Integrative and Comparative Biology

Integrative and Comparative Biology, volume 59, number 4, pp. 994–1004
doi:10.1093/icb/icz015

Society for Integrative and Comparative Biology

SYMPOSIUM

Hypothesized Evolutionary Consequences of the Alternative Oxidase (AOX) in Animal Mitochondria

Ryan J. Weaver[1]

Department of Biological Sciences, Auburn University, 331 Funchess Hall, Auburn, AL 36849, USA

From the symposium "Beyond the powerhouse: integrating mitonuclear evolution, physiology, and theory in comparative biology" presented at the annual meeting of the Society of Integrative and Comparative Biology, January 3–7, 2019 at Tampa, Florida.

[1]E-mail: RyanJWeaver1@gmail.com

Synopsis The environment in which eukaryotes first evolved was drastically different from what they experience today, and one of the key limiting factors was the availability of oxygen for mitochondrial respiration. During the transition to a fully oxygenated Earth, other compounds such as sulfide posed a considerable constraint on using mitochondrial aerobic respiration for energy production. The ancestors of animals, and those that first evolved from the simpler eukaryotes have mitochondrial respiratory components that are absent from later-evolving animals. Specifically, mitochondria of most basal metazoans have a sulfide-resistant alternative oxidase (AOX), which provides a secondary oxidative pathway to the classical cytochrome pathway. In this essay, I argue that because of its resistance to sulfide, AOX respiration was critical to the evolution of animals by enabling oxidative metabolism under otherwise inhibitory conditions. I hypothesize that AOX allowed for metabolic flexibility during the stochastic oxygen environment of early Earth which shaped the evolution of basal metazoans. I briefly describe the known functions of AOX, with a particular focus on the decreased production of reactive oxygen species (ROS) during stress conditions. Then, I propose three evolutionary consequences of AOX-mediated protection from ROS observed in basal metazoans: 1) adaptation to stressful environments, 2) the persistence of facultative sexual reproduction, and 3) decreased mitochondrial DNA mutation rates. Recognizing the diversity of mitochondrial respiratory systems present in animals may help resolve the mechanisms involved in major evolutionary processes such as adaptation and speciation.

Introduction

The origin of eukaryotes and their ubiquitous mitochondrial respiration has been proposed to be linked to the great oxygenation event nearly 2.5 billion years ago (Kump 2008; Lyons et al. 2014; Erwin 2015). Atmospheric oxygen went from essentially nonexistent to about 0.1% of present atmospheric levels (PALs) and set the course for the evolution of complex life (Lane 2014; Knoll and Nowak 2017). Evidence for the existence of animals didn't appear until after a second O_2 increase in the Neoproterozoic Era, where O_2 reached ~3% PAL (Och and Shields-Zhou 2012; Knoll and Nowak 2017). It is thought that O_2 limitation constrained the evolution of larger complex animals from their relatively simpler eukaryotic progenitors (Lane 2014). With these increases in O_2 levels came the vastly increased capacity to produce cellular energy (ATP) by oxidative phosphorylation (Lane and Martin 2010; Lane 2014). However, Earth's oxygenation did not occur instantaneously, nor continuously. Instead, geologic evidence shows a fluctuating temporal pattern of O_2 availability before a steady state was achieved (Fig. 1; Johnston et al. 2012; Lyons et al. 2014). The increased O_2 levels came with a concomitant reduction in hydrogen sulfide levels (Olson and Straub 2016). As the earliest metazoans evolved in response to the newly oxygenated world they faced stochastic oxygen and hydrogen sulfide environments which are agonists and antagonists of canonical mitochondrial respiration, respectively (Nicholls et al. 2013). How then, could early metazoans reap the benefits of mitochondrial respiration without incurring the energetic costs of a highly fluctuating oxygen environment? And what

Advance Access publication April 27, 2019

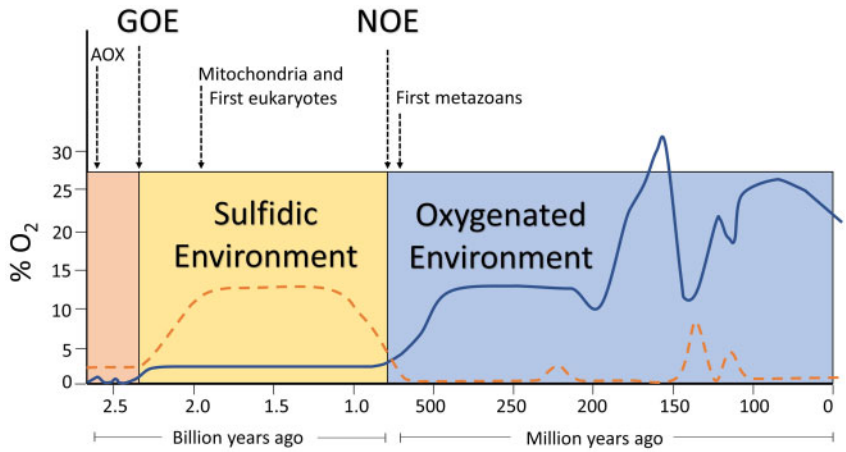

Fig. 1 A timeline of Earth's oceanic H_2S (dashed line) and atmospheric O_2 content (solid line). AOX, alternative oxidase; GOE, great oxygenation event; NOE, neoproterozoic oxygenation event. Redrawn from Olson and Straub (2016). This figure does not include all Earth events or timepoints at the local scale and is meant only to be a general timeline. A color version of this figure is available online.

physiological mechanisms facilitated aerobic respiration in such harsh conditions?

In this essay, I describe a perhaps lesser-known component of eukaryotic mitochondria that I argue was critical to the evolution of animals: the alternative oxidase (AOX). Next, I outline three evolutionary consequences of AOX in animals that are based on the functional outcomes of AOX respiration (described below). Specifically, that AOX mediated 1) adaption to harsh environments of early Earth, 2) the evolution of a facultatively sexual mode of reproduction, and 3) mutation accumulation rate, with a specific focus on "basal" metazoans. (I use the term "basal" to describe taxa from Porifera, Placozoa, Ctenophora, and Cnidaria phyla). I also propose some experiments to test these hypotheses and conclude with a call for the need to consider the role of AOX in physiology and mitochondrial research.

