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Review

Evolutionary perspectives on the links between mitochondrial genotype and disease phenotype☆

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ABSTRACT

Background: Disorders of the mitochondrial respiratory chain are heterogeneous in their symptoms and underlying genetics. Simple links between candidate mutations and expression of disease phenotype typically do not exist. It thus remains unclear how the genetic variation in the mitochondrial genome contributes to the phenotypic expression of complex traits and disease phenotypes.
Scope of review: I summarize the basic genetic processes known to underpin mitochondrial disease. I highlight other plausible processes, drawn from the evolutionary biological literature, whose contribution to mitochondrial disease expression remains largely empirically unexplored. I highlight recent advances to the field, and discuss common-ground and -goals shared by researchers across medical and evolutionary domains.
Major conclusions: Mitochondrial genetic variance is linked to phenotypic variance across a variety of traits (e.g. reproductive function, life expectancy) fundamental to the upkeep of good health. Evolutionary theory predicts that mitochondrial genomes are destined to accumulate male-harming (but female-friendly) mutations, and this prediction has received proof-of-principle support. Furthermore, mitochondrial effects on the phenotype are typically manifested via interactions between mitochondrial and nuclear genes. Thus, whether a mitochondrial mutation is pathogenic in effect can depend on the nuclear genotype in which is it expressed.
General significance: Many disease phenotypes associated with OXPHOS malfunction might be determined by the outcomes of mitochondrial–nuclear interactions, and by the evolutionary forces that historically shaped mitochondrial DNA (mtDNA) sequences. Concepts and results drawn from the evolutionary sciences can have broad, but currently under-utilized, applicability to the medical sciences and provide new insights into understanding the complex genetics of mitochondrial disease. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

The mitochondria are cornerstones to eukaryote life, providing the cell with a highly efficient means of converting biochemical energy stored in food, through the oxidation of nutrients to produce adenosine-5′-triphosphate (ATP). This process occurs via the metabolic pathway known as oxidative phosphorylation (OXPHOS), during which redox reactions, which take place over a series of five enzyme complexes (the mitochondrial respiratory chain), release energy that is used to form ATP [1]. This ability to harness energy via OXPHOS is likely to have been salient in enabling the evolution of complex and energy-demanding forms of life [2]. In addition to their central role in energy conversion, the mitochondria are heavily involved in other vital biological processes including thermogenesis via mitochondrial uncoupling [3], cellular apoptosis [4], calcium storage and signaling [5], and reactive oxygen species (ROS) production (used in signaling [6], albeit highly toxic when at surplus levels [7]).

As a legacy of their ancient endosymbiotic origin, the mitochondria retain their own diminutive genome (around 16 000 nucleotides, 37 genes in the typical metazoan) of mitochondrial DNA (mtDNA) [8]; although many of the genes essential for mitochondrial respiration have been translocated across to the nuclear genome throughout the course of evolutionary history [9]. As a result, of the more than 80 protein subunits that comprise the enzyme complexes of the mitochondrial respiratory chain, 13 are encoded by the mtDNA, and the remainder by genes within the nuclear genome [8]. Given their pivotal role in regulating essential biological function, one would logically infer that if mutations were to arise in these genes, then the phenotypic consequences would be serious. Thus, by implication, these genes will be subjected to the intense and perpetual force of natural selection to ensure their optimization. Yet, despite the prediction of strong and effective selection to remove such mutations, it is well known that mutations to OXPHOS-encoding genes do persist and can cause mitochondrial disease in humans. However, the link between these mutations and the expression of disease is complex [10], and for instance likely to be mediated by interactions between alleles spanning both mitochondrial and nuclear genomes (mito-nuclear interactions) [11], and predicting

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the presence or severity of mitochondrial disease of patients based on their genetic profile remains a major challenge in many cases.

