metabolic rates and rates of reproduction should age faster, since faster metabolism should augment ROS production. Indeed, this live fast–die young approach, to our understanding of the ageing process, seems ingrained in the public psyche as dogma, despite empirical efforts that demonstrate many exceptions to the rule (Khazaeli *et al.* 2005; Hulbert *et al.* 2007). Recently, Hulbert *et al.* (2007) have suggested that some exceptions to the rule might be explicable if variation documented in the fatty acid composition of cell membranes across species is taken into account. In short, cell membranes are prone to lipid peroxidation (i.e. oxidative degradation of the lipids in cell membranes due to free radical attack), with certain fatty acid types more prone than others. Given that the fatty acid composition of cell membranes varies systematically across species, some species may be more prone to autoimmunity via oxidative stress than others (Hulbert *et al.* 2007).

Although associations between metabolism, ROS production and lifespan are clearly complex, the integrated conceptual framework that links these traits is nonetheless well developed and worth pursuing empirically. Furthermore, the presumed trade-off between the rate of living and lifespan is consistent with a core genetic model of ageing: antagonistic pleiotropy. Under this model, alleles that increase the rate of ageing, and decrease lifespan, will accumulate if these same alleles confer high fitness early in life (Williams 1957).

It is a well-known premise in gerontology that dietary restriction extends lifespan, and this has been widely attributed to caloric restriction (Weindruch & Walford 1988; Masoro 2006). Yet, recent studies in *Drosophila* (Mair *et al.* 2005; Lee *et al.* 2008; Skorupa *et al.* 2008) and *Teleogryllus* crickets (Maklakov *et al.* 2008) have clearly indicated that in these systems, it is nutrient, rather than caloric, restriction that plays the major role in modulating lifespan. For instance, in both species, the ratios of protein

to carbohydrate that correspond to the longest lifespans differ from the ratios that correspond to highest reproductive success (Lee *et al.* 2008; Maklakov *et al.* 2008), consistent with the idea that a trade-off exists between lifespan and fitness. These results are particularly striking when linked to the recent finding that protein, rather than caloric, restriction decreases mitochondrial ROS production and subsequently the levels of oxidative damage to nuclear and mtDNA in rat liver, without inducing changes in mitochondrial oxygen consumption (Sanz *et al.* 2004). Thus, one of the central tenets of gerontology—that dietary restriction extends lifespan—may also hinge on the damaging effects of excess ROS production.

In sum, there appears an ample opportunity for ROS to play a hand in regulating trade-offs between lifespan, metabolic rates and fitness. If such ROS-mediated trade-offs are realized in nature, then this entails that reproduction is costly and implies that ROS will also be significant players in determining the *cost of reproduction*. We review the evidence for this below.

4. ROS AND THE COST OF REPRODUCTION

The *cost of reproduction* represents one of the most fundamental of life-history trade-offs. Reproduction is costly (Reznick 1985), and therefore investment in current reproduction must be traded off against one's future reproductive potential and survival prospects. Several timely studies have implicated ROS as non-trivial players in the cost of reproduction by demonstrating that increases in reproductive effort result in increased susceptibility to oxidative stress. Two complementary studies experimentally increased the costs of reproduction in female *Drosophila melanogaster* by stimulating egg production through supplementation with either yeast (Wang *et al.* 2001) or a juvenile hormone analogue—methoprene (Salmon *et al.* 2001). They then compared susceptibility to oxidative stress (tolerance to a ROS generating compound, 30 mM methyl viologen, measured via a survival assay) of these females compared with controls. Females supplemented with either yeast or methoprene proved consistently more susceptible to oxidative stress than control females, suggesting that boosting egg production lowered a female's ability to withstand a free radical attack.

