

## Mitonuclear match: Optimizing fitness and fertility over generations drives ageing within generations

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Many conserved eukaryotic traits, including apoptosis, two sexes, speciation and ageing, can be causally linked to a bioenergetic requirement for mitochondrial genes. Mitochondrial genes encode proteins involved in cell respiration, which interact closely with proteins encoded by nuclear genes. Functional respiration requires the coadaptation of mitochondrial and nuclear genes, despite divergent tempi and modes of evolution. Free-radical signals emerge directly from the biophysics of mosaic respiratory chains encoded by two genomes prone to mismatch, with apoptosis being the default penalty for compromised respiration. Selection for genomic matching is facilitated by two sexes, and optimizes fitness, adaptability and fertility in youth. Mismatches cause infertility, low fitness, hybrid breakdown, and potentially speciation. The dynamics of selection for mitonuclear function optimize fitness over generations, but the same selective processes also operate within generations, driving ageing and age-related diseases. This coherent view of eukaryotic energetics offers striking insights into infertility and age-related diseases.

### Keywords:

■ apoptosis; free-radical leak; mitochondria; mitonuclear coadaptation; respiratory chain

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### Introduction: Mitochondria are essential to complex life. . .

All complex life on Earth is composed of eukaryotic cells. All eukaryotes either possess mitochondria, or once did and later lost them via reductive evolution to hydrogenosomes or mitosomes [1, 2]. The acquisition of mitochondria and the origin of the eukaryotic cell were, therefore, plausibly the same event [3], an interpretation corroborated by genomic studies, which indicate that the eukaryotic cell originated as a genomic chimera between an archaeon and a bacterial endosymbiont, the ancestor of mitochondria [4–7]. Why mitochondria made the critical difference has long been unclear. It was not aerobic respiration, protection against oxygen toxicity, or compartmentalization, as prokaryotes are adept at all three. Instead the key to complexity resides, for bioenergetic reasons, in the mitochondria themselves, specifically the tiny but mighty mitochondrial genome [8, 9].

All eukaryotes capable of oxidative phosphorylation independently retained a remarkably similar core mitochondrial genome, invariably encoding respiratory chain subunits. This evolutionary conservation leaves little doubt that these few remaining mitochondrial genes are strictly required for the maintenance of inner membrane potential [10, 11]. Mitochondrial membrane potential is no trivial force, having a field strength of 30 million V/m, equal to that discharged by a bolt of lightning. This distinguishes mitochondria from other endomembrane systems, such as the endoplasmic reticulum. Endosymbiosis has a unique ability to place the right genes in the right place to maintain membrane potential, while at once eliminating superfluous genes by reductive evolution and gene transfer to the nucleus.

Protein synthesis is energetically expensive [8]. The total amount of protein-coding DNA lost from all the endosymbionts combined equates to the amount of protein-coding DNA that could be supported, energetically, in the nucleus. In other words, only the streamlining and specialization of mitochondrial DNA (mtDNA) enabled the evolution, maintenance and expression of many thousands of new genes in the nucleus [8, 9]. This massively expanded genome capacity was

central to the evolution of multicellular organisms, and was *strictly dependent* on the reductive evolution of mitochondria, such that they retained only the minimum number of genes needed for respiration within individual mitochondria. Possessing thousands of mitochondria enabled eukaryotic cells to generate thousands of times the energy of a bacterium at a fraction of their total running costs – the consequence of bioenergetic specialization. The essential difference between prokaryotes and eukaryotes, the basis of all complex life, is not the nucleus alone but the extreme genomic polarization of eukaryotic cells, with giant nuclear genomes supported energetically by multiple necessarily tiny mitochondrial genomes.

### ...but mitochondria demand genomic matching

This energetic dependence of eukaryotes on tiny mitochondrial genomes dictates two conditions of overriding importance to their physiology and evolution. First, eukaryotic respiration is made possible *only* by the retention of multiple copies of this core genome. Second, these core genomes, because of their minimal nature, *must* interact with genes in the nucleus for respiration to work. This gives rise to the obligate mosaic character of all eukaryotic respiratory chains, in which subunits encoded by mitochondrial genes interact directly with subunits encoded in the nucleus. Despite this requirement for mosaic respiratory chains, the mitochondrial and nuclear genomes evolve in quite different ways (sexual versus asexual), and at different speeds. Even the evolution rate of mtDNA (after purging by selection) is around 10–30 times faster than nuclear genes in most animals, and the mutation rate (before selection) can be several orders of magnitude faster: 10,000–100,000 times faster in *Saccharomyces* [12]. In plants, where the nucleotide substitution rate is usually much lower, aberrant recombinations nevertheless give rise to high mutation rates [13]. In the face of such divergent tempi and modes of evolution, the mitochondrial and nuclear genomes must adapt to each other, or respiration will not work.

There is compelling evidence across eukaryotic orders from fungi and plants to invertebrates and mammals that the mitochondrial and nuclear genomes have indeed adapted to each other over evolutionary time [14, 15]. This evidence includes a high proportion of neutral mutations in nuclear and mitochondrial genes encoding respiratory-chain subunits [16]; a concordance in evolutionary rates of these mitochondrial and nuclear genes [17–19]; a decline in respiratory function in nuclear cytoplasmic hybrids (cybrids) [20–22]; and hybrid breakdown in introgressed populations, caused by mitonuclear incompatibilities [23]. In plants, hybrid breakdown also frequently involves mitonuclear incompatibilities [24].