In this review, I draw on insights from evolutionary theory and empiricism to suggest new avenues for exploring the complex genetics underlying human mitochondrial disease. Research into mitochondrial genome evolution and research into mitochondrial disease share fundamental common ground, because both center on the study of mutations. While each field is undoubtedly driven by a different set of conceptual questions and end goals, a central shared aim is to understand the effects and role that mutations within the mitochondrial–nuclear (mito-nuclear) complexes of the respiratory chain have on expression of the organismal phenotype (Fig. 1). I start by introducing human mitochondrial disease (Section 1.1), and then outline the primary genetic factors that are well known by medical researchers to affect the expression and severity of disease symptoms (Section 2). I then discuss other genetic peculiarities and processes relevant to the mitochondrial genome (Sections 3 and 4), which have received focused attention from evolutionary theorists and empiricists, but for which the realized implications to mitochondrial disease expression remain elusive and not at the frontline of the biomedical mitochondrial research agenda, despite their potential key relevance.

1.1. Mitochondrial disease

Mitochondrial disease in humans encompasses a broad range of metabolic disorders that result in a range of disease phenotypes, from myopathies, to visual and hearing impairment, organ failure, respiratory and neurological disorders, dementia, aging [1,12–16] and even male infertility [17]. Mitochondrial genetics have furthermore been linked to several other common human diseases, including diabetes [18], autism spectrum disorders [19], cancer [16,20], Alzheimer's [21] and Parkinson's [22] disease. The first case of mitochondrial disease was not identified until the early 1960s [23], followed by the first identified mtDNA disease-causing mutations in the 1980s [24,25]. The present estimate is that mutations to genes affecting OXPHOS function cause mitochondrial disease in around one in every five thousand human births [26]. A caveat is that this number could well be much higher, since mitochondrial OXPHOS disorders are clinically heterogeneous in their symptoms, as well as genetically heterogeneous (pathogenic mutations at 30 of the 37 mtDNA encoded genes, and at more than 30 nuclear genes alone, have been linked to OXPHOS malfunction), making their accurate diagnosis difficult. It has been suggested that over 100 nuclear-encoded OXPHOS disorders might still await identification [26]. This estimate seems plausible, in light of recent estimates that at least one in every 200 humans in the general population harbors a pathogenic mutation in the mtDNA alone [27] (i.e. not including the possible suite of mutations at nuclear-encoded sites), and are hence at risk of developing mitochondrial disease.

2. Mitochondrial disease is governed by distinct mitochondrial genetics

There are many factors, acting synergistically, which can account for the difficulty that researchers and clinicians face in establishing clear and consistent linkages between pathogenic mutations in the mtDNA and resulting disease phenotypes. These factors can be directly ascribed to the distinct genetics of the mitochondrial genome [28,29], and while some of the factors have been the subject of intense research by biomedical researchers (e.g. heteroplasmy, mitochondrial bottlenecks, and critical energy thresholds), the details of others remain nearly completely elusive (sex-specific mutation accumulation, mitochondrial–nuclear interactions, environmental-dependent mitochondrial genetic effects). These factors are the focus of this review.

The peculiarities of mitochondrial genetics render it not only possible, but actually commonplace, for individuals to harbor known pathogenic point mutations in the mtDNA without actually expressing a disease phenotype at all [27], or alternatively expressing disease at just one of numerous possible tissues or organs known to be associated with that particular mutation [10]. In short, the fundamental principles of mitochondrial genetics are markedly different from the Mendelian principles governing the inheritance of nuclear genes and their associated phenotypic effects. To start with, in diploid metazoans such as humans, each somatic cell generally harbors two copies of the nuclear genome, and germ cells carry a single copy. This contrasts to the intra-cellular

