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Review

Integrating Mitochondrial Aerobic Metabolism into Ecology and Evolution

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Biologists have long appreciated the critical role that energy turnover plays in understanding variation in performance and fitness among individuals. Whole-organism metabolic studies have provided key insights into fundamental ecological and evolutionary processes. However, constraints operating at subcellular levels, such as those operating within the mitochondria, can also play important roles in optimizing metabolism over different energetic demands and time scales. Herein, we explore how mitochondrial aerobic metabolism influences different aspects of organismal performance, such as through changing adenosine triphosphate (ATP) and reactive oxygen species (ROS) production. We consider how such insights have advanced our understanding of the mechanisms underpinning key ecological and evolutionary processes, from variation in life-history traits to adaptation to changing thermal conditions, and we highlight key areas for future research.

Highlights

The study of mitochondrial aerobic metabolism provides a promising avenue through which ecologists and evolutionary biologists can explore mechanistic sources of variation in metabolic and life-history phenotypes, with important consequences for organismal performance and fitness.

Mitochondria vary not only in rate of respiration, but also in ATP production efficiency through OXPHOS, rate of ROS production, and amount of heat generated; the interplay among these factors underlies mechanistic trade-offs and constraints that can have cascading effects up to the level of organismal performance.

Growing interest in mitochondrial biology within ecology and evolutionary biology, has enriched understanding of how metabolic rate, energy availability, and the byproducts of mitochondrial aerobic metabolism, may interact to shape different life-history strategies and how organisms adapt to their environment.

Energy Metabolism Drives Ecological Processes

Aerobic respiration is a principal source of energy for most eukaryotes. Energy metabolism controls the amount of energy uptake from the environment and the relative allocation of this energy to life-history traits. Classical ecological approaches for quantifying energy flows have estimated metabolic rate by measuring whole-organism gas exchange [1–3], which has provided a powerful foundation for understanding variation in individual performance, population growth rate, and community structure [4]. However, respiration at the whole-organism level ultimately derives from energy flow at the subcellular level [5]. Aerobic respiration in the mitochondria is a central process by which organisms transfer chemical energy locked in substrates into the chemical bonds of ATP, the main form of chemical energy fueling cellular processes. The efficacy with which mitochondria execute this energy transfer can be a major determinant of organismal fitness [6–10].

Investigating variation in mitochondrial measurements provides an opportunity for integrating how mechanistic constraints and trade-offs at the cellular level, may shape fundamental evolutionary and ecological patterns [5,11–15]. Such constraints and trade-offs can involve interrelationships between ATP, **reactive oxygen species (ROS)** (see Glossary) production, and heat generated during cellular respiration—relationships that can be flexibly modulated in response to changing needs, but may also face physiological constraints (see later) [16,17]. Teasing apart these relationships has the potential to offer valuable insights into the nature of the physiological changes, that enable organisms to adapt and to optimize function across a broad range of conditions.

Here, we suggest that characterizing energy flow via measurements of **mitochondrial aerobic metabolism,** can provide new insights into differences in energy turnover and physiological performance among and within individuals, across time, and in response to environmental

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change (Figure 1) [18,19]. Meeting energetic demands at the subcellular level, while preventing the accumulation of potentially damaging byproducts under changing conditions, requires careful orchestration of biochemical processes in the mitochondria [20]. The effectiveness of mediating such processes is driven by both genes and environments (Box S1 in the supplemental information online), linking mitochondrial processes not only to individual fitness, but also to local adaptation (Figure 1) [21]. Our aim is to discuss how the study of mitochondria may yield new insights into evolutionary and ecological sciences. We will firstly summarize mitochondrial traits and their quantification, and then secondly discuss the implications of these processes for ecology and evolution.

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Mitochondrial Traits and Their Quantification

Mitochondria have a wide range of functions (e.g., immune signaling pathways, apoptosis, and epigenetics [22–24]), but we focus predominantly on the central mitochondrial process of **oxidative phosphorylation (OXPHOS)** and on how *in vitro* mitochondrial measurements can be interpreted in relation to animal performance and fitness. OXPHOS is the specific component of mitochondrial aerobic respiration, that harnesses potential energy from an electrochemical gradient, to drive the phosphorylation of ADP into ATP, consuming oxygen in the process. Specifically, electrons harvested from food-derived substrates flow through the protein complexes of the **electron transport chain (ETC)** to fuel the pumping of protons from the mitochondrial matrix into the intermembrane space (Figure 2). This creates a disequilibrium in both charge and proton concentration across the inner membrane (a source of potential energy called the **protonmotive force**). Protons passing down this gradient through ATP synthase power the phosphorylation of ADP into ATP (Figure 2) [25,26]. OXPHOS fluctuates in response to changes in substrate availability and composition, ATP demand, and environmental conditions. Flexibility in the activity of the ETC, the build-up of protonmotive force, and the movement of protons across the inner mitochondrial membrane, sets the rate of ATP production and affects metabolic heat generation as well as mitochondrial ROS production (Figure 2).

Mitochondrial ROS are formed when electrons escape the ETC and react directly with oxygen, and some ROS are always generated during mitochondrial aerobic metabolism. The contribution of ROS to **oxidative stress** plays a prominent role in many ecological and evolutionary hypotheses, such as theories of aging [27–29] and of reproductive costs [30–32]. While ROS serve important signaling functions and are not inherently harmful [33], excess ROS have the potential to cause oxidative damage and lead to cellular dysfunction. Rate of mitochondrial ROS generation may increase due to altered functionality of the ETC, such as mild dysfunction from oxidative damage, mutation, replication error, or genetic incompatibilities between genes encoding subunits of OXPHOS (**mitonuclear interactions)** (Box S1 in the supplemental information online) [34–36]); we can consider this structural variation as variation in mitochondrial 'quality' (Figure 3). In contrast, mitochondrial ROS production also changes inherently with changing of protonmotive force (Figures 2 and 3); specifically, more ROS are generated during high protonmotive force, such as may occur when demand for ATP is low (Figure 2B) [37]. This point is key because it highlights the misconception that increased metabolic rate will inherently increase rate of ROS production. Rather, ROS production increases with the build-up of protonmotive force, which can be countered either by a higher rate of ATP production (coupled with demand) [30,38,39] or a higher **proton leak** (i.e., protons flowing back to the mitochondrial matrix without passing through ATP synthase, dissipating energy as heat) (Figure 2) [39].

Proton leak across the inner mitochondrial membrane can occur through two means: (i) passive proton leak (movement of protons directly through the membrane, largely influenced by protonmotive force and membrane composition [40]); and (ii) inducible proton leak (regulated

movement of protons through protein channels that span the inner mitochondrial membrane [41]). Either form of proton leak is essentially '**uncoupling**' proton movement from the phosphorylation of ADP into ATP. Proton leak decreases rate of ATP synthesis relative to mitochondrial respiration rate, but it is not necessarily a dysfunction; rather, proton leak can be beneficial for respiratory function by lowering protonmotive force and thereby reducing mitochondrial ROS production (Figure 2) [42]. Inducible proton leak can also be an important means to generate extra metabolic heat for thermoregulation in endotherms (e.g., UCP1 in the brown adipose tissue of small mammals [43]), in addition to heat already produced during ATP synthesis.

