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Multilevel selection on mitochondrial genomes

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Mitochondria are vital organelles for life in eukaryotes, taking centre stage in the process of cellular respiration. This process is regulated via a series of finely coordinated obligate interactions of molecules encoded by two genomes: nuclear DNA and mitochondrial DNA. Both genomes are required to work harmoniously to provide cellular energy, with detrimental consequences occurring when there is miscommunication between them. Whilst the need for cooperation is strong, vast differences between genomes (ploidy, size, and inheritance) create an arena for conflict. Here, we examine the varying levels of selection operating on the mitochondrial genome and the consequences they have on all these levels. We conclude by highlighting the potential for conflict when selection at different levels is driven by different evolutionary forces.

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Introduction

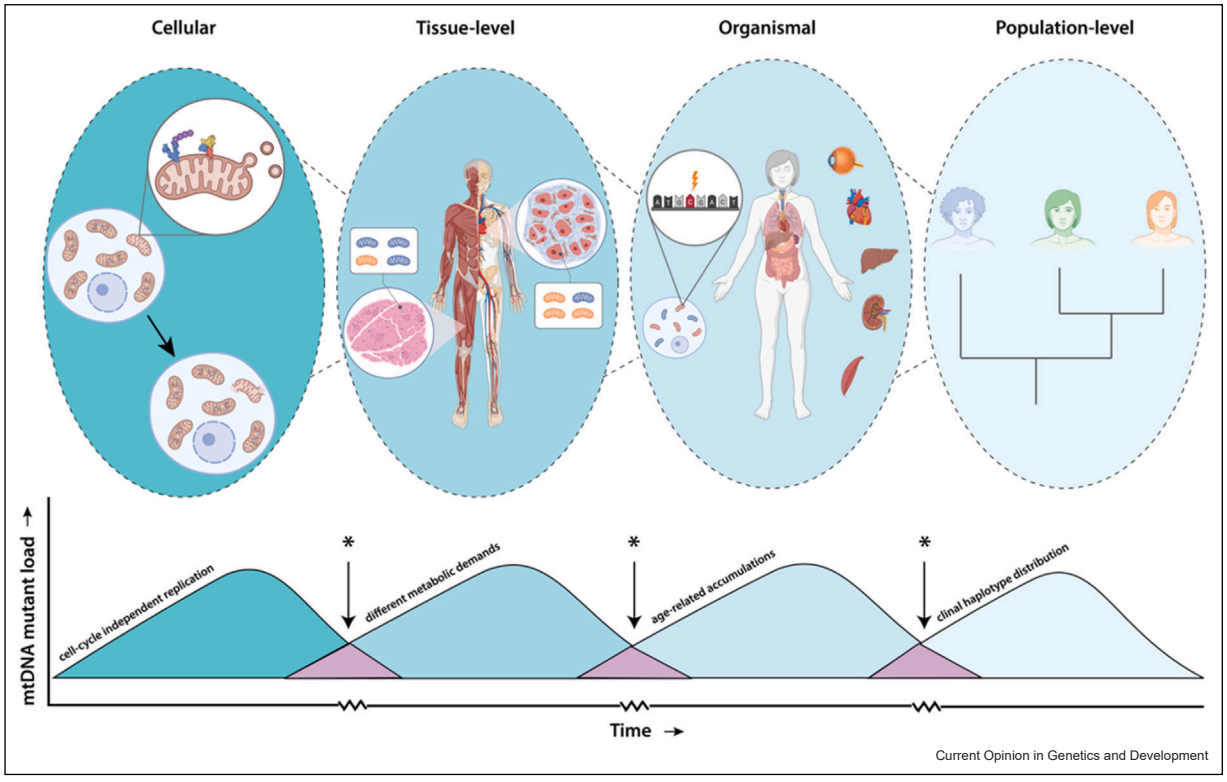
Mitochondria have long been recognised as mostly maternally inherited sources of genetic information across most eukaryotes [1]. Once thought to be a neutrally evolving bystander [2], the past couple of decades have highlighted the role of the mitochondrial DNA (mtDNA) genome as an important source of adaptive variation [3]. Aside from proteins encoded in the mtDNA, the functioning of mitochondria depends on imported proteins encoded in the nuclear genome. As a result, harmonious interaction between protein products from both genomes is vital for mitochondrial function. This duality produces a further selective force on the joint nature of mitochondrially encoded proteins [4].