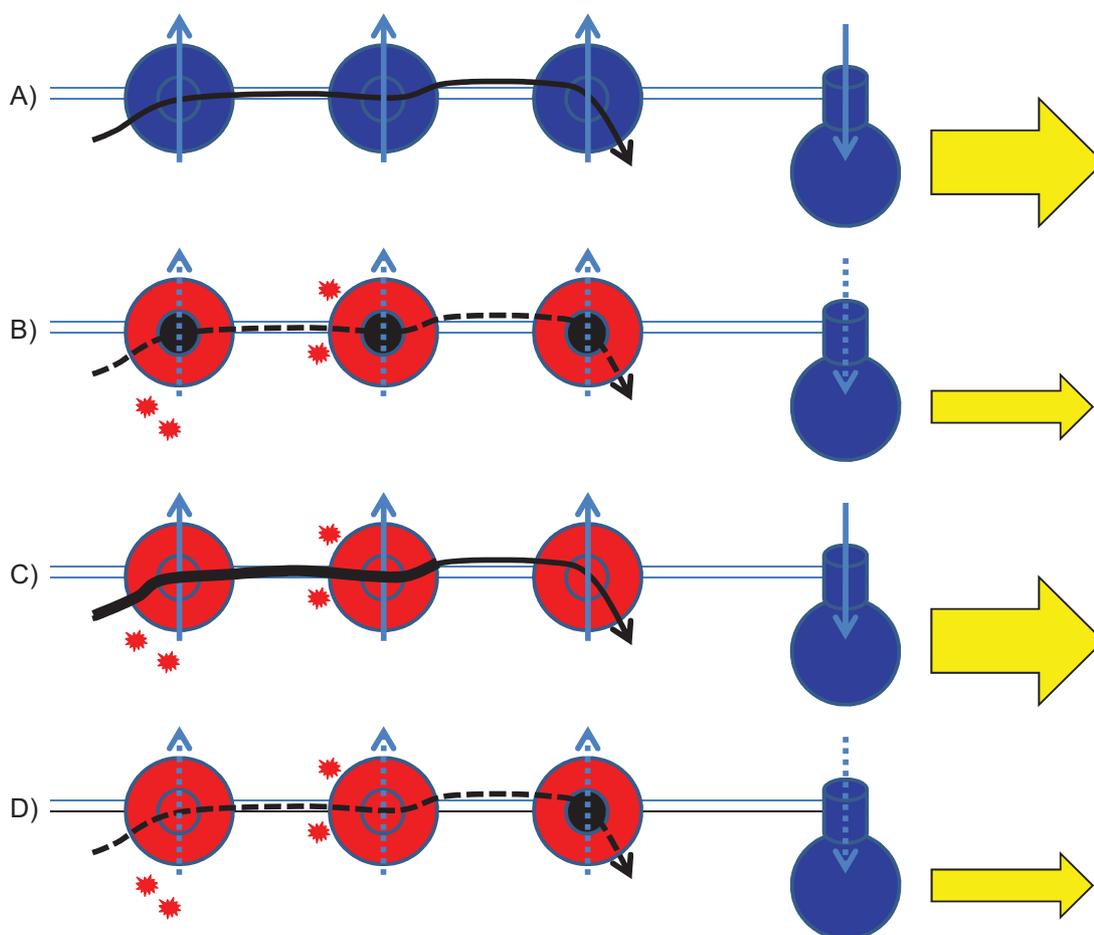
I propose here that selection for genomic match has played a defining role in the evolution of eukaryotes, potentially explaining not only the requirement for two sexes, but also playing an ongoing and critical role in fitness, fertility, adaptability, speciation, ageing and age-related disease. I argue that free-radical signalling is inescapably central to selection for genomic match.

### Selection for optimal mitonuclear function depends on free-radical leak

Oxidative phosphorylation involves the transfer of electrons through a succession of redox centres in respiratory chains, from an electron donor such as NADH, to a terminal acceptor such as oxygen. A slowing of electron transfer means that respiratory complexes become more highly reduced, which increases their reactivity with oxygen, corresponding to a rise in free-radical leak [25]. A slowing of electron transfer is the most likely outcome of any mismatch between mitochondrial and nuclear genomes. The reason relates to the mechanism of electron transfer. If the gap between adjacent redox centres in respiratory chains is increased by just 1 Å, electron transfer by quantum tunnelling slows down by an order of magnitude [26] and free-radical leak should rise accordingly. Given that hydrogen bonds and Van der Waal's forces act over distances in the range of 1–2 Å, it is likely that any changes to optimal subunit interactions would disrupt the distance between redox centres by more than 1 Å, slowing electron flow and increasing free-radical leak. Thus, a rise in free-radical leak is the predicted outcome of virtually any subunit mismatch, and indeed has been reported [27, 28].

