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SYMPOSIUM

A Mitochondrial Perspective on the Demands of Reproduction

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Synopsis The cost of supporting traits that increase mating opportunities and maximize the production of quality offspring is paid in energy. This currency of reproduction is enabled by bioenergetic adaptations that underlie the flexible changes in energy utilization that occur with reproduction. This review considers the traits that contribute to variation in the capacity of an organ to produce ATP. Further, it synthesizes findings from studies that have evaluated bioenergetic adaptations to the production of sexually selected traits and performance during reproduction and the role of change in mitochondrial respiratory performance in the tradeoff between reproduction and longevity. Cumulatively, these works provide evidence that in selecting for redder males, female finches will likely mate with a male with high mitochondrial respiratory performance and, potentially, a higher probability of mitonuclear compatibility. Females from diverse taxa allocate more to reproduction when the respiratory performance of mitochondria or density of the inner mitochondrial membrane in the liver or skeletal muscle is higher. Finally, reproduction does not appear to have persistent negative effects on mitochondrial respiratory performance, countering a role for mitochondria in the trade-off between reproduction and longevity. I close by noting that adaptations that improve mitochondrial respiratory performance appear vital for optimizing reproductive fitness.

Introduction

As eukaryotic life gained complexity, evolving from single cells to simple multicellular organisms to bilaterian animals with designated germ lines, reproduction became increasingly complex and increasingly energetically demanding (Lane 2006; Wallace 2010; Gao et al. 2022). Consequently, the race to contribute descendants to the next generation gave rise to an incredible diversity of reproductive strategies. In these complex forms, the challenge of meeting the energetic demands of reproduction requires flexible variation in the expression of traits that allow for greater nutrient intake, greater nutrient and oxygen delivery to supporting tissues, and an increase in the capacity of tissues to produce adenosine triphosphate (ATP) (Anderson et al. 1970; Speakman and McQueenie 1996; Selman et al. 2012; Stier et al. 2019). Further, because there are limits to the amount of ATP an individual can produce or energy it can expend (Hammond and Diamond 1994; Hammond et al. 1996; Speakman and Krol 2005), animals are thought to optimize allocation to reproduction by reducing support for other energetically demanding processes, including those that support longevity (Kirkwood 1990).

Over the last two decades, the search for traits that underlie individual variation in fitness has led investigators inside the cell to focus on mitochondria. The reasons for this are two-fold: (1) mitochondria are the key source of ATP required to support reproduction and survival and (2) mitochondria are the primary source of free radicals in the body that are thought to have a negative impact on the performance of the mitochondria and other cellular components (Murphy 2009). Numerous reviews have synthesized what we have learned from studies evaluating *oxidative stress* as a *cost of reproduction* (Selman et al. 2012; Speakman et al. 2015; Blount et al. 2016; Alonso-Alvarez et al. 2017), but none have simultaneously considered mitochondrial respiration. With this review, I will not reconsider whether oxidative stress is a cost of reproduction. But, when available, I will discuss *oxidative damage* markers when

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paired with mitochondrial respiration measurements, noting whether there could be a negative or positive interaction between mitochondrial respiration and oxidative damage. This review aims to describe the new insights we gained quantifying how animals have adapted to meet the energetic demands of reproduction and the consequences of that demand.

Mechanisms for increasing ATP production

Reproductive animals typically display greater food intake both in response to greater energy demand and in anticipation of such demand (Illius et al. 2002; Stephens et al. 2009). Greater nutrient intake or mobilization increases nutrient delivery to active tissues, providing substrate for increased ATP production and utilization by single or multiple organ systems within the reproductive adult. Increased nutrient intake also provides the building blocks for anabolic processes that support any morphology changes in the adult and for the production of gametes and developing young. These changes are reflected in well-recognized increases in basal metabolic rate and daily energy expenditure (Gittleman and Thompson 1988), which typically exceed those of their non-reproductive counterparts. While our understanding of the cost of specific traits that support reproduction is limited, it is expected that the energetic costs of supporting some traits vital to the reproduction effort will be trivial while others will be high.

Nutrients are mobilized throughout the body, where they are metabolized to *OXPHOS substrates* that ultimately donate electrons (NADH and $FADH_2$) to the electron transport system of the mitochondria to support *oxidative phosphorylation* (OXPHOS). When an organ is at rest, the mitochondria use a minimum level of nutrients and, thus, electron donors and oxygen to maintain the proton motive force across the inner membrane. Further, a limited amount of ATP will be used to support housekeeping functions (Willis et al. 2016). The amount of oxygen used to maintain the proton motive force is quantified as *state 4 respiration*. This is measured under the conditions of abundant oxygen and substrate but no ADP. State 4 respiration allows the mitochondria to overcome both the passive and active leak of protons across the inner membrane, and thus, state 4 respiration is also referred to as *leak respiration* (Koch et al. 2021). When the cells need more ATP, oxygen and substrate utilization increase. More protons are pumped into the intermembrane space, and those protons are used to power the synthesis of ATP from ADP and inorganic phosphate by the ATP synthase. Yet, each mitochondrion displays a limit to its capacity to produce ATP. The maximum capacity of mitochondria to use oxygen when provided abundant substrates and ADP is referred to as *state 3 respiration* (Brand and Nicholls 2011; Willis et al. 2016).