Mitochondrial respiration and the AOX

The catalytic core of mitochondrial respiration is the electron transport chain (ETC), which is made up of sets of protein complexes (Complexes I–IV) that reside in the inner mitochondrial membrane along with two mobile electron carriers, ubiquinone and cytochrome c (Fig. 2A). This system harvests electrons from reducing equivalents derived mostly from the tricarboxylic acid cycle to create a transmembrane proton gradient that is utilized by Complex V to synthesize ATP. In addition to producing ATP, the ETC is the primary source of reactive oxygen species (ROS). Any amount of ROS is, in principle, detrimental to cells by their potential for indiscriminate oxidation of macromolecules. However, eukaryotes have evolved mechanisms that integrate low levels of ROS as signals that regulate cellular function and moderate ROS levels have been shown to improve organismal and mitochondrial performance (Zhang et al. 2018a, 2018b) a process called hormesis (Costantini 2014). However, when ROS is produced in excessive amounts, it can lead to oxidative damage to proteins, lipids, and nucleic acids and has deleterious effects at the cellular and organismal levels.

The mitochondrial ETC is typically depicted as an unbranched respiratory chain in which electrons enter through Complexes I and II, are passed to the ubiquinone pool, then follow the cytochrome pathway to Complex IV (cytochrome c oxidase) where they are used to reduce oxygen to water (Fig. 2A). This textbook figure of the ETC is modeled after mammalian mitochondria, which misrepresents the diversity of ETC pathways that exist in eukaryotes, such as alternative electron entry and exit points (Matus-Ortega et al. 2011; McDonald and Gospodaryov 2019), and perhaps has limited our capacity to explore some fundamental aspects of animal evolution.

Basal metazoan taxa have distinct mitochondrial characteristics that deviate from typical patterns described in later-evolving animal lineages. One such deviation of basal metazoan mitochondria is the presence of a branched respiratory chain that utilizes a nuclear-encoded alternative terminal oxidase (AOX) in addition to cytochrome c oxidase (Complex IV) (Fig. 2B; Abele et al. 2007; McDonald and Gospodaryov 2019). AOX creates a branching point in the ETC; electrons passed to the ubiquinone pool can follow either the classic cytochrome pathway

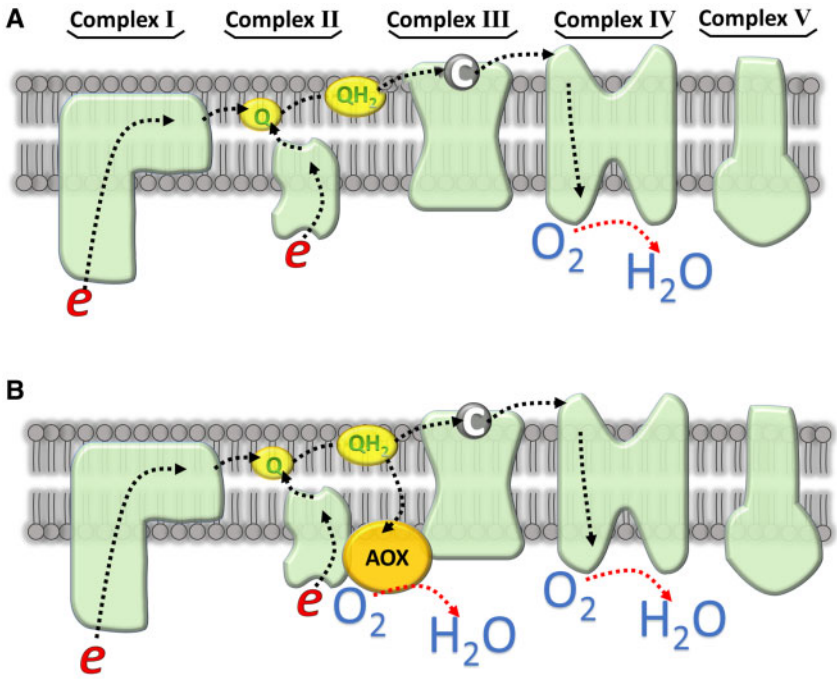

Fig. 2 Depiction of the mitochondrial ETC showing the typical unbranched (**A**) and branched (**B**) composition. AOX is an alternative terminal oxidase found in mitochondria of many metazoan taxa; e is an electron; QH_2 is ubiquinol; and C is cytochrome c. A color version of this figure is available online.

(Complexes III and IV) or the AOX pathway to reduce molecular oxygen to water (Arnholdt-Schmitt et al. 2006; Abele et al. 2007; Vanlerberghe 2013).

AOX was discovered in animals in the early 2000s (McDonald and Vanlerberghe 2004); previously AOX was thought to be restricted to bacteria, plants, and fungi (McDonald and Vanlerberghe 2006; McDonald et al. 2009, 2015). AOX is present in some modern α-proteobacteria (McDonald et al. 2003; Stenmark and Nordlund 2003; Atteia et al. 2004), the proposed source of the mitochondrion, which suggests that eukaryotic AOX originated from the endosymbiosis that gave rise to the mitochondrion and eukaryotes (Finnegan et al. 2003; McDonald 2008). The observation that the putative earliest diverged eukaryote, a jakobid *Reclinomonas americana*, has AOX further supports the endosymbiotic origin of AOX (McDonald 2008). To date, AOX sequences have been detected in over 150 animal taxa with representatives from every metazoan phylum, but AOX appears to be absent from vertebrates (McDonald and Gospodaryov 2019). Such a wide distribution of AOX across the evolutionary tree suggests that, if these sequences code for functional proteins, AOX respiration is adaptive and selection has favored its retention in certain animal taxa.

Much of what is known of the physiological roles of AOX comes from studies on non-animal taxa (Maxwell et al. 1999; Van Aken et al. 2009; Vanlerberghe 2013; Rogov et al. 2014; Selinski et al. 2018). The overarching consequence of AOX activation is preventing the overproduction of ROS (Maxwell et al. 1999; Fernandez-Ayala et al. 2009; McDonald et al. 2009; Van Aken et al. 2009; Cvetkovska and Vanlerberghe 2012; Munro et al. 2013). Because it bypasses proton-pumping Complexes III and IV, AOX respiration is partially uncoupled from the generation of ATP (McDonald and Vanlerberghe 2006). Therefore, Complex I is the sole contributor to the requisite proton-gradient across the inner mitochondrial membrane and as a result, ATP production from AOX respiration is about one-third of that from the cytochrome pathway (Millenaar and Lambers 2003). Additionally, plants and some animals also have NADH dehydrogenases that act as an alternative to Complex I; when AOX and these alternative dehydrogenases are activated together, electron transport can be completely decoupled from ATP production (Matus-Ortega et al. 2011; McDonald and Gospodaryov 2019). However, the diminished capacity for energy production comes with the benefit of preventing the overproduction of ROS. Several papers have described the known details of the biochemistry of AOX-mediated decrease in ROS production (e.g., Maxwell et al. 1999; Van Aken et al. 2009; Dahal and Vanlerberghe 2017; Szibor et al. 2017), so I will only briefly summarize it here. When the cellular

