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REVIEW

Current versus future reproduction and longevity: a re-evaluation of predictions and mechanisms

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ABSTRACT

Oxidative damage is predicted to be a mediator of trade-offs between current reproduction and future reproduction or survival, but most studies fail to support such predictions. We suggest that two factors underlie the equivocal nature of these findings: (1) investigators typically assume a negative linear relationship between current reproduction and future reproduction or survival, even though this is not consistently shown by empirical studies; and (2) studies often fail to target mechanisms that could link interactions between sequential life-history events. Here, we review common patterns of reproduction, focusing on the relationships between reproductive performance, survival and parity in females. Observations in a range of species show that performance between sequential reproductive events can decline, remain consistent or increase. We describe likely bioenergetic consequences of reproduction that could underlie these changes in fitness, including mechanisms that could be responsible for negative effects being ephemeral, persistent or delayed. Finally, we make recommendations for designing future studies. We encourage investigators to carefully consider additional or alternative measures of bioenergetic function in studies of life-history trade-offs. Such measures include reactive oxygen species production, oxidative repair, mitochondrial biogenesis, cell proliferation, mitochondrial DNA mutation and replication error and, importantly, a measure of the respiratory function to determine whether measured differences in bioenergetic state are associated with a change in the energetic capacity of tissues that could feasibly affect future reproduction or lifespan. More careful consideration of the life-history context and bioenergetic variables will improve our understanding of the mechanisms that underlie the life-history patterns of animals.

KEY WORDS: Life-history traits, Cost of reproduction, Reactive oxygen species, Oxidative stress, Mitochondrial function, Longevity

Introduction

Nearly a century ago, Fisher (1930) suggested that the high energetic demand of reproduction should have a negative impact on future reproduction and longevity. In subsequent decades, understanding the interactions among life-history variables became a major focus of evolutionary biology, ecology and physiology. Great strides have been made in our comprehension of why life-history patterns vary among species (Stearns, 1976; Martin, 1995; Hamrick and Godt, 1996; Sæther and Bakke, 2000; Ricklefs, 2008), but despite numerous studies and reviews, physiologists have been less successful in describing the mechanisms that link reproduction, bioenergetics and survival within species (McKinnon and Caldecott, 2007; Monaghan et al., 2009; Metcalfe and Monaghan, 2013; Costantini, 2014; Speakman and Garratt, 2014; Blount et al., 2015; Speakman et al., 2015). We propose that progress in understanding the interactions between life-history events has been hindered, at least in part, by two factors: (1) the assumption that reproduction will necessarily entail a reduction in future reproductive performance and survival, and (2) a lack of mechanisms that accurately explain the observed interactions between sequential life-history events.

Our understanding of the factors that contribute to variation in life-history strategies of individuals is rooted in the idea that the way in which an animal uses limited resources plays an important role in determining its fitness. When resource allocation to one trait that benefits fitness comes at the expense of resource allocation to another potentially beneficial trait, those traits are said to 'trade off' (Cody, 1966; Reznick, 1985; Stearns, 1992; Roff, 2002). For example, if a juvenile allocates significant resources to growth and, as a consequence, does not develop a functional reproductive system and forgoes early first reproduction, growth can be said to trade off with reproduction. Because it can be difficult to measure the direct fitness consequences of life-history traits, many investigators have used proxies for fitness, such as relative energy expenditure (Stearns, 1992). In using energy expenditure as a proxy for fitness, it is assumed that because reproduction is energetically demanding it will necessarily have a negative impact on future fitness. However, there is limited empirical evidence to support the assertion that energy expenditure reduces future reproductive performance or survival (Ryser, 1989; Kotiaho and Simmons, 2003; Ricklefs and Cadena, 2007; Skibiel et al., 2013). If higher energy expenditure was associated with reduced longevity, our health care professionals would encourage us to relax in our favorite armchair rather than get some exercise.

Oxidative damage (see Glossary) has been proposed as a likely mediator of the assumed trade-offs between current and future reproduction (Ołdakowski et al., 2012, 2015), and between reproduction and longevity (Speakman and Garratt, 2014). This line of thinking is based on the theory that oxidative damage causes senescence (Cui et al., 2012), and the idea that production of adenosine triphosphate (ATP) is positively related to the production of reactive oxygen species (ROS; see Glossary) (Harman, 1956; Barja, 2007). Recent reviews by Speakman and Selman (2011), Selman et al. (2012), Metcalfe and Monaghan (2013), Speakman and Garratt (2014), Blount et al. (2015) and Speakman et al. (2015) show that there is limited support for the idea that oxidative damage mediates trade-offs between current reproduction and future reproduction or longevity. Instead, studies focusing on the function of the mitochondrial electron transport system (ETS) have shown that ROS production is typically greater when energy expenditure is low (rather than high), the proton motive force (see Glossary) is high and the ETS complexes are reduced with electrons (Brand, 2000; Ricquier and Bouillaud, 2000; Murphy, 2009;

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List of symbols/abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
ETS	electron transport system
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
P/O	phosphate/oxygen ratio
RCR	respiratory control ratio
ROS	reactive oxygen species
UCP	uncoupling protein

Jastroch et al., 2010; Speakman and Garratt, 2014; Salin et al., 2015b). Under these conditions, electron transport through the ETS is slowed by a high proton gradient across the inner mitochondrial membrane. Electrons become 'backed up' within the ETS complexes, reducing the ability of the ETS complexes to accept addition electrons. It is thought that electrons that are transported to complexes I and III but are not immediately accepted are responsible for most of the ROS that is generated by the ETS, but several additional sights have recently been described (Murphy, 2009; Brand, 2016). In addition, although limited data are available, there appears to be little evidence that oxidative damage increases with age in free-living populations (Selman et al., 2012). Nevertheless, several authors have recently argued that advancing our understanding of life-history trade-offs requires more study of the role that oxidative damage plays in the interaction among life-history traits (Nussey et al., 2009; Isaksson et al., 2011; Selman et al., 2012; Speakman et al., 2015). By contrast, we argue that to understand the mechanisms that underlie the interactions among life-history variables, it is critical to take a broader look at bioenergetic processes – such as those influencing mitochondrial ROS generation, oxidative damage repair, biogenesis, replication error and, importantly, a measure of the respiratory function of mitochondria – to determine whether measured differences are associated with a change in the energetic capacity of tissues.

Although reproduction may alter bioenergetic processes that support future reproduction and longevity in both males and females (Georgiev et al., 2015; Sharick et al., 2015), we focus on females in this Review, because the demands of reproduction are often greater and less variable in females than in males (Trivers, 1972; Clutton-Brock, 1991). As a result, the physiological cost of reproduction in females has received more attention and is better understood than the cost of reproduction in males. To understand the mechanisms responsible for interactions between life-history events for females of a species, it is critical that predictions be rooted in an understanding of how reproductive performance (fecundity) and survival change over time and with parity (see Glossary). In addition, predictions should be based on a strong understanding of the bioenergetic processes that both support and prevent females from maximizing energy allocation to reproduction. An important, but often neglected, consideration is that predictions should be based on an understanding of the homeostatic processes responsible for maintaining the bioenergetic capacity of cells and tissues.