Both state 3 and state 4 respiration are measures of oxygen utilization by the cell *ex vivo*. Importantly, an increase in oxygen utilization by the *electron transport chain* (ETC) is commonly coupled to an increase in ATP production, with the ETC pumping proteins into the intermembrane space that create a proton gradient and the potential energy to fuel the synthesis of ATP from ADP (Stryer 1999). It is important to note that *coupling efficiency*, or the ratio of ATP production relative to oxygen utilization, is variable (Brand 2005), and in rare instances, the production of ATP can be bypassed altogether (i.e., brown adipose tissue using uncoupling protein-1 to produce heat, Williamson et al. 1970). Thus, an increase in oxygen utilization may not be coupled with an equally high increase in ATP production (Brand 2005). Nevertheless, I will interpret an increase in the number of mitochondria and an increase in state 3 respiration as having the potential to increase ATP production. Further, *ex vivo* measurements of mitochondrial respiration provide information about the relative changes that occur under different conditions but do not provide measures of absolute change in oxygen use or ATP production in a cell or organ, given that *ex vivo* conditions do not match the endogenous conditions experienced by mitochondria *in vivo*.

Organ systems have multiple processes, which can be used when the energy demands of reproduction are high (Somero et al. 2016). For example, across female mammals, the liver appears to play an important role in providing glucose and fatty acids needed by the uterus and mammary glands during gestation and lactation (Rook 2000; Schlumbohm and Harmeyer 2008; Zhang et al. 2017; Wuyts et al. 2021; Ramos-Roman et al. 2022; Arlt et al. 2023). During reproduction, the liver of mammals commonly increases in size (Hollister et al. 1987; Parra et al. 2014; Bartlett et al. 2021). Assuming mitochondrial volume and coupling efficiency by the mitochondria are held constant, this change in liver size can increase the organ's capacity for ATP production and, in some species, could even be sufficient to support the liver's increased demand for ATP during reproduction. In addition to increasing mitochondrial volume, the efficiency of processes that carry and modify nutritional substrates that ultimately deliver electrons to the *electron transport system* (ETS) as NADH or $FADH_2$ can also be modified, as can the performance of the ETS (Stucki 1976) (Fig. 1).

Changes in the performance of the ETS are most commonly evaluated by characterizing proton leaks and the consequences of electron leaks (Koch et al. 2021).

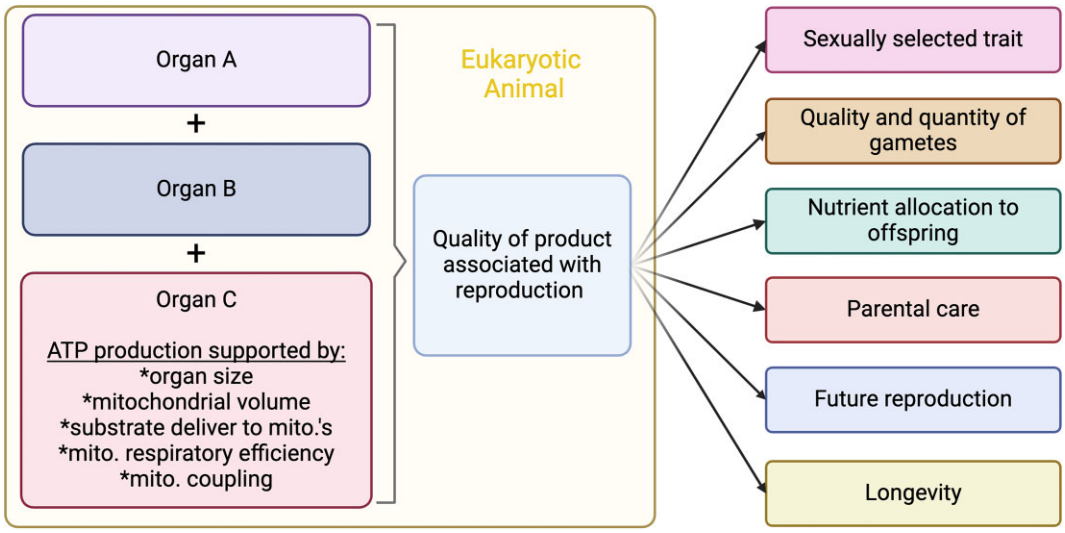

Fig. 1. Organs vital to producing products or behaviors necessary for successful reproduction (including those shown on the right of the figure) may act synergistically or even trade off resources. Within any given organ, several processes can interact to alter the capacity of the organ to produce sufficient ATP to support the change in demand during reproduction. These processes are listed in organ C (mito. = mitochondria).