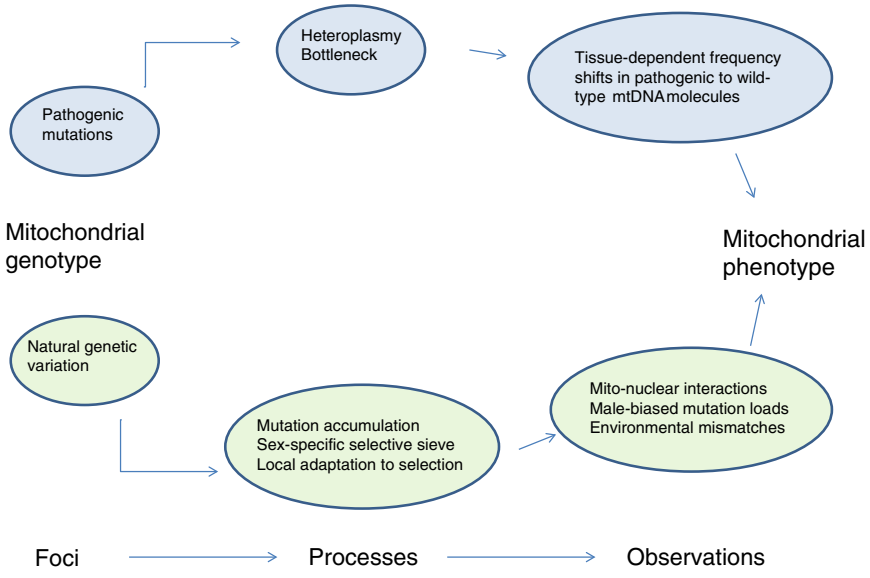

Fig. 1. Prospective routes to mitochondrial disease. The figure illustrates the general field-specific focus of researchers studying the link between mitochondrial genotype and phenotype. In the biomedical domain (top half of figure — blue bubbles), research has focused on known pathogenic mtDNA mutations, and effects of frequency changes of mutant relative to healthy mtDNA molecules under heteroplasmy (i.e. intra-individual mtDNA variation). Such frequencies can change rapidly as a consequence of the mitochondrial bottleneck during oogenesis, and these dynamics determine incidences and expression of mitochondrial disease. In the evolutionary biological domain (bottom half of figure — green bubbles), research has focused on adaptive (selection) and non-adaptive (mutation accumulation, drift) processes shaping naturally-occurring mitochondrial genetic variation (i.e. inter-individual mtDNA variation), across generations. These studies have highlighted the ubiquity by which mitochondrial effects on phenotype are manifested via mito-nuclear interactions, uncovered empirical evidence for a set of male-harming mutations within the mtDNA sequence, and provided evidence that mtDNA mutations beneficial in one environment (e.g. arctic climates), may be harmful in another (e.g. tropical climates).

content of the mitochondrial genome, which is typically present in thousands of copies per cell [30], and in over 150 000 copies per mature oocyte in mammals [31]. These mtDNA molecules replicate independently of the nuclear DNA and the cell division cycle [28]. The implication is that within any one cell, and indeed within the one mitochondrion (of which there are many per cell), there can be different mtDNA molecules that are comprised of slightly different sequences. This state is called heteroplasmy, and the widespread prevalence of heteroplasmy in mtDNA sequences within human individuals [32], and across taxa [32–36], has now been established.

2.1. Heteroplasmy, the mitochondrial bottleneck, and critical energy thresholds

From the biomedical point of view, heteroplasmy means that an individual can harbor two distinct mtDNA molecules per cell — one healthy wild-type, and the other containing a pathogenic mutation. The recent realization that heteroplasmy in human mtDNA is omnipresent [32,37–39] is notable, because mitochondrial genomes follow a strict mode of maternal inheritance, are haploid, and were traditionally not considered to recombine [28] — factors that should theoretically restrict the opportunity for the accumulation and maintenance of heteroplasmy [40]. Heteroplasmy was documented in a study of Holstein cows more than three decades ago [41], with the authors noting rapid shifts in mitochondrial genotype frequencies across short time periods, from within the one maternal lineage. This led to the formulation of the mitochondrial bottleneck theory, since a bottleneck during oogenesis would enable the amplification and segregation of a subsample of heteroplasmic mtDNA [41]. This could lead to random increases, via 'founder' effects, in the frequency of a pathogenic mtDNA mutation as it passes from mother to offspring [42].