Glossary

Free radicals
Atoms or groups of atoms with unpaired electrons that can be formed when oxygen interacts with certain molecules.

Oxidative damage
Change to the structure of a molecule caused by reaction with ROS or other free radicals.

Oxidative stress
An imbalance between the production of ROS from the ETS or other sources and the capacity of antioxidant mechanisms to control the damaging effects of ROS.

Parity
Number of reproductive bouts carried to a viable age.

Proton motive force
The potential energy generated by a combination of the proton and voltage gradient that occurs within the intermembrane space of the mitochondria. The proton motive force regulates the movement of protons across inner mitochondrial membrane.

Reactive oxygen species
Chemically reactive molecules generated primarily as by-products of mitochondrial electron transport. ROS not only refers to oxygen-centred radicals but also to non-radical but reactive derivatives of oxygen.

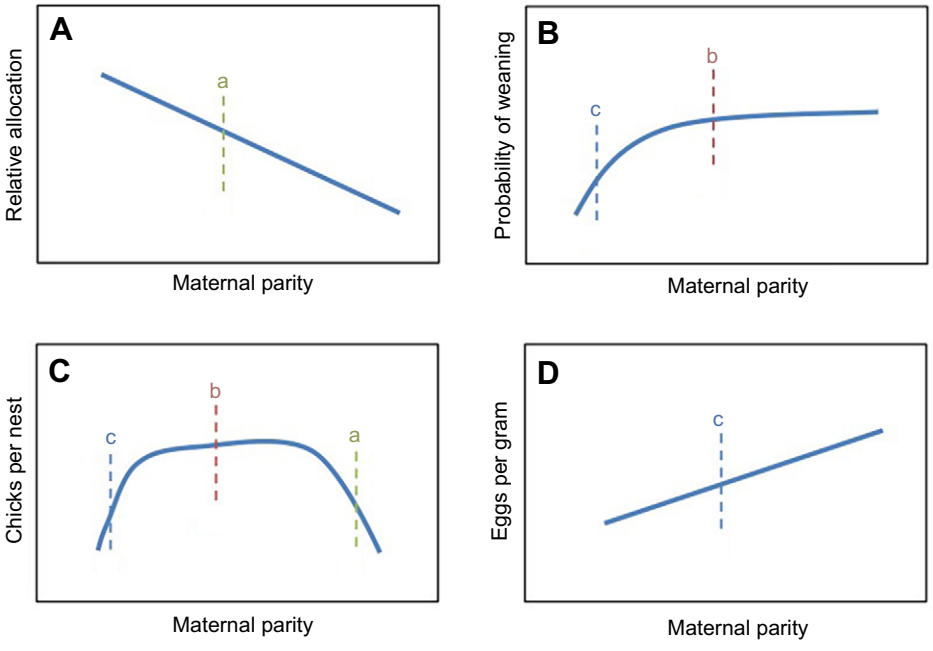

Fig. 1. Common patterns of reproductive performance versus parity. (A) As current and future reproduction are often though to trade off, this panel represents the hypothetical relationship that would be expected if trade-offs were to occur at each reproductive event. (B–D) Each of the species listed display a pattern of reproduction that is observed in numerous species (additional references given in text). (B) An increase and later plateau in reproductive performance is observed in the northern elephant seal (Le Boeuf and Reiter, 1988). (C) An increase, plateau and late-life decline in reproductive performance is observed in the Eurasian sparrowhawk (Newton et al., 1981). (D) An increase in reproductive performance with parity is common among species with indeterminate growth such as the black rockfish (Bobko and Berkeley, 2004). In these examples, the relationship between current and future reproduction changes with parity, dashed line 'a' indicates negative interactions, dashed line 'b' indicates neutral interactions, and dashed line 'c' indicates positive interactions between current and future reproduction.

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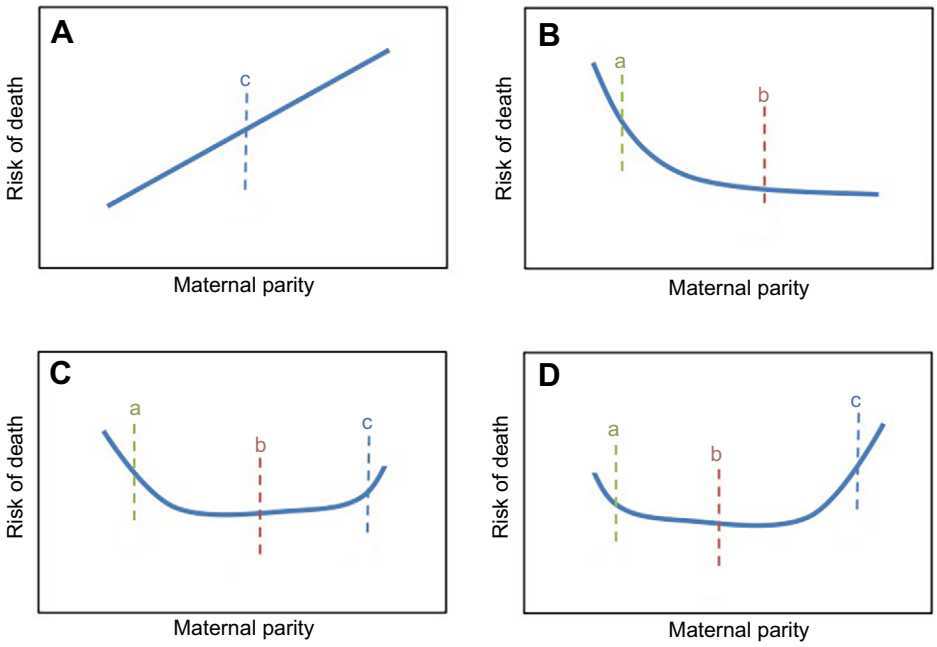

Fig. 2. Common patterns of maternal parity versus risk of death. (A) This panel represents the hypothetical relationship that would be expected if females experienced increased risk of death with each reproductive event. All other panels represent the most common relationships between female parity and risk of death observed among vertebrate species. (B) Exponential decay curve is found for 58% of the species that have been investigated. (C) A left-skewed U-curve is found for 24% of the investigated species. (D) A right-skewed U-curve is found for 12% of the species that have been investigated. In many of these examples, the relationship between parity and risk of death changes over time: dashed line 'a' indicates negative interactions, dashed line 'b' indicates neutral interactions and dashed line 'c' indicates positive interactions between parity and risk of death. Modified from Proaktor et al. (2007).