Efficient mitochondrial function is crucial for cellular viability, affecting all aspects of organismal life history [5–7]. Intergenomic cooperation is further challenged by the vast differences between these two genomes [8]; animal mitochondrial genomes are small, circular, maternally inherited, nonrecombining and haploid but with multiple copies in each cell. Biological processes also generate variance in mtDNA mutant load over time, which alters the composition and distribution of mtDNA variants across levels of biological organisation. All these differences have the potential to generate intergenomic conflict [9]. Furthermore, given the plastic nature of energetic demands, selection on mtDNA (and its epistatic interactions with the nuclear genome) should happen across most — if not all — levels of selection (Figure 1). The quasi-independent dynamics of mtDNA versus nuclear DNA (nucDNA) variation create potential for multilevel selection, a phenomenon where selection can simultaneously favour different traits across levels of biological hierarchy. Opposing selection pressures on the mtDNA between different levels can lead to conflicting optimal states. Here, we review the forces that shape mitochondrial evolution at different biological hierarchies, and then highlight recent studies that have examined how multilevel selection can generate conflict for the mtDNA.

Selection at the cellular level

Selection at the cellular level for mtDNA genotypes has important implications for both evolutionary biology and biomedical research [10]. It is essential for maintaining mitochondrial quality and preventing cellular dysfunction and disease. Research has shown that the selective removal of damaged mtDNA is crucial for maintaining cellular health and preventing disease [11]. Mitophagy, a type of autophagy that targets mitochondria, is considered an important process for maintaining mitochondrial quality and preventing damage [12]. The PINK1–PRKN pathway is the most extensively studied pathway for mitophagy [13], along with mitochondrial-assisted degradation (MAD) [14] and associated processes involving the ubiquiting-proteasome system (UPS) [15]. When mitochondria undergo stress or damage, such as loss of electrical potential, it can cause a blockage in protein transfer into the organelle and trigger the breakdown of import machinery components [16].

Figure 1



Multilevel selection on mtDNA. Top: Natural selection acts on mtDNA at multiple levels of biological organisation: cellular, tissue level, organismal, and population level. At the cellular level, dysfunctional mitochondria are selectively degraded through mitophagy via several different pathways such as the PINK1–PRKN pathway, UPS and MAD. At the tissue level, mtDNA segregates nonrandomly in a tissue-specific manner suggesting selection plays a role in maintaining a tissue-specific haplotype composition. Mutations in the mtDNA are associated with a variety of multisystemic disorders that impair fitness at the organismal level. At the population level, mtDNA exhibits clinal variation with factors such as temperature and altitude. Bottom: mtDNA genetic diversity (mutant load) within each level of biological organisation increases partially independent of other levels. At the cellular level, mtDNA replicates independently of the cell cycle. At the tissue level, differing metabolic demands of the tissues lead to differential mtDNA replication. At the organismal level, mtDNA mutations accumulate with age. At the population level, heteroplasmy levels often differ in different populations. Competition amongst entities within a level then selects against the dysfunctional entities. A potential for conflict (* in figure) then arises whenever there are opposing pressures between two levels of biological organisation. Image created with BioRender.com.

Many researchers have aimed to understand how mtDNA genome quality is maintained during germline development. This is because mtDNA is known to have elevated mutation rates compared to the nuclear genome in animals [17], with mutations having the potential to cause drastic consequences on all aspects of organismal viability. The idea that mitochondrial selection occurs via a bottleneck is pervasive in the field [18,19], however, bottleneck estimations are not consistent with empirical data. More recently, it has been suggested that primary oocytes carrying deleterious mtDNA mutations (or possibly mtDNA mutations that generate conflict with the nuclear genome) are eliminated by follicular atresia. This process involves nurse cells preferentially localising healthy mitochondria to the Balbiani body, a non-membrane-bound compartment packed with mitochondria and other organelles [20]. These processes thus reduce the amount of variance produced via replication of the mtDNA during early development, avoiding further conflict with the nuclear genome. Overall, these studies illustrate the dynamic and complex nature of selection for mtDNA genotypes at the cellular level, and its implications for cellular function, organismal fitness and disease.