The movement of protons from the intermembrane space back into the matrix, independent of ATP synthase, increases state 4 respiration (Brand 1990). Proton leak reduces the proton motive force and, thus, the amount of ATP produced from a given amount of NADH or $FADH_2$. Such a loss of membrane potential can result from the passive leak of protons across the inner mitochondrial membrane or from the active transfer of protons out of the inner membrane space by uncoupling proteins and adenine nucleotide translocase (Jastroch et al. 2010; Koch et al. 2021). In contrast, leaked electrons can interact with oxygen, nitrogen, halogens, or sulfur. Yet, interaction with oxygen is most common, forming reactive oxygen species (ROS) (Murphy 2009; Halliwell and Gutteridge 2015; Shields et al. 2021). ROS can give rise to oxidative stress, which occurs when ROS production exceeds the cell's capacity to produce antioxidants that can quench it (Costantini 2014; Skrip and McWilliams 2016). The oxidative damage is a consequence of oxidative stress. ROS that are not quenched by antioxidants can damage lipids, proteins, and nucleic acids and have the potential to damage molecules throughout the cell, particularly in mitochondria, due to their proximity to a key (but not exclusive, Zhang and Wong 2021) source of ROS and, thus, are thought to contribute to reduced *mitochondrial respiratory performance* and reduced ATP production (Cui et al. 2011).

While ROS have the capacity to cause damage, it is important to realize that a change in production of ROS can signal the upregulation of antioxidants that prevent damage and potentially oxidative stress, repair processes that mend damaged molecules, mitophagy that removes damaged mitochondria, and mitochondrial biogenesis that produces new mitochondria (Ray et al. 2012; Ristow and Schmeisser 2014). Further, an oxidative event may lower baseline ROS levels (Zhang et al. 2018; Yap et al. 2022). As a consequence of these signaling processes, modest levels of ROS production have been shown to have proximate benefits on the respiratory performance of mitochondria and have been proposed to have ultimate benefits on the fitness and longevity of animals (Zhang and Hood 2016; Hood et al. 2018; Zhang et al. 2018). The theory that modest levels of ROS are beneficial while high levels of ROS induce reduced mitochondrial performance and longevity associated with persistent oxidative damage has been termed mitochondrial hormesis (Tapia 2006; Ristow and Schmeisser 2014). By measuring mitochondrial or cellular function and oxidative damage simultaneously, it is possible to determine if the damage that has accumulated has altered the performance of the cell or organelle.

It is also important to note that other mechanisms can also be up- or down-regulated to alter mitochondrial respiration, including the enzymatic activity of mitochondrial complexes (Stram and Payne 2016; Mathers and Staples 2019), mitochondrial fission, fusion, and rate of mitophagy (i.e., mitochondrial dynamics) (Benard et al. 2011; Wai and Langer 2016), various aspects of mitochondrial morphology (Heine and Hood 2020), mitochondrial membrane composition and fluidity (Schon 2018), and changes in the relative portion of ETC complexes forming supercomplexes (Dudkina

et al. 2010; Hutchinson et al. 2022). Most of these processes are unstudied in the context of the energetic demands and consequences of reproduction.

Bioenergetics and sexual selection

Through sexual selection, many species have evolved traits that indicate the quality of the individual to rivals and potential mates. While researchers have identified which traits have been selected as armaments and ornaments in many species, it has been more challenging to identify why many of these traits are indicators of individual quality. For example, female House Finches (*Haemorhous mexicanus*) have been shown to prefer mates with breast feathers that display a redder hue and a more intense red coloration (Hill 1991). Decades of research has shown that males with more intense, redder carotenoid-based coloration outperform males with duller red or orange-to-yellow hues in multiple ways. Redder males are more resistant to and recover from infection more quickly, provision offspring more frequently, and are more likely to survive the winter than less red males (Hill 2002). The key question is: what is the mechanism that links the redness of feathers to individual quality?

The most prominent theory for how sexually selected traits are linked to energetically demanding activities is the resource tradeoff hypothesis (Halliday 1987; Zera and Harshman 2001; Koch and Hill 2018), which posits that energy allocation to sexual selection necessitates a tradeoff between allocating resources to the sexually selected trait and self-maintenance. Yet, the findings of Hill's lab suggest that vital maintenance processes appeared to be positively, not negatively, correlated with the expression of sexually selected traits. Hill and colleagues gained greater support for this pattern by directly examining the relationship between mitochondrial respiratory performance and coloration. They found that the liver mitochondria of redder males displayed a higher respiratory control ratio when provided complex I substrates (pyruvate, malate, and glutamate), higher mitochondrial membrane potential (MMP), and lower peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)—which stimulates mitochondrial replacement (Hill et al. 2019). Combined, these data suggest that the mitochondria of redder animals have the potential to produce more ATP (higher RCR and high MMP) and mitochondria of higher quality, given that they have a reduced need for replacement (lower PGC-1α). These findings suggest that mitochondrial respiratory performance lies at the heart of condition-dependent signaling. A positive tie to the performance of mitochondria supports a new model—the shared pathway hypothesis—which suggests that the production of colorful ornaments arises as honest signals of male condition because of their physiological links between vital cellular processes and coloration (Hill 2011; Weaver et al. 2017; Powers and Hill 2021). While mitochondria are maternally inherited, the nucleus produces ~1200 nuclear genes (mt-N genes) that function in the mitochondria (Hill 2019). Thus, selecting males with high-quality mt-N genes would increase the probability that a female would produce young with similar high-quality alleles and display a more robust phenotype (Hill and Johnson 2013).