Armed with this information, it will then be possible to design effective experiments and to evaluate the bioenergetic mechanisms that could contribute to individual differences in performance within a population. To this end, in this Review, we describe common patterns of reproductive performance versus parity, and parity versus survival (Figs 1 and 2). We also describe how changes in mitochondrial function during reproduction could have negative, neutral or positive effects on a female's bioenergetic capacity, and we suggest processes that could be responsible for negative effects being ephemeral, persistent or delayed. Finally, we end by giving recommendations for the design of future studies.

Proposed physiological basis for life-history patterns

Animals display diverse patterns of reproduction (Fig. 1) and survival (Fig. 2) (Proaktor et al., 2007; Jones et al., 2014), but despite this diversity, investigators often assume a simple linear trade-off between reproduction and survival. However, evidence from a number of studies suggests that reproduction during one event does not necessarily influence a female's probability of survival to the next reproductive event or performance during that event (Murie and Dobson, 1987; Shine and Schwarzkopf, 1992; Humphries and Boutin, 2000).

When we evaluate data on how allocation to reproduction and risk of death varies with age and/or parity, it becomes clear that the relationship between reproduction and survival is not consistently negative or linear. Reproductive performance and risk of death can increase, remain stable or decline between sequential reproductive events. Improved reproductive performance and reduced probability of death between sequential reproductive events commonly occurs early in life (Fig. 1B,C; Fig. 2B–D) (Newton et al., 1981; Le Boeuf and Reiter, 1988; Proaktor et al., 2007; Weladji et al., 2010; Billman and Belk, 2014; Jones et al., 2014). Improved reproductive performance may persist indefinitely in species with indeterminate growth (Fig. 1D) (Schultz and Warner, 1991; van Buskirk and Crowder, 1994; Bobko and Berkeley, 2004). Following a female's initial one to three bouts of reproduction, reproductive performance and probability of death often plateaus and may remain constant until her final reproductive event (Fig. 1B; Fig. 2B) (Le Boeuf and Reiter, 1988; Wheelwright and Schultz, 1994; Robertson and Rendell, 2001; Proaktor et al., 2007; Jones et al., 2014) or until she nears her final few reproductive attempts (Fig. 1C). Reproductive performance may increase (Andrade and Kasumovic, 2005; Isaac and Johnson, 2005; Creighton et al., 2009; Weladji et al., 2010) or it may decline (Fig. 1C) (Finn, 1963; Newton et al., 1981; Descamps et al., 2009; Hammers et al., 2015; Warner et al., 2016) during a female's final reproductive event(s).

Under poor environmental conditions or when relative allocation to reproduction is experimentally elevated, trade-offs are more likely to be observed, suggesting that conditions that push females to their physiological limit may be important in the evolution of species-specific life-history patterns (Sinervo and Licht, 1991; Zera and Harshman, 2001; Jacobs et al., 2011). However, such conditions may not be typical for females within a population. Age also plays a central role in reproductive declines, but survival following reproduction for old females is undescribed in many species because only a small (and non-random) percentage of adults survive to the age where such an effect is evident.

It is important to recognize, however, that ecological factors can also affect female survival and the ability to support sequential reproductive events (Speakman, 2008). Although a female's relative risk of death may change with her age or reproductive experience, her risk of death commonly may be lower if she does not reproduce than if she does (Clutton-Brock, 1984). Ecological effects, such as increased predation risk, rather than physiological consequences of reproduction, could drive such differences (McNamara and Houston, 2008; Speakman, 2008). Given that recent reviews suggest that reproduction does not lead to an accumulation of oxidative damage, we emphasize the short-term effects of reproduction on bioenergetic performance but, importantly, we also provide a mechanism by which negative consequences of reproduction could be delayed. Thus we encourage investigators to consider both the short-term and long-term fitness consequences of reproduction.

When one measures the physiological impact of reproduction on bioenergetic processes, data from a single point in the life cycle can sometimes be misleading. Oxidative damage can be repaired or removed before fitness is affected, and other forms of damage may

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Box 1. Possible consequences of reproduction on an individual's bioenergetic capacity

Here, we define bioenergetic capacity as the ability to efficiently produce ATP. The bioenergetic capacity of a cell can be reduced by damage or dysfunction – these effects have the potential to affect an individual's capacity for future reproduction and longevity.

Negative effects – a consequence of reproduction that has a negative impact on a female's bioenergetic capacity.

a. **Ephemeral** – a negative consequence that does not persist long enough to affect an animal's future survival or reproduction. Effects are typically ephemeral because oxidative damage can be repaired and removed or metabolic compensation can occur as a result of processes such as mitochondrial biogenesis.
b. **Persistent** – a negative consequence that lasts long enough to reduce an animal's probability of survival or reproductive performance in a subsequent reproductive bout. Persistent effects are expected to be most common when oxidative damage is high, environmental conditions are poor, or when repair, replacement and compensation mechanisms decline with age.
c. **Delayed** – a negative consequence that takes time and/or many reproductive events before it has a detectable impact on a female's reproductive capacity or survival. Delayed effects are expected to be associated with mitochondrial DNA mutation.

Neutral effects – a consequence of reproduction that has no impact on a female's bioenergetic capacity. Neutral effects are a consequence of processes that prevent damage within a cell.

Positive effects – a consequence of reproduction that enhances the bioenergetic capacity of an animal. This could include cell and mitochondrial proliferation, which would increase the density of mitochondria within an organ. The respiratory capacity of tissue could be elevated by mitochondrial hormesis, where moderate levels of ROS signal increase mitochondrial biogenesis, repair and antioxidant production.

take time to accumulate before they have a detectable impact on bioenergetic processes and fitness. When investigators detect negative effects of reproduction on bioenergetic processes, the effect that is detected may be either ephemeral or persistent (Box 1). When negative effects are not detected, it is still possible that reproduction will negatively affect fitness – delayed negative effects may take time and/or many reproductive events before they have a detectable impact on a female's bioenergetic capacity (Box 1) and fitness.

Based on our current knowledge of mitochondrial function, life history trade-offs should not be considered an inevitable consequence of cellular bioenergetic processes, because neutral and even positive interactions between sequential life-history events are possible, in addition to negative effects (Box 1). Although there has been an overwhelming emphasis on understanding the physiological mechanisms that underlie the purported negative impacts of reproduction on the body, there has been little consideration of how transient these effects may be, and little emphasis on understanding the mechanisms that could allow for delayed negative effects. The mechanisms that support neutral or positive interactions between reproduction and future performance have largely been ignored. Below, we review the context and mechanisms that could be responsible for each of these interactions.

