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Mitonuclear interactions: evolutionary consequences over multiple biological scales

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Fundamental biological processes hinge on coordinated interactions between genes spanning two obligate genomes—mitochondrial and nuclear. These interactions are key to complex life, and allelic variation that accumulates and persists at the loci embroiled in such intergenomic interactions should therefore be subjected to intense selection to maintain integrity of the mitochondrial electron transport system. Here, we compile evidence that suggests that mitochondrial–nuclear (mitonuclear) allelic interactions are evolutionarily significant modulators of the expression of key health-related and life-history phenotypes, across several biological scales—within species (intra- and interpopulational) and between species. We then introduce a new frontier for the study of mitonuclear interactions—those that occur within individuals, and are fuelled by the mtDNA heteroplasmy and the existence of nuclear-encoded mitochondrial gene duplicates and isoforms. Empirical evidence supports the idea of high-resolution tissue- and environment-specific modulation of intraindividual mitonuclear interactions. Predicting the penetrance, severity and expression patterns of mtDNA-induced mitochondrial diseases remains a conundrum. We contend that a deeper understanding of the dynamics and ramifications of mitonuclear interactions, across all biological levels, will provide key insights that tangibly advance our understanding, not only of core evolutionary processes, but also of the complex genetics underlying human mitochondrial disease.

1. Introduction

One of life's most important biological functions—ATP production via oxidative phosphorylation (OXPHOS)—critically depends on the concerted and synchronized interaction between genes encoded over two obligate genomes—nuclear and mitochondrial. Respiratory function and its regulation in response to cellular and environmental cues (e.g. nutrients, exercise, temperature, hypoxia, etc.) necessitates the recruitment of over 500 proteins into the mitochondria [1–4]. The very essence of complex eukaryotic life depends on the interactions that take place between these imported nuclear-encoded proteins, and those encoded by the mitochondrial DNA (mtDNA) [5]. At its core, therefore, the mitochondrial–nuclear (mitonuclear) genomic interaction is essential for the assembly and function of the mitochondrial electron transport system (mETS). The mETS is the site of OXPHOS, and in eukaryotes entails five multi-subunit enzyme complexes, four of which are composed of subunits encoded by both mitochondrial (13 subunits) and nuclear (approx. 73 subunits) genomes [6]. Protein subunits from the two genomes must be highly compatible—akin to a 'lock and key' principle—in order to maintain structural and biochemical properties to ensure uncompromised enzymatic function.

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The genetic architecture (in terms of genes involved), and genomic location (in terms of in which genome each gene resides), of the genes that encode subunits of the mETS are remarkably conserved across metazoans [7]. What is, however, subjected to considerable heterogeneity is the level of nucleotide variation observed across these OXPHOS-coding genes. In particular, variation in the mtDNA sequence has long been known to exist over several biological scales—between congeneric species, between populations of the same species, within panmictic populations, and even within individuals. With the exception of the intraindividual level of variation [8], this sequence variation was historically neglected in terms of its functional and evolutionary relevance. Rather, the variation found within the sequence was typically considered to accumulate under a neutral equilibrium model, by-and-large silent to selection, and with little if any relevance as a mediator of population evolutionary processes.

The past two decades have seen a substantial shift away from this traditional view. This shift is founded on accumulating evidence, spanning vertebrate and invertebrate model systems, which indicates that the sequence polymorphism observed in mtDNA is ubiquitously tied to sizeable phenotypic effects [9–13]. Much of this evidence comes from studies that have associated putatively healthy mitochondrial genetic variants to phenotypic variation [14–22]. Other evidence comes from the medical sciences, where studies have reported associations between mutations in the human mitochondrial genome and deficiencies in mitochondrial function and the expression of disease [8,23]. In certain cases, disease phenotypes can be traced to specific mitochondrial polymorphisms (e.g. mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes [24] or Leber hereditary optic neuropathy (LHON) [25]). In many cases, however, while particular diseases are linked to mitochondrial function (e.g. diabetes [26], Alzheimer's [27], Parkinson's [28], cancer [29,30], ageing [31–33], etc.), direct links to specific disease-causing mtDNA polymorphisms remain elusive, a shortcoming further complicated by the varying nature, penetrance and severity of mitochondrial degenerative diseases [8,32]. We contend that a non-trivial amount of the currently unexplained variance in penetrance and expression of mitochondrial diseases might ultimately be traced to the unit of the mitonuclear interaction.