redox state or the accumulation of some retrograde signal (e.g., purines, α-keto acids) reaches a sufficient threshold (McDonald 2008; Rogov et al. 2014), AOX is activated and electrons are partitioned away from the cytochrome pathway. AOX activation has been shown to prevent excessive ROS during stress, in part by preventing the over-reduction (i.e., charged with electrons) of the ubiquinone pool by maintaining electron flow through the ETC where AOX passes electrons to oxygen (Maxwell et al. 1999; McDonald et al. 2009; El-Khoury et al. 2013; Vanlerberghe 2013; Dominiak et al. 2018). At the same time, the inner mitochondrial membrane potential is decreased by uncoupling electron transport from ATP production. AOX functions similarly to uncoupling proteins by preventing an excessively high mitochondrial membrane potential and thus decreases ROS production (Brand 2000; Brookes 2005; Woyda-Ploszczyca and Jarmuszkiewicz 2017). AOX-mediated ROS decrease has been shown to protect against oxidative damage to proteins and also to maintain mitochondrial function in plants under drought conditions that caused the cytochrome pathway to shutdown (Dahal and Vanlerberghe 2017).

The key attribute I will focus on in this essay is that AOX respiration prevents the overproduction of ROS under stress conditions. While the term "stress" is used in a variety of contexts, I am using "stress" to describe conditions under which the cytochrome c pathway is unavailable. In the canonical ETC, when the cytochrome pathway is unavailable, respiration halts because electron flow is stalled at ubiquinone or Complex III. As a result, electrons either escape Complex III, or electron transport is reversed and flow back to Complex I and ROS production is increased. For example, overproduction of ROS and ETC dysfunction are a common consequence of environmental stressors that disrupt the cytochrome pathway such as hypoxia, xenobiotic exposure, and temperature, salinity, and pH fluctuations (Maxwell et al. 1999; Arnholdt-Schmitt et al. 2006; Clifton et al. 2006; Sierra-Campos et al. 2009; Van Aken et al. 2009; Sussarellu et al. 2013). Taken together, AOX activation has physiological effects that are hypothesized to be adaptations to environmental stress.

The role of AOX in the evolution of animals and adaptation to stressful environments

The first evolutionary consequence of AOX I propose is that it facilitated the evolution of animals as we know them. AOX is commonly noted for its capacity to catalyze oxidative metabolism in the presence of cyanide because Complex IV is inhibited by cyanide, whereas AOX is not (Vanlerberghe and McIntosh 1997; Siedow and Umbach 2000). But an attribute that deserves more emphasis from an evolutionary perspective is AOX's resistance to sulfide inhibition (Volkel and Grieshaber 1996; McDonald and Vanlerberghe 2004; Searcy 2006; McDonald et al. 2009). Both cyanide and sulfide block the cytochrome c pathway by inhibiting Complex IV, shutting off the utilization of oxygen as the terminal electron acceptor (Vanlerberghe and McIntosh 1997; Nicholls et al. 2013). I propose that during the evolution of early metazoans, sulfide-resistant respiration was perhaps the most critical function of AOX, which would allow for oxidative metabolism—and more production of ATP—under otherwise inhibitory conditions.

Early animals likely had a complex array of cellular solutions to the hypoxic, anoxic, and euxinic (sulfide-rich) environments they faced (Johnston et al. 2012; Erwin 2015; Knoll and Nowak 2017) and many of those traits have been retained in extant taxa that occupy similar environments (Grieshaber and Volkel 1998; Lushchak 2011; Mentel et al. 2014; Tobler et al. 2016). These include anaerobic metabolic pathways, hypoxia-inducible factors, and sulfide detoxification. The latter requires both oxygen and a functional ETC (Volkel and Grieshaber 1996; Searcy 2006). Sulfide detoxification by sulfide: quinone oxidoreductase (SQR) yields thiosulfate and passes electrons from sulfur to Complex IV through ubiquinone and Complex III to reduce O_2 to water. However, relatively high concentrations of sulfide (10–20 μM) inhibit Complex IV and *t* (Volkel and Grieshaber 1996; Searcy 2006; Kelley et al. 2016); without an alternative path for electrons to flow from SQR, sulfide detoxification halts, as does oxidative metabolism (Volkel and Grieshaber 1996; Searcy 2006). Relatively high sulfide levels and transitions between anoxic and hypoxic conditions are the environment in which early metazoans are predicted to have faced (Johnston et al. 2012; Och and Shields-Zhou 2012; Erwin 2015; Reinhard et al. 2016; Knoll and Nowak 2017). Therefore, because AOX is insensitive to hydrogen sulfide (Volkel and Grieshaber 1996; McDonald and Vanlerberghe 2004), AOX respiration allows for oxidative metabolism and sulfide detoxification under sulfide concentrations that inhibit Complex IV (Volkel and Grieshaber 1996; Grieshaber and Volkel 1998; Searcy 2006). This suggests that AOX likely played a critical role in mediating the balance between enhanced ATP production from aerobic respiration

and the environmental conditions that would otherwise allow only anaerobic metabolism (McDonald and Vanlerberghe 2004; Searcy 2006; McDonald et al. 2009). The enhanced ATP production by oxidative metabolism relative to anerobic metabolism has been proposed to be a causal factor in the evolution of multicellular complex animals (Lane 2014). Simply put, without AOX, oxidative metabolism using the classical mitochondrial ETC—which relies on the cytochrome pathway—would not be possible in early metazoan environments.

Given the proposed role of AOX in mediating the transition from sulfide-rich to oxygen-rich environments, it may not be surprising that many extant animals that experience hypoxic and or sulfidic conditions also have AOX. For example, burrowing and benthic organisms such as marine worms and bivalves have AOX sequences in their genomes (Volkel and Grieshaber 1996; Tschischka et al. 2000; Abele et al. 2007; Huang et al. 2013; Munro et al. 2013; Robertson et al. 2016). McDonald and Gospodaryov (2018) hypothesized that animals that occupy dynamic oxygen environments, such as benthic and intertidal habitats, have retained AOX because of its protective role against ROS-based damage during the hypoxic–reoxygenation transition. Recently, the marine copepod, *Tigriopus californicus*, which lives in shallow splash pools that fluctuate wildly in temperature, salinity, and oxygen content, has had AOX detected in its genome, and that AOX transcription is upregulated under stress conditions which resulted in increased AOX protein levels (Tward et al. 2019). As a greater diversity of genomic sequence data become available for non-model organisms, the extent to which AOX is distributed among animal taxa and how it plays a role in mediating environmental stress will become clearer.