Negative effects on bioenergetic capacity and their proposed mechanisms

Although recent papers (Mappes et al., 1995; Knops et al., 2007; Skibiel et al., 2013) and reviews (Speakman and Garratt, 2014; Blount et al., 2015) have questioned the ubiquity of trade-offs between current and future reproduction and between reproduction and longevity, both correlational and experimental data suggest that negative effects of reproduction (i.e. trade-offs) do occur. For example, the number of eggs produced by the carabid beetle *Notiophilus biguttatus* declines precipitously during the second to fifth month of egg production when females are maintained at an optimal temperature and have *ad libitum* access to prey (Ernsting and Isaaks, 1991). Female northern water snakes (*Nerodia sipedon*) that are in poor condition (low length-adjusted body mass) following parturition do not survive to reproduce the next year (Brown and Weatherhead, 1997). Glaucous-winged gulls (*Larus glaucescens*) (Reid, 1987) and blue tits (*Cyanistes caeruleus*) (Nur, 1984) with low body mass are less likely to survive to the next reproductive event when rearing (artificially) large clutches. A variety of physiological mechanisms could be responsible for these and other observed negative effects of current reproduction on future reproduction and survival – some potential mechanisms are discussed in more detail below.

Loss of somatic stores

Reproduction can negatively affect a female's bioenergetic capacity to support future reproduction and/or survival by mobilizing somatic tissue stores and/or partitioning resources away from somatic tissue maintenance (Kirkwood, 1992). Poor body condition could reduce a female's probability of survival by reducing tissue function and/or the amount of tissue that can be mobilized as a source of energy to support the demand of avoiding predators (Curio, 2012) or coping with environmental stressors (Zhang et al., 2015). Poor body condition can also reduce the amount of substrate that a female can mobilize from her body to synthesize milk (Berry et al., 2007) and/or reduce the condition of skeletal muscle and other organs that are necessary to capture prey for dependent young (Bachman, 1993). Physiological indicators of body condition, such as the levels of the adipose and gastrointestinal hormones leptin and ghrelin, appear to play an important role in permitting or inhibiting ovulation (Comninos et al., 2014), and thus could reduce future reproductive fitness. Finally, both correlational and experimental studies have shown that reproduction can reduce immune function, which is predicted to reduce the probability of survival (Cichoń et al., 2001; Kortet et al., 2003; Lozano and Lank, 2003; Ardia, 2005). Thus a loss of somatic stores can reduce the capacity of females to support survival and future reproduction because of the reduction in the amount of readily mobilizable substrate that could otherwise be used to maintain fitness.

Production of free radicals

Reproduction has also been proposed to negatively affect female survival and future reproductive performance because increased cellular respiration associated with reproduction has been assumed to increase free radical production (see Glossary; a brief overview of ROS production is provided in Box 2) (Harman, 1956; Speakman and Garratt, 2014). Yet recent evidence suggests that this assumption is incorrect, as no consistent relationship has been found between oxygen consumption and ROS production (Barja, 2007; Salin et al., 2015b). Nevertheless, reproduction has been associated with increased oxidative damage to lipids and proteins in select species under certain conditions. For example, damage to plasma proteins is elevated during late reproduction in female Northern elephant seals (*Mirounga angustirostris*; Sharick et al., 2015), and lipid peroxidation is elevated during late pregnancy and lactation in the liver and kidney of laboratory rats (*Rattus norvegicus*; Upreti et al., 2002). Thus variable(s) other than metabolic rate are likely to determine whether a female accumulates oxidative damage during a reproductive event.

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Box 2. Reactive oxygen species: formation, targets and damage

Free radicals are produced when electrons escape transport within the ETS and react with either free oxygen or free nitrogen. The more common reaction with free oxygen leads to the production of ROS (Powers and Jackson, 2008). If ROS are not quickly quenched by antioxidants, they can oxidize lipids, proteins and both mitochondrial and nuclear DNA; thus ROS are said to inflict oxidative damage (Cooke et al., 2003).

Direct damage from ROS has been intensively studied as a mechanism of cellular decline and ageing (Beckman and Ames, 1998; Harman, 2006). ROS damage proteins by oxidizing amino acid residue side chains, forming protein–protein cross-linkages and oxidizing protein backbones, resulting in protein fragmentation that reduces or hinders function (Berlett and Stadtman, 1997). Oxidized proteins that are not repaired are prone to aggregation, and protein aggregates can disrupt cellular functions and serve as nucleation sites for the aggregation of other proteins (Squier, 2001). ROS rapidly oxidize lipids in membranes due to their high solubility in the lipid bilayers. Membrane polyunsaturated fatty acids are particularly prone to oxidation (Pamplona, 2008). If not repaired, peroxidized membranes become rigid, lose selective permeability and, under extreme conditions, lose integrity (Pacifici and Davies, 1991; Özben, 2013). Lipid peroxidation can also generate hydroperoxides, which fragment into reactive carbonyl species that react with proteins, DNA and aminophospholipids to form advanced lipoxidation end-products. Advanced lipoxidation end-products can diffuse throughout the cell, cross cell membranes and ultimately damage targets that are far away from the site of formation (Pamplona, 2008).

Not only is there potential for reproduction to affect oxidative status, but the oxidative status of a female as she begins to reproduce also appears to affect when she reproduces and how much she allocates to reproduction. Costantini et al. (2015) experimentally induced oxidative stress (see Glossary) just before breeding in canaries and found that this can delay the onset of egg laying and reduce clutch size. This finding suggests that oxidative stress generated during one reproductive bout could affect performance in the next reproductive bout. However, just because oxidative damage is detected does not assure that it will persist to the next reproductive bout, because oxidative damage to lipids and proteins can be repaired and/or removed.

The oxidation of proteins can cause significant unfolding, and the partial denaturation of protein molecules can expose hydrophobic patches that would otherwise be hidden. Because of protein repair mechanisms, these potentially damaging effects can be ephemeral. Oxidized proteins can be repaired, in part, by re-reduction of oxidized sulfhydryl groups (Costa et al., 2007) and through the action of heat-shock proteins that regulate protein re-folding and protect cells against the accumulation of damaged proteins by transporting them to sites of degradation (Whitley et al., 1999). If oxidized proteins are not removed through protein repair pathways, they can be recognized by proteases (or proteasomes in the cytoplasm) and degraded. *De novo* replacement protein molecules are then synthesized (Jung et al., 2014).

Damage to lipids can also be short-lived. Protection of membranes is achieved by a complex system that involves three steps: lipid repair, lipid replacement and scavenging of lipoperoxidation-derived end-products. When ROS react with membrane phospholipids, lipid hydroperoxides are produced. Peroxidized acyl chains are removed from damaged phospholipids *in situ* by phospholipid hydroperoxide glutathione peroxidase (Imai and Nakagawa, 2003). Once released from the membrane, peroxidized acyl chains can either be metabolized to fatty acid alcohols by glutathione peroxidase (Hulbert et al., 2007), or phospholipase A2 can remove the peroxidized end of the acyl chain from the damaged fatty acid so that the remaining acyl chain can be used to synthesize new membrane phospholipids. The reactive peroxidized end is then detoxified by glutathione *S*-transferases (Ayala et al., 2014).