Because oxygen consumed during aerobic respiration can be uncoupled from ATP synthesis through proton leak (Figure 2), two individuals (or more specifically, two mitochondria) with the same rate of oxygen consumption, can have quite different rates of ATP synthesis. Indeed, the proportion of oxygen consumed that leads to ATP synthesis versus the proportion that leads to proton leak (e.g., as captured in measures of **mitochondrial efficiency**; Box 1 and Figure 3) can vary considerably across species, individuals, and time (see Outstanding Questions) [11,44]. Such variation can be due to strategic adjustment of mitochondrial processes to best respond to current conditions(e.g., increasing mitochondrial efficiency to enhance energy savings during prolonged fasting [45]) (Figure 3). However, such variation may also be indicative of structural differences in, for example, the ETC; differences that could constrain ATP production capacity during times of high demand (Figure 3). Only limited research to date has focused on the evolutionary implications of within-individual variation in mitochondrial aerobic respiration, across tissues and life stages.

Understanding the interconnections between mitochondrial oxygen consumption, ATP and ROS production, and proton leak, is critical for understanding physiological trade-offs operating at the cellular level that may influence animal phenotype and performance (Box 2). The importance of these interactions has been recognized for decades, including their relevance to the evolution of body size [46], thermal biology [47], and more recently, even sexually selected displays [48]. Exploring these processes, particularly with recently developed techniques that allow for nonterminal and even repeated sampling (Box 1), offers ecologists insights into the long-term costs and constraints of the metabolic processes that underlie life-history strategies. An important outstanding area of research with relevance to both ecological and evolutionary questions, is how and why individuals differ in their mitochondrial phenotype [parameters such as the efficiency with which ATP is produced from mitochondrial aerobic metabolism, or density of mitochondria within cells (Figure 3 and Box 2)] and the higher-order effects of such subcellular variation on broader aspects of organismal performance.

While a full overview of the practical and methodological considerations required to collect informative mitochondrial measures is beyond the scope of this review, we provide a brief overview of several of the most well-established methods by which mitochondrial performance can be quantified in Box 2 (though none are without limitations), and important considerations for experimental design in Box 1. In the following section, we seek to translate the broad applications of the study of mitochondrial aerobic metabolism to evolutionary and ecological processes.

Applications of Mitochondrial Aerobic Metabolism to Ecological and Evolutionary Research

Mitochondrial Processes Underlie Whole-Organism Metabolic Rate

An understanding of mitochondrial aerobic metabolism can enhance our understanding of variation in whole-organism traits. Whole-organism metabolic rates have been found to vary severalfold among species and individuals [49,50], and variation in metabolic rate can be a

Glossary

Electron transport chain (ETC): a series of protein complexes in the inner mitochondrial membrane that transfer electrons via oxidation/reduction reactions. These reactions are coupled to the pumping of protons out of the mitochondrial matrix to create a protonmotive force.
LEAK respiration: oxygen used to offset proton leak. Also called state 4 respiration rate.
Mitochondrial aerobic metabolism: the metabolic (anabolic and catabolic) reactions that occur within the mitochondria, that involve direct and indirect use of oxygen. This comprises activity of the electron transport chain, protonmotive force, ATP production, and ROS generation.
Mitochondrial efficiency: efficiency of mitochondria to convert food-derived energy substrate into ATP. Efficiency is often estimated *in vitro* with P:O ratios and respiratory control ratios (RCR).
Mitonuclear interactions: functional associations between the products of the mitochondrial and nuclear genomes that can affect mitochondrial aerobic metabolism.
Oxidative phosphorylation (OXPHOS): phosphorylation of ADP into ATP in the presence of oxygen. Conversion of ADP to ATP is performed by ATP synthase, and uses the energy provided by the protonmotive force.
Oxidative stress: imbalance between the production of pro-oxidants (such as ROS) and the ability of a biological system to neutralize them through antioxidant defenses, leading to oxidative damage to biomolecules.
OXPHOS respiration: oxygen used when mitochondria are actively producing ATP. Also called state 3 respiration rate.
Proton leak: protons that flow back across the inner mitochondrial membrane into the matrix outside of ATP synthase. Proton leak may be passive, particularly when membrane potential is high, or inducible (and regulated through transmembrane proteins).
Protonmotive force: the potential energy stored across the inner mitochondrial membrane, that is established by pumping protons from the mitochondrial membrane to the intermembrane space; the protonmotive force involves both the chemical gradient formed by the difference in proton

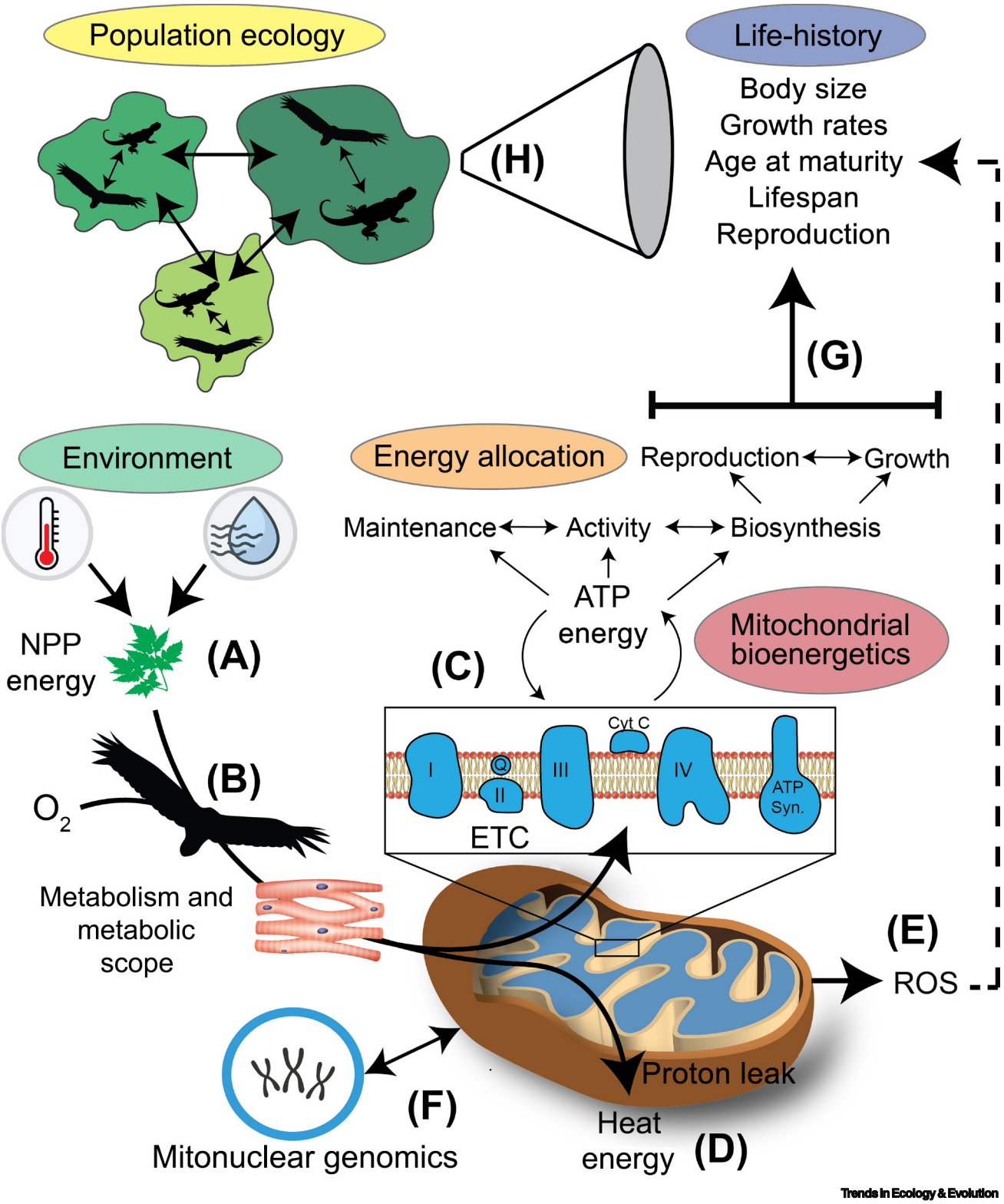
functional determinant of variation in fitness and fitness-related traits [51–53]. Field and laboratory studies have identified strong relationships between whole-organism metabolic rate and survival, and reproductive success, but the strength and form of selection varies among species and ecological contexts [53]. Variation in mitochondrial aerobic metabolism is likely an important underlying source of the observed among-individual variation in whole-organism metabolic rate and is likely to influence the relationship between metabolic rate and fitness [44,54,69]. Almost all measurements of metabolic rate are made through the measurement of O_2 or CO_2 fluxes (e.g., the data for thousands of species summarized in [55]). Such measurements are important estimates of overall energy flow, but as described above, variation at the mitochondrial level (e.g., uncoupling) alters the proportion of that energy that ultimately results in ATP, heat, or ROS (Figures 2 and 3) [11].