Facultative sexual reproduction in animals is facilitated by AOX

Just as mitochondria are a hallmark of eukaryotes, so too is sexual reproduction; with rare exception, every eukaryotic lineage undergoes sexual reproduction, at least at some point along its evolutionary history (Goodenough and Heitman 2014; Speijer et al. 2015). However, the mode of reproduction an animal uses presents a conundrum in evolutionary biology. Sexual reproduction is thought to be beneficial primarily because it can create new nuclear genomic combinations through meiotic recombination (Muller 1932; Maynard Smith 1978; Hamilton et al. 1990). On the other hand, asexual reproduction is faster and allows genes to be inherited intact, and theory suggests that asexuals should quickly outcompete sexuals if all else is equal (Maynard Smith 1978). However, the fitness of asexual reproduction is dependent on how well-suited the genotype of asexual individuals is to the current environment (Morran et al. 2011; Luijckx et al. 2017). Moreover, asexual populations accumulate mildly deleterious mutations which cannot be removed by natural selection unless the entire genotype (i.e., population) is selected against leading to an evolutionary dead-end (Muller 1964; Maynard Smith 1978; Engelstädter 2008). In contrast, in sexual taxa, when "unfavorable" conditions arise and cause a mismatch between genotype and environment, new nuclear gene sets created by meiotic recombination and nuclear fusion of haploid gametes (i.e., sex) can be filtered for compatibility with the current environmental conditions by natural selection without selecting on unrelated parts of the genome (Hill and Robertson 1966; Felsenstein 1974).

Another shared characteristic among basal metazoans is a facultatively sexual reproductive strategy (Fautin 2002; Maldonado and Riesgo 2008). In contrast to obligate sex, taxa that are facultatively sexual undergo asexual reproduction without recombination (e.g., budding, fission) under certain conditions, and switch to sexual reproduction after the occurrence of some environmental or endogenous cue. Terms commonly used to describe conditions under which asexual and sexual reproduction takes place are "favorable" and "unfavorable," respectively (Loomis and Lenhoff 1956; Felsenstein 1974; Nedelcu and Michod 2003; Neiman et al. 2014). For example, temperature stress, nutrient limitation, UV exposure, and hypoxia have been shown to trigger the switch from asexual to sexual reproduction in some animals (Loomis and Lenhoff 1956; Park and Ortmeyer 1972; Nedelcu and Michod 2003; Burke and Bonduriansky 2017).

While different for many taxa, these unfavorable conditions generally describe instances that are likely to cause increased ROS production, and mitochondria—specifically via the ETC—are the primary generators of endogenous ROS (Liu et al. 2002; Indo et al. 2007; Zapico and Ubelaker 2013). Elevated ROS levels have been proposed to be one of the signals that indicate environmental stress and thus initiates the switch to sex (Goodenough and Heitman 2014; Speijer et al. 2015; Hörandl and Speijer 2018). The link between ROS and sex could be due to the notion that ultimately, sex is a repair mechanism (Bernstein et al. 1981; Goodenough and Heitman 2014; Speijer et al. 2015), and environmentally-

induced ROS-based damage to cellular components, including double strand breaks and other DNA damage (Shadel and Clayton 1997; Nedelcu et al. 2004), could act as a trigger for genomic repair by meiotic recombination (i.e., sex).

In a series of studies, Nedelcu and colleagues experimentally showed that the switch from asexual to sexual reproduction in a facultatively sexual green alga (*Volvox carteri*) was triggered in response to increased endogenous ROS production by heat stress (Nedelcu and Michod 2003; Nedelcu et al. 2004; Nedelcu 2005). Administration of exogenous antioxidants under the same conditions prevented the switch to sexual reproduction. *Volvox* have AOX (Neimanis et al. 2013) and AOX has been shown to prevent overproduction of ROS in plants (Siedow and Girvin 1980; Considine et al. 2002; Watanabe et al. 2008; Cvetkovska and Vanlerberghe 2012), but how this alternative electron pathway might mediate the relationship between oxidative stress and sexual reproduction in this species or any other taxa has not been investigated.

I propose that AOX acts to increase the breadth of environmental/physiological conditions under which asexual reproduction is beneficial by keeping ROS levels below the "trigger point" that initiates the switch to sex. Under this model, a facultatively sexual organism that lacks AOX reproduces asexually until an environmental stressor increases to some level, *s*. At *s*, ROS production reaches the point that triggers the switch to sex, denoted as *i* (Fig. 3). A facultatively sexual organism that has AOX also switches to sex at *i*; however, the switch point does not occur until environmental stressor level $s + x$ (Fig. 3). AOX keeps ROS below the trigger point to switch to sex under a wider range of environmental conditions.

The evolution of mitochondria is linked to the evolution of sex as both originated with the eukaryotes (Lane 2014; Speijer et al. 2015; Speijer 2016), and sex has been proposed to have evolved in part to respond to oxidative stress (Nedelcu and Michod 2003; Nedelcu et al. 2004; Hörandl and Speijer 2018). Several hypotheses have proposed that mitochondria played a direct role in the evolution of sex (Havird et al. 2015; Radzvilavicius and Blackstone 2015; Garg and Martin 2016; Hörandl and Speijer 2018). Similar to the idea that sex is triggered as a part of a stress response, AOX respiration mediates the response to stress in plants, fungi, and animals by decreasing ROS production (Clifton et al. 2006; Van Aken et al. 2009; Sussarellu et al. 2013) and may play a role in the maintenance of a facultative sexual reproductive strategy.

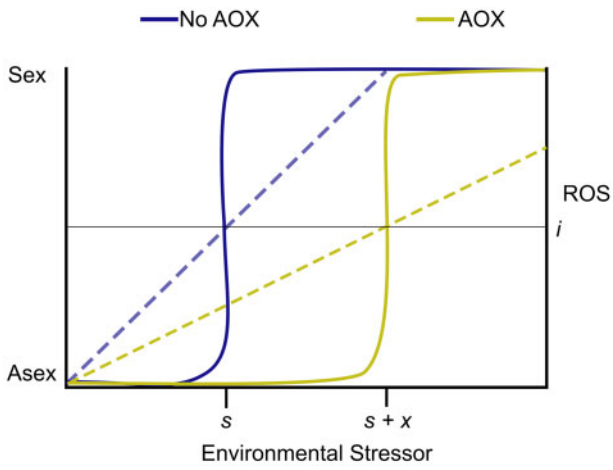

Fig. 3 A model for how AOX mediates the response to stress and the switch to sex in facultative species. The amount of environmental stress that triggers sex with AOX present ($s + x$, right, solid curve) is higher than that at which sex is triggered in the absence of AOX (*s*, left, solid curve). This model predicts that ROS levels (dashed lines) will be the same at both switch points (*i*, right axis). A color version of this figure is available online.