Mitochondrial DNA damage

ROS can also directly induce strand breakage and point mutations in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA; Cooke et al., 2003). Because mtDNA is situated near the ROS-generating ETS, it is more susceptible to oxidative damage than nDNA (Shokolenko et al., 2009); as a result of damage to mtDNA, the assembly and function of proteins in the ETS could be reduced. However, DNA damage has rarely been measured in studies where reproduction was associated with enhanced oxidative stress. At least one study quantifying DNA damage during a reproductive event found no change in the cumulative markers of nDNA and mtDNA damage (Sharick et al., 2015). Moderate levels of mtDNA damage appear to be effectively repaired by base-excision repair and gap-filling pathways. The base-excision repair pathway acts on 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-oxo-7,8-dihydroguanine, products of guanine damage (David et al., 2007). The presence of metabolites of these guanine derivatives in urine is an indication that damage has been repaired (Fraga et al., 1990). When damaged mtDNA is not repaired, point mutations occur if damaged bases are replaced with an incorrect base during replication (Park and Larsson, 2011). In the gap-filling pathway, a poly (ADP-ribose) polymerase rapidly detects single-strand breaks to mtDNA. After detection, DNA polymerase β and other DNA polymerases are involved in gap filling, typically by single-nucleotide insertion. After gap filling, DNA ligase is responsible for DNA ligation (McKinnon and Caldecott, 2007).

Mitochondrial DNA can also be mutated via replication error. Mitochondria replicate by fission every 2–14 days, depending on tissue type (Miwa et al., 2008). Each time replication occurs, errors can arise through point mutations due to proofreading errors by the mtDNA polymerase or due to replication stalling, which can contribute to deletions. Like oxidative damage-induced mutations, this damage can impair the respiratory function of mitochondria. As replication is frequent, replication errors can be compounding and can contribute to a substantial change in the mtDNA sequence over time (Larsson, 2010). The impact of reproduction on the rate of mtDNA replication errors has not been evaluated, yet this would be particularly interesting to determine, given that reproduction is typically associated with organ hypertrophy, hyperplasia and increased mitochondrial biogenesis (Richardson, 1959; Speakman and McQueenie, 1996; Kunz and Orrell, 2004). Waves of tissue accretion and regression that occur with sequential reproduction could increase the rate at which errors occur relative to non-reproductive individuals or individuals with lower resource allocation to reproduction (Capuco and Ellis, 2013). Recent work in *Drosophila* suggests that the age-related decline in mitochondrial function might be more related to replication errors rather than oxidative damage (Lagouge and Larsson, 2013). By evaluating the relative number of G:C to T:A transversions (associated with oxidative damage) versus the number of G:C to A:T transitions (associated with replication errors) in young versus old flies, Itsara et al. (2014) were able to deduce that only 8% of mtDNA mutations in old flies were associated with oxidative damage, whereas 86% were associated with replication errors. These results suggest that –

at least under the conditions of their study – the impact of replication errors on mtDNA may be more important than the effects of oxidative damage.

The relative importance of accumulating mtDNA damage – whether via oxidative damage or replication error – to the function of mitochondria is an area of intense debate (Pinto and Moraes, 2015). Some studies have shown the predicted negative change in mitochondrial respiratory function with moderate levels of mtDNA damage (Trounce et al., 1989). However, other studies suggest that heteroplasmy, the condition in which one cell possesses more than one mitochondrial genome, must increase to 70–90% of mtDNA within a tissue (Fayet et al., 2002; Trifunovic and Larsson, 2008), before mitochondrial respiratory function is reduced. This level of change in heteroplasmy is not typically observed with ageing (Rossignol et al., 2003; Pinto and Moraes, 2015). As mtDNA damage does not appear to affect the bioenergetic capacity of an individual until significant damage has accumulated, mtDNA damage would be predicted to have delayed negative consequences on the bioenergetic capacity of an individual, whether it occurred through oxidative damage or mtDNA replication error. The relative importance of mtDNA damage in life-history trade-offs has been largely ignored in free-ranging animals. Although controversial, evidence that mtDNA mutations accumulate slowly over time, and only affect mitochondrial respiratory function after significant damage occurs, should be particularly intriguing for those studying the mechanistic basis for life-history interactions. The predicted pattern of change in mitochondrial respiratory function associated with mtDNA mutation mimics the pattern of change in reproductive performance and probability of survival observed in many species (Fig. 1C; Fig. 2C,D), with no immediate negative consequence of reproduction for relatively young and middle-aged females, but a subsequent reduction in reproductive performance and reduced survival in older breeders.

Finally, negative effects of reproduction on mitochondria may be masked because cells can maintain consistent ATP production when mitochondrial respiratory function is reduced by up-regulating mitochondrial biogenesis (Haden et al., 2007). Mitochondrial biogenesis is the process that stimulates the growth and division of mitochondria within a cell (Jornayvaz and Shulman, 2010). Biogenesis is typically up-regulated when the demands placed on the cell are high and/or when mitochondria display reduced function or dysfunction (Suliman et al., 2003). Biogenesis has the potential to mask both persistent and delayed negative changes in the function of mitochondria, thereby preventing mtDNA damage from negatively affecting the reproductive performance or survival of individuals. However, the capacity of an individual to produce new mitochondria may decrease with age (Piantadosi and Suliman, 2012; Chistiakov et al., 2014), thus revealing prior and new damage that may not have contributed to a reduction in performance earlier in life.

Neutral effects of reproduction on bioenergetic capacity

In many iteroparous species, females often display relatively consistent reproductive performance across multiple reproductive bouts, particularly when environmental conditions are generally favorable. In keeping with this observation, many studies have found no impact of current reproduction on the probability of survival to the next reproductive event or on future reproductive performance. For example, non-experimental studies have shown no impact of current reproduction on probability of survival or reproductive performance during the next reproductive event in Columbian ground squirrels (*Urocitellus columbianus*; Murie and Dobson, 1987), banner-tailed kangaroo rats (*Dipodomys spectabilis*; Waser and Jones, 1991), wandering albatross (*Diomedea exulans*; Weimerskirch, 1992), common side-blotched lizards (*Uta stansburiana*) and eastern fence lizards (*Sceloporus undulates*; Tinkle and Hadley, 1975; Shine and Schwarzkopf, 1992). Indeed, bearing up to 900 young seems to have no impact on longevity in naked mole-rat queens that appear to have lifespans comparable to those of sterile workers (Buffenstein, 2000; Sherman and Jarvis, 2002). In addition, a handful of studies have also shown no effect of reproduction on future performance, even when litter size was experimentally elevated, as in Columbian ground squirrels (Skibiel et al., 2013) and red squirrels (*Sciurus vulgaris*; Humphries and Boutin, 2000). The relative consistency of this pattern across many species implies that there are many processes in place that maintain homeostasis during reproduction.