2. Mitonuclear interactions and coevolutionary processes

The mitonuclear interaction governs the integrity of OXPHOS and ensuing metabolic functions, and therefore should be under intense selection. All else being equal, deleterious mutations that arise in either genome should be swiftly eliminated to ensure uncompromised function, and selection should optimize combinations of alleles located on mitochondrial and nuclear genomes that work particularly well together, resulting in these combinations increasing in frequency within populations over evolutionary time [10]. Because the pool of genetic variation—created through de novo mutations in each genome, and the standing genetic variation—will differ across populations, and because each population is exposed to differing regimes of natural selection as a result of inhabiting distinct spatial and temporal environments, coevolutionary trajectories of mitonuclear coadaptation are predicted to be population-specific.

Yet, the general dynamics of mitonuclear interactions manifested in nature, and modes of ensuing coevolution, remain enigmatic and largely uncharacterized [10,34]. This shortcoming is most likely fuelled by different tempi and modes at which the two genomes evolve, requiring swift and efficient selective processes to fix or purge allelic variation arising in either genome to uphold OXPHOS function. The simple verbal model discussed above, whereby coevolution involves adaptations selected over both genomes to promote optimized function, is certainly plausible. However, several asymmetries in the genetics of the mitochondrial and nuclear genomes would suggest that the adaptive component of mitonuclear coevolution might commonly be left in the hands of the nuclear genome [10,34]. In particular, the mitochondrial genome is thought to have a small effective population size (N_e) as a result of its maternal inheritance and haploidy, relative to its nuclear counterpart [35]. The implication is that the efficacy of selection in shaping the mtDNA sequence across generations should be dampened, and the effects of genetic drift amplified [10,12,36]. When coupled with absence of recombination [37, but see 38–40] and the observations that mutation rates within the metazoan mitochondrial genomes are typically high, then it can be expected that non-adaptive processes will play a pervasive role in driving mitochondrial genome evolution, and facilitate the perpetual accumulation of deleterious mutations within mtDNA sequences [36,41]. The accumulation of mtDNA mutations, which threaten OXPHOS integrity and associated phenotypes, should therefore place intense selection on the nuclear genome for counter-adaptations that restore compromised function. This model describes a coevolutionary process that is primarily compensatory—where the nuclear genome must repeatedly come to the evolutionary rescue of mtDNA-induced mitochondrial dysfunction [10,34], a notion congruent with the genetic footprint of compensatory adaptation observable in nuclear genes encoding subunits of the cytochrome *c* oxidase complex in primates [42].

While the relative contributions of adaptive to non-adaptive processes in shaping mitochondrial genome evolution are unclear, it is likely that each of the coevolutionary models (co-adaptive and compensatory) described above contribute to shaping the genomic landscape of the mitonuclear genomic interaction. And regardless of the specific drivers involved, we predict that the footprint of mitonuclear coevolution will be significant, and leave its mark over multiple taxonomic scales. Evolutionary forces acting within populations will drive population-specific evolutionary trajectories of mitonuclear genotypes, which over evolutionary time will promote reproductive isolation caused by mitonuclear allelic incompatibilities between disjunct populations, with these incompatibilities ultimately serving as engines of speciation [43].

In this paper, we outline evidence that suggests that the expression of key health parameters, and life-history phenotypes, are routinely shaped by mitonuclear interactions. Furthermore, these interactions are concurrently taking place, and are evolutionarily relevant, over several tiers of biological structure (intrapopulation to interpopulation, and interspecies). We argue that a deeper understanding of the ubiquity and magnitude of these interactions has potential to feed into our general understanding of core evolutionary concepts, and to also provide insights into the complex genetic basis of

mitochondrial diseases. We then conclude by introducing a fourth tier on which mitonuclear interactions could well exert significant effects, but remain completely unstudied—the 'intra-individual' tier. Individuals often carry heteroplasmic mtDNA sequences (a non-clonal population of mitochondrial genomes, typically consisting of wild-type and mutant mtDNA molecules) [44,45]. Furthermore, many nuclear-encoded mitochondrial genes are also present in duplicates, and these duplicates often exhibit tissue-specific expression [46–48]. Thus, allelic variation within particular mtDNA- and nuclear-encoded genes can co-segregate, and thus potentially interact, even within an individual, and this might have hitherto unrealized medical and evolutionary implications.