Another compelling case for a ROS-induced effect on the cost of reproduction is provided by a collection of studies that explored the effects of oxidative stress on various breeding parameters in zebra finches, *Taeniopygia guttata* (Alonso-Alvarez *et al.* 2004*a*,*b*, 2006; Bertrand *et al.* 2006). Alonso-Alvarez *et al.* (2004*b*) explored the relationship between reproductive effort and oxidative stress by manipulating the brood size, hence breeding effort, of captive finches. They then assayed the time taken to haemolyse 50 per cent of the red blood cells exposed to a controlled free radical attack, to provide a general indication of the total antioxidant defence capability of whole blood. Increasing the brood size induced a reproductive cost in the form of decreased parental body mass, and males co-raising the largest broods experienced sizeable decreases in resistance to oxidative stress. In another paper, the same set of authors presented a negative relationship between the number of breeding events by birds and resistance to oxidative stress in the captive finches, reinforcing the idea that ROS plays an important role in the cost of reproduction in this species (Alonso-Alvarez *et al.* 2006). In a third study, the authors reported a negative association between the number of eggs laid by a breeding pair during the study and the resistance to oxidative stress of both members of the breeding pair. This association was absent in the breeding pairs that were supplemented with antioxidant carotenoids in their drinking water (Bertrand *et al.* 2006).

5. ROS, IMMUNITY AND MALE FITNESS

ROS are not only produced as by-products of OXPHOS, but can also be harnessed to provide benefits when it comes to immune defence. The exploitation of ROS as a weapon for fighting invading microbes represents a core and evolutionary ancient innate immune response among both animals and plants (Nappi & Ottaviani 2000). Various cells, collectively termed phagocytes, engulf microbes and produce ROS via a rapid non-mitochondrial oxidative burst, which is used to kill these invaders (Hampton *et al.* 1998; Nappi & Ottaviani 2000; Bender *et al.* 2005). The production of such ROS as part of the innate immune response is clearly beneficial, and the enzyme complex NADPH oxidase plays a major role in this production, although other complexes such as xanthine oxidase, glucose oxidase and enzymes involved in arachidonic acid metabolism probably also contribute here (Nappi & Ottaviani 2000). Phagocytes are also known generators of RNS, through NO synthase, which similarly act as killing molecules against foreign microbes. Other enzyme complexes important in innate immunity such as phenoloxidase, responsible for the melanization/encapsulation response in insects, also produce ROS and reactive quinoid intermediates of melanin as by-products, which might also be harnessed to combat pathogenic microbes (Nappi & Ottaviani 2000; Kumar *et al.* 2003).

The use of non-specific reactive metabolites in mounting an immune response is obviously fraught with potential hazards. Left uncontrolled, ROS will be highly reactive and damaging not only to the pathogens but also to the host (Sadd & Siva-Jothy 2006). Naturally, several enzymatic and non-enzymatic mechanisms, discussed above, are in place to detoxify surplus ROS (Nappi & Ottaviani 2000; Apel & Hirt 2004). Nonetheless, there is obvious scope for condition-dependent variation to occur between individuals in their ability to deal with surplus ROS, and therefore variation in how they might resolve ROS-mediated trade-offs involving immunity. Any benefits of mounting an immune response (and the degree to which it is mounted) towards a specific pathogen may need to be traded off against the costs of autoimmunity, and any such trade-offs might be amplified during periods of stress (e.g. periods of antioxidant deficiency) or heavy investment in other functions. Support for this idea comes from a recent study that implicated ROS as a regulator in the trade-off between investment in immune protection and investment in somatic growth in zebra finch nestlings (Alonso-Alvarez *et al.* 2007).

(a) *Pre-copulatory sexual selection*

The importance of the immune system in shaping life histories has not been neglected by evolutionary biologists. Resistance to parasites formed the central premise

around Hamilton & Zuk's famous (1982) hypothesis of parasite-mediated sexual selection. In 1992, Folstad and Karter reworked this idea into the *immunocompetence handicap hypothesis*, proposing that testosterone regulates the trade-off between investment in male secondary sexual trait expression and immunity. This was based on empirical findings that demonstrated testosterone to be both a stimulator of male sexual trait elaboration and also an immunosuppressant (Folstad & Karter 1992). This hypothesis, however, lacks generality given the absence of male-specific hormones, such as testosterone, in most invertebrates (Nijhout 1994; Rolff 2002). Sheldon & Verhulst (1996) and Zuk & Stoer (2002) provided such generality by suggesting that life-history trade-offs involving immune defence, including those involved in male sexual trait elaboration, can be visualized well within a framework of resource limitation.