The bottleneck theory has since received widespread empirical support in vertebrates [31,36,43–46], although it remains contentious whether the bottleneck involves a physical reduction per se in copy number [46] or a genetic bottleneck resulting from the replication of a random subsample of mtDNA molecules during oocyte maturation [31,43,44]. The traditional view was that mtDNA copy number was reduced by a physical bottleneck to as few as 10 to 200 segregating molecules in the primordial germ cells (PGCs) [47], prior to a rapid expansion to over 150,000 mitochondria in the mature oocyte [31,40]. Regardless of the mechanism underlying the bottleneck, its capacity to rapidly shift the frequency of a pathogenic mtDNA mutation across generations is thought to play a profound role in the unpredictability and complexity of mitochondrial disease expression [48]. This is primarily because the expression of mitochondrial disease, for any given tissue-type, depends on the percentage of pathogenic mtDNA mutations, within the total heteroplasmic pool of molecules, exceeding a critical threshold level [1,12,49]. Typically, mitochondrially-dependent tissue function is remarkably robust to the presence of pathogenic mtDNA mutations. For example, for pathogenic tRNA point mutations within the mtDNA, the threshold of mutant mtDNAs must typically exceed 85–90% of the total mtDNA pool, to affect the phenotype [12]. Once the threshold is surpassed, however, small incremental increases in the frequency of mutant mtDNA can result in large decreases in phenotypic function [12]. Furthermore, different tissues might be more likely to express a disease phenotype [50,51]. Firstly, because certain tissues, such as the brain, heart, skeletal muscle and retina, are more heavily OXPHOS-reliant than others [1,50]. Secondly, because initial frequencies of mutant to wild-type mtDNA molecules will vary across tissues, owing to variation in segregation of wild-type and mutant mtDNA during embryogenesis and tissue-differentiation [47,49,52]. Thirdly, because mutant to wild type mtDNA molecules may exhibit consistent differences in their replication rates. Fourth, because different tissues may exhibit different rates of mutation accumulation of somatic mtDNA mutations with advancing age [50], and these rates might be higher in OXPHOS-reliant tissues or tissues that generate higher levels of mutagenic reactive oxygen species (ROS). And finally because, even if faced with the same frequencies of mutant mtDNA across tissue types, certain tissues are known to exhibit higher levels of nuclear-encoded OXPHOS gene-expression, and such transcripts might function in a tissue-specific compensatory manner to buffer against the presence of disease-causing mtDNA molecules [50].

The literature summarizing research on heteroplasmy and the mitochondrial bottleneck is well covered, and here I will only highlight recent developments that highlight the potential for natural selection to shape mtDNA allele frequencies across generations. Observed patterns of mtDNA segregation in humans [53], and early experimental studies of mice made heteroplasmic for two non-pathogenic mtDNA genotypes [54], were thought to be largely consistent with the idea that shifts in mtDNA frequencies during the bottleneck were randomly determined (i.e. by genetic drift). Traditionally, the argument was posed that the mitochondrial bottleneck could provide an efficient means of removing mutant mtDNA molecules from a lineage, simply because such variants when present at low frequencies within the germ line would be unlikely to be represented in a 'random sample' of the total available molecules during the bottleneck. Via this model, drift would be expected to remove these mutations before they accumulated to high levels within a population. However, by the same logic, the bottleneck could also explain why such mutations occasionally did survive the bottleneck and accumulate to levels above the critical threshold required for mitochondrial disease expression [55]. Such a verbal model seemed congruent with the complex epidemiology of mitochondrial disease, and the curious genetic feature that mutant mtDNAs need to surpass a critical threshold to affect the phenotype — and thus be exposed to selection.

Such findings, and arguments, are consistent with the prediction that the mitochondrial bottleneck will decrease the effective population size (*Ne*) of mitochondrial genomes relative to a hypothetical situation involving mtDNA segregation in the absence of a bottleneck. This is of clinical consequence because reductions in *Ne* should amplify the contribution of random genetic drift to determining changes in mitochondrial allele frequencies across generations, and decrease the efficacy with which natural selection can shape the mtDNA sequence. By this logic, the bottleneck could well enable the accumulation of deleterious mutations within the mtDNA [56]. Yet, to the contrary, theoretical models have shown that the bottleneck can actually act to reduce mutation accumulation within mitochondrial genomes by increasing the level of mitochondrial genetic variance at both the cellular and individual levels, and thereby providing the 'fuel' on which selection can act [57]. Thus, the question of whether or not the mitochondrial bottleneck is generally a facilitator or inhibitor of adaptive evolutionary change in mtDNA frequencies across generations remains outstanding.