concentration, and the electrical gradient formed by the difference in charge.
Reactive oxygen species (ROS): derivatives of oxygen that are chemically unstable and quickly react with different kinds of biomolecules; ROS are important cellular signals but can also cause altered function through oxidative damage.
Uncoupling: the dissociation of mitochondrial protonmotive force generation (i.e., the 'uncoupling' of electron transport chain activity) from ATP synthesis.

Exploring variation in mitochondrial aerobic metabolism can therefore help better pinpoint the significance of variation (or lack thereof) in energy flow at the whole-organism level. For example, two animals with the same whole-organism metabolic rate can differ in energy available as ATP (e.g., if they differ in mitochondrial efficiency; Box 2); similarly, two animals that differ in whole-organism metabolic rate can have the same energy available as ATP, but different levels of heat or ROS generation (Figures 2 and 3). Differences in strategy or quality at the mitochondrial level thereby can alter the relationships between whole-organism metabolic rate and physiological performance or life-history trait expression. Measurements of mitochondrial aerobic metabolism (e.g., mitochondrial efficiency; Boxes 1 and 2) offer a means to further contextualize measurements of whole-organism energy flow by providing information on whether that energy may be put toward ATP or instead toward heat or ROS production, alternatives that have ramifications for the hypotheses we formulate for how metabolic rate relates to fitness. Combining measurements of fitness with measurements of mitochondrial aerobic metabolism (e.g., from repeated, nonterminal sampling; Box 1) and whole-organism metabolic rate will provide an opportunity to grow our understanding of the functional bases of variation.

Can Variation in Mitochondrial Aerobic Metabolism Explain Life-History Trade-Offs?

The study of metabolism has also played a central role in explaining fundamental life-history trade-offs, such as that between growth rate and lifespan. Applying an understanding of mitochondrial aerobic metabolism and the relationships between metabolic rate, ATP production, and ROS production, offers a new tool for further exploring such life-history trade-offs. The 'oxidative stress life-history hypothesis', for example, proposes that energy-demanding phases of life that necessitate increased metabolism, such as growth or reproduction, come at the cost of increased generation of mitochondrial ROS, which in turn would have ultimate consequences to longevity (Figure 1) [56,57]. However, as described previously, increased metabolism does not necessarily increase the production of mitochondrial ROS, and can actually have the opposite effect (Figure 2); so, it is perhaps unsurprising that empirical evidence for this hypothesis is currently mixed (reviewed in [31,56,58]). More comprehensive comparisons of mitochondrial and organismal respiration across species, individuals, and tissues, has the potential to provide new insights into longstanding questions regarding major physiological and life-history patterns, such as trade-offs surrounding whole-organism metabolic rate [54] or growth [59]. For example, an individual could potentially maintain growth rate during times of food scarcity without altering oxygen consumption by instead increasing efficiency of ATP production (Box 2), but such increases in coupling between electron flow and ATP synthesis at lower metabolic rates, may come at the potential cost of the increases in mitochondrial ROS production (Figures 2 and 3) [17,60,61]. Mitochondrial processes can therefore provide testable predictions for how and why individuals differ in performance when whole-organism measures do not fully explain observed variation.



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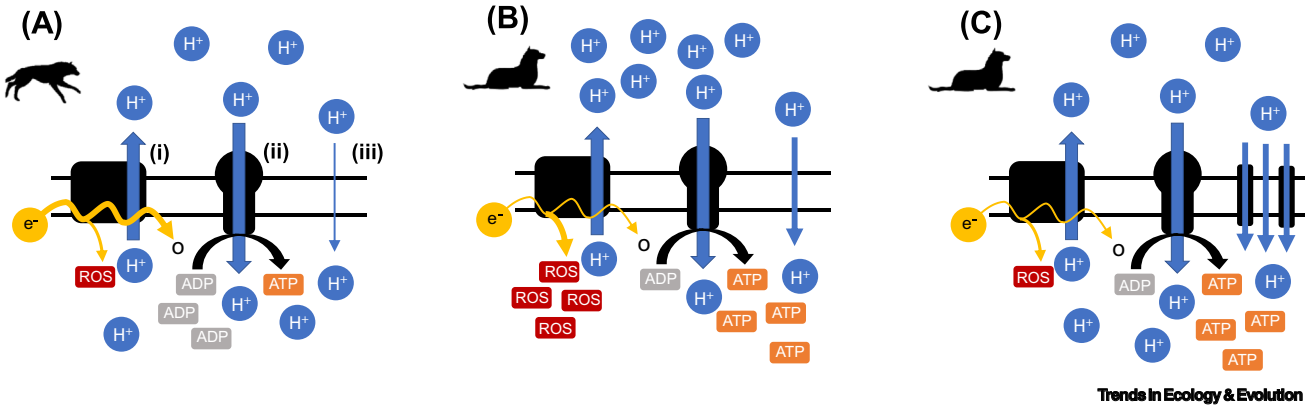

Figure 2. Simplified Diagrams Illustrating Relationships among ATP Demand, Protonmotive Force, Mitochondrial ROS Production, and Proton Leak. Here, we illustrate electron movement through the ETC, ATP synthesis from ADP via ATP synthase, and proton movement across the inner mitochondrial membrane through the ETC (i), ATP synthase (ii), or proton leak (iii). The top region of each graphic represents the intermembrane space, and the bottom region represents the mitochondrial matrix. The three panels represent conditions of high ATP demand (A), low ATP demand (B), and low ATP demand with increased inducible proton leak (C). In (A), high demand for ATP prevents the build-up of protonmotive force as protons are quickly passed through ATP synthase, which limits mitochondrial ROS production. In (B), decreased ATP demand has increased protonmotive force because proton movement through ATP synthase has decreased; under these conditions, electrons 'escape' the ETC at higher rates, forming ROS. In (C), proton leak, both basal and induced (black bars across the membrane represent upregulated protein channels), prevents the build-up of protonmotive force and thereby controls ROS generation even while demand for ATP is low, producing heat in the process. The differences between (B) and (C) are key to the concept of how the strategic adjustment of proton leak may be beneficial, but also underlie trade-offs; for example, greater proton leak in (C) compared to (B) decreases mitochondrial efficiency, but also decreases mitochondrial ROS production even when ATP demand is low. Abbreviations: ETC, electron transport chain; ROS, reactive oxygenated species.