Free radicals oxidize membrane lipids, notably cardiolipin, releasing cytochrome *c* [29]. Disruptions in electron flow impair ATP synthesis, so without compensation ATP levels fall. This triumvirate (high free-radical leak, cytochrome *c* release and low ATP levels) is the classic trigger for apoptosis, reported to 'general stupefaction' [30] in the mid 1990s. In fact, the combination emerges naturally from the biophysics of respiratory chains [31] – apoptosis can be seen as an intrinsic form of functional selection against cells with compromised respiration.

But genomic mismatch is not the only cause of respiratory insufficiency. Stoichiometry is important too (Fig. 1). If respiratory capacity is low relative to supply and demand, electrons back up in the respiratory chains (because they enter faster than they can exit), making the complexes more highly reduced, and increasing free-radical leak. This reduction state is equivalent to that arising from genomic mismatch, but in this case (low capacity), apoptosis would be the 'wrong' outcome. In principle, electron flow could be improved by raising capacity: expressing more respiratory complexes. This is achieved simply if mtDNA transcription is controlled by redox-sensitive transcription factors within mitochondria. While there is as yet little indication of the mechanism, intra-mitochondrial glutathione depletion does increase the transcript abundance of mtDNA-encoded proteins such as ND1 and COX1 [32], as would be predicted. Likewise, the mtDNA topoisomerase I is redox sensitive, with the oxidation of critical cysteine residues increasing rates of transcription and replication [33]. Mild chronic inhibition of complex III with antimycin A produces a modest rise in free-radical leak, which roughly doubles mitochondrial mass and transcript abundance via inactivation of mt Topo I (Fritz Boege, personal communication). These postulated free-radical signals are *intra-mitochondrial*, up-regulating mtDNA transcription locally, and potentially not affecting the redox state of the cell as a whole, or retrograde signalling to the nucleus. Such modulation of mtDNA transcription without changes in nuclear gene expression has been reported [34].



**Figure 1.** Free-radical leak depends on reduction state of respiratory chain. **A:** Normal ATP synthesis. Flow of electrons down respiratory chain to oxygen (black arrow) powers proton pumping across the membrane (blue arrows). The reverse flow of protons through the ATP synthase powers ATP synthesis (yellow arrow). Respiratory complexes are blue, depicting oxidized redox state and low free-radical leak. Inner circles depict subunits encoded by mtDNA, which are adapted to outer circles of nuclear-encoded subunits (hence same colour). **B:** Mitonuclear mismatch. Black inner circles depict core subunits encoded by mtDNA, which are not adapted to outer (nuclear-encoded) subunits. This slows electron flow (dashed black line). Respiratory complexes are shown in red, depicting reduced redox state, with high free-radical leak (miniature explosions). ATP synthesis (yellow arrow) is low as a result of slow electron flow and proton pumping (dashed blue lines). **C:** Low capacity relative to demand. Flow of electrons into respiratory chain outstrips capacity to transfer them to oxygen (thick black arrow). Respiratory complexes are red, depicting reduced redox state, with high free-radical leak (miniature explosions). ATP synthesis (yellow arrow) is equivalent to that in (A) but not sufficient to meet higher demand. Inner and outer circles (depicting mtDNA and nuclear-encoded subunits, respectively) are well adapted (hence same colour). **D:** Age-related heteroplasmy. Mutations in mtDNA give rise to mitonuclear mismatch, in which some mtDNA-encoded subunits (black inner circle) are not matched to nuclear background (hence different colour). This slows electron flow (dashed black arrow) and proton pumping (dashed blue arrows), renders the respiratory chain reduced (red circles) and increases free-radical leak (red explosions). ATP synthesis (yellow arrow) falls.

As noted, maintaining membrane potential over a wide area of internal bioenergetic membranes is the most plausible reason for mitochondria to retain genes at all [10, 11]. In essence, the reduction state of the inner membrane is the signal giver and output sensor for the *reactive* (as opposed to *proactive*) expression of mitochondrial genes. Certainly, translational regulation,

protein stability, phosphorylation and super-complex assembly all modulate respiratory rate; but these mechanisms ultimately revolve around transcriptional control, as demonstrated by the near-linear correlation between mtDNA copy number and respiratory rate [35]. This system enables respiratory stoichiometry to be calibrated locally to need, tailoring supply to demand. Such a system could only work if the transcription of mtDNA controls the rate of assembly of new respiratory complexes; and this is indeed the case [36–38]. Thus, free-radical leak signals respiratory insufficiency, activating mtDNA transcription factors, and culminating in the expression of new respiratory complexes exactly where needed. Because mitochondrial fusion and fission blurs the distinction between individual mitochondria, and because transcription and replication of mtDNA are frequently linked [36], a

rise in mtDNA transcription rate equates to mitochondrial biogenesis, termed here *reactive biogenesis*. In essence, reactive biogenesis is stimulated by intra-mitochondrial free-radical leak, and works to optimize respiration. Such optimization of respiration by free-radical leak has been demonstrated in mouse hybrids [28], cultured cells and whole animals [39].