In a landmark paper, von Schantz *et al.* (1999) formulated a new and exciting framework for immuno-dependent pre-copulatory sexual selection, mediated by the costs of oxidative stress, rather than around a currency of resource limitation. The idea is based on several premises. First, females may improve offspring survival by selecting males on the basis of sexual ornaments that reliably signal their health status (this is a good-genes model of sexual selection; Andersson 1994). Second, the expression of these sexual ornaments is sensitive to oxidative stress. Third, activation of the immune system results in the production of ROS that may cause oxidative damage in the absence of a tightly coordinated control system to detoxify such metabolites, and fourth, the level of resistance to oxidative stress is heritable. If all of these premises hold true, then in the quest to procure matings, males should face a trade-off between their investment in the immune system and investment in showy ornamentation, since investing heavily in the former will result in the ROS production that will negatively affect the latter. Such ornaments should thus serve as reliable indicators of a male's immuno-health status, and his ability to detoxify reactive metabolites and resist oxidative stress.

Since its inception, the idea of ROS-mediated sexual selection has received substantial attention. Many studies have tested these ideas indirectly by experimentally manipulating antioxidants in the diet of males, and observing changes in the degree of sexual trait expression and various immune parameters. In particular, the effect of dietary carotenoids on a range of carotenoid-dependent sexual traits has received much empirical attention, and has been reviewed elsewhere (Blount 2004; Catoni *et al.* 2008). Carotenoids are a class of dietary pigments responsible for producing the brilliant red, orange and yellow sexual ornaments commonly seen among birds, fishes and reptiles. Given that they are also known antioxidants required to scavenge surplus ROS and stimulate the immune system, individuals might face an allocation trade-off when it comes to investing carotenoids into sexual ornamentation or immune function (Olson & Owens 1998).

Recently, studies have sought to directly manipulate or quantify the levels of oxidative stress/damage and ROS production, and then to examine associations between these parameters and sexual trait expression and immune defence, thereby providing more direct tests of ROS-mediated sexual selection (Alonso-Alvarez *et al.* 2004*a*; Constantini & Dell'Omo 2006; Constantini *et al.* 2007; Hõrak *et al.* 2007; Kurtz *et al.* 2007; Pike *et al.* 2007; Torres & Velando 2007; Isaksson & Andersson 2008; Olsson *et al.* 2008*a*). For instance, a recent study of blue-footed booby, *Sula nebouxii*, by Torres & Velando (2007) reported that experimentally increasing the levels of ROS through immunizing males with lipopolysaccharide (an immune activator) resulted in increased levels of oxidative damage and decreased foot coloration among males that were 10 years of age or older. With the exception of this study, almost all the other direct tests of ROS-mediated sexual selection have focused on carotenoid-based sexual traits. Although some of these have found that the expression of carotenoid-based sexual ornamentation is sensitive to oxidative stress triggered upon experimental immune activation (Alonso-Alvarez *et al.* 2004*a*; Pike *et al.* 2007), and others have found links between indices of oxidative stress or oxidative damage and carotenoid levels (Constantini & Dell'Omo 2006; Hõrak *et al.* 2007), other studies have failed to find compelling links between sexual trait expression, oxidative stress and carotenoid intake (Isaksson & Andersson 2008; Olsson *et al.* 2008*a*). Experiments in three-spined sticklebacks (*Gasterosteus aculeatus*) have revealed fascinating links between circulating testosterone levels, expression of carotenoid-based coloration, immunity, major histocompatibility complex (MHC) gene expression and levels of oxidative damage. In one study, males implanted with testosterone exhibited redder body coloration, decreased immunocompetence (measured as the respiratory burst upon phagocytosis, and as the size of immune system organs—head kidney index and relative spleen mass) and increased oxidative damage (Kurtz *et al.* 2007). A second study found that sticklebacks with higher levels of the MHC gene expression (a highly polymorphic gene region in vertebrates important in immune defence) were in poorer body condition and experienced higher levels of oxidative damage (Kurtz *et al.* 2006). Clearly, there is a need for further experiments across a more diverse range of taxa (i.e. not only birds, fishes and lizards) and sexually selected trait types (i.e. not only those traits that are carotenoid based) to establish the generality with which such traits are sensitive to ROS generated via immune activation.