3. Intrapopulation interactions

Given the haploid nature of the mitochondrial genome, all de novo mutations appearing in the mtDNA will be continually exposed to the full force of natural selection. When this observation is coupled with the knowledge that the mtDNA encodes some of life's most essential gene products, one could conclude that directional selection will generally efficiently purge (when pathogenic) or fix (when beneficial) any phenotype-changing (i.e. functional) mutations, contingent on the magnitude of their associated effects [10]. This would suggest, *prima facie*, that most polymorphisms segregating in the mitochondrial genome of metazoan species are likely to be selectively neutral, or near-neutral. However, as discussed above, the diminished efficacy of selection in shaping the mitochondrial genome that directly results from a low N_e, should confer an upward shift in the equilibrium frequencies at which deleterious mutations are maintained within the mitochondrial genome, under mutation–selection balance, relative to that which might be expected for mutations of equivalent effect in the nuclear genome.

Theoreticians have addressed the potential for stable joint polymorphisms in the mitochondrial and nuclear genomes to be maintained within populations via mitonuclear interactions (a form of balancing selection) [49–53]. These studies have shown a range of conditions under which protected mitonuclear or mitochondrial polymorphisms could be upheld, such as under frequency-dependent or sex-specific selection [49], or sex linkage of the interacting nuclear genes [50]. Based on empirical results derived from the interpopulation level, other authors have suggested that gene-by-environment interactions involving polymorphisms in the mtDNA, including complex mtDNA-by-nuclear-by-environment effects, could plausibly uphold mitochondrial genetic variance within populations [54,55]. Relevant empirical work at the intrapopulation scale is scarce [50,54,56,57], and all comes from work on *Drosophila melanogaster*. These studies generally did not have the required resolution to home their evidence of cytonuclear interactions to the level of the mtDNA sequence, but they nonetheless provide some proof of concept for the idea that mtDNA polymorphisms could be maintained within populations via mitonuclear interactions for fitness [50,54].

Much research remains to be done at the intrapopulation level to empirically validate the possibility and extent to which polymorphisms in mtDNA are maintained by mitonuclear interactions. To date, the few studies conducted at the intrapopulation scale would suggest that the links between the mitochondrial genotype and phenotype are highly contingent on the particular nuclear background with which the mtDNA is coexpressed. These results give credence to the hypothesis that mitonuclear allelic variation can be maintained under adaptive balancing selection. Thus, selection may act to preserve levels of functional mitochondrial genetic variance in nature, and to maintain multiple mitonuclear allelic combinations, which regulate OXPHOS function. We believe that this might be important to long-term sustainability of our biodiversity in the face of directional change in environmental parameters such as temperature, and the creation of novel environmental, thus, selection pressures. The existence of functional allelic variation underlying OXPHOS function, which relies on uncompromised function of highly temperature-sensitive enzyme complexes, might provide the fuel on which populations can mount adaptive evolutionary responses to preserve their metabolic capacity during bouts of sustained changes to their environments.

4. Interpopulation interactions

In the absence of high levels of gene flow, conspecific populations are expected to coevolve along population-specific trajectories, with each population accumulating its own set of adaptations in interacting regions of each of the mtDNA and nuclear DNA, as well as deleterious mutations that arise in the mtDNA, and their nuclear restorer alleles. The result is that mitonuclear interactions—and OXPHOS function—will evolve towards population-specific optima, and the mitonuclear componentry of incipient populations may become increasingly incompatible as populations diverge, under a Dobzhansky–Muller model of hybrid incompatibilities (DMIs) [58–60] in which the mitonuclear unit takes centre stage [61]. Considering the fundamental role of OXPHOS function to components of organismal fitness, any disruption to the coevolved mitonuclear allelic combination is thus expected to invoke deleterious fitness consequences.