The reason for the relative consistency in reproductive performance and limited survival consequences of reproduction could be that negative effects are ephemeral (Box 1) and/or negative effects are prevented by the capacity of cells and mitochondria to adapt to changing bioenergetic conditions. As outlined above, increased ROS production occurs when electrons do not move efficiently between the ETS complexes, the proton motive force becomes high and the complexes become saturated with electrons (Murphy, 2009). As long as the inflow of electrons matches the outflow of ATP, ROS production remains relatively low (Barja, 2007; Murphy, 2009). At least under relatively stable environmental and social conditions (Garratt et al., 2011), there is little reason to expect that ROS levels should increase, or that damage should accumulate, during reproduction. ROS can also induce a variety of intracellular signalling cascades that affect antioxidant capacity (D'Autreaux and Toledano, 2007), oxidative damage repair pathways (Morimoto and Santoro, 1998), cell proliferation (Le Belle et al., 2011), cell migration (Kim et al., 2009), mitochondrial biogenesis (Sano and Fukuda, 2008) and cellular apoptosis (Fleury et al., 2002). These responses to moderate ROS production have the potential to prevent or neutralize negative effects on bioenergetics and, in some cases, these responses may be responsible for positive effects (see below).

ROS-mediated induction of redox-sensitive signalling cascades adjusts the expression of antioxidant enzymes to match the redox state of the cell through nuclear factor E2-related factor 2 signalling (Nguyen et al., 2009; Ma, 2013). ROS activate the expression of several gene products involved in antioxidant defence, including superoxide dismutases, the glutathione system, catalases, hydroperoxidase I, peroxiredoxins and a regulatory RNA referred to as oxyS (Ma, 2013). When ROS levels are high and unregulated, the production of antioxidants is less effective at preventing damage; thus lipid, protein and DNA damage accumulates (Rahman, 2007). Antioxidant activities have been shown to vary between organ systems, and antioxidant enzymes have been found to display increased, stable or reduced expression during reproduction in different species (Wiersma et al., 2004; Alonso-Alvarez et al., 2006; Garratt et al., 2011, 2013). However, without data on ROS production (which is rarely reported in ecological and evolutionary physiology studies), the relative significance of antioxidant levels is difficult to interpret. Low antioxidant capacity could indicate that the defences are depleted by a high rate of ROS production, or it could indicate that defences have been down-regulated because they are not needed (Speakman and Selman, 2011; Selman et al., 2012; Metcalfe and Monaghan, 2013). However, antioxidant capacities have been shown to play an important part in reducing premature ageing processes and may play

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an important role in reducing both immediate and delayed effects of ROS production (Cui et al., 2012). In short, both low ROS production and ROS-induced changes in antioxidant capacity are at least partially responsible for the neutral effects of reproduction.

ROS-induced changes in the expression of mitochondrial uncoupling proteins (UCP1, UCP2 and UCP3) reduce the proton motive force by allowing protons to flow out of the mitochondrial intermembrane space and back into the matrix, which decreases mitochondrial production of ROS. ROS activates UCPs through the lipid peroxidation product 4-hydroxynonenal, which – along with its homologues – has been shown to induce uncoupling of mitochondria through UCP1, UCP2 and UCP3, and also through the adenine nucleotide translocase. Although the thermogenic function of UCP1 has been well characterized, a function for its homologues (UCP2, UCP3 and avian UCP) has yet to be unambiguously defined (Talbot et al., 2003; Pamplona, 2008). Some authors argue that mitochondrial uncoupling seems to be an unlikely mechanism by which animals would decrease the efficiency of energy production when metabolic demands are high, such as during reproduction (Blount et al., 2015). Two independent studies support this idea, showing that UCP levels are unchanged or even down-regulated during lactation in the rat and in the mouse (Xiao et al., 2004; A. Mowry, Energetic tradeoffs between reproduction and longevity in the house mouse (*Mus musculus*), MSc thesis, Auburn University, 2015).

Positive effects of reproduction on bioenergetic capacity

According to the predictions of life-history trade-off theory, positive correlations among energetically expensive life-history traits are unexpected (Reznick, 1985; Stearns, 1989). However, both empirical data and models that incorporate differential resource acquisition among individuals support the idea that a reproductive event could have a positive impact on future survival and reproduction (Van Noordwijk and de Jong, 1986; Houle, 1991; Olijnyk and Nelson, 2013). For example, allocation towards reproduction increases over the first few reproductive bouts in several species (Fig. 1B,C), and can increase during a female's terminal reproductive bout (Fig. 1D). The queens of both bees and ants display very high fecundity and longevity that exceeds that of sterile workers (De Loof, 2011). Greater parity is associated with lower risk of mortality in recent populations of Norwegian (Grundy and Kravdal, 2008), but not Israeli, women (Dior et al., 2013). Thus we believe that it is both important to recognize the potential for positive effects to occur and to consider the bioenergetic mechanisms that are likely to support their occurrence.

Improved reproductive performance, in many cases, is attributable to mechanisms independent of prior reproduction. For example, allocation to reproduction can increase throughout the lifespan in species with indeterminate growth (Fig. 1D), as shown in the water flea (*Daphnia pulicaria*), bluehead wrasse (*Thalassoma bifasciatum*), black rockfish (*Sebastes melanops*) and sea turtles (Schultz and Warner, 1991; van Buskirk and Crowder, 1994; Love et al., 2002; Mangel et al., 2007; Olijnyk and Nelson, 2013). These effects are primarily due to increased size and egg-carrying capacity of the reproducing female. In addition, under differential resource acquisition, some individuals acquire more resources than others, which benefits all aspects of their life history (van Noordwijk and de Jong, 1986; Houle, 1991).

Beyond accommodating changes in food availability, the physiological basis for a positive relationship between current and future reproduction and between reproduction and longevity is virtually unstudied. In species that breed continuously, individuals may incur energetic savings associated with not having to support organ hypertrophy, hyperplasia and/or biogenesis, which may contribute to improved future reproductive performance. The cost of these processes may be high in a female's first reproductive bout, but low in her subsequent bout(s), allowing her to allocate more resources towards offspring development. It is also feasible that a female could experience a positive effect that is immediately evident while simultaneously accumulating damage that ultimately contributes to a delayed impact on bioenergetic capacity. In addition, it is feasible that bioenergetic capacity can be improved when moderate levels of ROS up-regulate antioxidants, repair mechanisms and mitochondrial biogenesis (as described above).