A second hypothesis more specifically considers how regulation of protonmotive force to adjust ROS production may itself be a key to life-history trade-offs. This 'uncoupling to survive' hypothesis proposes that proton leak is carefully controlled to decrease protonmotive force and thereby to regulate mitochondrial ROS generation (Figures 2 and 3) [42,44,62,63]. Studies have tested this hypothesis by experimentally manipulating uncoupling and/or by comparing uncoupling levels to life-history trait performance and oxidative stress [14,64–66]. For example, the administration of an experimental mitochondrial uncoupler to captive zebra finches (*Taeniopygia guttata*) caused whole-organism metabolic rate to increase, likely in response to reduced ATP production efficiency caused by the treatment but did not alter oxidative stress. However, finches receiving the uncoupling treatment had decreased reproductive investment, which supports the potential existence of a trade-off between possible benefits of uncoupling and maximizing current fitness [67]. Further studies are needed to better understand how uncoupling varies in wild animals that are balancing ATP and ROS production, lifespan, and reproduction across constantly varying environmental conditions.

Figure 1. Mitochondria Link Energy Flow through the Environment to Organism Life-History, Impacting Meta-Population and Community Dynamics. Environmental factors, such as temperature and water availability, affect net primary productivity (NPP) (A), and thus food availability. Organisms ingest food (B) and liberate the organic molecules that are absorbed, transporting them to tissues and cells. Carbohydrates, fats, and proteins are each independently catabolized and the energy stored within their chemical bonds is captured to produce ATP through aerobic respiration. The final steps take place in the electron transport chain (ETC) within the inner membrane of the mitochondria. Protonmotive force across the inner mitochondrial membrane is used to fuel ATP production through ATP synthase (C) but can also be dissipated through proton leak; this decreases ATP production efficiency and increases the amount of energy released as heat (D). Mitochondria are also a source for reactive oxygen species (ROS), which can damage proteins, lipids, and nucleic acids, potentially causing mitochondrial and cellular dysfunction and affecting life-history traits (E). Mitochondrial and nuclear genes coding for enzymes in the ETC (F) interact with environmental factors to play critical roles in determining the efficiency of mitochondria in producing ATP, heat, and ROS. Mitochondrial aerobic metabolism is therefore important in determining the amount of ATP available to fuel metabolic processes underlying cellular and tissue maintenance, activity, growth, and reproduction, all critical underpinnings of an organism's life-history repertoire (G). Changes in life-history traits at the individual level 'funnel' up to affect the dynamics of populations, and ultimately, metapopulations and communities (H).

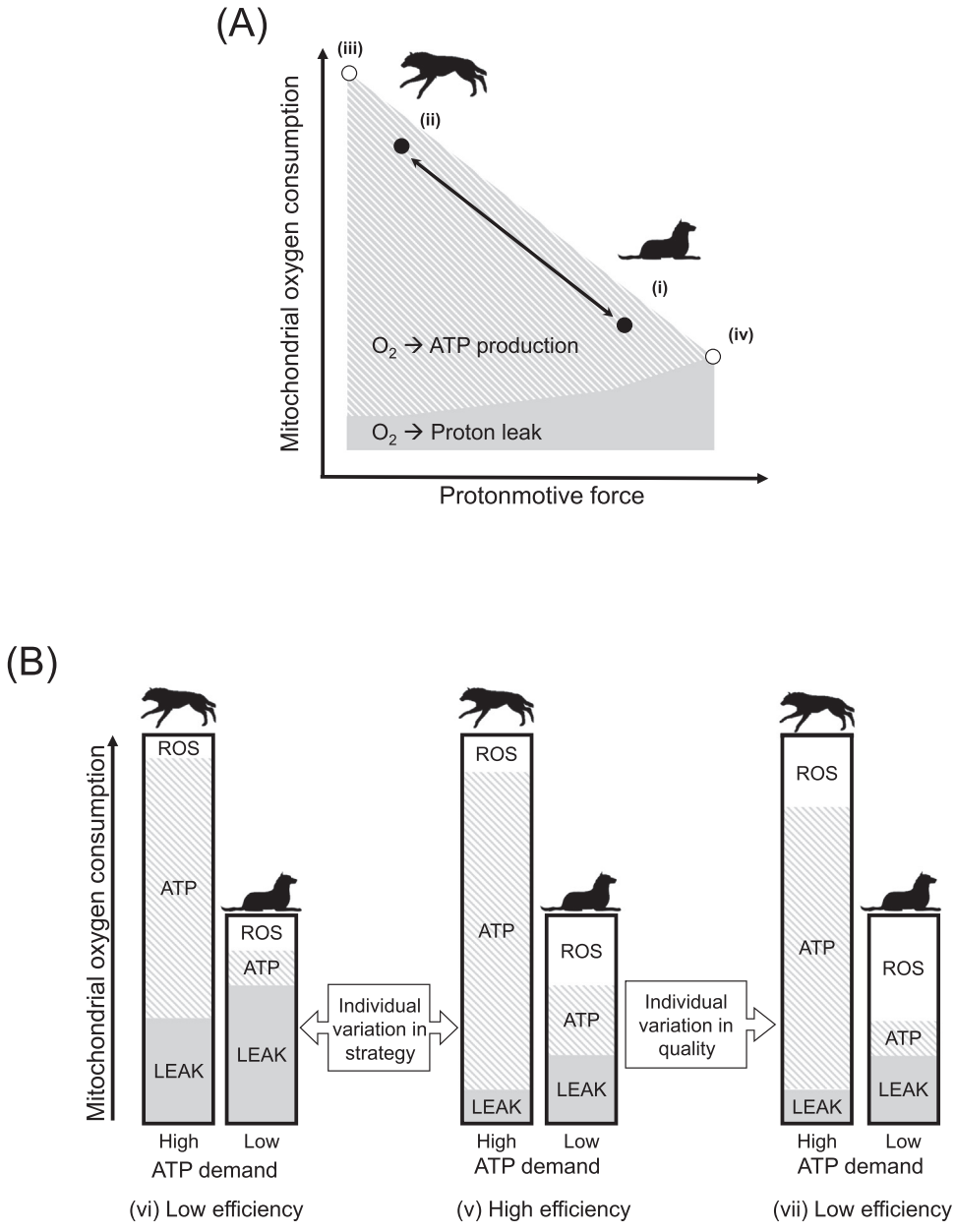

Figure 3. Illustration of Within- and Among-Individual Variation in Mitochondrial Aerobic Metabolism.