However, recent studies [39,58,59], particularly two experimental studies in mice [58,59], strongly support the contention that selection can effectively eliminate at least the most debilitating mtDNA mutations present in the germ line [60]. Fan and colleagues [58] introduced mtDNA containing a pathogenic mutation of severe effect into the germ line of mice, in heteroplasmy. Their experiment was replicated twice — one with a founder female possessing a severe ND6 mutation at a frequency of 47% and another at 14%. The mutation was lost within four generations, and evidence was presented that selection against the mutant took place prior to ovulation. At the same time, Stewart and colleagues [59] reported a similar finding, in which they harnessed 'mtDNA mutator-mice'. These mice are homozygous for a proof-reading deficient subunit of mitochondrial DNA polymerase [61], and this results in the accumulation of high levels of random mutations in the mtDNA sequences. The authors reported the rapid elimination of mtDNA mutations in the female germ line across generations. This pattern of elimination was heavily biased to non-synonymous (amino-acid, i.e. clearly 'function'-changing) mutations, with synonymous mutations observed more frequently in the next generation, and with less intense selection against mutations in rRNA and tRNA genes [59].

These two studies raise the question of how selection can rapidly target and then eliminate these mutations, given that their effects on the disease phenotype should not typically be realized when maintained at low frequencies within an individual, owing to the critical thresholds in disease expression. While this remains an open question, selection can theoretically act on mitochondrial genetic variation at multiple levels — the individual, cellular and mitochondrion-specific levels [62]. In such a scenario, the energy threshold at which mutants, in heteroplasmy, express their effects might only be pertinent to the individual level and somatic cells. That is, the effects associated with a particular pathogenic mtDNA variant within the primordial oogonia of the germline might well differ tangibly to that of the same mutation expressed within the soma. For example, it is possible that PGCs that carry inefficient mitochondria burdened by mtDNA mutations might divide at a slower rate, and thus be swamped by PGCs that are loaded with competent mtDNA and thus optimized OXPHOS machinery [60]. The typical mammalian female will produce millions of primordial oogonia derived from PGCs, but ovulate only a tiny fraction (a few hundred) of these as mature oocytes [58]. It has been argued that it is during this process of PGC death – known as atresia – that selection may effectively purge cells burdened by underperforming mitochondria [60,63,64].

The two studies outlined above suggest that purifying selection can efficiently target debilitating mtDNA molecules in PGCs during atresia, but might struggle to purge variants that are less pathogenic in their effects. Nonetheless, selection can operate at multiple tiers on mtDNA molecules, and is potentially able to discriminate against mildly pathogenic mutations at other life stages. Evidence for this comes from a study by Freyer and colleagues [36], who examined the frequency of a newly-discovered single base-pair deletion in the mitochondrial $tRNA^{met}$ gene, when in heteroplasmy in the mutator mice. They reported that the mutation did not confer biochemical dysfunction in the respiratory chain or affect fecundity, and that the distribution of mutant molecules in PGCs and oocytes was consistent with that expected under random drift. However, they also reported strong evidence of purifying selection pushing the frequency of this mutation downward in the offspring, after the oocyte stage [58,60]. Specifically, mothers carrying high frequencies of the mutant molecules (>70%) produced offspring that exhibited a negative shift in the frequency of the mutation of around 10%.

3. Evolutionary genetic processes relevant to mitochondrial disease

3.1. Haploidy, maternal inheritance and high mutation rates

Above (Section 2.1), I discussed the notion of the mitochondrial bottleneck depressing the *Ne* of mitochondrial genomes, and debated the idea of the bottleneck acting as either a facilitator or inhibitor of selection. Here, I highlight research from the evolutionary sciences that indicates that the significance of the *Ne* of mitochondrial genomes will extend far beyond the issue of heteroplasmy and the bottleneck.

The mitochondrial genome is both haploid and maternally transmitted, in mammals [65], as well as in the typical sexually-reproducing eukaryote [66]. Consequently, it has generally been thought to lack recombination [11], although this latter assumption has been subject to increasing challenge given that heteroplasmy provides the fuel for these molecules to recombine heterologously [28,40,67]. Nonetheless, recombination between mtDNA molecules is regarded as the exception rather than the norm [40]. The haploidy and maternal inheritance of the mtDNA in theory confers a fourfold reduction in *Ne* of the mitochondrial genome relative to its nuclear counterpart [68,69]. This reduction in *Ne* should dampen the efficacy via which selection can purge deleterious mutations from a mitochondrial lineage. Furthermore, the lack of recombination limits the scope for selection to eliminate mutations without purging the entire lineage (since all genes will be physically linked within the mitochondrial genome). Finally, mitochondrial genomes generally exhibit much higher mutation rates than most genomic regions of the nuclear genome [70,71], but lack an efficient means of removing deleterious mutations [40]. In sum, these features essentially predispose mitochondrial genomes to the non-adaptive accumulation of deleterious mutations via a process akin to Muller's Rachet [72,73].