Another criterion required to facilitate a good-genes model of ROS-mediated sexual selection is that a male's ability to detoxify ROS, or resist oxidative stress, must be heritable. In the first test of this criterion, Olsson *et al.* (2008*b*) established that various measures of the ROS production, and in particular the reactive metabolite superoxide, exhibited high heritability in the painted dragon lizard, *Ctenophorus pictus*.

The *phenotype-linked fertility hypothesis* of sexual selection predicts that there will be a link between a male's sexual ornamentation and his fertilizing ability, such that females choosing to mate with males with highly expressed ornaments will receive the direct benefit of fertility insurance (Sheldon 1994). These predictions have been tested on several occasions, and have been supported in some (Matthews *et al.* 1997; Gomendio *et al.* 2000; Pizzari *et al.* 2004; Malo *et al.* 2005; Locatello *et al.* 2006), but not all (Birkhead & Fletcher 1995; Birkhead *et al.* 1997; Skinner & Watt 2007; Peters *et al.* 2008) cases. Blount *et al.* (2001) realized that oxidative stress, imposed by ROS

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production, could well provide a mechanistic link regulating any association between sexual ornamentation and fertilizing ability. This is based on two assumptions—that sexual ornaments are potentially susceptible to oxidative stress as are the sperm that will determine male fertilizing ability. Although the first assumption has been widely tested (with the evidence reviewed above), the second assumption has received scant empirical attention by evolutionary ecologists. Fortunately, the medical literature has made substantial progress here, and much is known about the links between ROS and male fertility in mammals (Aitken & Baker 2002, 2004; Aziz *et al.* 2004; Baker & Aitken 2004; Liu *et al.* 2006).

In humans, the sperm midpiece is loaded with mitochondria that are used to power sperm motility (Mitchell *et al.* 1976), and thus sperm are themselves a site of ROS production via OXPHOS (Aitken & Baker 2004). Furthermore, the testes (the site of spermatogenesis) will also generate ROS as by-products of cellular metabolism. In addition to this, leucocytes are known to infiltrate the semen and constitute a primary source of ROS production in the ejaculate (Whittington & Ford 1999). The upshot is that sperm will be routinely exposed to ROS. The logical prediction is that sperm will therefore have evolved an array of defence mechanisms to protect themselves from oxidative stress. Recent proteomic analysis supports this prediction, with 11 per cent of proteins found in human spermatozoa involved in protection against oxidative damage, apoptosis and cell cycling (Martinez-Heredia *et al.* 2006). Nonetheless, sperm have a high polyunsaturated fat content, making them highly vulnerable to lipid peroxidation. They also possess a limited arsenal of cytosolic antioxidant defences relative to somatic cells, although this shortfall seems to be counterbalanced by a high antioxidant capacity of the seminal fluid (Aitken 1994; Smith *et al.* 1996; Baker & Aitken 2004), together with a range of seminal proteins (Schöneck *et al.* 1996; Collins *et al.* 2004) and testes-specific proteins that probably play a role in protecting sperm (Hüttemann *et al.* 2003; Liu *et al.* 2006). For example, in mice, a testes-specific form of cytochrome *c* (T-C*c*), an electron-carrying protein central to OXPHOS, is able to catalyse the reduction of the ROS, H_2O_2, three times faster than its somatic counterpart (S-C*c*), and T-C*c* is also more resistant to degradation by H_2O_2 than S-C*c* (Liu *et al.* 2006). These findings would suggest that T-C*c* has evolved to counter the damaging effects of H_2O_2 on sperm integrity within the testis.

Thus, the main assumptions of a *ROS-mediated phenotype-linked fertility hypothesis*—that sexual ornamentation and sperm quality are both vulnerable to oxidative damage—appear to hold up to scrutiny. The hypothesis, then, assumes that males with highly expressed antioxidant-dependent ornamental displays should possess ample amounts of circulating antioxidants, and hence also exhibit effective antioxidant protection of sperm. Females may then receive fertility assurance by mating with such males (Blount *et al.* 2001). Recently, Peters *et al.* (2004) have provided the first empirical support for this integrated hypothesis, by finding associations between plasma carotenoid levels, bill colour and sperm velocity in mallards, *Anas platyrhynchos*. Further controlled tests of this idea are certainly warranted.