Testing this AOX–Sex hypothesis could be as simple as inhibiting or knocking out AOX in a facultatively sexual animal and comparing the stress and ROS levels at which gametogenesis occurs to the wild type animal that has AOX. For example, the sea anemone, *Nematostella vectensis* (which has AOX, McDonald et al. 2009), can be experimentally induced to produce gametes in part by a 10°C increase in temperature for 6 h (Fritzenwanker and Technau 2002; Genikhovieh and Technau 2009). The AOX–Sex hypothesis predicts that without AOX, the switch to sex would occur at a lower temperature stress and/or a shorter duration of exposure, but that ROS production would reach the same level as the wild type animal (Fig. 3).

AOX, mitochondrial mutation rates, and why DNA barcoding fails in basal metazoans

The advent of DNA barcoding that was popularized during the early 2000s took advantage of the observation that in many animals, a portion of the mitochondrial gene cytochrome c oxidase subunit I (COI, a part of Complex IV of the ETC) is strikingly similar among individuals within a taxonomically described species (~3% genetic distance divergence, Hebert et al. 2003). Individuals from a diverged population or species have its own COI sequence similarity (i.e., barcode) that differs from other species (10–25% genetic distance divergence, Hebert et al.

2003). Thus, species could be delimited by a barcode "gap"; where the interspecific genetic distances of COI are greater than the COI sequence variation observed among individuals within a species (Meyer and Paulay 2005).

COI barcode gaps match species boundaries in the majority of cases, especially among vertebrates (Bucklin et al. 2011). Notably, however, basal metazoans do not follow this same pattern; interspecific COI variation overlaps considerably with variation within species. DNA barcoding fails so remarkably in basal metazoans that they have been described as "the problem children" (Bucklin et al. 2011). Closely related species within these groups lack the distinct barcode gaps observed among other taxa. At the proximate level, there is an obvious reason why this pattern is observed: mtDNA mutation rates are much slower among basal metazoans than bilaterian animals. For example, mutation rates in COI are up to 20 times lower for poriferans than bilaterians (Shearer et al. 2002) and very low levels of COI variation exist within most cnidarian taxa, particularly in anthozoans (Huang et al. 2008). COI sequences were found to be invariant in close to 50% of the cases examined by Huang et al. (2008). But at the ultimate level, why is this the case? Here, I am proposing that AOX respiration and the protection it confers from ROS is the basis for low mtDNA mutation rates in basal metazoans.

mtDNA mutation accumulation has previously been linked to oxidative damage from ROS and insertion or deletion errors during the repair of oxidized nucleotides. Excessive ROS accumulation within cells catalyzes chain-reactions that produce DNA-damaging hydroxyl radicals that induce double strand breaks and oxidative lesions (Lagouge and Larsson 2013; Saki and Prakash 2017). Damaged DNA must be repaired before replication and errors in the repair process are one way that mutations accumulate (Shadel and Clayton 1997; Kazak et al. 2012; DeBalsi et al. 2017). Moreover, oxidative stress from excessive ROS has been shown to increase basal mutation rates by inducing downregulation of repair processes involved in correcting mismatched nucleotides (Gutierrez et al. 2013). ROS production contributes to the background oxidation state of a cell (Kamata and Hirata 1999; Ray et al. 2012; Sies 2015) and prior work has shown that the cellular redox state significantly mediates mutation rates (Jee et al. 2016). Jee et al (2016) found that experimentally decreasing ROS production in *Escherichia coli* under antibiotic stress decreased mutation rates by a factor of 8 compared with wildtype bacteria.

Slowly-evolving mtDNA has been argued to be the ancestral state in metazoans (Shearer et al. 2002; Huang et al. 2008) as has the presence of AOX (discussed above). It is enticing to speculate that AOX may play a role in the observed low mtDNA mutation rates of some basal metazoans. One way to assess this putative relationship would be to map mtDNA mutation rates onto the phylogeny of animals that have functional AOX. Do those animals that have low mutation rates also have AOX? For example, *Acropora* coral species have low mtDNA mutation rates (Fukami et al. 2000; van Oppen et al. 2001) and also have AOX sequences in their genome (Technau et al. 2005; McDonald et al. 2009). The same holds true for many poriferans, anthozoans, hydrozoans, and placozoans. While this relationship, if it exists, precludes inference of any mechanism that may link the two characters it would provide some support to the idea that AOX may be playing a role. However, I note that high mutation rates do not require an absence of AOX. These patterns could emerge from a number of mechanisms: differences in mtDNA base-repair efficiencies, differences in environmental fluctuations/stress, and differences in the strength of purifying selection on mtDNA. A perhaps better test of the AOX-mutation rate hypothesis would be to experimentally remove AOX from a taxon that normally expresses it and also has low mtDNA mutation rates. For example, would AOX genetic knockout lineages of *N. vectensis* accumulate mtDNA mutations at a higher rate than wild type anemones? Sequence-based approaches for estimating mutation rates may be a powerful method to test this AOX-mutation rate hypothesis (Jee et al. 2016; Sloan et al. 2018).

Concluding remarks

The relatively recent discovery of AOX in animals perhaps belied its role in adaptation to stressful environments. Further, its likely ancient origin puts AOX at the inception of eukaryotes and I suggest AOX played a critical role in the evolution of complex life during the transition to a fully oxygenated Earth. A major challenge in assessing the contribution of AOX to the above hypothesized arenas is the disconnect between detecting AOX at the sequence level and that sequence coding for a functional protein. In addition, while the details of AOX regulation have been revealed to be complex and variable in plants and fungi (Vanlerberghe and McIntosh 1997; Szal et al. 2003; McDonald et al. 2009), very little information is available on the post-transcriptional and post-translational regulation of AOX in animals.

Non-invasive isotope-discriminating techniques that quantify metabolic flux through AOX and cytochrome pathways (Del-Saz et al. 2018) are well-suited to fill in these gaps. While physiological experiments such as oxygen consumption assays in the presence of known AOX inhibitors or using AOX knockout lineages could be useful to understanding how AOX mediates the response to environmental stress. Aside from the hypotheses I proposed above, I hope that future research will consider the contribution of AOX and other alternative mitochondrial proteins (e.g., Matus-Ortega et al. 2011) to animal physiology and mitochondrial performance.

Acknowledgments

I thank three anonymous reviewers for their insightful comments, which improved the quality of this manuscript.

Funding

This work was supported by the National Science Foundation [IOS-1839203], the Company of Biologists [EA1694], the Crustacean Society, and the Divisions of Comparative Physiology and Biochemistry, Invertebrate Zoology, and Phylogenetics and Comparative Biology in the form of travel and support funds to attend the 2019 meeting of the Society for Integrative and Comparative Biology.

References

Abele D, Philipp E, Gonzalez PM, Puntarulo S. 2007. Marine invertebrate mitochondria and oxidative stress. Front Biosci 12:933–46.