Currently, there is a lack of experimental evidence to clarify the relative contributions of adaptive and non-adaptive processes to shaping mtDNA sequences. While studies based on molecular inferences of mutational changes at non-synonymous and synonymous sites in the mtDNA clearly indicate that purifying selection is undoubtedly a pervasive force shaping mtDNA sequences across taxa [62,74], comparative analyses by Lynch in the 1990s [75,76] strongly supported the prediction that mitochondrial genomes will perpetually accumulate deleterious mutations. Comparison of nucleotide substitutions in transfer RNAs (tRNAs) of animal mitochondrial genomes and nuclear genomes indicate that the mitochondrial tRNAs accumulate mutations more rapidly, and that these substitutions are likely to be mildly pathogenic [75,76]. These mutations are presumably escaping selection, and thus it can be said that mitochondrial genomes will evolve under a 'selective sieve' [75,77] — the wider the sieve, the lower the efficiency of selection in shaping the mtDNA sequence, and the more mutations can thus perpetuate non-adaptively through generations.

3.2. Evidence for adaptive mitochondrial genome evolution

The results of other studies are consistent with the contention that positive "Darwinian" selection has been an important contributor to mtDNA sequences. Studies of mutational patterns within the OXPHOS complexes of invertebrates [78], mammals [79,80], and specifically primates [81], are consistent with the conclusion that some mitochondrially-encoded genes have evolved adaptively under positive selection, and in particular via adaptive co-evolution with the nuclear-encoded mitochondrial counterparts. Further evidence for Darwinian selection on the mtDNA comes from a study that compared mitochondrial genetic diversity across taxa of differing *Ne*. Under the neutral theory of molecular evolution, positive covariation between genetic diversity and population size would be predicted. While the authors confirmed these patterns for nuclear genetic diversity across taxa exhibiting different *Ne*, mitochondrial genetic diversity was remarkably homogenous across taxa, which would be expected under a scenario of recurrent fixation of beneficial mutations [82].

Other evidence for Darwinian adaptation of mtDNA sequences has come from studies exploring mutational patterns in human mtDNA sequences [83–85]. Human mtDNA sequences exhibit many differences across geographic and climatic zones — and these differences are broadly categorized as mitochondrial haplotypes. It had traditionally been assumed that these differences across haplotypes were generated by founder events and genetic drift. But, using standard genetic tests for selection on a sample of about 100 mtDNA sequences, in 2003 Mishmar and colleagues [83] argued that the distribution of mtDNA sequence variants across geographic regions was unlikely to have been generated via genetic drift. They analyzed ratios of non-synonymous mutations to synonymous mutations in mtDNA-encoded genes across the sequence variants, and found that mtDNA genes that are usually observed to be highly evolutionary-conserved across species actually exhibited high amino acid variation among human populations. In fact, particular mtDNA genes exhibited higher amino acid sequence variation in certain global climatic zones. Moreover, they revealed striking associations between particular amino acid substitutions in mtDNA genes and climatic zones, suggestive of climate-driven adaptive evolution. These findings were soon backed up by those of Ruiz-Pesini et al. [84], who found that certain amino acid substitutions were highly conserved at the roots of multiple mtDNA lineages from the arctic zones, implying that these substitutions were essential in allowing humans to adapt to

cold climates. These two studies invoked a strong reaction from biologists and were soon challenged by other studies, which employed larger datasets or examined different populations but were unable to detect any patterns between amino acid substitutions in mtDNA and climatic zones [85–88]. Some studies did however find patterns consistent with climatic-driven selection [85]. Then, further support for the idea of climate-driven mtDNA adaptation in humans was presented by Balloux et al. in 2009 [89]. Rather than focusing on ratios of non-synonymous to synonymous mutations across climatic zones, or on associations between particular amino acid mutations and climatic zones as had previous studies, they directly modeled the distribution of worldwide mitochondrial sequence diversity against geographic (distance from sub-Saharan Africa — the most likely origin of modern humans) and climatic (minimum temperature) variables. They found that geographic distance from Africa had a large bearing on within-population genetic diversity of mtDNA sequences (explaining about 18% of the variance). However, after statistically controlling for geographic distance, they also reported that the minimum temperature that a population experiences explained around 8% of the variance in mtDNA diversity. They reported that human populations living in colder climates have lower mtDNA diversity, and genetic differentiation in mtDNA between pairs of populations correlates with the difference in temperature between those populations. These patterns between genetic diversity and temperature were unique to the mitochondrial genome, with all the other genetic markers tested (namely DNA microsatellites on the nuclear autosomes, X and Y chromosomes) failing to exhibit the signature of climate-driven evolution [89].