Recent evidence supports the notion that tight mitonuclear coevolution underpins organismal fitness. This has been most prominently illustrated by observations of intergenomic incompatibilities leading to mitochondrial dysfunction following experimental hybridization in the marine copepod, *Tigriopus californicus*. Such hybridization results in fitness breakdown in the F_2 generation, marked by decreased survivorship of larvae [62], slower development [63], reduced fecundity and viability [64], as well as decreased cyclooxygenase (COX) activity and ATP production [64–66]. The decrease in COX activity and ATP production is plausibly triggered by a set of mutations in the mitochondrial cytochrome *c* [67,68]. These mtDNA mutations appear to be compensated by restorer counter-adaptations within the population-specific coevolved nuclear backgrounds, which become 'unmasked' in F_2 hybrids [68]. Reduced fitness of interpopulation hybrids in this model is further correlated with elevated oxidative damage [69], and associated with differential expression of mitochondrial and nuclear OXPHOS genes [70], indicating the complexity of consequences triggered by the disruption of the coevolved mitonuclear lineages. Fitness of these hybrids can be restored via backcrossing to the maternal, not paternal, populations of origin, heavily implicating negative mitonuclear epistasis as the driver of the hybrid breakdown [71]. The impairment of fundamental functions—foremost the

expression of reproductive traits—in this model, highlights the critical role of mitonuclear interactions in upholding organismal fitness, and also the potential significance of these epistatic interactions to the evolution of reproductive isolation between incipient, allopatric populations [10,43,72].

Another model that has provided valuable insights into the role of interpopulation mitonuclear allelic interactions in organismal fitness, uses isonuclear fly lines of the fruit fly *D. melanogaster*, whereby distinct mitochondrial haplotypes sourced from different populations around the globe have been placed into a standardized foreign nuclear background [14,17]. Segregating nuclear allelic variation across these lines has been eliminated, such that modifications to the expression of phenotypes across the lines can be traced directly to variation in the mitochondrial genotype [17]. Fertility assessments among these lines suggest that all mitochondrial haplotypes confer lower male competitive fertility when expressed alongside an evolutionary novel nuclear background in comparison with the coevolved nuclear background [14]. Disruption of the coevolved mitonuclear genotype of one line rendered males, but not females, sterile when placed alongside one particular nuclear background [16,73]. These patterns of decreased fitness upon mitonuclear disruption are consistent with the hypothesis that coevolved nuclear genomes host compensatory counter-adaptations that offset deleterious mutations in the mtDNA. One study that harnessed these isonuclear lines reported that nuclear genome-wide gene expression patterns were influenced by cryptic polymorphisms within the mitochondrial genome, with these patterns virtually completely limited to males. Specifically, the expression of approximately 10% of all nuclear transcripts surveyed (approx. 1500) was sensitive to the mitochondrial haplotype in males, but only seven genes in total in females [73]. The stark male specificity of these mtDNA-induced effects is in line with the hypothesis that the maternal inheritance of the mitochondria will facilitate the build-up of mutations that are male-biased in their phenotypic effects under a sex-specific selective sieve, often referred to as *mother's curse* [15,73–76]. Even though the study did not explicitly examine mitonuclear interactions (i.e. the nuclear background was held isogenic), it revealed a candidate list of around 1500 nuclear genes whose expression is sensitive to interference by mutations harboured within the mitochondrial genome, but only in males. This list contains many genes whose expression is limited to the male reproductive tissues, and which serve essential roles in encoding male reproduction function, enriched for expression in the male reproductive tissues, and the gene list does not overlap considerably with the list of genes that are currently annotated as having mitochondrial-related function. Thus, these nuclear genes greatly increase the number of candidate nuclear genes for involvement in mitonuclear interactions, and extend the significance of the mitonuclear interaction well beyond that of simply being a regulator of core OXPHOS function [77].