This work sparked additional studies that suggest mitochondrial respiratory performance may underlie the differential expression of other sexually selected traits. Focusing on another bird that displays red-carotenoid-based coloration, Cantarero et al. (2020) evaluated the relationship between mitochondrial phenotype and red coloration in male red crossbills (*Loxia curvirostra*) by injecting the birds with mimetics of ubiquinone (an OXPHOS electron carrier) (mitoQ) or superoxide dismutase (an antioxidant) (mitoTEMPO), or a control solution. They found that male birds exposed to mitoQ reduced circulating yellow carotenoids, while mitoTEMPO increased circulating red carotenoid canthaxanthin, which is important in red pigmentation. The mitoTEMPO males that had redder coloration before the onset of this study were able to take advantage of the increased circulating canthaxanthin and transfer it into their feathers to display a redder color, while birds that had been duller before the study did not express greater red coloration. The authors speculated that improved mitochondrial respiratory performance in redder males may have allowed those individuals to begin ketolating yellow carotenoids into canthaxanthin more quickly than the duller birds. This would have allowed the redder birds to maintain high circulating canthaxanthin for longer than dull birds, which would have given them a greater opportunity to transfer the pigments into their feathers before feather development ended (Cantarero et al. 2020); unfortunately, this was not measured directly.

Crino et al. (2022) showed that the development environment of zebra finches (*Taeniopygia guttata*) impacts both male song quality and mitochondrial respiratory performance of whole red blood cells (RBC). Specifically, they showed that male zebra finches exposed to corticosterone during development have mitochondria with a lower maximum capacity and display higher-frequency calls. These results suggest that mitochondrial performance may modulate this sexually selected trait, although it remains unclear what components of the male call are preferred by females (Crino et al. 2022).

Bioenergetics and offspring provisioning

The energetic demands of reproduction are best studied, and often most costly, among species with extended parental care. Research on the mitochondrial strategy for supporting that demand has largely been biased toward endotherms, which commonly have extended parental care and relatively large body sizes. With this, I provide examples of how reproductive animals support the need for greater ATP than virgin non-reproductive individuals. I describe the role that differences in the ability to support mitochondrial respiration needed for ATP production may underlie individual variation in reproductive performance. Finally, I refer to evidence that change in the respiratory performance of a female's mitochondria before reproduction may have persistent effects, both impacting her ability to allocate to reproduction and the condition of her mitochondria after reproduction has ended.

In mammals, the daily energetic demands of lactation typically exceed those of pregnancy. For females of many species, energetic demands peak when the volume of milk produced is highest (Oftedal 1984). Quantification of the bioenergetic adaptations supporting milk synthesis has been limited to extra-mammary organs, where the mechanisms responsible for meeting the demand for fuel appear to vary by species. Two independent studies evaluated mitochondrial respiration in the liver of lactating house mice derived from different wild populations. In companion papers by Garratt et al. (2013) and Pichaud et al. (2013), mitochondrial volume (based on citrate synthase activity), mitochondrial respiration, and oxidative stress were compared between the livers of non-reproductive mice, mice that suckled two, and mice that suckled eight young. Data were collected at peak lactation. They found that the liver mitochondria of both groups of reproductive females displayed higher mitochondrial volume and a trend toward lower maximum mitochondrial respiratory capacity (state 3) when presented on an organ mass-specific basis and normalized to citrate synthase activity (Pichaud et al. 2013). Further, there was no difference in oxidative damage to proteins (protein carbonyls) between groups (Garratt et al. 2013).

In another study, Mowry et al. (2017) found no significant difference in liver mitochondria's *respiratory control ratio* (RCR, state 3/state 4) or leak (state 4) in age-matched lactating and non-reproductive mice. These values were normalized to mitochondrial protein content, but they were not presented relative to the mass of the liver. While no markers of mitochondrial volume were evaluated, PGC-1α, which stimulates mitochondrial biogenesis, was not upregulated. Nevertheless, the size of the liver itself was more than double that of non-reproductive mice (Mowry et al. 2017).