The evidence described above clearly links surplus ROS production with decreases in sperm quality. Although the ROS-mediated phenotype-linked fertility hypothesis acknowledges the ramifications that unwanted ROS production might have on sperm quality, the hypothesis is nonetheless based squarely within the framework of pre-copulatory sexual selection, based on direct fertility benefits. We suggest that condition dependence between males in their ability to deal with surplus ROS in the ejaculate will also drive post-copulatory sexual selection, via the evolution of female mating behaviour and sperm competition.

(b) *Post-copulatory sexual selection*

Females of a vast number of species commonly mate more than once within the same reproductive cycle (Simmons 2001). ROS may play a role in the evolutionary origin and maintenance of multiple mating by females. There is accumulating experimental evidence that females benefit from mating with multiple males via an increase in the viability of embryos they produce (Simmons 2005). These effects are generally interpreted in terms of genetic benefits obtained, either from avoiding fertilizations by sperm from genetically incompatible males (Zeh & Zeh 1997), or from promoting fertilizations by sperm from males of intrinsically high genetic quality (Yasui 1997). In hamsters, the male accessory glands are a major source of antioxidant enzymes in the seminal fluid (Chen *et al.* 2003) that preserve sperm DNA integrity from oxidative damage (O *et al.* 2006). Ablation of the major accessory sex glands results in sperm with relatively greater DNA damage than sperm produced by control males (Chen *et al.* 2002). These ROS-damaged sperm are capable of fertilization, but the resulting embryos are inviable, suffering decreased rates of implantation and higher rates of post-implantation loss for those that do implant (O *et al.* 1988, 2006). Generally, across mammals, polyandry is associated with increased embryo viability (Stockley 2003). We suggest then that variation between males in their ability to buffer sperm from ROS renders monogamous females at risk of reproductive failure, thus favouring the evolution of multiple mating. Furthermore, females will presumably stand to benefit by biasing fertilization, through cryptic choice mechanisms, in favour of those males that are better able to shield their sperm from ROS. The resulting competition between the sperm of different males for fertilizations represents a major driving force behind the evolution of male fitness (Parker 1970; Birkhead & Møller 1998; Simmons 2001), and males with adaptations that enable their ejaculate constituents (sperm and seminal fluid proteins) to withstand the damaging effects of ROS, generated during spermatogenesis and sperm motility, stand to gain higher fitness under sperm competition.

Furthermore, owing to the risks of autoimmunity, ROS may be mediators of trade-offs involving sperm numbers and sperm quality. Increasing the rate of spermatogenesis, or the motility of sperm, might entail an upregulation of metabolic activity (Parapanov *et al.* 2008), which would presumably augment ROS production in the ejaculate and potentially adversely impact on the expression of other sperm traits important in sperm competition, such as sperm viability, integrity and longevity. Thus, ROS biology may well play a hand in accounting for the negative associations sometimes found between sperm

traits (Pitnick 1996; Levitan 2000; Gage *et al.* 2002; Moore *et al.* 2004; Schulte-Hostedde & Millar 2004; Birkhead *et al.* 2005; Dowling *et al.* 2007), particularly those associations between sperm numbers and sperm quality (Pitnick 1996; Moore *et al.* 2004; Schulte-Hostedde & Millar 2004), as well as trade-offs between sperm quality and immune function (Simmons & Roberts 2005). We believe that the role of ROS in post-copulatory sexual selection deserves immediate empirical assessment.

6. CONCLUSIONS

We have integrated the present state of knowledge regarding ROS-induced effects, and oxidative stress, on a variety of life-history traits in animals. The studies that we have reviewed support the notion that ROS production stands as a central mediator in the evolution of life-history trade-offs. That is, the evolutionary trajectory taken by any given life-history trait will be limited by the trajectories taken by a whole suite of other traits with which it is entwined. In many cases, ROS production appears to be the limiting factor preventing the unconstrained evolution of such traits. The examples that we have documented may not represent an exhaustive list of ROS-induced biological effects, and most of our conclusions are based on studies biased towards insects, birds and humans. The generality by which ROS impact on life-history evolution remains to be fully explored across a range of other taxa. However, the examples that we have highlighted do act to serve our suggestion that ROS might well be entrenched as cornerstones of life-history evolution, and we hope that this review will inspire the additional empirical effort required to fully assess this suggestion.

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