Further compelling evidence for the profundity of mitonuclear interactions and their impact on key fitness traits comes from research in seed beetles [55,78] and yeast [79]. Arnqvist *et al*. [78] generated 25 fully crossed mitonuclear genotypes, via introgressive backcrossing of mtDNA haplotypes sourced from five distinct populations into the nuclear backgrounds associated with the same five populations, and subsequently measured the carbon dioxide production under two different temperature regimes. While no overall differences in metabolic performance between mitochondrial haplotypes or nucleotypes were detected, complex gene-by-gene-by-environment interactions existed in the form of mitonuclear interactions whose outcomes were thermally sensitive. This finding fully aligns with the results of a similar experiment, harnessing the same 25 mitonuclear genotypes, which found that the development rates associated with particular mitonuclear genetic combinations were contingent on the temperature at which the beetles were reared [55]. That is, certain combinations of mitochondrial and nuclear genotype conferred relatively faster development time at the lower temperature, whereas other combinations had a relatively faster development time at the higher temperature. Generally, the superior mitonuclear genotypes at one temperature were not the superior performers at the other temperature. Interestingly, in four of five cases, the development times associated with coevolved mitonuclear combinations was slower compared with those associated with disrupted mitonuclear gene complexes, counter to prediction.

Together, these studies demonstrate that mitonuclear interactions affect core fitness traits, but that the outcomes of these epistatic interactions depend on the prevailing environment. These findings fit the expectation that the mitonuclear interaction coevolves along population-specific trajectories, and towards population-specific optima in response to natural selection as a result of inhabiting distinct spatial and temporal environments. Considering the temperature sensitivity of OXPHOS enzyme activity, it is not surprising, in retrospect, that temperature has a strong effect on the efficiencies at which different combination of mitochondrial haplotype and nucleotype perform. The mix-and-matching that naturally occurs in panmictic populations, combined with the allelic variation that is known to persist within populations, will by default generate a plethora of distinct mitonuclear combinations, some of which will be most likely more efficient regulators of OXPHOS than others under certain environmental conditions.

While variation in mitochondrial genes, and thus in metabolic rate, is likely to play a pivotal role in the adaptability and evolvability of single populations to changing environments, mitochondrial incompatibilities will eventually invoke hybrid breakdown effects, ultimately marking the early stages of reproductive isolation between incipient populations. Because of this common pattern of negative mitonuclear epistasis in hybrids, and the role of mitochondrial function/dysfunction in organismal and reproductive fitness, mitochondrial bioenergetics has been hypothesized as a major driver of speciation [72]. Negative mitonuclear epistasis might act as an efficient barrier between species to reinforce reproductive isolation manifested as predicted by DMI [58–60]. Under this model, a deleterious mutation that reduces fitness can theoretically persist within allopatric populations as long as this mutation is rescued by a compensatory mutation at a second locus within respective populations. However, the compensatory effect may relinquish once breeding between individuals of these population occurs, leading to allelic mixtures that fail to combine the deleterious mutation with the compensatory allele, and conferring hybrid breakdown, at least of latter generation hybrids (F_2 and beyond). Considering the complexity of mitonuclear interaction, the distribution of mitochondrial components over two obligate genomes, and the different rates at which mitochondrial and nuclear loci evolve, the mitonuclear bond should be highly susceptible to DMI.

5. Interspecies interactions

Species-level evidence for mitonuclear mismatches must fundamentally be driven by evolutionary processes that are particular to populations. That is, the occurrence of macroevolutionary species-level mitonuclear incompatibilities must be the outcome of coevolutionary processes that take place within species and populations. As such, DMIs between allopatric populations are likely to progress through to speciation if isolation between populations persists over long enough evolutionary timescales to enable macroevolutionary patterns to manifest [31,60,61,80]. It is difficult to predict whether the degrees of mitonuclear mismatch, driven by DMI will increase as we move from the within species to between species levels. Intuitively, one might expect this increase to arise, given that the levels of genetic divergence increase markedly as one transitions through these scales.