For a Figure360 author presentation of Figure 3, see the figure legend at https://doi.org/10.1016/j.tree.2020.12.006.

The shading of each region represents whether oxygen is put toward ATP production (broken-lined region), proton leak (filled gray region), or ROS production (unfilled region). In (A), the filled circles represent possible measurements of an animal at rest (i) or the same animal in a state of high activity (ii), informed by data in [78]. In a highly active state (ii), the high demand for ATP causes ATP synthase to rapidly use up the proton gradient, so protonmotive force and rate of proton leak become relatively low. In contrast, the slowing of ATP synthase activity when an active animal shifts to rest (i) causes an increase in membrane potential. The open circles represent the *in vitro* measurements of OXPHOS (iii) or LEAK (iv) respiration (Box 2), though these extremes would not occur *in vivo*; it is worth noting that using these extremes to calculate the proportion of oxygen resulting in proton leak (i.e., RCR; Box 2) would result in an misleading estimate of the actual proportions of oxygen resulting in leak or ATP production at either during either high or low activity [79,80]. In (B), within- and among-individual variation in

(Figure legend continued at the bottom of the next page.)

Box 1. Designing Experimental Tests of Mitochondrial Aerobic Metabolism

Measures of mitochondrial aerobic metabolism tend to be relatively low-throughput and most have to be measured in freshly collected biological samples (especially efficiency proxies) within a few hours of sampling ([81], but see [82,83]). Such constraints may limit the number of animals sampled and require that tissue collection be organized strategically. In addition, the sampling approach (e.g., choice of tissue and frequency of sampling), the mitochondrial properties to assess (e.g., mitochondrial respiration rates, efficiency, and density; Box 2), and the experimental conditions (e.g., assay temperature [20], substrates [84]) should each be chosen with care based on the focus and constraints of a given study.

Since mitochondrial aerobic metabolism varies considerably among tissues (e.g., [17,54]), conclusions based on a single tissue should always be considered within context, and extrapolation to additional tissues without validation should be avoided. When selecting which tissue to measure, researchers should consider both the physiology that underlies the question being evaluated and the practical and ethical constraints of destructive sampling. For example, a study of ornamental red coloration in male house finches (*Haemorhous mexicanus*) measured the performance of mitochondria in the liver because the process of converting dietary yellow to red feather pigments is thought to occur specifically within the liver in that species [48]. When studies focus on small species, sample collection often must be destructive, making repeated sampling of an individual impossible. In larger animals, it may be possible to biopsy organs such as skeletal muscle [26], but the consequences of such invasive procedures should be carefully considered. Beyond ethical considerations, it is important to consider how the stressors animals experience prior to death or during sampling can impact their physiology [85].

Evaluating mitochondrial aerobic metabolism from blood cells has emerged over the last decade as a potential alternative to circumvent some of these issues. This approach using blood cells is becoming more prevalent in biomedical studies (e.g., [86]), and is starting to be implemented in ecological studies on non-model and wild animals [14,15,26,87–89]. Several studies have revealed moderate positive associations between measurements of mitochondrial aerobic metabolism in blood cells and other tissues ([26,90–92]; but see [93,94]). Additionally, a study in wild female pied flycatchers (*Ficedula hypoleuca*) during reproduction showed that mitochondrial measurements in blood cells were moderately to highly repeatable within individuals over a period of 10 days, despite those measurements being highly plastic across reproductive stage [88]. Such results suggest that measurements from blood cells have the potential to be relevant and individual-specific markers of mitochondrial parameters.

Mitochondrial Aerobic Metabolism and Temperature: Thermoregulation, Climatic Adaptation, and Acclimation

Uncoupling also has important ramifications for thermoregulation, as proton leak can be an important endogenous heat source. In developing chicken embryos, for example, the transition from ectothermy to endothermy is accompanied by increased mitochondrial uncoupling [68], suggesting an important role of proton leak in mechanisms of thermoregulation. In addition, a particular uncoupling protein in mammals (UCP1) has been found to drive heat production through nonshivering thermogenesis in the brown adipose tissue of many (but not all) cold-adapted mammals [43,69]. Increased proton leak may also potentially be a means to generate extra metabolic heat while decreasing ROS production [70]; for example, a study subjecting zebra finches to acute cold stress found that untreated birds had increased oxidative damage to DNA, while birds treated with an experimental mitochondrial uncoupler showed no such increases [71]. Such examples provide insight into the potential for mitochondrial measurements to provide new mechanistic explanations for the potential costs or benefits of different means of endogenous heat production.

mitochondrial efficiency, is illustrated by tracing hypothetical relative proportions of different endpoints of mitochondrial oxygen consumption during states of high or low demand for ATP. Mitochondria with high efficiency (v) will produce more ATP at a given level of oxygen consumption than those with low efficiency (vi and vii). However, higher efficiency mitochondria can also generate higher levels of ROS under states of low ATP demand (v, right column), so decreased efficiency may hypothetically be an adaptive 'strategy' to lower ROS (e.g., shift from v to vi). In contrast, lower efficiency can also be caused by decreased mitochondrial 'quality' (e.g., due to mild dysfunction from damage or mitonuclear incompatibility), potentially both reducing ATP production and increasing ROS generation (vii). Note that the relative proportions allocated to ROS are inflated to be more visually clear.

Abbreviations: OXPHOS, oxidative phosphorylation; RCR, respiratory control ratio; ROS, reactive oxygen species.

Box 2. The Biological Implications of Methodological Choices

(i) Sample Type and Preparation

Mitochondrial aerobic metabolism can be measured *in vitro* from isolated mitochondria, permeabilized cells/tissues, or intact cells [95]. While isolated mitochondria provide precise control of cellular conditions (i.e., substrates and ADP availability), they lack cellular context. Intact cells have a preserved cellular and mitochondrial network and are able to inform on endogenous mitochondrial aerobic metabolism but lack control of substrates and ADP availability. Permeabilized cells/tissues share some of the advantages and drawbacks of both isolated mitochondria and intact cells [95]. Isolated mitochondria are considered a better choice for mechanistic studies, while intact cells have a greater ecological relevance [95].

(ii) Mitochondrial Aerobic Metabolism Parameters

Different mitochondrial aerobic metabolism parameters can be measured (Table I); the two main categories are respiration rates (e.g., **OXPHOS** and **LEAK respiration**) and proxies of mitochondrial efficiency (e.g., P:O, the amount of ADP used, or ATP produced per atom of oxygen consumed; or, respiratory control ratio (RCR), the ratio of oxygen used for OXPHOS to oxygen used for proton leak). Although RCR (OXPHOS/LEAK respiration) is widely used, it has several mathematical and interpretation limitations; a related metric has recently been proposed to alleviate these limitations, called OXPHOS Coupling Efficiency (abbreviated here as OxCE). OxCE reflects the proportion of total mitochondrial oxygen consumption being allocated to ATP synthesis (1-LEAK/**OXPHOS respiration**) [79,96]. Yet, both RCR and OxCE are biased by the fact that the proton leak estimated when no ATP synthesis occurs (i.e., LEAK respiration) over-estimates the proton leak occurring when active ATP synthesis takes place (Figure 3A). Respiration rates indicate potential metabolic capacity levels, while efficiency proxies indicate how efficient mitochondria are at producing ATP [80,95]. Both are likely to be relevant for organismal performance, but the *in vivo* relevance of such *in vitro* measurements must be carefully considered.