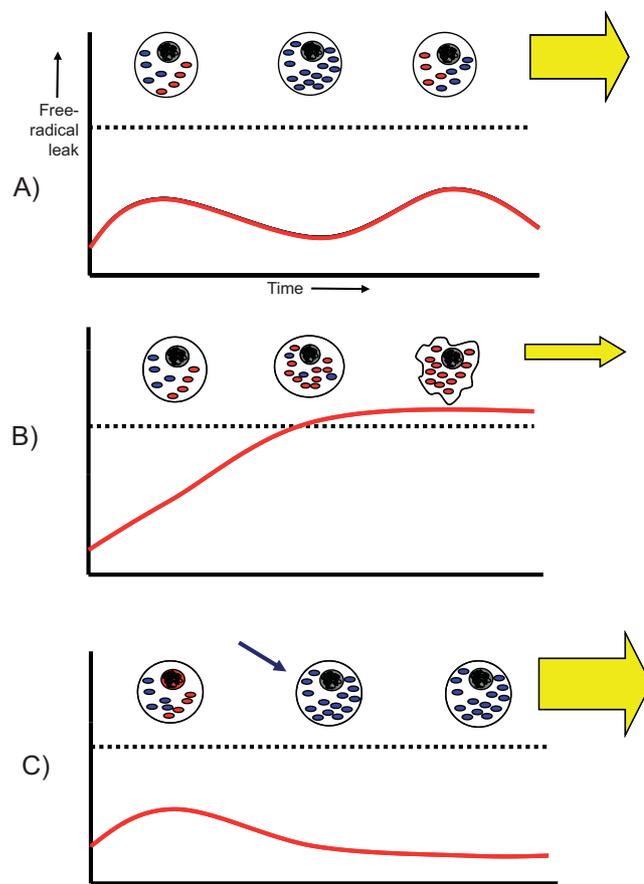
This brings us to the central postulate. The two conditions necessary for the existence of eukaryotic cells – the need for multiple cytoplasmic genomes to keep control of respiration, and the need for these minimal genomes to interact with nuclear genes – both give rise to the same type of free-radical signal, which derives inescapably from the biophysics of the respiratory chain. Presumably there is a threshold. Below the threshold, free-radical leak stimulates the expression of new respiratory complexes (reactive biogenesis, Fig. 2A); above it, apoptosis (Fig. 2B). So free-radical leak signals a problem. If the problem can be fixed by reactive biogenesis, good; if not, the cell dies. The major proposal here is that the dynamics of this system optimize fitness, fertility and adaptability over generations, but at the cost of ageing and age-related disease within each generation.

## The origin of species and evolution of sexes

Consider what happens when individuals from reproductively isolated populations are crossed. The best example is the marine copepod *Tigriopus californicus*. Introgression between individuals from nearby but isolated populations causes hybrid breakdown by the F2 generation: ATP synthesis is reduced by about 40%, as is larval production, time to metamorphosis and survival [23]. Back-crossing to the maternal population restores wild-type fitness: hybrid breakdown is attributable to the mismatch of mitochondrial and nuclear genes [40]. Given the fast mutation rate of mitochondrial genes, and the requirement for coadaptation with nuclear genes, mitonuclear mismatch might well contribute to reproductive isolation and speciation [41, 42], most notably in species with faster mtDNA mutation rates [43]. Mitonuclear incompatibilities cause reproductive isolation in yeast [44, 45], and varying degrees of hybrid breakdown in *Drosophila* [46, 47] *Nasonia* wasps [48], centrarchid fishes [49], beetles [50], and various plants, including sunflower [51], partridge pea [52], *Arabidopsis*, maize and rice [24, 53].

The penalty for serious mitonuclear mismatch – a reduction in fitness of 40% or more – is a significant evolutionary cost. This cost is incurred because compromised electron flow translates into low ATP, high free-radical leak, and a greater probability of apoptosis, which in turn compromises fertility, embryonic development and adult survival. There is presumably a scale between individuals with good mitonuclear match and those with serious mismatch. Hybrid breakdown is offset by, but ultimately outweighs, the well-known genetic advantages of hybrid vigour (heterosis). The greater the evolutionary distance, the greater the likelihood of hybrid breakdown, hence the relationships with mtDNA mutation rate, duration of reproductive isolation, and geographical distance. Hybrid breakdown in the partridge pea *Chamaecrista fasciculata*, for example, depends on geographical distance, with outbreeding depression only occurring in crosses between populations more than 1,000 km distant [51].

But outcrossing between populations is not the only way to generate mitonuclear mismatch: uncontrolled mitochondrial heteroplasmy (where there are no safeguards against additive mixing of different mtDNA haplotypes in the same cell) should