Comparing these studies, we can assume that the reproductive mice in Pichaud's study displayed enlarged livers, as was found by Mowry et al. (2017), given that increased liver mass is a consistent change observed with reproduction in wild-derived and laboratory strains of *Mus musculus* (Speakman and McQueenie 1996; Mowry et al. 2017). Since the energetic demands on the liver are undoubtedly high for the lactating versus non-reproductive mice in both studies, these observations suggest that the increased energetic demands placed on the liver during lactation in wild-derived mice are primarily met by increasing the size of the liver, rather than with a large change in the performance of the OXPHOS system.

Hyatt et al. (2018) evaluated changes in the bioenergetic capacity of laboratory rats across pregnancy and lactation. They found that the mass of the liver was greater in females who experienced pregnancy than those who did not and was greater still in those who experienced lactation, showing that a change in liver mass is also important to meet the metabolic demands placed on the liver during pregnancy and lactation in the rat. They showed that the liver of lactating mice displayed a higher PGC-1α than the other groups. While citrate synthase activity, a marker of mitochondrial volume, was not reported directly, higher PGC-1α suggests there is a signal to produce more mitochondria during lactation. In contrast, the RCR of liver mitochondria with complex I substrates (pyruvate and malate) was lowest during pregnancy and highest during lactation. RCR was also lower during pregnancy when provided a complex II substrate (succinate), but succinate was not elevated following parturition for either group. There was no difference between groups in oxidative damage to lipids (4HNE) or proteins (protein carbonyls) (Hyatt et al. 2018). These results indicate that, unlike the wild-derived mice, the livers of lab rats display a mix of strategies for supporting pregnancy and lactation. Specifically, a reduction in mitochondrial respiratory performance during pregnancy may have helped reduce lipolysis and increase lipid storage as adipose, which is later mobilized during lactation (Stuebe and Rich-Edwards 2009). Females support the energetic demands of the liver during lactation by increasing liver mass, mitochondrial turnover, and RCR, which are expected to increase the capacity of the mitochondria to produce ATP. While it is possible that artificial selection for improved reproductive performance contributed to the multi-pronged approach to supporting energetic demands, we do not have lab mouse or wild rat data available for comparison. Given that artificial selection has increased relative allocation to reproduction in outbred

laboratory mice and rats relative to their wild counterparts, a comparison between these populations of animals would provide interesting insight into how selection can impact bioenergetic capacity.

Variation among individuals in the processes that underlie the capacity to produce ATP by organs supporting parental care appears to be an important determinant of reproductive performance. For example, in a predatory wasp, European beewolf, females provide their developing offspring with honeybees as a source of nutrition. Female beewolves hunt for bees on flowers, sting their prey to paralyze it, and then carry the bee while in flight back to its nest to its offspring (Strohm and Daniels 2003). Female beewolves display high variation in hunting and reproductive success, and this variation appears to be determined in part by the capacity of flight muscle mitochondria. More specifically, Strohm and Daniels (2003) showed that the density of the ETS-hosting inner mitochondria membrane in beewolf flight muscle mitochondria is positively correlated with hunting success, as indicated by the outcome of its provisioning efforts, the number of bees per brood cell. From this, we can deduce that inner mitochondria membrane density likely increases the capacity of the flight muscles to produce ATP and, in turn, the reproductive performance in this species.

In dairy cows, an increase in the relative mass of the liver (Reynolds et al. 2004) and the relative volume of mitochondria in the liver (Laubenthal et al. 2016) are important for transitioning from the energetic demands of pregnancy to lactation. As was found in female beewolves, however, variation in traits that have the potential to support greater ATP production in the cow, that is, milk synthesis, appears to be responsible, at least in part, for individual differences in performance. Specifically, Favorit et al. (2021) found that the RCR of liver mitochondria is predictive of the volume of milk produced by the individual. This is particularly interesting in dairy cows, where artificial selection has maximized milk yields. Despite an increase in ROS production in mid- to late-lactation, there were no differences in oxidative damage (MDA and protein carbonyls) to the liver with the stage of lactation (Favorit et al. 2021). An important missing observation in the study of how mammals support the energy demands of their young is an evaluation of mitochondrial respiration in mammary tissue, where the final steps in milk synthesis are completed, and milk is transferred directly from the mother to the young. Such data would be incredibly valuable in understanding the mitochondrial response to selection.