Table I. Logistical Considerations When Measuring Mitochondrial Aerobic Metabolism Parameters

–		Respiration rates	RCR or OxCE	ADP:O	ATP:O ratio	
Equipment required		Oxygraphy	Oxygraphy	Oxygraphy	Oxygraphy + colorimetry	Oxygraphy + fluorimetry
Mitochondrial preparation	Isolated mitochondria	Feasible	Feasible	Feasible	Feasible	Feasible
	Permeabilized cells/tissues	Feasible	Feasible	Not feasible	Not feasible	Feasible
	Intact cells	Feasible	Feasible	Not feasible	Not feasible	Not feasible
Start-up effort required		Low	Low	Low	High	High
Able to measure ATP production?		No	No	No	Yes	Yes
Relative processing time per sample		Low to moderate	Low to moderate	Low to moderate	Very high	Moderate
Cost (equipment + consumables)		Low to moderate	Low to moderate	Low to moderate	Low to moderate	High
Refs		[97]	[97,98]	[97,98]	[97,98]	[99]

(iii) Normalization of Mitochondrial Respiration Rates

Ratios like P:O, RCR, and OxCE are unitless, but mitochondrial respiration rates (e.g., OXPHOS or LEAK respiration rates) need to be normalized relative to tissue/mitochondrial content in order to be compared. Normalizing respiration rates of isolated mitochondria is usually done by expressing oxygen consumption per amount of mitochondrial protein content. Normalizing mitochondrial respiration rates from permeabilized and intact cells can either be achieved using mass of tissue or number of cells, or a proxy of mitochondrial abundance/density, and this choice has critical consequences on data interpretation [79]. For instance, two groups of animals could exhibit similar respiration rates expressed per unit of tissue, but one group may require more mitochondria to reach the same respiratory capacity; contextualizing measures in this way is important to reveal hidden complexity, which in turn can suggest the possibility of further underlying patterns to explore (e.g., increased mitochondrial density to compensate for dysfunction, at the possible cost of increased ROS generation [100]).

Mitochondria are not only important to regulating internal temperature, but also to responding to changes in external temperature, on the scales of both long-term adaptation and short-term acclimation. For example, a study of wild mayfly larvae (*Baetis* and *Drunella* spp.) found that high-and low-elevation-adapted populations differed in multiple measures of mitochondrial aerobic metabolism when tested under common conditions [72]. This study demonstrates the potential for the evolution of mitochondrial phenotypes (over generations) to prevailing climatic conditions, with implications for local population adaptation. On shorter-term scales, mitochondrial aerobic metabolism also appears to be key to organisms' response to varying temperatures, such as may occur during seasonal acclimation (e.g., [73]) or short-term thermal stress (e.g., [74]). Thermal conditions can themselves have fundamental thermodynamic effects on reaction rates in the mitochondria as cellular temperature changes, and organisms may respond by changing mitochondrial efficiency or density (potentially facing trade-offs in the process; e.g., [75,76]). Importantly, the limits of mitochondria to respond to changing conditions are expected to be a key determinant of thermal limits [72,76,77], with implications for how populations will respond to a changing climate.

Concluding Remarks

There is a growing awareness among ecologists and evolutionary biologists that mitochondrial phenotype can play a central role in shaping the fundamental characteristics of organisms. At the heart of this awareness is an understanding that selection acts on individual variation, and yet we know relatively little about how selection acts on mitonuclear genotype and mitochondrial phenotype. To understand how variation in mitochondrial aerobic metabolism has contributed to shaping selection on organisms, we need to understand how mitochondrial phenotype constrains and/or enables response to environmental change. Fruitful lines of research include quantifying individual variation in mitochondria, including individual consistency in responses to changing environmental conditions, and tissue-specific responses for which fitness is dependent on managing periods of intense energy demands (e.g., rapid growth or migration). As interest in mitochondrial biology expands, there emerges an increasing need for a common foundation in the best practices for studying mitochondrial aerobic metabolism, grounded in a careful understanding of mitochondrial biology. Such a foundation will promote a more unified understanding of how cellular processes may shape the evolution of life-history traits, and support new research avenues that probe the broader ecological and evolutionary implications (see Outstanding Questions).

Outstanding Questions

Does early developmental environment alter mitochondrial aerobic respiration, and how may such effects carry across life-history stages?

To what extent does variation in mitochondrial aerobic respiration underlie variation in whole-organism metabolic rate, and how does incorporation of a mitochondrial perspective alter our understanding of the role of metabolic variation in ecology and evolution?

Do constraints on mitochondrial aerobic respiration shape the expression of condition-dependent, sexually selected traits?

When is mitochondrial ROS production beneficial and when is it harmful? How do the answers to these questions change our understanding of the evolutionary consequences of variation in mitochondrial bioenergetics?

Is blood a useful tissue in many species for noninvasive, repeated sampling, for understanding the evolutionary consequences of variation in mitochondrial aerobic respiration?

Can we establish links from genotype to mitochondrial aerobic respiration phenotype, then ultimately to the fitness of individuals?

When does heterogeneity in mitochondrial aerobic respiration have a selective advantage that is context-specific (i.e., mitochondria function well in one environment but not another)?

To what extent does mitochondrial 'quality' (i.e., levels of function vs dysfunction) vary in natural populations, and what mechanisms underlie this variation?

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References

1. Ruiz, T. *et al.* (2018) A microcalorimetric approach for investigating stoichiometric constraints on the standard metabolic rate of a small invertebrate. *Ecol. Lett.* 21, 1714–1722
2. Pettersen, A.K. *et al.* (2016) Metabolic rate covaries with fitness and the pace of the life history in the field. *Proc. R. Soc. B Biol. Sci.* 283, 20160323
3. Glazier, D.S. (2015) Is metabolic rate a universal 'pacemaker' for biological processes? *Biol. Rev.* 90, 377–407
4. Brown, J.H. *et al.* (2004) Toward a metabolic theory of ecology. *Ecology* 85, 1771–1789
5. Delhaye, J. *et al.* (2016) Interspecific correlation between red blood cell mitochondrial ROS production, cardiolipin content and longevity in birds. *Age (Omaha)* 38, 433–443
6. Ballard, J.W. and Youngson, N.A. (2015) Review: can diet influence the selective advantage of mitochondrial DNA haplotypes? *Biosci. Rep.* 35, e00277