Arnholdt-Schmitt B, Costa JH, de Melo DF. 2006. AOX—a functional marker for efficient cell reprogramming under stress? Trends Plant Sci 11:281–7.

Atteia A, Van Lis R, Van Hellemond JJ, Tielens AGM, Martin W, Henze K. 2004. Identification of prokaryotic homologues indicates an endosymbiotic origin for the alternative oxidases of mitochondria (AOX) and chloroplasts (PTOX). Gene 330:143–8.

Bernstein H, Byers GS, Michod RE. 1981. Evolution of sexual reproduction: importance of DNA repair, complementation, and variation. Am Nat 117:537–49.

Brand MD. 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Exp Gerontol 35:811–20.

Brookes PS. 2005. Mitochondrial H+leak and ROS generation: an odd couple. Free Radic Biol Med 38:12–23.

Bucklin A, Steinke D, Blanco-Bercial L. 2011. DNA barcoding of marine metazoa. Ann Rev Mar Sci 3:471–508.

Burke NW, Bonduriansky R. 2017. Sexual conflict, facultative asexuality, and the true paradox of sex. Trends Ecol Evol 32:646–52.

Clifton R, Millar AH, Whelan J. 2006. Alternative oxidases in *Arabidopsis*: a comparative analysis of differential expression in the gene family provides new insights into function of non-phosphorylating bypasses. Biochim Biophys Acta Bioenerg 1757:730–41.

Considine MJ, Holtzapffel RC, Day DA, Whelan J, Millar AH. 2002. Molecular distinction between alternative oxidase from monocots and dicots. Plant Physiol 129:949–53.

Costantini D. 2014. Oxidative stress and hormesis in evolutionary ecology and physiology: a marriage between mechanistic and evolutionary approaches. Berlin Heidelberg: Springer.

Cvetkovska M, Vanlerberghe GC. 2012. Alternative oxidase modulates leaf mitochondrial concentrations of superoxide and nitric oxide. New Phytol 195:32–9.

Dahal K, Vanlerberghe GC. 2017. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. New Phytol 213:560–71.

DeBalsi KL, Hoff KE, Copeland WC. 2017. Role of the mitochondrial DNA replication machinery in mitochondrial DNA mutagenesis, aging and age-related diseases. Ageing Res Rev 33:89–104.

Del-Saz NF, Ribas-Carbo M, McDonald AE, Lambers H, Fernie AR, Florez-Sarasa I. 2018. An in vivo perspective of the role(s) of the alternative oxidase pathway. Trends Plant Sci 23:206–19.

Dominiak K, Koziel A, Jarmuszkiewicz W. 2018. The interplay between mitochondrial reactive oxygen species formation and the coenzyme Q reduction level. Redox Biol 18:256–65.

El-Khoury R, Dufour E, Rak M, Ramanantsoa N, Grandchamp N, Csaba Z, Duvillié B, Bénit P, Gallego J, Gressens P, et al. 2013. Alternative oxidase expression in the mouse enables bypassing cytochrome c oxidase blockade and limits mitochondrial ROS overproduction. PLoS Genet 9:1–11.

Engelstädter J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138–50.

Erwin DH. 2015. Early metazoan life: divergence, environment and ecology. Philos Trans R Soc B Biol Sci 370:20150036.

Fautin DG. 2002. Reproduction of cnidaria. Can J Zool 80:1735–54.

Felsenstein J. 1974. The evolutionary advantage of recombination. Genetics 78:737–56.

Fernandez-Ayala DJM, Sanz A, Vartiainen S, Kemppainen KK, Babusiak M, Mustalahti E, Costa R, Tuomela T, Zeviani M, Chung J, et al. 2009. Expression of the *Ciona intestinalis* alternative oxidase (AOX) in *Drosophila* complements defects in mitochondrial oxidative phosphorylation. Cell Metab 9:449–60.

Finnegan PM, Umbach AL, Wilce JA. 2003. Prokaryotic origins for the mitochondrial alternative oxidase and plastid terminal oxidase nuclear genes. FEBS Lett 555:425–30.

Fritzenwanker JH, Technau U. 2002. Induction of gametogenesis in the basal cnidarian *Nematostella vectensis* (Anthozoa). Dev Genes Evol 212:99–103.

Fukami H, Omori M, Hatta M. 2000. Phylogenetic relationships in the coral family Acroporidae, reassessed by inference from mitochondrial genes. Zoolog Sci 17:689–96.

Garg SG, Martin WF. 2016. Mitochondria, the cell cycle, and the origin of sex via a syncytial eukaryote common ancestor. Genome Biol Evol 8:1950–70.

Genikhovieh G, Technau U. 2009. Induction of spawning in the starlet sea anemone *Nematostella vectensis*, in vitro fertilization of gametes, and dejellying of zygotes. Cold Spring Harb Protoc 4:1–4.

Goodenough U, Heitman J. 2014. Origins of eukaryotic sexual reproduction. Cold Spring Harb Perspect Biol 6:a016154.

Grieshaber MK, Volkel S. 1998. Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53.

Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, Baharoglu Z, Mazel D, Darfeuille F, Vogel J, et al. 2013. β-Lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. Nat Commun 4:1610.

Hamilton WD, Axelrod R, Tanese R. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proc Natl Acad Sci U S A 87:3566–73.

Havird JC, Hall MD, Dowling DK. 2015. The evolution of sex: a new hypothesis based on mitochondrial mutational erosion. BioEssays 37:951–8.

Hebert PDN, Ratnasingham S, deWaard JR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Biol Lett 270:S96.

Hill W, Robertson A. 1966. The effect of linkage on limits to artificial selection. Genet Res 8:269–94.

Hörandl E, Speijer D. 2018. How oxygen gave rise to eukaryotic sex. Proc Biol Sci 285:20172706.

Huang D, Meier R, Todd PA, Chou LM. 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J Mol Evol 66:167–74.

Huang J, Zhang L, Li J, Shi X, Zhang Z. 2013. Proposed function of alternative oxidase in mitochondrial sulphide oxidation detoxification in the Echiuran worm, *Urechis unicinctus*. J Mar Biol Assoc UK 93:2145–54.

Indo HP, Davidson M, Yen H-C, Suenaga S, Tomita K, Nishii T, Higuchi M, Koga Y, Ozawa T, Majima HJ. 2007. Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. Mitochondrion 7:106–18.

Jee J, Rasouly A, Shamovsky I, Akivis Y, Steinman SR, Mishra B, Nudler E. 2016. Rates and mechanisms of bacterial mutagenesis from maximum-depth sequencing. Nature 534:693–6.