Empirical evidence supporting the notion that mitonuclear incompatibilities increase with genetic distance comes from research using human cell lines stripped of mtDNA and then repopulated with the mtDNA from increasingly distantly related taxa [81,82]. A first set of experiments paired a human nucleotype with the mtDNA haplotype of the common chimpanzee, pigmy chimpanzee and gorilla [82]. Interruption of the coevolved mitonuclear lineage resulted in decreases in the activity of complex I of the mETS of around 40% in cybrids where the human nuclear background was paired with foreign mitochondrial genotypes in comparison with the native mtDNA genotype, whereas complexes II, III, IV and V were not affected [82]. In a second study, mitonuclear combinations were extended by combining the human cell line with mitochondrial haplotypes from even more distant taxa—orangutan, species representative of Old-World monkeys, New-World monkeys and lemurs—all of which failed to restore any tangible OXPHOS activity [81]. Similar mitonuclear incompatibilities have been documented in mtDNA-depleted cybrid cell lines of mouse species, repopulated with mtDNA from increasingly distant murine species [81–85]. By assessing respiratory capacities of single cell lines, these experiments reported varying decreases of activity for mETS complexes, with complex III most affected, displaying a striking decline in electron transport capacity with increasing species divergence [84]. Collectively, these experiments suggest that the disruption of the coevolved mitonuclear lineage leads to mitonuclear incompatibilities, reducing either OXPHOS function, complex assembly or increasing abnormally reacting oxygen species, and that the mismatch indeed increases with the level of taxonomic divergence in these groups.

The susceptibility of OXPHOS function to mitonuclear incompatibilities between species is perhaps best illustrated considering that all mitochondrial functions depend on nuclear factors that require import into the mitochondrion. These include mitochondrial transcription factors, transcription termination factors, RNA processing and modifying enzymes, ribosomal proteins, translation factors, all of which interact with and rely on specific sequence motifs in mitochondrial DNA or RNA to fulfil their functions [86], and which are expected to be sensitive to increasing genetic divergence between mitochondrial and nuclear genomes. Changes in recognition sites can thus readily disrupt mitochondrial translational machinery, leading to a breakdown in OXPHOS function. One such example comes from studies, which used an *in vitro* system, combining factors of human and mouse mitochondrial transcription machinery, and found that mouse mitochondrial RNA polymerase (POLRMT) performs poorly when transcribing mtDNA using human promoters [87]. This work strongly suggests that the poor performance is linked to altered binding motifs of the transcription system establishing contact between POLRMT and mtDNA that have coevolved with their counterpart mtDNA recognition sites between the two species [70,87]. Another such example comes from a yeast hybrid model between *Saccharomyces cerevisiae* and *Saccharomyces bayanus* identifying the inability of the nuclear translation factor *AEP2* (*S. bayanus*) to regulate the translation of the mitochondrial F0-ATP synthase subunit c (*OLI1*, *S. cerevisiae*), causing sterility and sporulation defects [88,89]. Similar translational breakdown between species was observed between hybrids of *S. cerevisiae*, *S. bayanus* or *Saccharomyces paradoxis*, revealing the inability to properly splice mtDNA-encoded *COX1* via the interaction with the nuclear factor *Mrs1*, thus leading to sterility [89]. Similarly, recent hybridization studies in the fruit fly revealed a pronounced mitonuclear incompatibility, which was traced specifically to the mtDNA encoded $tRNA^{TYR}$ of *Drosophila simulans* and the nuclear-encoded mitochondrial tyrosyl-tRNA synthetase of *D. melanogaster* [90]. This specific incompatibility decreases the activity of mETS complexes I, II and IV, compromises bristle formation, delays development and decreases fecundity [90]. A similar decline in OXPHOS capacity was previously documented in backcrosses of *D. simulans* and *Drosophila mauritiana* [91], and decreased fertility, fecundity and offspring viability, ultimately leading to hybrid breakdown in parasitoid wasps [92–94], and extensive mitonuclear epistatic interaction in the fruit fly [95].

Collectively, the empirical evidence outlined is compelling in its message that the phenotypic expression associated with specific mitochondrial genotypes is contingent upon the nuclear background alongside which these are expressed, and that these two genomes do not evolve along independent routes, but exhibit evolutionary trajectories that are tightly entwined with each other. Thus, the mitonuclear unit is a profound evolutionary unit, and mitonuclear coevolutionary processes are likely to impact on the general evolutionary dynamics of populations. What this means is that some mtDNA genotypes may perform well when coexpressed alongside certain nucleotypes, but may perform poorly alongside others, thereby significantly modulating key fitness traits. The reliance of physiological and reproductive traits on mitochondrial performance is nothing new, but what is unexpected is the extent to which coadaptive processes underpinning the mitonuclear interaction modify these traits. In hindsight, this may not be surprising, considering the different tempi and modes at which the interacting genomes evolve, and the perpetual process of coadaptation in response to selective pressure exerted by de novo mutations, standing genetic variation as well as distinct spatial and temporal environments. These epistatic interactions between mitochondrial and nuclear loci shape the adaptive landscapes and genetic architecture following population- and species-specific trajectories, and define mitonuclear complexes. The upshot of these processes is that nuclear and mitochondrial alleles of mitonuclear complexes are not freely interchangeable genetic elements, but rather act as a single, highly adapted and optimized functional unit. Efficient respiration is thus highly dependent on a good matching of mitochondrial and nuclear alleles, with suboptimal matches