There is substantial evidence showing that the exogenous conditions females experience before reproduction can have lasting effects on their subsequent reproductive performance (Clutton-Brock 1988; Bronson 1989). Yet, the extent to which bioenergetic processes underlie such effects is almost entirely unstudied. To determine if processes known to stimulate a mitochondrial hormetic response could impact subsequent reproductive performance, Zhang et al. (2018) evaluated the impact of relative activity before reproduction on subsequent reproductive performance by providing lab mice with a running wheel or not for one month prior to breeding. After one month, animals provided the opportunity to run voluntarily had a greater volume of mitochondria in their skeletal muscle, liver, and heart, but not brain, and no difference in oxidative damage to lipids (4HNE) or proteins (protein carbonyls) relative to mice that did not have a wheel. Further, skeletal muscle mitochondria displayed a higher RCR in mice that ran versus those that did not. Wheels were then removed, and the reproductive fitness of animals with access to a running wheel and those without was compared. Mice that had access to a running wheel for 1 month before reproduction gave birth to a larger litter, and despite a standardization of litter size to 8, mothers that had access to a running wheel before reproduction weaned a heavier litter than those that did not have a wheel. Finally, 1 week after weaning, mothers were sacrificed to evaluate mitochondrial performance, and it was found that the liver of females that had a running wheel before reproduction retained a greater volume of mitochondria, higher PGC-1α levels that stimulate mitochondrial biogenesis, and higher mitochondrial RCR. As a consequence of these differences, it was predicted that these females would display a higher performance during a subsequent breeding event if given the opportunity (Zhanget al. 2018), implying that the bioenergetic consequence of reproduction can impact future performance, as discussed in the next section.

Bioenergetics and the costs of reproduction

The cost of reproduction hypothesis suggests that individuals that allocate more toward reproduction will have a reduced capacity for future reproduction and reduced longevity (Williams 1966). The mechanisms proposed to underlie this tradeoff have included differential use of resources and somatic stores that would reduce those available to support future reproductive events and self-maintenance, as well as an accumulation of damage during reproduction that could impact future performance (Reznick et al. 2000; Shanley and Kirkwood 2000; Zera and Harshman 2001; Zhang and Hood 2016). Given that across species, mitochondrial respiratory performance declines with age, the mitochondria are a valuable place to look for evidence of

a tradeoff between reproduction and longevity (Zhang and Hood 2016).

Biogerontologists have identified the reduction in mitochondrial respiratory performance and mitochondrial membrane potential that occurs with age, known as mitochondrial dysfunction, as a key hallmark of aging (López-Otín et al. 2013, 2023; Miwa et al. 2022). In humans, it has been shown that RCR and the ATP/O ratio of liver mitochondria decline linearly between ages 31 and 76 (Yen et al. 1989), while capacity for ATP production and mtDNA content declines linearly between ages 18 and 89 in skeletal muscle mitochondria (Short et al. 2005). While a gradual decline may have limited impacts on human survival throughout their lives, maintaining high muscle function to support foraging and to evade predators is paramount for any free-ranging animal (Austad 1993).

Several investigators have searched for mechanisms responsible for the tradeoff between reproduction and longevity by evaluating the physiology of animals during reproduction. What is observed is that reproduction is often associated with considerable physiological flexibility. Thus, my lab has argued that the consequences of a reproductive event are best measured after the reproductive event has ended and reproductive tissues have regressed (Zhang and Hood 2016). By taking this approach, investigators can determine if the basal physiology of an animal has been altered in a manner that could impact future fitness and longevity. I will bias papers presented in this section toward studies that have taken this approach.

In a study on female lab rats, Hyatt et al. (2017) compared three groups: one that did not reproduce, one that did not suckle their young, and a third that completed lactation. They then evaluated the persistent impact of treatment on mitochondrial respiratory function and oxidative damage in the liver and skeletal muscle 15 weeks after parturition or 12 weeks after weaning. They found that liver mitochondria in the lactating group displayed higher state 3 and state 4 respiration via complex I (pyruvate, malate, and glutamate substrates) than in the other groups. Interestingly, complex II respiration (succinate) was significantly lower in skeletal muscle, but there was no difference in oxidative damage to proteins (protein carbonyls) or lipids (4HNE) between groups. These results suggest that lactation may protect a mother's condition, presumably improving the capacity of the liver to support its basic functions, such as gluconeogenesis and lipogenesis (Hyatt et al. 2017). Lower activity is associated with a lower maximum respiratory performance in skeletal muscle mitochondria (Krieger et al. 1980), and increased time spent running (training) increases running speed and duration (Kemi et al. 2005). Thus, lower complex II respiration could be associated with reduced muscle performance and put females that lactated at greater risk for depredation.

Park et al. (2020) evaluated variation in mitochondrial respiratory performance in the liver and skeletal muscle of age-matched outbred lab mouse females that did not reproduce, females that reproduced once, and females that reproduced four times. Tissues were collected at least two weeks after reproduction had ended. In these mice, body mass varied substantially among individuals, and the relationship between body mass and mitochondrial respiration in the liver varied with reproductive status. RCR decreased with increasing body mass in non-reproductive females, and RCR increased with increasing body mass in females that reared four litters (Park et al. 2020). A significant interaction between body mass and RCR in the liver indicated that RCR was greatest for females with the highest body mass that bred four times but lowest in females that did not breed. In mice, females that have reproduced maintain a persistently larger liver mass and larger blood volume after reproduction has ended (Speakman and McQueenie 1996). Thus, higher body masses in non-reproductive mice are more likely to be associated with obesity than in reproductive mice. Interestingly, within the four-bout treatment group, females with the highest allocation to reproduction also had the highest liver mitochondrial respiratory performance, as indicated by a near-significant cumulative mass of all young weaned and liver RCR. Further, females that had allocated more to reproduction also displayed greater oxidative damage to liver proteins, suggesting that the detected damage did not hinder the relative mitochondrial respiratory performance or the reproductive performance of these females (Park et al. 2020). With high RCR after reproduction had ended, it is likely that the performance of these females would also have been greater during future reproductive events. These findings provided strong support for the idea that the negative consequences of prior reproduction are lowest in high-condition females (Reznick et al. 2000) and that high variance in condition among individuals can obscure potential tradeoffs between reproduction and somatic maintenance (van Noordwijk and de Jong 1986).