**Figure 2.** Free-radicals signal mitochondrial biogenesis, increasing capacity or triggering apoptosis. **A:** Reactive biogenesis. Below a threshold, free-radical signals optimize respiration by stimulating mitochondrial biogenesis. Deficient mitochondria (with low respiratory capacity relative to demand) are reduced (depicted in red) and leak free radicals. These activate redox-sensitive transcription factors, up-regulating the expression of mtDNA and nuclear genes (equating to biogenesis), thereby improving respiratory capacity and redox poise (blue mitochondria). Mitochondrial populations decline with falling demand, thus balancing ATP production and mitochondrial density over time. Red line depicts fluctuations in free-radical leak over time; yellow arrow depicts ATP synthesis. Dotted line depicts the apoptotic threshold. **B:** Reactive biogenesis with a mismatch between mtDNA and the nuclear background. The mismatch results in small misalignments of respiratory chain subunits, which slow electron transfer, thus decreasing respiratory capacity and increasing free-radical leak. As in (A) high free-radical leak induces the biogenesis of deficient mitochondria, but this does not solve the problem, as in this case respiratory deficiency is caused by genetic mismatch. In fact, free-radical leak preferentially amplifies the most mismatched (i.e. most deficient) mitochondria. Free-radical leak rises above the threshold, leading to apoptosis. **C:** Proactive biogenesis. A signal from outside the cell (e.g. calorie restriction; blue arrow) stimulates 'proactive' mitochondrial biogenesis without respect to local redox conditions or demand; the entire population is amplified, without selection for deficient mitochondria, reducing demand per mitochondrion, so improving both respiratory capacity and redox poise (blue mitochondria). This situation remains stable as long as the external signal persists (e.g. duration of calorie restriction) lowering free-radical leak and extending lifespan.

also lead to fitness loss. Heteroplasmy could result from the biparental inheritance of mitochondria (mitochondria inherited from both gametes), or the absence of a maternal mitochondrial bottleneck (which usually generates a clonal population of mitochondria in the oocyte) or both. In the absence of uniparental inheritance and a bottleneck, heteroplasmy is unavoidable and should generate at least a mild mismatch within a few generations. The problem is that heteroplasmy interferes with selection. If there are two or more different haplotypes of mtDNA, the respiratory capacity depends on their average. The greater the heteroplasmy, the closer this average tends towards mismatch (the variance between cells falls, hindering selection). Mathematical modelling suggests that uncontrolled heteroplasmy can indeed lower fitness significantly, with approximately 90% of homoplasmic populations achieving 95% fitness, compared with barely 50% of heteroplasmic populations (manuscript in preparation).

Uncontrolled heteroplasmy rarely occurs because there are two sexes. Females normally pass on a clonal population of mitochondria in the oocyte, whereas males generally do not pass on mitochondria. Uniparental inheritance of mtDNA has a good claim to being the deepest distinction between the two sexes [54, 55]. Various exceptions prove this rule. Biparental inheritance in yeast (*S. cerevisiae* and *S. pombe*) is offset by non-random segregation of mitochondria, resulting in swift restoration of homoplasmy [56, 57]. Protists such as *Chlamydomonas* actively destroy male mtDNA after syngamy [58], as does the slime mould *Physarum polycephalum*, in dominant hierarchical order of its 13 sexes [54]. Fungi with multiple mating types such as *Schizophyllum commune* do not fuse cytoplasm, hence mitochondria are not transferred with the nucleus [55]. Exceptions, such as the heteroplasmic mycelia in *Basidiomycetes* are usually homoplasmic in individual hyphae, showing a non-random sorting of mitochondria that depends on the nuclear background [59]. Even the doubly uniparental inheritance in bivalve molluscs such as *Mytilus* does not involve serious heteroplasmy. Males receive mitochondria from both parents, but only the male mitochondria enter the germline; female mitochondria are restricted to somatic tissues [60]. Thus restricted heteroplasmy equates to local homoplasmy, and does not obscure selection for mitonuclear match.

If the sexual barriers to heteroplasmy were removed, fitness would decline by mitonuclear mismatch alone (regardless of other potential factors like selfish conflict). This fitness penalty may be partly responsible for the maintenance, if not the origin, of two sexes [55]. Thus, the existence of two sexes helps to optimize mitonuclear match by generally promoting homoplasmy. Nonetheless, even with two sexes, limited heteroplasmy is not uncommon [13, 61]. I propose that the occurrence of heteroplasmy relates to the costs and benefits – in the explicit, specific, and tangible currency of free-radical leak – of optimal matching across species.

## A variable apoptotic threshold optimizes mitonuclear match

If selection for optimal mitonuclear match requires homoplasmy, the key question arises: How good does mitonuclear

match need to be? Plainly, if a poor match slows electron flow, then animals with high aerobic demands, such as birds and bats, would require a good match to maximize their aerobic capacity. Conversely, animals with lower fitness requirements could get by with a worse match, and this would be beneficial if there are any costs to selecting for mitonuclear match. Such putative costs would depend on the timing and mechanism of selection.

While severe mtDNA mutations are eliminated during oocyte maturation [62, 63], at low cost to the organism, selection for mitonuclear match can only commence after fertilization, when the new nuclear background is established: during embryonic development or after birth. Mismatches severe enough to cause apoptosis compromise embryonic development. The scale, while unknown, could be substantial. In humans, some 40% of pregnancies terminate in early occult miscarriage [64], some of which might have a bioenergetic basis, as ATP availability is a critical factor in embryo viability [65]. Even in nematodes, mutations in the mitochondrial ND5 subunit reduce fecundity [66]. Mild mitonuclear mismatches that do not compromise development may nonetheless be unmasked as mitochondrial disease after birth, when metabolic demand can rise several-fold (in mammals).