Tissue-specific selection

A growing body of evidence suggests that tissue-specific selection can occur for different mtDNA variants within an individual, which has important implications for understanding the evolution of mitochondria and their functional consequences in different tissues. Studies in mice and in humans have provided evidence for tissue-specific selection, with certain mtDNA genotypes or haplogroups being overrepresented in specific tissues. The magnitude of the segregation bias does vary according to the nuclear genetic environment [21]. Specifically, studies have found evidence for tissue-specific selection for different mtDNA variants in mice, with

certain genotypes being preferentially selected in certain tissues [22–24]. More recent work has demonstrated in mice with nonpathogenic heteroplasmy, that nonrandom segregation of mtDNA is an intracellular process based on organelle selection [22]. Similar work on humans has found that specific mtDNA haplogroups were over-represented in muscle compared to blood, suggesting that selection was acting differently on mtDNA in these two tissues [25,26]. The implications of tissue-specific selection for mtDNA extend beyond evolutionary processes and may have important implications for human health. For example, recent studies have suggested that tissue-specific selection may contribute to differential susceptibility to certain diseases [27,28]. These findings have important implications for understanding the mechanisms utilised for resolving intergenomic conflict, by partitioning mtDNA genetic variation across tissues. Together, these studies highlight the importance of considering tissue-specific effects when studying mtDNA evolution and suggest that selection may act differently on mtDNA in different tissues.

How this tissue-specific segregation bias occurs is still a largely unanswered question, however, there are many hypotheses at play. One explanation is the existence of a mtDNA balance between replication and transcription. Using modelling approaches, the authors identify the formation of secondary structures consisting of stacked planar guanine tetrads (G-quadruplex) that can successfully predict the segregation patterns in the observed literature [28]. Another explanation is the existence of mechanisms enabling selective degradation of deleterious mitochondria, which has been shown to reduce mtDNA load in cultured cells [29]. A third hypothesis involves segregation based on tissue-specific metabolic demands. Mitochondria harbouring defective genomes could produce signals that stimulate compensatory upregulation of mitochondrial biogenesis, leading to the rapid increase of a defective mtDNA [30]. More recently, however, it was found that metabolic interventions (via environmental and pharmacological treatments) that modulate oxidative phosphorylation (OXPHOS) performance, played a significant role in tissue-specific haplotype retention [22], highlighting the fact that these processes are highly labile to the environment. By shedding light on the role of tissue-specific selection in mtDNA evolution and function, these studies may ultimately inform new strategies for preventing and treating mitochondrial diseases.

Individual-level selection

Deleterious mutations in mtDNA (and associated nucDNA proteins) often result in dysfunctions of the mitochondrial respiratory chain, manifesting as multisystemic disorders that impair individual fitness. This direct link to organismal fitness creates potential for selection to act on the individual level, with several studies reporting signatures of purifying selection on mtDNA [31,32]. In humans, mitochondrial disorders have been observed to affect all organs of the body and tissues with high-energy demands such as the brain, liver, and muscle demonstrating pronounced defects. Defects in the OXPHOS pathway were linked with increases in energy consumption, leading to hypermetabolism, and shortened lifespans both at the cellular and the individual level [33].

Most mitochondrial disorders are associated with increased levels of heteroplasmy and often require the pathogenic mtDNA variant to exceed a critical threshold before the biochemical defects resulting from the variant are expressed (threshold effect) [34]. Variation in the patterns of heteroplasmy could arise due to the accumulation of *de novo* mutations in later life by clonal expansion or via inheritance through the maternal lineage [35]. While random genetic drift by relaxed selection appears to be sufficient for explaining the accumulation of point mutations but not mtDNA deletions [36], recent studies of mother–offspring pairs [37], trios and multigenerational families revealed evidence of selection against novel heteroplasmic variants in human populations (but see Refs. [38,39] for difficulties in measuring heteroplasmy and detecting nuclear mtDNA segments).

The detrimental effects of incompatible interactions between mtDNA and nucDNA have been observed across a wide range of taxa [40–44]. The classic example for these incompatibilities uses the copepod *Tigriopus californicus*, where populations that are relatively close have 20–30% mitochondrial divergence [45]. Genetic crosses between populations and closely related species result in most life history traits being affected, from mitochondrial bioenergetics [46], immunity–fecundity tradeoffs [47], to developmental time [4] and fitness [48].

Selection across populations

Several studies have investigated selection of mtDNA genotypes at the population level. These involve two main experimental designs: (1) tracking alleles in natural populations in time/space, (2) using experimental techniques to simulate populations in lab-controlled environments. Differences in mtDNA genotype across populations may be the result of genetic drift, population migration, natural selection, and/or adaptation to different environments. Studies have found that environmental factors, such as latitude, can also influence the distribution of mtDNA haplogroups in populations, which has large consequences on the mtDNA playing a more important role in adaptation than previously thought. Many studies in this field have examined the consequences of population admixture on mitonuclear dynamics, with some studies finding signatures of

mitonuclear coadaptation many generations after the admixture event [49,50].