7. Heine, K.B. and Hood, W.R. (2020) Mitochondrial behaviour, morphology, and animal performance. *Biol. Rev.* 95, 730–737
8. Burton, R.S. *et al.* (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annu. Rev. Ecol. Evol. Syst.* 44, 281–302
9. Hill, G.E. *et al.* (2019) Assessing the fitness consequences of mitonuclear interactions in natural populations. *Biol. Rev.* 94, 1089–1104
10. Melvin, R.G. and Ballard, J.W.O. (2006) Intraspecific variation in survival and mitochondrial oxidative phosphorylation in wild-caught *Drosophila simulans*. *Aging Cell* 5, 225–233
11. Salin, K. *et al.* (2015) Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B Biol. Sci.* 282
12. Hill, G.E. (2014) Cellular respiration: the nexus of stress, condition, and ornamentation. *Integr. Comp. Biol.* 54, 645–657
13. Hood, W.R. *et al.* (2018) Life history trade-offs within the context of mitochondrial hormesis. *Integr. Comp. Biol.* 58, 567–577
14. Casagrande, S. *et al.* (2020) Increased glucocorticoid concentrations in early life cause mitochondrial inefficiency and short telomeres. *J. Exp. Biol.* 223, jeb222513
15. Gangloff, E.J. *et al.* (2020) Mitochondria as central characters in a complex narrative: linking genomics, energetics, pace-of-life, and aging in natural populations of garter snakes. *Exp. Gerontol.* 137, 110967
16. Gyllenhammer, L.E. *et al.* (2020) Developmental programming of mitochondrial biology: a conceptual framework and review. *Proc. R. Soc. B Biol. Sci.* 287, 20192713
17. Salin, K. *et al.* (2019) Differences in mitochondrial efficiency explain individual variation in growth performance. *Proc. R. Soc. B Biol. Sci.* 286, 8
18. Mahalingam, S. *et al.* (2020) Chronic cold exposure induces mitochondrial plasticity in deer mice native to high altitudes. *J. Physiol.* 598, 5411–5426
19. Scott, G.R. *et al.* (2018) The mitochondrial basis for adaptive variation in aerobic performance in high-altitude deer mice. *Integr. Comp. Biol.* 58, 506–518
20. Treberg, J.R. *et al.* (2018) Comparing electron leak in vertebrate muscle mitochondria. *Integr. Comp. Biol.* 58, 495–505
21. Hill, G.E. (2016) Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap. *Ecol. Evol.* 6, 5831–5842
22. Angajala, A. *et al.* (2018) Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. *Front. Immunol.* 9, 1605
23. Spinelli, J.B. and Haigis, M.C. (2018) The multifaceted contributions of mitochondria to cellular metabolism. *Nat. Cell Biol.* 20, 745–754
24. Wallace, D.C. and Fan, W. (2010) Energetics, epigenetics, mitochondrial genetics. *Mitochondrion* 10, 12–31
25. Sokolova, I. (2018) Mitochondrial adaptations to variable environments and their role in animals' stress tolerance. *Integr. Comp. Biol.* 58, 519–531
26. Stier, A. *et al.* (2017) How to measure mitochondrial function in birds using red blood cells: a case study in the king penguin and perspectives in ecology and evolution. *Methods Ecol. Evol.* 8, 1172–1182
27. Balaban, R.S. *et al.* (2005) Mitochondria, oxidants, and aging. *Cell* 120, 483–495
28. Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300
29. Salmon, A.B. *et al.* (2010) Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radic. Biol. Med.* 48, 642–655
30. Speakman, J.R. and Garratt, M. (2014) Oxidative stress as a cost of reproduction: beyond the simplistic trade-off model. *BioEssays* 36, 93–106
31. Zhang, Y. and Hood, W.R. (2016) Current versus future reproduction and longevity: a re-evaluation of predictions and mechanisms. *J. Exp. Biol.* 219, 3177–3189
32. Monaghan, P. *et al.* (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements, and interpretation. *Ecol. Lett.* 12, 75–92
33. Zuo, L. *et al.* (2015) Biological and physiological role of reactive oxygen species - the good, the bad and the ugly. *Acta Physiol.* 214, 329–348
34. Hood, W.R. *et al.* (2019) An ecologist's guide to mitochondrial DNA mutations and senescence. *Integr. Comp. Biol.* 59, 970–982
35. Hill, G.E. (2019) *Mitonuclear Ecology*, Oxford University Press
36. Lane, N. (2011) Mitonuclear match: optimizing fitness and fertility over generations drives ageing within generations. *BioEssays* 33, 860–869
37. Jastroch, M. *et al.* (2010) Mitochondrial proton and electron leaks. *Essays Biochem.* 47, 53–67
38. Willis, W.T. *et al.* (2016) A simple hydraulic analog model of oxidative phosphorylation. *Med. Sci. Sports Exerc.* 48, 990–1000
39. Korshunov, S.S. *et al.* (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett.* 416, 15–18
40. Chung, D.J. *et al.* (2018) Mitochondria, temperature, and the pace of life. *Integr. Comp. Biol.* 58, 578–590
41. Brand, M.D. (2005) The efficiency and plasticity of mitochondrial energy transduction. *Biochem. Soc. Trans.* 33, 897–904
42. Brand, M.D. (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Exp. Gerontol.* 35, 811–820
43. Nedergaard, J. *et al.* (2001) UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta Bioenerg.* 1504, 82–106
44. Speakman, J.R. *et al.* (2004) Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* 3, 87–95
45. Monternier, P.-A. *et al.* (2014) Mitochondrial phenotypic flexibility enhances energy savings during winter fast in king penguin chicks. *J. Exp. Biol.* 217, 2691–2697
46. Speakman, J.R. (2005) Body size, energy metabolism, and lifespan. *J. Exp. Biol.* 208, 1717–1730
47. Seebacher, F. (2020) Is endothermy an evolutionary by-product? *Trends Ecol. Evol.* 35, 503–511
48. Hill, G.E. *et al.* (2019) Plumage redness signals mitochondrial function in the house finch. *Proc. R. Soc. B Biol. Sci.* 286, 20191354
49. White, C.R. and Kearney, M.R. (2013) Determinants of interspecific variation in basal metabolic rate. *J. Comp. Physiol. B.* 183, 1–26
50. Konarzewski, M. and Książek, A. (2013) Determinants of intraspecific variation in basal metabolic rate. *J. Comp. Physiol. B.* 183, 27–41
51. Auer, S.K. *et al.* (2017) Resting vs. active: a meta-analysis of the intra- and inter-specific associations between minimum, sustained, and maximum metabolic rates in vertebrates. *Funct. Ecol.* 31, 1728–1738
52. Mathot, K.J. *et al.* (2019) The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biol. Rev.* 94, 1056–1074
53. Pettersen, A.K. *et al.* (2018) Understanding variation in metabolic rate. *J. Exp. Biol.* 221, jeb166876
54. Salin, K. *et al.* (2016) Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. *Physiol. Biochem. Zool.* 89, 511–523
55. White, C.R. *et al.* (2019) The origin and maintenance of metabolic allometry in animals. *Nat. Ecol. Evol.* 3, 598–603
56. Costantini, D. (2019) Understanding diversity in oxidative status and oxidative stress: The opportunities and challenges ahead. *J. Exp. Biol.* 222, jeb194688
57. Blount, J.D. *et al.* (2016) Oxidative shielding and the cost of reproduction. *Biol. Rev.* 91, 483–497
58. Speakman, J.R. *et al.* (2015) Oxidative stress and life histories: unresolved issues and current needs. *Ecol. Evol.* 5, 5745–5757
59. Barneche, D.R. *et al.* (2019) Warming increases the cost of growth in a model vertebrate. *Funct. Ecol.* 33, 1256–1266
60. Salin, K. *et al.* (2018) Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Funct. Ecol.* 32, 2149–2157