The above evidence nonetheless remains contentious by nature, simply because the inferences are all based on correlations, rather than being derived from manipulative experiments. The possibility always remains that the molecular patterns above were generated by demographic factors rather than thermal selection. Nonetheless, results of experimental studies in invertebrates are consistent with the above inferences, by indicating that the performance associated with different mito-nuclear genomic combinations is at least sensitive to the thermal environment [90–92]. Furthermore, researchers have found consistent effects of temperature [93–96], often in interaction with the nuclear background [94,96], on inter-generational shifts in intra-individual frequencies of distinct mtDNA types, expressed in heteroplasmy. In these studies, the heteroplasmy was artificially induced using germ plasm transplantation, thus effectively placing mtDNA of one species of the *Drosophila melanogaster* subgroup, alongside mtDNA of another species, inside of individual flies [93–96].

Clarifying the role of climatic selection, both in terms of driving divergence across mitochondrial haplotypes, and in shaping the finer molecular architecture of mito-nuclear gene complexes, is a salient question deserving of empirical attention into the future [11]. While experimental studies cannot be conducted to validate the patterns reported in human mtDNA sequences, proof-of-principle experimental studies in invertebrates can yield essential insights into whether such adaptation is possible, or indeed ever realized, in natural populations. In this regard, invertebrate model species can provide ideal test-beds for addressing these questions because they often have cosmopolitan global distributions (like humans), and they share the same core mitochondrial genetic parameters (in terms of inheritance, ploidy and recombination patterns, and in terms of gene number and function – i.e. 37 genes – 13 protein coding, 22 tRNA, 2 rRNA) as do humans. I return to the significance of climate-driven mtDNA adaptation for mitochondrial disease later in the review (Section 4).

3.3. The sex-specific selective sieve in mitochondrial genome evolution

Maternal inheritance of the mtDNA invokes an added evolutionary complexity to the evolution of mitochondrial genomes, for it means that the mtDNA hits an evolutionary dead-end in males. While all males carry mtDNA, and are affected by the pool of function-changing allelic variants that exist within the mitochondrial genome [97–101], they generally will never pass these alleles onto their own offspring [28]. The implications are potentially profound. Evolutionary adaptation occurs when allele frequencies change across generations because of the differential performance of alleles at any given gene locus. If males do not transmit their mitochondrial genes onto their offspring, this then may in many scenarios (but see [102]) preclude them from direct involvement in mitochondrial genomic adaptation. Thus, the evolutionary fate of any given allele within the mitochondrial genome might hinge not only on the relative contributions of non-adaptive to adaptive forces that I described above (Section 3.1), but when it comes to the adaptive component, primarily on the performance of the allele when carried inside of females. That is, in addition to the selective sieve described in Section 3.1 [100], a "sex-specific selective sieve" should contribute to mitochondrial genome evolution. This hypothesized process was termed "Mother's Curse" within the evolutionary literature [103]. As a simple illustration of point, if a particular mtDNA variant was to confer superior fertility in males relative to other variants segregating within a population (or within an individual), then the evolutionary fate of this variant (i.e. whether its frequency increases or decreases) would presumably depend on the allele also conferring a benefit to female function. Thus, when it comes to evolutionary-optimized mitochondrial function for any given trait or tissue, in theory males will have to rely on the female-specific adaptation of the mitochondrial genome [100,104]. This should not pose any problem when it comes to the typical sexually-monomorphic trait, since selection on the mtDNA for optimized mitochondrial requirements in females should also result in optimized mitochondrial functioning in males, for that trait in question. Potential implications arise when focusing on traits and tissues that are sexually-dimorphic or sex-limited in their expression. Many traits fall into these categories, but two of the most obvious candidates are the gonads and gametes. The male version of the gonads is the testis — a tissue that is highly metabolically reliant [105], and the gamete is the spermatozoan, whose fertilizability critically hinges on its motility, which in mammals is driven by the mitochondria packed inside the sperm midpiece [106]. A critical question, then, is how will males optimize their mitochondrial requirements in the testes and gonads, when mitochondrial function will depend in part on the performance of mtDNA-encoded alleles that are exclusively maternally inherited, and presumably optimized for function within the female gonads – the ovaries, and gametes – the ova [100,104]. Theoretically, the benefits that males can salvage from relying on the female-specific adaptation of the mitochondrial genome should diminish as the level of sexual dimorphism increases for any particular trait, and the intersexual genetic correlation decreases [100].