We know from studies of moderate exercise, moderate calorie restriction and hypothermia in a diver that elevated energy expenditure can also have protective effects on the body due to both a reduction in ROS production (Powers and Jackson, 2008; Ristow and Schmeisser, 2014; mechanisms described above) and the beneficial signalling properties of ROS that occur when they are produced in a regulated manner (Fig. 3; D'Autreaux and Toledano, 2007; Ristow and Schmeisser, 2011; Schieber and Chandel, 2014; Rey et al., 2016). Indeed, both moderate exercise and moderate calorie restriction are well known to improve longevity, while sustained intense exercise and starvation can have the opposite effect, reducing an individual's lifespan. The idea that ROS can have beneficial effects with moderate production and negative effects with high production is known as mitochondrial hormesis (i.e. mitohormesis; Fig. 3). Mitohormesis could feasibly be responsible for reproduction having a positive impact on an animal's bioenergetic capacity that could allow a female to maintain or improve performance during sequential reproductive

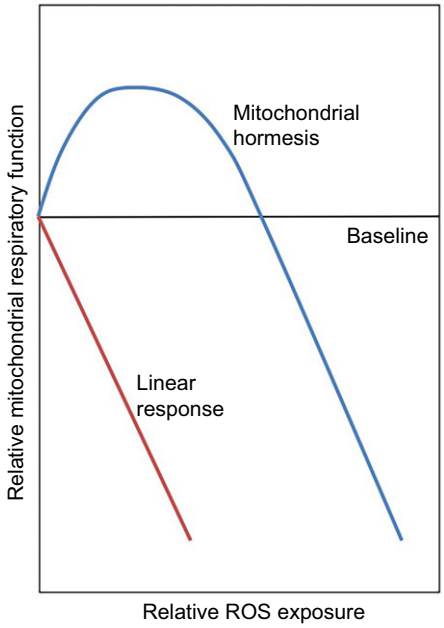

Fig. 3. The relationships between reactive oxygen species production and relative mitochondrial respiratory function under a linear response and mitochondrial hormesis. Traditional predictions suggest a linear negative relationship between increasing amounts of ROS and mitochondrial respiratory function (indicated by the red line). The concept of mitohormesis (blue curve) indicates a non-linear dose–response relationship, where low doses of ROS exposure increase mitochondrial repiratory fucntion, whereas higher doses decrease mitochondrial repiratory function. Figure modified from Ristow and Schmeisser (2014), incorporating the findings of Schulz et al. (2007), Schmeisser et al. (2013).

bouts (Romero-Haro et al., 2016). It is also possible that mitohormesis could be responsible for a positive impact of breeding on longevity in young breeders that display a U-shaped relationship between parity and mortality risk. The challenge for life-history studies is determining whether measured levels of ROS or oxidative damage are indicative of positive or negative effects on bioenergetic capacity.

Several authors have also predicted that allocation to reproduction should be enhanced during a female's last reproductive bout, i.e. terminal investment theory, as maintaining somatic tissue beyond the reproductive period will no longer improve fitness. This increase in resource allocation would allow females to incur a fitness benefit if they mobilized relatively more somatic tissue to support reproduction during this final bout than in prior reproductive events (Clutton-Brock, 1984). In support of this idea, several studies have observed enhanced reproductive performance during the terminal reproductive bout (Clutton-Brock, 1984; Poizat et al., 1999; Andrade and Kasumovic, 2005; Isaac and Johnson, 2005; Creighton et al., 2009; Weladji et al., 2010; Fisher and Blomberg, 2011). The physiological mechanisms that would allow females to increase somatic tissue mobilization in old age are unclear and warrant future study. As older individuals are expected to display a decline in several aspects of mitochondrial capacity (Balaban et al., 2005), it seems unlikely that mitochondrial hormesis would play a role in this context.

Understanding interactions between life-history variables: suggestions for future research

In this Review, we have discussed patterns of reproduction and survival over time in females and reviewed the bioenergetic mechanisms that could contribute to the observed patterns. We conclude that, based on bioenergetic mechanisms, there is no reason that reproduction must entail a cost and lead to life-history trade-offs. Indeed, in a survey of the literature, we find examples in which allocation to reproduction and probability of death are unchanged, increased or decreased between sequential reproductive events. What becomes clear from the diversity of observed patterns related to reproduction and survival is that studies evaluating the mechanisms responsible for life-history interactions should be rooted in a solid understanding of the life-history pattern of the animal so that researchers know which mechanisms to evaluate, when to collect measurements and how to interpret results.

An important caveat of this argument is that the costs of reproduction are often revealed when a female is subjected to unexpected conditions, such as poor food availability or poor food quality or a litter/clutch size that is too large for current conditions, as could occur in variable environments (Reznick, 1992; Zera and Harshman, 2001). Indeed, naturally occurring mismatches between the demands of reproduction and the amount of exogenous or endogenous resources that a female has available are thought to play an important role in the evolution of species–specific life-history patterns (Harshman and Zera, 2007). Under most conditions, females should adjust their relative investment in reproduction to a level at which the cost and benefits of breeding are optimally balanced. In many cases, the optimal level of investment will enable females to compensate for many costs associated with the event – as we have seen above – allowing a female to maintain her reproductive performance over time and potentially masking the physiological costs of the event. Thus one could argue that if a female's reproductive effort increased between sequential events, the cost of the first event could be masked by compensatory mechanisms, such as maintaining an increased mitochondrial density in cells and tissues. With an experimental increase in litter size or reduction in food availability, an animal's probability of survival and future reproductive performance will often be lower than that of non-reproductive individuals (Nur, 1984; Gustafsson, 1988; Zera and Harshman, 2001), suggesting that reproduction is costly (but see Humphries and Boutin, 2000; Skibiel et al., 2013). Yet the physiological mechanisms that underlie such experimentally induced costs may differ from the mechanisms responsible for the interactions between life-history events under natural conditions. Thus we encourage investigators to carefully consider the goals of their study and ask whether their goal is to describe the typical changes in bioenergetics that occur in a natural population or to evaluate the response to extreme conditions that are likely to be important drivers of natural selection.

To determine whether a reproductive event has had a negative, neutral or positive impact on bioenergetic capacity, it is necessary to measure mitochondrial respiratory function to determine how relative ROS levels, oxidative damage, antioxidant production, repair, replication errors and mitochondrial biogenesis have affected bioenergetic capacity. Several techniques have been used for measuring the functional capacity of mitochondria. Two of most commonly applied methods include the respiratory control ratio (RCR) and the ADP/oxygen ratio (also known as P/O ratio). The RCR is the ratio of state 3 (maximum respiratory rate) to state 4 (basal respiratory rate) respiration, representing the ability of the mitochondria to increase respiratory rate in response to newly available adenosine diphosphate (ADP; Brand and Nicholls, 2011). This method is valuable because it is sensitive to any changes in redox state and the functional capacity of the ETS. However, as substrate must be added to the mitochondria to fuel respiration, the responses that are measured will be substrate specific, and may or may not directly reflect the nutrients used to fuel activity *in vivo* (Kuzmiak et al., 2012). Moreover, because RCR is a ratio between state 3 and state 4 respiration, simultaneous changes to both state 3 and state 4 could be overlooked by RCR. As a result, we encourage researchers to report both state 3 and state 4 levels with RCR. Another method often used to investigate mitochondrial function is the P/O ratio. The P/O ratio measures the relative coupling efficiency of the mitochondria, which constrains mechanistic models of the electron-transport chain and ATP synthase (Brand, 2005; Salin et al., 2015a). Mitochondrial coupling may contribute to individual differences in performance for select life-history variables, such as growth and ageing (Speakman et al., 2004; Salin et al., 2012; but see Stier et al., 2014) – its role in the interaction among life-history variables warrants further study. Another advantage of the P/O ratio is that it can allow identification of the energy substrate used by mitochondria owing to the different respiratory quotients of different substrates (Salin et al., 2015a). The P/O ratio method characterizes the functional capacity of ATP synthase but does not characterize changes in other mitochondrial complex activities. Thus measuring enzymatic activity of individual complexes can also provide valuable information on changes in the functional capacity of the ETS (Spinazzi et al., 2012). Moreover, measurements involving proton circuit, mitochondria proton current and modular kinetics can also provide valuable information for interpreting differences in mitochondrial function (Brand and Nicholls, 2011). We encourage researchers to include multiple measures of mitochondrial function in their studies. In sum, measurements of mitochondrial bioenergetics will allow investigators to determine whether a reproductive event – and associated changes in apoptosis, biogenesis, repair, oxidative

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damage, replication error and antioxidants – has had negative, neutral or positive effects on the energetic capacity of mitochondria.