Using experimental techniques, one can better control all other environmental and genetics variables, albeit in a semi-synthetic manner. Several studies have used this technique to compete mtDNA genomes in a population against each other, with their frequency being tracked across multiple generations. For instance, studies have examined how two mtDNA frequencies changed over time in beetles [51] and *Drosophila* [52] when the founder generation started with very different frequencies. Interestingly, the authors found signatures of negative frequency-dependent selection, whereby the haplotype seeded at the lower frequency showed a significant increase in all cases. Further work has been done to examine how the environment shapes these dynamics, and the mtDNA genome has been shown to respond (by changes in frequency) to both thermal [53] and nutritional environments [54]. Taken together, these results are quite significant as the maintenance of mitochondrial genetic diversity can be predicted to stem from selection at different biological levels, although it is not yet tested in these systems.

Overall, studying the selection of mtDNA genotype across populations has important implications for evolutionary biology and biomedical research. By analysing the distribution of mtDNA haplogroups and their effects on various phenotypic traits, researchers can gain insight into the mechanisms underlying human evolution and the genetic basis of disease susceptibility. Moreover, understanding mtDNA diversity across different populations can shed light on the processes that drive genetic adaptation to different environments. Ultimately, this knowledge can help improve the prevention and treatment of diseases associated with mtDNA mutations.

Potential for conflict generated by multilevel selection

Having varying levels of selection has the potential to generate conflict both within and between individuals. For instance, an advantageous trait at one level of selection might be disadvantageous at another. It is difficult to track mitochondrial mutations across many levels of selection, given the strong purifying selection acting on mtDNA genomes and the low heteroplasmy levels across most species. Consequently, more synthetic approaches have been used to answer these questions, with some of the classic examples in this area involving highly defective mtDNA genomes, allowing them to track mitochondrial heteroplasmy in time and space.

Drosophila models utilise strains that harbour two different mtDNA haplotypes: a functional (wild-type) variant that improves mitochondrial function and a 'selfish' variant that increases its own transmission in the germline [55]. This transmission advantage is achieved via a large section of the mtDNA being deleted, affording it a replication advantage although at the cost of overall mitochondrial function. Studies have found that the selfish haplotype quickly increases in frequency in the germline, however, this comes at a cost [56]. These studies also found that the selfish mtDNA haplotype led to a decrease in overall mitochondrial function and impaired the flies' physical abilities [56]. The findings suggest that selfish mtDNA can persist in populations, even when it reduces overall mitochondrial function, with potential implications for our understanding of the evolution and inheritance of mtDNA mutations in animals.

A similar system has been described in the nematode *C. elegans*, where it introduced a 'cheater' mitochondrial genome that had a large deletion to compete with the wild-type mtDNA [57]. Worms with high levels of 'selfish' mtDNA have decreased levels of mitochondrial function and lay fewer viable eggs. And so, while cheater mtDNA has a drive to invade the next generation, it does so at the expense of the hosts. The researchers then looked at how these dynamics play out across many generations. They show that although cheater mtDNA has an advantage within the germline, competition against strictly wild-type hosts reduces the prevalence of cheater mtDNA across the population [58].

In the last decade, the role that the environment plays on mitonuclear outcomes has been highlighted, with many of these interactions being context-dependent [59]. Furthermore, genetic defects at one level of biological organisation can be buffered by physiological responses at a higher level of organisation [60]. The question that arises here is: does the environment constrain multilevel selection? For instance, in the *C. elegans* system, restricting nutrients in the environment inhibits germline development (reducing the total pool of mtDNA in the germline), and hampering the mtDNA cheating strategies. One would predict that decreasing the efficacy of replication of the cheat mtDNA would lead it to extinction quickly. However, population-level experiments demonstrate that the rate of decline of cheater mitochondria is similar to that of the control [58]. These results suggest that the ability to survive a stressful environment can foster tolerance to cheating, unintentionally prolonging the persistence of selfish genomes.

To summarise, we aimed to showcase some of the complexities regarding mitonuclear interactions. Owing to the central role that mitochondria play in eukaryotes, there is strong need for coadaptation across all levels of selection, and we highlight examples of conflict generated by opposing forces at different levels. Moving

forward, research in this area should investigate how the environment influences these dynamics [61], as it has the potential to accentuate or ameliorate this conflict in a currently unpredictable manner.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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