Johnston DT, Poulton SW, Goldberg T, Sergeev VN, Podkovyrov V, Vorob NG, Bekker A, Knoll AH. 2012. Late Ediacaran redox stability and metazoan evolution. Earth Planet Sci Lett 335–336:25–35.

Kamata H, Hirata H. 1999. Redox regulation of cellular signalling. Cell Signal 11:1–14.

Kazak L, Reyes A, Holt IJ. 2012. Minimizing the damage: repair pathways keep mitochondrial DNA intact. Nat Rev Mol Cell Biol 13:659–71.

Kelley JL, Arias-Rodriguez L, Patacsil Martin D, Yee MC, Bustamante CD, Tobler M. 2016. Mechanisms underlying adaptation to life in hydrogen sulfide-rich environments. Mol Biol Evol 33:1419–34.

Knoll AH, Nowak MA. 2017. The timetable of evolution. Sci Adv 3:1–14.

Kump LR. 2008. The rise of atmospheric oxygen. Nature 451:277–8.

Lagouge M, Larsson NG. 2013. The role of mitochondrial DNA mutations and free radicals in disease and ageing. J Intern Med 273:529–43.

Lane N. 2014. Bioenergetic constraints on the evolution of complex life. Cold Spring Harb Perspect Biol 6:a015982.

Lane N, Martin W. 2010. The energetics of genome complexity. Nature 467:929–34.

Liu Y, Fiskum G, Schubert D. 2002. Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 80:780–7.

Loomis WF, Lenhoff HM. 1956. Growth and sexual differentiation of hydra in mass culture. J Exp Zool 132:555–73.

Luijckx P, Ho EKH, Gasim M, Chen S, Stanic A, Yanchus C, Kim YS, Agrawal AF. 2017. Higher rates of sex evolve during adaptation to more complex environments. Proc Natl Acad Sci U S A 114:534–9.

Lushchak VI. 2011. Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101:13–30.

Lyons TW, Reinhard CT, Planavsky NJ. 2014. The rise of oxygen in Earth's early ocean and atmosphere. Nature 506:307–15.

Maldonado M, Riesgo A. 2008. Reproduction in the phylum Porifera: a synoptic overview. Treballs de la SCB 59:29–49.

Matus-Ortega MG, Salmerón-Santiago KG, Flores-Herrera O, Guerra-Sánchez G, Martínez F, Rendón JL, Pardo JP. 2011. The alternative NADH dehydrogenase is present in mitochondria of some animal taxa. Comp Biochem Physiol Part D Genomics Proteomics 6:256–63.

Maxwell DP, Wang Y, McIntosh L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci U S A 96:8271–6.

Maynard Smith J. 1978. The evolution of sex. Cambridge (UK): Cambridge University Press.

McDonald A, Vanlerberghe G. 2004. Branched mitochondrial electron transport in the Animalia: presence of alternative oxidase in several animal phyla. IUBMB Life 56:333–41.

McDonald AE. 2008. Alternative oxidase: an inter-kingdom perspective on the function and regulation of this broadly distributed "cyanide-resistant" terminal oxidase. Funct Plant Biol 35:535–52.

McDonald AE, Amirsadeghi S, Vanlerberghe GC. 2003. Prokaryotic orthologues of mitochondrial alternative oxidase and plastid terminal oxidase. Plant Mol Biol 53:865–76.

McDonald AE, Costa JH, Nobre T, de Melo DF, Arnholdt-Schmitt B. 2015. Evolution of AOX genes across kingdoms and the challenge of classification. In: Gupta KJ, Mur LAJ, Neelwarne B, editors. Alternative respiratory pathways in higher plants. 1st ed. West Sussex, UK: John Wiley & Sons, Ltd. p. 267–72.

McDonald AE, Gospodaryov DV. 2019. Alternative NAD(P)H dehydrogenase and alternative oxidase: proposed physiological roles in animals. Mitochondrion 45:7–17.

McDonald AE, Vanlerberghe GC. 2006. Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase. Comp Biochem Physiol Part D Genomics Proteomics 1:357–64.

McDonald AE, Vanlerberghe GC, Staples JF. 2009. Alternative oxidase in animals: unique characteristics and taxonomic distribution. J Exp Biol 212:2627–34.