When animals are exposed to environmental stressors during reproduction, the balance between reproduction and self-maintenance is altered (Roff 1992; Stearns 1992; Hegemann et al. 2013). To determine if changes in mitochondrial respiration and oxidative damage underlie these shifts, Yap et al. (2022) compared wild-derived house mice (*Mus musculus domesticus*) provided corticosterone daily during mid-lactation to females that only received the vehicle and evaluated the impact of treatment on mitochondrial respiratory performance in liver and skeletal muscle

after reproduction had ended. Despite experimental adjustment of litter size at birth so that it was consistent between groups, females that received the corticosterone treatment weaned litters that weighed less than those that did not receive corticosterone, suggesting that corticosterone-treated females allocated less nutrients to their young during lactation or their young did not grow as efficiently during the suckling period (Yap et al. 2022). Interestingly, the maximum mitochondrial respiratory capacity (state 3) and leak respiration (state 4) of liver and skeletal mitochondria provided complex I and complex II substrates were comparable between the corticosterone and vehicle groups ten days after reproduction had ended. Further, oxidative damage to lipids (4HNE) and proteins (protein carbonyls) was also similar. These results suggest that there were no persistent differences in the cost of reproduction between treatment groups (Yap et al. 2022).

Conclusions and future direction

Here, I have reviewed a handful of studies that evaluated the bioenergetic mechanisms that underlie the production of sexually selected traits, the energy demands of offspring production, and the persistent consequences of energy allocation to reproduction. From these works, we have learned that assessment of a sexually selected trait displayed by males may provide females with information about the respiratory performance of a male's mitochondria and, therefore, information about the quality of the mt-N genes that a prospective mate would contribute to her offspring (Hill and Johnson 2013). We learned that different species, different lineages of animals, and different organ systems likely vary in the mechanisms they use to support the increased demand for ATP during reproduction. Further, individual differences in the ATP production capacity of organs vital to reproduction appear to underlie individual variation in reproductive performance. Finally, studies have found little evidence that reproduction negatively impacts the bioenergetic performance of organs vital to the reproductive effort. Indeed, the findings by Park et al. (2020) suggest that female mice with the highest lifetime allocation to reproduction continue to have the highest functioning liver mitochondria after four reproductive events, despite bearing any potential burden of greater oxidative damage. These findings support a broader application of the shared pathway hypothesis (Hill 2011), where greater cellular—and, in this case, mitochondrial—performance is associated with greater allocation to reproduction.

Much of what we know about the mechanisms that underlie variation in mitochondrial respiration that impacts the capacity for ATP production has come from studies of exercise physiology and various metabolic diseases. These studies provide valuable insight but little information on the bioenergetic adaptations that support the diversity of life-history patterns observed among animals today. Yet, research across taxa is starting to provide strong evidence that bioenergetic adaptations support many aspects of variation in individual performance, including variation in growth rates (Salin et al. 2019), capacity to hibernate (Staples 2014), avian migration (Rhodes et al. 2024), and capacity to survive under conditions of periodic anoxia (Sokolova 2018), among others. While the papers reviewed herein have improved our understanding of the role of bioenergetics in reproductive performance, there is much to learn about how evolutionary processes have acted on and are constrained by the ability of animals to support the energetic demands of reproduction.

Studies to date provide little evidence for a mitochondrial basis for a tradeoff between reproduction and longevity. While this failure to support reproductive tradeoffs from a mitochondrial respiration perspective may reflect the challenge of uncoupling the effect of individual condition and life history tradeoffs (van Noordwijk and de Jong 1986), it is important to remember that for many species, including the mice and rats highlighted herein, early life reproductive performance is paramount. Thus, selection on processes that optimize, if not maximize, reproductive performance must be strong. Given that among those studies completed to date, we find evidence that female finches select for males with high-quality mitochondria, processes that improve mitochondrial performance before reproduction in mice improve fitness, wasps, mice, rats, and cows with higher mitochondrial performance allocate more to reproduction, mice that previously allocated the most to reproduction continue to have the highest mitochondrial respiratory performance well after reproduction, I suggest that where selection on mitochondrial bioenergetics really comes into play is in improving reproductive fitness. Thus, I propose that mitochondrial respiratory performance is vital to variance in reproductive performance among individuals.