invoking declines in fitness levels, because all fitness traits rely on mitochondrial function [77].

6. Intraindividual interactions and biomedical implications

The ubiquity of mitonuclear effects across multiple taxonomic scales suggests that mitonuclear interactions and incompatibilities will arise, and exert important consequences, wherever allelic variation in participating genes exists to fuel potential conflicts. We note that these conditions indeed exist at one further, but as yet unstudied, scale—within individuals. Intraindividual mitonuclear allelic variation can be fuelled by heteroplasmy in the mitochondrial genome, by heterozygosity and by the existence of gene duplicates (i.e. multiple varying copies of the same gene at different loci), and gene isoforms (i.e. splicing or length variants of the same gene) in the nuclear genome. What this means is that alternate mtDNA alleles can potentially be placed alongside alternate variants of nuclear-encoded mitochondrial genes, within one and the same individual. Assuming that all alleles are expressed, this gives rise to the possibility that mitochondrial complexes are assembled by a variety of different combinations of slightly varying subunits (protein isoforms), with the potential for enzymatic activities to be modified, exerting selective pressure on single complexes and mitonuclear combinations.

Support for this concept comes from two lines of research. First, mitonuclear allelic interactions have been observed in heteroplasmic individuals of fruit fly, where temperature-dependent selection of mitochondrial haplotypes, within individuals, has been shown to be contingent on the individual's coexpressed nuclear background [96]. Second, many species possess duplicates of nuclear-encoded mitochondrial genes, which exhibit tissue-specific (generally testis) expression. In one study on fruit flies, the expression of some mitochondrial gene duplicates was exclusively limited to testes and the expression of these duplicates outweighed the expression of the original parental genes threefold in the testes, whereas in all other tissues, gene duplicates were not expressed at all, instead fully relying on the expression of the parental variant [97].

An interesting case in point, which exemplifies the potential requirement of sex-specific functional variants of mitonuclear complexes, comes from the system of doubly uniparental transmission of mtDNA in some bivalves [98–100]. In this system, heteroplasmy is the norm: a maternal haplotype is transmitted to all offspring, and a paternal haplotype is exclusively transmitted to male offspring. In males, the maternal haplotype is predominant in all somatic tissues, but is outweighed by the paternal haplotype in the gonads, a system that may have evolved in response to asymmetries in functional requirements of reproductive tissues [101,102]. Interestingly, sequence divergence between the two haplotypes can exceed 40%, yet mitochondrial function is maintained, suggesting that mtDNA-encoded components may be matched alongside sex-specific isoforms or duplicates of nuclear-encoded mitochondrial components [101,102].

In mammals, the expression patterns of tissue-specific, other than testis-specific, variants is currently being elucidated. At least five complex IV protein subunits have tissue-specific isoforms. These are Cox4i1/Cox4i2; Cox6a1/Cox6a2; Cox6b1/Cox6b2; Cox7a1/Cox7a2; Cox8a/Cox8b/Cox8c. Cox7a1, for example, is a heart/muscle isoform, whereas Cox7a2 is a liver isoform [103]. Moreover, expression patterns associated with particular isoforms, which confer different catalytic properties, do not only change across tissues, but also upon certain metabolic stimuli. Thus, hypoxia induced the replacement of the complex IV subunit 4–1 by its isoform 4–2, conferring a higher turnover rate to the activity of the enzyme [104]. A similar phenomenon was previously described in yeast mitochondria [105].