Another variable that we encourage investigators to measure directly is ROS production. Essentially no studies evaluating the relative costs of reproduction have included direct measures of ROS production. Studies have instead focused on the downstream responses to ROS production (antioxidants) and its effects (oxidative damage). Measuring ROS is important because oxidative damage and antioxidant data are difficult to interpret without this information. For example, low antioxidant production by an organ can be associated with either low ROS production or a downregulation of antioxidants, thus conserving energy and amino acids that would be used for antioxidant production for another activity. ROS production is most commonly measured using an Amplex® red hydrogen peroxide/peroxidase assay that quantifies the amount of hydrogen peroxide produced by fresh tissue. This method is limited in that it only measures hydrogen peroxide and not other forms of ROS (Degli Esposti, 2002). Alternative methods exist, but they are considerably more challenging to apply and/or are less accurate (Degli Esposti, 2002). A relatively new technique, using the probe MitoB, holds promise in allowing investigators to measure ROS production *in vivo* (Cochemé et al., 2012). Animals are injected with a ratiometric mass spectrometry probe, MitoB, that localizes in the mitochondria (Murphy and Smith, 2007). Within the mitochondria, MitoB is converted by hydrogen peroxide to a stable phenol product, MitoP. The relative amount of MitoB converted to MitoP can then be quantified to determine ROS production (Cochemé et al., 2012). An important drawback of this method is that the animal must be sacrificed to recover MitoP.

To successfully evaluate putative interactions between life-history traits, careful experimental design is vital. The timing of data collection is essential in understanding the impact that current reproduction has on future reproduction and performance of an animal. Most studies designed to evaluate the mechanisms responsible for trade-offs between reproduction and longevity have either compared reproductive and non-reproductive animals or animals with varying levels of reproductive performance (Speakman and Garratt, 2014; Blount et al., 2015), and samples have largely been collected during the reproductive event for reproductive animals (Speakman and Garratt, 2014; Blount et al., 2015). Although negative consequences of reproduction may be initiated during this period, reproduction is typically a period of high physiological and morphological plasticity for females, with some organs experiencing hypertrophy or hyperplasia (Martin et al., 1973; Yu and Marquardt, 1974; Shynlova et al., 2010), while the function of other organs is down-regulated. As a result, it is impossible to untangle changes that support the metabolic demand of reproduction from changes that could have persistent effects on a female's probability of survival or on her fitness after the reproductive bout has ended. At the end of the reproductive event, enlarged tissues and other physiological adaptations return to their pre-reproduction state (Dawson et al., 2001; T. Vaskivuo, Regulation of apoptosis in the female reproductive system, PhD dissertation, University of Oulu, 2002). If animals or tissues can only be sampled once, we recommend that investigators carefully consider the timing of sample collection, taking into account the life-history pattern of their study animal and whether they are interested in targeting neutral, positive, persistent negative or delayed negative effects of reproduction when designing their studies. For the first three effects, we recommend sampling after the reproductive event has ended and reproductive tissues have regressed. We also recommend quantifying survival and reproductive performance of the same individuals, or a subset of similar individuals, through the next reproductive event. However, to evaluate delayed effects, the impact of breeding should be measured in relatively old females, and effects could be compared between reproductive and non-reproductive females or among females with differences in levels of lifetime reproductive performance. If it is possible, longitudinal sampling provides the most informative data and could allow investigators to track the onset of delayed negative effects of reproduction. For many species, longitudinal sampling can only be accomplished by collecting blood. Some of the products of oxidative damage in organ systems are detectable in the blood; thus blood may allow for a cumulative assessment of what is going on throughout the body. However, it is important to keep in mind that different organs may have different bioenergetic responses to a reproductive event, which limits our ability to interpret data collected from blood (Speakman and Garratt, 2014; Blount et al., 2015). Birds, reptiles and amphibians all appear to have active mitochondria within their erythrocytes, whereas mammals do not (Stier et al., 2013). Thus changes in the bioenergetic capacity of erythrocytes could provide valuable data for evaluating bioenergetic responses to reproduction (Stier et al., 2015); however, interpretation would remain limited to the effect of reproduction on erythrocyte mitochondria.

'Big data' analysis, such as transcriptomic, proteomic and metabolomic analyses, should also help to uncover the mechanisms responsible for life-history interactions. High-throughput screening, next-generation sequencing and pathway analysis could reveal physiological differences between and within species (Macarron et al., 2011; Khatri et al., 2012), as well as highlighting genetic correlates with life-history patterns, which undoubtedly play an important role in individual differences in reproductive output and pace of life, but are beyond the scope of this Review. Nevertheless, such approaches should complement more traditional assays. A recent proteomics analysis revealed that oxidative balance-related proteins in the livers of laboratory mice were not affected by a litter size manipulation, whereas both calcium metabolism (controlling cellular apoptosis) and cell growth are negatively affected in females with larger litters (Plumel et al., 2014). It is not known whether the changes to the cell growth and apoptosis pathways that were negatively affected in females with large litters persisted after the reproductive event had ended. Unfortunately, this study was conducted at weaning; thus processes associated with tissue regression at the end of the reproductive event cannot be uncoupled from any impact that reproduction will have on future performance. The timing of this study highlights the importance of carefully considering the appropriate time to collect samples, even when using these big data approaches.

Conclusions

The goal of this Review was to articulate two key points. Firstly, life-history patterns are highly variable among species. Studies that attempt to explain the interactions among life-history variables should acknowledge this diversity and not assume that reproduction inevitably has negative consequences on future performance. Secondly, when negative effects do arise, it is important to consider that these effects could be ephemeral or associated with a delayed response that has accumulated over time. We hope that this Review will encourage investigators to consider including measures of oxidative repair, mitochondrial biogenesis, cell proliferation, mtDNA mutation and replication error and, importantly, a measure of the respiratory function of mitochondria (e.g. RCR) to determine whether measured differences among groups are associated with a change in the energetic capacity of tissues. By embracing the diversity in the

life-history patterns of animals and carefully considering the mechanisms that could underlie this diversity, a better fundamental understanding of life-history strategies is possible.

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