One of the major limitations of our ability to understand how animals meet the bioenergetic demands of reproduction is that we often compare relative differences between groups. Quantitative assessments of how much ATP is needed or used to support a specific process are lacking, and thus, all we can do is look for patterns in related but complex variables and predict how they interact to influence ATP production and animal performance. Further, much of the work that has been completed has been limited to small laboratory animals and animals of agricultural importance. Many of these animals have been subject to artificial selection.

Comparisons of free-living species within a closely related taxonomic group that varies in reproductive performance would be particularly valuable in understanding how different bioenergetic adaptations evolved to optimize reproductive fitness. Other processes, such as the quality and quantity of gametes and parental care that are not described here, should also be considered (**Fig. 1**).

Glossary of terms

Cost of reproduction hypothesis suggests that individuals who allocate more toward reproduction will have a reduced capacity for future reproduction and reduced longevity (Williams 1966).

Coupling efficiency is the ratio of ATP produced relative to oxygen used.

Electron transport chain **(ETC)** versus *electron transport system* **(ETS)**. The ETC specifically references the electron-transporting enzymes that contribute to oxidative phosphorylation in the mitochondria, including complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome c reductase), and complex IV (cytochrome c oxidase). The term electron transport system is used when referencing the electron transport chain plus the ATP synthase (complex V), which is necessary to complete OXPHOS.

Mitochondrial respiratory performance is the efficiency of oxygen utilization by the ETC. (I have intentionally used this term rather than more specific terminology in some instances because the conditions under which mitochondrial respiration is quantified often vary with the instrument used for quantifying mitochondrial respiration).

Oxidative damage is a consequence of oxidative stress. ROS that are not quenched by antioxidants can damage lipids, proteins, and nucleic acids (Cui et al. 2011).

Oxidative phosphorylation **(OXPHOS)** is the primary source of ATP for eukaryotic cells. During OXPHOS, the ETC complexes couple the movement electrons with the production of a protein gradient that powers the phosphorylation of ADP to ATP by the ATP synthase, making ATP available as a source of fuel for the organism (Stryer 1999; Box 1).

Oxidative stress occurs when reactive oxygen species production exceeds the cell's capacity to produce antioxidants that can quench ROS (Costantini 2014; Skrip and McWilliams 2016).

OXPHOS substrate or *substrate*. In the context of OXPHOS, a substrate is an organic molecule used to induce NADH or $FADH_2$ production that donates electrons to the ETC. *In vivo*, ingested or stored macronutrients are catabolized to provide mitochondria with substrates to supply the citric acid cycle and the ETC. *In vitro*, researchers can use a variety of substrates. Pyruvate, malate, and glutamate are often supplied to mitochondria to support the production of NADH, and succinate is used to provide $FADH_2$. Palmitoylcarnitine is also often used in avian studies because of the high use of fatty acids as fuel by members of this taxonomic group. It is a fatty acid attached to a carrier protein that allows the fatty acid to be transported into the mitochondria, where the fatty acid must go through β-oxidation before being supplied to the TCA cycle, where the electron donors are produced (Stryer 1999; Kuzmiak et al. 2012).

Reactive oxygen species **(ROS)** are a type of reactive species generated by the reduction of an oxygen-containing molecule by a free electron. The leak of electrons from the ETS is commonly reactive with oxygen to form superoxide. Reactive species include all products of reduction by a free electron, including reactive oxygen species, reactive nitrogen species, reactive halogen species, and reactive sulfur species (Halliwell and Gutteridge 2015). Importantly, the production of ROS is the most important (but not necessarily exclusive) reactive species produced as a product of electron leaks from the ETS (Murphy 2009).

Respiratory control ratio **(RCR)** is the ratio of state 3 and state 4 respiration. A high RCR indicates that mitochondria have the capacity for high substrate oxidation while maintaining low proton leak. A low RCR is considered a sign of dysfunction in a biomedical context (Brand and Nicholls 2011).

State 3 respiration is the maximum performance of coupled mitochondria when substrate, oxygen, and ADP are not limited.

State 4 respiration (i.e., **leak**) is a measure of basal respiratory performance of the mitochondria when ADP has been depleted and is generally considered a measurement of leak respiration (Koch et al. 2021). Specifically, (proton) leak is the passive or induced movement of protons from the intermembrane space back into the mitochondrial matrix. To compensate for the leak, the electron transport system must continuously use substrate and oxygen to maintain the proton gradient between the intermembrane space and the matrix and, thus, maintain a minimum proton motive force. Thus, state 4 respiration measures the amount of oxygen used to compensate for leak (Koch et al. 2021). Note that when the term leak is used, it is almost invariably used to describe proton leak, but electrons leak from the ETS.

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Conflict of interest

The author declares no conflict of interest.

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