Similar patterns are found in plants, notably cytoplasmic male sterility (CMS). In sunflower, the CMS mutation is an aberrant recombination of ORFB, a subunit of ATP synthase, the effect of which is to suppress ATP synthesis [51]. This is decisive only in tissues with high metabolic rate, notably pollen development in the anthers, where mitochondrial density per cell rises 20–40-fold [52]; the CMS mutation leads to selective apoptosis of anthers, thereby sterilizing males. The nuclear modifier gene restores ATP synthesis by breaking down aberrant subunits [51, 52].

Selection for mitonuclear match during embryogenesis and early life depends on calibration of free-radical leak in relation to the threshold mentioned earlier. Below the threshold, free-radical leak signals mitochondrial biogenesis; above it, apoptosis. The threshold could in principle be raised or lowered, altering sensitivity to apoptosis. A low threshold gives good mitonuclear match with low free-radical leak; even a slight rise in leak should trigger apoptosis and early developmental failure, eliminating any embryos with poor match. Conversely, a high threshold equates to greater tolerance of free-radical leak before apoptosis. The seemingly paradoxical physiology of endogenous signalling gases such as nitric oxide (NO) might be interpreted in these terms. NO, by binding to cytochrome c oxidase, amplifies free-radical signals and lowers the threshold for apoptosis [67]. Such amplification of free-radical signals may be crucial during embryonic development, enabling the selective termination of mismatched embryos without compromising viability later in life (albeit perhaps shedding light on the pathophysiological effects of NO in degenerative diseases).

Alterations in the threshold setting should influence fitness, for example litter size, aerobic capacity and adaptability (Table 1). Intriguingly, litter size correlates with oocyte loss (atresia) during oocyte maturation across different species – the heavier the atresia, the smaller the litter size [68]. As severe mitochondrial mutations are eliminated during atresia [62, 63], oocytes may be subject to some kind of quality

Table 1. Some consequences of a moveable apoptotic threshold

	Low apoptotic threshold	High apoptotic threshold
Example (hypothetical)	Pigeon	Rat
Mitonuclear match	↑	↓
Aerobic capacity	↑	↓
Free-radical leak	↓	↑
Heteroplasmy	↓	↑
Mitochondrial diseases	↓	↑
Inflammation	↓	↑
Reproduction	K strategy	r strategy
Fertility	↓	↑
Litter size	↓	↑
Adaptability	↓	↑
Lifespan	30 years	3 years
Rate of ageing	↓	↑
Age-related diseases	↓	↑

control. The degree of atresia also corresponds to the tightness of the mitochondrial bottleneck – the greater the atresia, the tighter the bottleneck [68]. A tighter bottleneck generates a more clonal population of mitochondria, enhancing selection for mitonuclear match. Thus, litter size and fertility should vary with mitonuclear fitness.

Theoretically, if the apoptotic threshold is high, litter size can be high. A poor mitonuclear match is not a problem if investment per offspring is low; the fittest offspring with the best mitonuclear match will tend to survive. Fertility should be correspondingly high. Another advantage of a higher threshold is greater tolerance for heteroplasmy, which can aid adaptation to changing environments (albeit at increased risk of mitochondrial disease). Mitochondria are central to both climatic and dietary adaptation, as any differences in temperature, calorie intake or dietary balance (protein, fat or carbohydrate source) likely require mitochondrial adaptation. Heteroplasmy provides a greater variety of mtDNA, some of which may be better adapted to the new environment or diet [69]. Such adaptation might even account for the regular introgression of mitochondria between species. Overall, then, high fertility, larger litter size and greater adaptability arise naturally from a high apoptotic threshold. These traits correspond to, and perhaps even dictate, a reproductive r strategy.

Conversely, a low apoptotic threshold, in which tolerance of free-radical leak is low, enables high aerobic capacity, as in powered flight in birds and bats (Table 1). The incidence of mitochondrial diseases should be lower. The costs of a lower apoptotic threshold are lower fertility, lower litter size and lower adaptability, corresponding to a K strategy – simply because more embryos are sacrificed during development to secure an optimal mitonuclear match. Interestingly, birds do seem to be less adaptable to changing conditions than many mammals, preferring to migrate rather than to over-winter or hibernate. But regardless of such behavioural patterns, a clear prediction is that sensitivity to apoptosis, measurable *in vitro* or *in vivo* (especially during embryonic development, and possibly linked to NO) should relate to the fertility, fitness and adaptability of organisms.

## The apoptotic threshold dictates the rate of ageing

The idea of an apoptotic threshold is central to ageing and lifespan, at least in animals. The reason is that lifespan across species correlates closely with free-radical leak (but not metabolic rate) [26]. While the free-radical theory of ageing as originally stated is at best incomplete, the relationship between lifespan and free-radical leak persists. It is hard to prove a causal link because dietary antioxidants do not prolong lifespan or ameliorate age-related diseases [70, 71]. Conversely, oxidative stress and free-radical signalling (produced, for example, by RNAi directed to respiratory chain components) can actually prolong lifespan in nematode worms [72] and *Drosophila* [73]. The most plausible interpretation is that physiological stress modulates lifespan around mean longevity for a particular species; but that mean, ultimately, is set by the rate of free-radical leak. Thus, while it is possible to double or treble the lifespan of simple organisms such as nematodes, the differences between species, over many orders of magnitude, cannot be bridged by physiology; the set point (i.e. the comparative phylogenetic rate of free-radical leak) itself becomes critical.