Mentel M, Rottger M, Leys S, Tielens AGM, Martin WF. 2014. Of early animals, anaerobic mitochondria, and a modern sponge. BioEssays 36:924–32.
Meyer CP, Paulay G. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biol 3: 1–10.
Millenaar FF, Lambers H. 2003. The alternative oxidase in roots of Poa species: in vivo regulation and function. Plant Biol 5:2–15.
Morran LT, Schmidt OG, Gelarden IA, Parrish RC, Lively CM. 2011. Running with the Red Queen: host–parasite coevolution selects for biparental sex. Science 333:216–8.
Muller HJ. 1932. Some genetic aspects of sex. Am Nat 66:118–38.
Muller HJ. 1964. The role of recomination to mutational advance. Mutat Res Mol Mech Mutagen 1:2–9.
Munro D, Pichaud N, Paquin F, Kemeid V, Blier PU. 2013. Low hydrogen peroxide production in mitochondria of the long-lived Arctica islandica: underlying mechanisms for slow aging. Aging Cell 12:584–92.
Nedelcu AM. 2005. Sex as a response to oxidative stress: stress genes co-opted for sex. Proc R Soc B 272:1935–40.
Nedelcu AM, Marcu O, Michod RE. 2004. Sex as a response to oxidative stress: a twofold increase in cellular reactive oxygen species activates sex genes. Proc R Soc B Biol Sci 271:1591–6.
Nedelcu AM, Michod RE. 2003. Sex as a response to oxidative stress: the effect of antioxidants on sexual induction in a facultatively sexual lineage. Proc R Soc B Biol Sci 270:S136–9.
Neiman M, Sharbel TF, Schwander T. 2014. Genetic causes of transitions from sexual reproduction to asexuality in plants and animals. J Evol Biol 27:1346–59.
Neimanis K, Staples JF, Hüner NPA, McDonald AE. 2013. Identification, expression, and taxonomic distribution of alternative oxidases in non-angiosperm plants. Gene 526:275–86.
Nicholls P, Marshall DC, Cooper CE, Wilson MT. 2013. Sulfide inhibition of and metabolism by cytochrome c oxidase. Biochem Soc Trans 41:1312–6.
Och LM, Shields-Zhou GA. 2012. The Neoproterozoic oxygenation event: environmental perturbations and biogeochemical cycling. Earth-Sci Rev 110:26–57.
Olson KR, Straub KD. 2016. The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling. Physiology 31:60–72.
Park HD, Ortmeyer AB. 1972. Growth and differentiation in Hydra II. The effect of temperature on budding in *Hydra littoralis*. Q Rev Biol 179:283–8.
Radzvilavicius AL, Blackstone NW. 2015. Conflict and cooperation in eukaryogenesis: implications for the timing of endosymbiosis and the evolution of sex. J R Soc Interface 12:20150584.
Ray PD, Huang B-W, Tsuji Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–90.
Reinhard CT, Planavsky NJ, Olson SL, Lyons TW, Erwin DH. 2016. Earth's oxygen cycle and the evolution of animal life. Proc Natl Acad Sci U S A 113:8933–8.
Robertson A, Schaltz K, Neimanis K, Staples JF, McDonald AE. 2016. Heterologous expression of the *Crassostrea gigas* (Pacific oyster) alternative oxidase in the yeast *Saccharomyces cerevisiae*. J Bioenerg Biomembr 48:509–20.
Rogov AG, Sukhanova EI, Uralskaya LA, Aliverdieva DA, Zvyagilskaya RA. 2014. Alternative oxidase: distribution, induction, properties, structure, regulation, and functions. Biochemistry 79:1615–34.
Saki M, Prakash A. 2017. DNA damage related crosstalk between the nucleus and mitochondria. Free Radic Biol Med 107:216–27.
Searcy DG. 2006. Rapid hydrogen sulfide consumption by *Tetrahymena pyriformis* and its implications for the origin of mitochondria. Eur J Protistol 42:221–31.
Selinski J, Scheibe R, Day DA, Whelan J. 2018. Alternative oxidase is positive for plant performance. Trends Plant Sci 23:588–97.
Shadel GS, Clayton DA. 1997. Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem 66:409–35.
Shearer TL, van Oppen MJH, Romano SL, Worheide G. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol 11:2475–87.
Siedow JN, Girvin ME. 1980. Alternative respiratory pathway—its role in seed respiration and its inhibition by propyl gallate. Plant Physiol 65:669–74.
Siedow JN, Umbach AL. 2000. The mitochondrial cyanide-resistant oxidase: structural conservation amid regulatory diversity. Biochim Biophys Acta Bioenerg 1459:432–9.
Sierra-Campos E, Velázquez I, Matuz-Mares D, Villavicencio-Queijeiro A, Pardo JP. 2009. Functional properties of the *Ustilago maydis* alternative oxidase under oxidative stress conditions. Mitochondrion 9:96–102.
Sies H. 2015. Oxidative stress: a concept in redox biology and medicine. Redox Biol 4:180–3.
Sloan DB, Broz AK, Sharbrough J, Wu Z. 2018. Detecting rare mutations and DNA damage with sequencing-based methods. Trends Biotechnol 36:729–40.
Speijer D. 2016. What can we infer about the origin of sex in early eukaryotes? Philos Trans R Soc B Biol Sci 371:20150530.
Speijer D, Lukeš J, Eliáš M. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Proc Natl Acad Sci U S A 112:8827–34.
Stenmark P, Nordlund P. 2003. A prokaryotic alternative oxidase present in the bacterium *Novosphingobium aromaticivorans*. FEBS Lett 552:189–92.
Sussarellu R, Dudognon T, Fabioux C, Soudant P, Moraga D, Kraffe E. 2013. Rapid mitochondrial adjustments in response to short-term hypoxia and re-oxygenation in the Pacific oyster, *Crassostrea gigas*. J Exp Biol 216:1561–9.
Szal B, Jolivet Y, Hasenfratz-Sauder MP, Dizengremel P, Rychter AM. 2003. Oxygen concentration regulates alternative oxidase expression in barley roots during hypoxia and post-hypoxia. Physiol Plant 119:494–502.
Szibor M, Dhandapani PK, Dufour E, Holmström KM, Zhuang Y, Salwig I, Wittig I, Heidler J, Gizatullina Z, Gainutdinov T, et al. 2017. Broad AOX expression in a genetically tractable mouse model does not disturb normal physiology. Dis Model Mech 10:163–71.
Technau U, Rudd S, Maxwell P, Gordon PMK, Saina M, Grasso LC, Hayward DC, Sensen CW, Saint R, Holstein TW, et al. 2005. Maintenance of ancestral complexity and

non-metazoan genes in two basal cnidarians. Trends Genet 21:633–9.
Tobler M, Passow CN, Greenway R, Kelley JL, Shaw JH. 2016. The evolutionary ecology of animals inhabiting hydrogen sulfide-rich environments. Annu Rev Ecol Evol Syst 47:239–62.
Tschischka K, Abele D, Portner HO. 2000. Mitochondrial oxyconformity and cold adaptation in the polychaete *Nereis pelagica* and the bivalve Arctica islandica from the Baltic and White Seas. J Exp Biol 203:3355–68.
Tward CE, Singh J, Cygelfarb W, McDonald AE. 2019. Identification of the alternative oxidase gene and its expression in the copepod *Tigriopus californicus*. Comp Biochem Physiol Part B Biochem Mol Biol 228:41–50.
Van Aken O, Giraud E, Clifton R, Whelan J. 2009. Alternative oxidase: a target and regulator of stress responses. Physiol Plant 137:354–61.
Vanlerberghe GC. 2013. Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci 14:6805–47.
Vanlerberghe GC, McIntosh L. 1997. Alternative oxidase: from gene to function. Annu Rev Plant Physiol Plant Mol Biol 48:703–34.
van Oppen MJH, Mcdonald BJ, Willis B, Miller DJ. 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence?. Mol Biol Evol 18:1315–29.
Volkel S, Grieshaber MK. 1996. Mitochondrial sulfide oxidation in Arenicola marina: evidence for alternative electron pathways. Eur J Biochem 235:231–7.
Watanabe CK, Hachiya T, Terashima I, Noguchi K. 2008. The lack of alternative oxidase at low temperature leads to a disruption of the balance in carbon and nitrogen metabolism, and to an up-regulation of antioxidant defence systems in *Arabidopsis thaliana* leaves. Plant Cell Environ 31:1190–202.
Woyda-Ploszczyca AM, Jarmuszkiewicz W. 2017. The conserved regulation of mitochondrial uncoupling proteins: from unicellular eukaryotes to mammals. Biochim Biophys Acta Bioenerg 1858:21–33.
Zapico SC, Ubelaker DH. 2013. mtDNA mutations and their role in aging, diseases and forensic sciences. Aging Dis 4:364–80.
Zhang Y, Brasher AL, Park NR, Taylor HA, Kavazis AN, Hood WR. 2018a. High activity before breeding improves reproductive performance by enhancing mitochondrial function and biogenesis. J Exp Biol 221:jeb177469.
Zhang Y, Humes F, Almond G, Kavazis AN, Hood WR. 2018b. A mitohormetic response to pro-oxidant exposure in the house mouse. Am J Physiol Integr Comp Physiol 314:R122–34.