61. Salin, K. *et al.* (2015) Individuals with higher metabolic rates have lower levels of reactive oxygen species *in vivo*. *Biol. Lett.* 11, 2015–2018
62. Brookes, P.S. (2005) Mitochondrial H + leak and ROS generation: an odd couple. *Free Radic. Biol. Med.* 38, 12–23
63. Mookerjee, S.A. *et al.* (2010) Mitochondrial uncoupling and lifespan. *Mech. Ageing Dev.* 131, 463–472
64. Caldeira Da Silva, C.C. *et al.* (2008) Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance, and longevity. *Aging Cell* 7, 552–560
65. Salin, K. *et al.* (2012) Alteration of mitochondrial efficiency affects oxidative balance, development, and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.* 215, 863–869
66. Stier, A. *et al.* (2020) Short Communication: long-term intake of the illegal diet pill DNP reduces lifespan in a captive bird model. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 108944
67. Stier, A. *et al.* (2014) Mitochondrial uncoupling as a regulator of life-history trajectories in birds: An experimental study in the zebra finch. *J. Exp. Biol.* 217, 3579–3589
68. Walter, I. and Seebacher, F. (2009) Endothermy in birds: underlying molecular mechanisms. *J. Exp. Biol.* 212, 2328–2336
69. Gaudry, M.J. *et al.* (2017) Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades. *Sci. Adv.* 3, e1602878
70. Stier, A. *et al.* (2014) Mitochondrial uncoupling prevents cold-induced oxidative stress: a case study using UCP1 knockout mice. *J. Exp. Biol.* 217, 624–630
71. Stier, A. *et al.* (2014) Chronic mitochondrial uncoupling treatment prevents acute cold-induced oxidative stress in birds. *J. Comp. Physiol. B.* 184, 1021–1029
72. Havird, J.C. *et al.* (2020) Powerhouses in the cold: mitochondrial function during thermal acclimation in montane mayflies. *Philos. Trans. R. Soc. B Biol. Sci.* 375, 20190181
73. Hu, S.N. *et al.* (2017) Temperature and photoperiod as environmental cues affect body mass and thermoregulation in Chinese bulbuls, *Pycnonotus sinensis*. *J. Exp. Biol.* 220, 844–855
74. Jimenez, A.G. and Williams, J.B. (2014) Rapid changes in cell physiology as a result of acute thermal stress House sparrows, *Passer domesticus*. *J. Therm. Biol.* 46, 31–39
75. Loughland, I. and Seebacher, F. (2020) Differences in oxidative status explain variation in thermal acclimation capacity between individual mosquitofish (*Gambusia holbrooki*). *Funct. Ecol.* 34, 1380–1390
76. Chung, D.J. and Schulte, P.M. (2015) Mechanisms and costs of mitochondrial thermal acclimation in a eurythermal killifish (*Fundulus heteroclitus*). *J. Exp. Biol.* 218, 1621–1631
77. Harada, A.E. *et al.* (2019) Variation in thermal tolerance and its relationship to mitochondrial function across populations of *Tigriopus californicus*. *Front. Physiol.* 10, 213
78. Roussel, D. *et al.* (2019) Threshold effect in the H2O2 production of skeletal muscle mitochondria during fasting and refeeding. *J. Exp. Biol.* 222, jeb196188
79. Gnaiger, E. *et al.* (2019) Mitochondrial respiratory states and rates. *MitoFit Preprint Arch* https://doi:10.26124/mitofit:190001.v6–Published in BEC: 2020-05-20 Mitochondrial physiology. *Bioenerg Commun* 2020.1. https://doi:10.26124/bec:2020-0001.v1
80. Salin, K. *et al.* (2018) The RCR and ATP/O indices can give contradictory messages about mitochondrial efficiency. *Integr. Comp. Biol.* 58, 486–494
81. Krajčová, A. *et al.* (2020) High resolution respirometry to assess function of mitochondria in native homogenates of human heart muscle. *PLoS One* 15, e0226142
82. Acin-Perez, R. *et al.* (2020) A novel approach to measure mitochondrial respiration in frozen biological samples. *EMBO J.* 39, e104073
83. Lagido, C. *et al.* (2008) Bridging the phenotypic gap: real-time assessment of mitochondrial function and metabolism of the nematode Caenorhabditis elegans. *BMC Physiol.* 8, 7
84. Goncalves, R.L.S. *et al.* (2015) Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed *ex vivo* under conditions mimicking rest and exercise. *J. Biol. Chem.* 290, 209–227
85. Hill, G.E. *et al.* (2013) Changes in concentrations of circulating heat-shock proteins in House Finches in response to different environmental stressors. *J. F. Ornithol.* 84, 416–424
86. Sjövall, F. *et al.* (2013) Mitochondrial respiration in human viable platelets-methodology and influence of gender, age, and storage. *Mitochondrion* 13, 7–14
87. Gutiérrez, J.S. *et al.* (2019) Oxidative status and metabolic profile in a long-lived bird preparing for extreme endurance migration. *Sci. Rep.* 9, 1–12
88. Stier, A. *et al.* (2019) Plastic but repeatable: rapid adjustments of mitochondrial function and density during reproduction in a wild bird species. *Biol. Lett.* 15, 9–13
89. Dawson, N.J. and Salmón, P. (2020) Age-related increase in mitochondrial quantity may mitigate a decline in mitochondrial quality in red blood cells from zebra finches (*Taeniopygia guttata*). *Exp. Gerontol.* 133, 110883
90. Karamercan, M.A. *et al.* (2013) Can peripheral blood mononuclear cells be used as a proxy for mitochondrial dysfunction in vital organs during hemorrhagic shock and resuscitation? *Shock* 40, 476–484
91. Tyrrell, D.J. *et al.* (2016) Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol.* 10, 65–77
92. Tyrrell, D.J. *et al.* (2017) Blood-based bioenergetic profiling reflects differences in brain bioenergetics and metabolism. *Oxidative Med. Cell. Longev.* 2017, 1–9
93. Rose, S. *et al.* (2019) A comparative study of mitochondrial respiration in circulating blood cells and skeletal muscle fibers in women. *Am. J. Physiol. Endocrinol. Metab.* 317, E503–E512
94. Hedges, C.P. *et al.* (2019) Peripheral blood mononuclear cells do not reflect skeletal muscle mitochondrial function or adaptation to high-intensity interval training in healthy young men. *J. Appl. Physiol.* 126, 454–461
95. Brand, M.D. and Nicholls, D.G. (2011) Assessing mitochondrial dysfunction in cells. *Biochem. J.* 435, 297–312
96. Doerrier, C. *et al.* (2018) High-resolution fluorespirometry and OXPHOS protocols for human cCells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. *Methods Mol. Biol.* 1782, 31–70
97. Estabrook, R.W. (1967) Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Methods Enzymol.* 10, 41–47
98. Fontaine, E.M. *et al.* (1997) The yield of oxidative phosphorylation is controlled both by force and flux. *Biochem. Biophys. Res. Commun.* 232, 532–535
99. Salin, K. *et al.* (2016) Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates and calculation of effective P/O ratios. *Physiol. Rep.* 4, e13007
100. Passos, J.F. *et al.* (2007) Mitochondria and ageing: winning and losing in the numbers game. *BioEssays* 29, 908–917