Free-radical leak, while necessary, is nonetheless hazardous. Over a lifetime, respiration mutates mtDNA. This in turn causes age-related heteroplasmy [74], and a slow loss of mitonuclear coadaptation. The speed at which this happens depends on the rate of free-radical leak; and this is set by the apoptotic threshold. A low threshold equates to a low leak, slow accumulation of mtDNA mutations and long lifespan. A high threshold equates to higher leak, faster accumulation of mtDNA mutations, and shorter lifespan (Table 1). For example, pigeons and rats are a similar size, with similar basal metabolic rates, but free-radical leak is substantially lower in pigeons, and their lifespan much longer (30 years versus 3 years; [25]). This is predicted on the basis that selection for tight mitonuclear match requires low free-radical leak, which translates into long lifespan. In other words, selection for low free-radical leak may be primarily related to mitonuclear match, and only secondarily to the rate of ageing, which is in fact a side effect of selection for aerobic fitness. In this regard it is notable that selection for aerobic fitness over multiple generations does indeed lead to increases in lifespan [75].

As an aside, these considerations bear on the aerobic capacity hypothesis for the origin of endothermy [76]. Despite having field metabolic rates that are typically elevated some 30-fold relative to reptiles, mammals and birds have surprisingly similar lifespans: they are not reduced by 30-fold as would be predicted on the basis of metabolic rate and body size alone. Here again, selection for aerobic capacity appears to have prolonged lifespan relative to resting metabolic rate.

This scenario – that free-radical leak optimizes fitness in youth, with costs that are deferred to later in life – is a variation of the theory of antagonistic pleiotropy, as developed by Williams [77], and so is not dissimilar to standard evolutionary theories of ageing. However, the hypothesis developed here does not trade directly in genes. Specifically, Williams argued that certain genes may have more than one effect (pleiotropy) some beneficial, others detrimental

(antagonistic). Genes that enhance sexual success early in life will tend to be selected even if they produce detrimental effects later in life, after sexual decline, when selection is weaker. Williams's hypothesis works in theory, but has enjoyed little empirical backing: very few antagonistically pleiotropic genes have been discovered, despite several decades of searching [78]. But free-radical leak is a different matter. The perspective developed here shows that free-radical leak is under strong selection early in life and indeed throughout life. Specifically, the benefits of a high rate of free-radical leak during early life – high fertility, high litter size and high adaptability, plus signalling benefits that optimize ATP synthesis – have costs later in life, notably a fast rate of ageing and disease. This perspective is particularly valuable because it does more than just predict the rate of ageing – it also predicts the spectrum of age-related diseases, and suggests possible avenues of treatment.

### Loss of mitonuclear match drives age-related diseases

Recall that free-radical leak corrects respiratory insufficiency by promoting the expression of mtDNA, tailoring supply to demand by way of reactive mitochondrial biogenesis (Fig. 2A). In youth, mtDNA is usually homoplasmic (the result of uniparental inheritance and a developmental bottleneck), which means that reactive biogenesis is driven solely by fluctuations in supply and demand.

The problem occurs with heteroplasmic mtDNA, which accumulates during normal ageing. Now the alternative developmental mechanism for eliminating mismatched mitonuclear genomes comes into play. The mitochondria with the worst match to the nuclear background leak the most free radicals, ultimately promoting apoptosis. Beneath the apoptotic threshold, however, reactive biogenesis *selectively amplifies the worst mitochondria*, giving rise to a clonal takeover of cells and tissues (Fig. 2B). There is a proviso here: autophagy may target precisely the mitochondria with the highest free-radical leak and lowest membrane potential [79]. Nevertheless, reactive biogenesis, as discussed here, involves *intra-mitochondrial* free-radical signals, which act to maintain mitochondrial membrane potential; so mildly compromised mitochondria may be invisible to the autophagosome. Regardless of autophagy, the clonal expansion of mutant mitochondria is common in aged tissues as well as mitochondrial diseases and has never been properly explained [80, 81]. Reactive biogenesis, as advanced here, can explain it. This mechanism also potentially accounts for the clonal expansion of mtDNA in cancer cells [74] and following HIV treatment [82]. Because different nuclear alleles of genes encoding mitochondrial proteins are expressed in different tissues, with varying metabolic demands, mitonuclear mismatch ought to change from tissue to tissue, potentially explaining the selective expansion of differing mitochondrial populations in different tissues [83]. In short, reactive mitochondrial biogenesis, which optimizes respiratory function in youth, inherently tends to select the worst mitochondria with age.

If a cell depends on oxidative phosphorylation, the clonal expansion of mutant mitochondria leads ultimately to apoptosis, as in development. During ageing, apoptosis drives

tissue loss, notably the loss of post-mitotic cells such as neurons; brain atrophy is one of the earliest signs of Alzheimer's disease [84]. Without stem cell replacement, the surviving tissue is placed under a greater burden to match energy supply to demand; even with replacement, mutations in stem cell mtDNA drive the same processes more slowly in other tissues. Deficient energy metabolism alters the NAD/NADH, and ADP/ATP ratios, resulting in sweeping epigenetic changes, including changes to chromatin phosphorylation, acetylation and methylation [85]. Such global epigenetic changes have major implications for both signalling and transcriptional regulation with ageing.