The presence of heteroplasmy in mtDNA, coupled with such context-dependence in expression of alternative mitochondrial gene isoforms, thus provides explicit scope for tissue-dependent intraindividual mitonuclear interactions, [97]. The existence of differential tissue-specific or physiologically induced expression of genes means that different combinations of mitochondrial components—with different catalytic properties—are expressed and assembled in different tissues or in response to particular stimuli. This componentry is labile and responsive to tissue-specific necessities, environmental changes or more generally to specific functional needs. In this respect, one must note that the variety of functions associated with the mETS is diverse and context-dependent, with different tissues requiring different, even contradictory, set-ups. For example, the main role of mETS in brown adipose tissue is to provide heat, whereas, in heart muscle, it is to provide ATP. However, maximizing ATP production efficiency implies reducing heat production. Less evident, but equally important, the physiological role of hepatocyte mETS is substantially different to that of neurons, to the extent that neurons cannot use fatty acids as a fuel because they lack β-oxidation [106].

Together, these observations strongly indicate that the within-individual level is not exempt from the effects of the mitonuclear interaction, because similar mechanisms, involving alternative variants of mitochondrial genes, exist within or across tissues in response to local or modified functional needs, or spontaneous mtDNA polymorphisms. Considering that heteroplasmy [11,40,44,45,107] and gene duplication of mitochondrial genes [46–48] are pervasive across taxa, the intraindividual level provides a new frontier for exploration of the evolutionary and medical significance of the mitonuclear interaction. A very striking example of the potential for intraindividual mitonuclear interaction was found in mice with artificially induced heteroplasmy between two wild-type mtDNAs, that of the Balb/cByJ mouse and that of the NZB/BINJ mouse [108]. It was robustly shown that selection on the mtDNA was tissue-specific, favouring NZB/BINJ mtDNA in the liver and kidney, but Balb/cByJ mtDNA in the blood and spleen, whereas other tissues showed no preference for either mtDNA type [108]. Differences in OXPHOS performance have been proposed to account for this phenomenon [21], which likely reflect the outcomes of mitonuclear interactions between the respective mtDNA types and different tissue-specific nuclear transcript isoforms.

Finally, we raise the prospect that mitonuclear interactions, at all scales from the intraindividual to between population levels, will not only be relevant to, but might help to explain much of the unaccounted for variation in the penetrance, variable array of symptoms and severity of human mitochondrial disease. The pervasiveness of the mitonuclear effects that we have outlined above, across numerous taxa and multiple biological scales, in itself suggests that humans will not be

exempt from the processes shaping mitonuclear coevolution and the ensuing effects. For instance, variance in mitonuclear compatibility could arise between divergent mitochondrial haplogroups and associated nucleotypes, which might predispose themselves to multifaceted and complex disease phenotypes [109]. It is well established that mitochondria are central to a multitude of disease phenotypes, yet, in many instances, straightforward links between mtDNA mutation and disease expression do not exist, and it remains largely unknown how this influence is exerted. We contend that in numerous instances mitochondrial disease may not be expressed through additive effects exerted by mitochondrial polymorphisms alone, but rather through epistasis between these polymorphisms and interacting nuclear alleles, and that this context-dependent expression of mitochondrial phenotypes may be key to understanding mitochondrial degenerative diseases. Particularly intriguing are those pathogenic mtDNA mutations that can reach homoplasmy and yet show very different penetrance among patients. It is puzzling that, with these homoplasmic mutations, some individuals suffer a severe life-threatening disease, whereas others are healthy [110]. It was proposed that functional epistasis between some nucleotypes and the mutant mtDNA may prevent disease [111].

Mitonuclear incompatibilities have previously been suggested to contribute to the expression of mitochondrial disease, and been associated with impairment of the mitochondrial translational machinery and OXPHOS assembly in LHON syndrome [112], Leigh syndrome [113], complex I-specific neurodegenerative disease [114] and in the phenotypic expression of deafness in humans and mice [20,115]. Heteroplasmy [44,45], gene duplication of nuclear-encoded mitochondrial genes [48,116], as well as the genetic footprint of adaptive selection in mitochondrial gene trees [117–119] are pervasive in humans, and all of these factors have the potential to fuel mitonuclear interactions for key phenotypes, as outlined above. Thus, the coevolutionary processes underpinning the mitonuclear interaction may provide tangible insights into our understanding of the origins of mitochondrial disease, and ultimately help us to predict incidences and severity of disease expression.

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