Cells able to meet their ATP demands by glycolysis, despite dysfunctional mitochondria, undergo a glycolytic switch and persist in a state of oxidative stress [67, 86]. This in turn activates transcription factors such as NF- $\kappa$ B [87] and the inflammasome [88], driving a pro-inflammatory transcriptional state linked with chronic low-grade inflammatory conditions, such as cardiovascular disease and Alzheimer's disease [87–89]. Dysfunctional mitochondria also cause chromosomal instability through perturbed pyrimidine synthesis and changes in DNA methylation [90]. The combination of resistance to apoptosis, glycolytic ATP synthesis, chromosomal instability and pro-inflammatory transcriptional state is the classic pro-carcinogenic state [67, 91, 92].

Thus, the major traits of ageing are all predicted as the outcome of free-radical signalling for mitochondrial function: the correlation between lifespan and free-radical leak; tissue atrophy via apoptosis, especially in postmitotic tissues with a high metabolic demand; changes in epigenetics related to the energy deficit; a low overall burden of mtDNA mutations, due to apoptotic cell loss; clonal takeover of some cells by mutant mitochondria; a generally increased mitochondrial density and mtDNA copy number in healthier cells (which must compensate for the loss of apoptotic cells); a glycolytic switch in apoptosis-resistant cells; a chronic low-grade inflammatory state that promotes degenerative diseases; and an increased risk of cancer. Moreover, the fact that dietary antioxidants do not prolong lifespan or protect against age-related diseases [70, 71] is predicted on the basis that the benefits of free-radical signalling in youth (optimizing respiration, aerobic fitness, etc.) outweigh any harm they cause with age – i.e. free radicals are antagonistically pleiotropic. Organisms therefore regulate intracellular antioxidant levels tightly and excrete excess antioxidants that could interfere with signalling [87], rendering megadose antioxidant supplements worthless.

In contrast, mitochondrial biogenesis has the potential to prolong lifespan and ameliorate disease. I have noted that there are two ways to raise mitochondrial density: reactive and proactive biogenesis. The distinction between the two is critical.

Reactive biogenesis, as discussed above, is beneficial in youth but ultimately selects for the worst mitochondria (Fig. 2B). In contrast, proactive biogenesis is dictated by signals from outside the cell (e.g. falling IGF-1 levels) and can be imposed by non-redox changes in nuclear gene transcription. Proactive commands should not amplify the worst mitochondria (Fig. 2C) and this does seem to be the case in calorie restriction [93, 94] and the ketogenic diet [95], although the role of mitochondrial biogenesis in calorie restriction has been challenged recently [96]. In theory, so long as electron flow

into the respiratory chains is controlled physiologically, proactive biogenesis cuts free-radical leak by lowering the reduction state of respiratory complexes [53, 97, 98], prolonging life and postponing or even avoiding age-related diseases.

As noted earlier, selection for aerobic capacity over generations in rats leads to increases in mitochondrial density, which in turn are associated with improvements in cardiovascular health and lifespan [75]. High mitochondrial density and low free-radical leak may also be written into the genes of birds, contributing to their long lifespan. Birds retain high mitochondrial density at all times, even in their visceral organs, contributing to their high body temperature (40 °C). Nascent chicks switch to endothermy by up-regulating mitochondrial biogenesis via the transcription factor PGC<sub>1α</sub> [99]. The changes orchestrated by PGC<sub>1α</sub> in mitochondrial function during the onset of endothermy in chicks may have similarities and differences with the changes occurring in calorie restriction in mammals, and offer valuable clues to how we can mimic birds pharmacologically to live longer, healthier lives.

## Conclusions

Mitochondrial genes are necessary for the existence of cells with large complex genomes, but must adapt to the nuclear background in each generation. A life-and-death switch involving mitochondrial electron transfer – efficient respiration or apoptosis – derives inescapably from the biophysics of mosaic respiratory chains. The rate of free-radical leak is central to this switch – high free-radical leak betrays a poor mitonuclear match and triggers apoptosis, removing cells, and by extension organisms, with poorly matched mitochondrial and nuclear genomes. The threshold for apoptosis depends on fitness requirements. High aerobic demands, such as flight in bats and birds, require closely matched genomes, with low free-radical leak, whereas animals with low aerobic capacity can tolerate poor match and high free-radical leak. Selection for optimal respiratory function involves a pleiotropic trade-off, which predicts many aspects of eukaryotic physiology and evolution from first principles, including two sexes, fertility, aerobic capacity, speciation, ageing and the spectrum of age-related diseases. The centrality of free-radical leak to these evolutionary tradeoffs explains why dietary antioxidants do not prolong lifespan or ameliorate age-related diseases. The perspective developed here suggests that ‘proactive’ mitochondrial biogenesis, by increasing aerobic capacity, may solve some of the problems associated with ageing.

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