From a biomedical standpoint, the maternal inheritance of the mitochondria can facilitate the accumulation of mutations in the mtDNA that are male-biased in their pathogenicity [100,103,107,108]. That is, males should suffer a greater 'mutation load' in their mitochondrial genomes than do females, because they will express the mtDNA mutations that are pathogenic to both sexes, and that have accumulated non-adaptively [75], but also an extra set of mutations that are male-biased in their pathogenicity [100,103,107]. Three evolutionary processes are predicted to contribute to a male-biased mitochondrial mutation load. First, population genetic models show that maternal inheritance will facilitate the build-up of male-pathogenic mtDNA mutations under mutation-selection balance [107]. If a mutation arises with a detrimental effect on male function (e.g. sperm function), to the extent that it cuts male fertility in half, but that only has slightly deleterious effects on female function, this mutation could be maintained at a low equilibrium frequency within the population [107]. Second, given the expectation that the role of genetic drift in shaping mitochondrial genome evolution will be amplified, relative to nuclear genomes, some of these male-harming mtDNA mutations, could plausibly be driven to fixation within a population [69,100,103]. Third, it is possible that some mutations that arise within the mitochondrial genome might be directly antagonistic

in their effects on each of the sexes [11,100,108,109]. While purifying selection would be expected to quickly purge any mutations that were harmful to female function, while beneficial to male function, mutations that were to benefit females at the expense of male evolutionary fitness would be under direct positive selection, and presumably increase in frequency, even if the benefits to females were modest relative to the costs to male fitness.

Direct support for the sex-specific selective sieve recently came from experiments in *D. melanogaster* [100]. The authors harnessed strains of fly in which the only genetic difference separating the strains lay in the nucleotide sequence of the mtDNA. [98,100]. Microarray analyses were used to compare patterns of genome-wide nuclear gene expression when the isogenic nuclear background associated with the strains was co-expressed alongside distinct and naturally occurring mitochondrial haplotypes. Remarkably, around 10% of nuclear genes in males (over 1000 genes in total) were differentially expressed across the mitochondrial haplotypes, whereas only seven genes in total were mitochondrially-sensitive in females. These differentially expressed genes were more male-biased in expression than expected by chance, and exhibited enrichment hotspots in the testes and reproductive accessory glands [100]. Thus, as predicted under a sex-specific selective sieve, the affected male tissues were the sexually dimorphic/sex-limited male reproductive tissues. These effects on the male transcriptome also had realized downstream consequences for male fertility. One of the five mitochondrial haplotypes was completely male sterile in the isogenic background used [98–100], but functioned normally in females [100], and the different mitochondrial haplotypes in this model system have been shown to affect the outcomes of male competitive fertility, in vivo (when sperm from two males compete inside the female reproductive tract) [110]. Thus, these studies suggest that purifying selection optimizes the mtDNA sequence for female function, but that maternal inheritance has enabled sex-specific mutations in the mtDNA sequence to accumulate that interfere with male function.

Theoretically, any sexually-dimorphic trait should be prone to accumulate a male-biased mitochondrial mutation load. Longevity is one such trait — across many animal species, females generally outlive males [111] (including populations of fruit flies and humans [112]). Recently, Camus and colleagues [97] used a collection of 13 *Drosophila* strains — created identically to, and including, the five strains described above [98]. They assayed longevity and patterns of aging in each sex, and found greater mitochondrial haplotypic variation for the expression of these traits in males than in females. Furthermore, the authors showed that the greater the molecular divergence separating any two mitochondrial haplotypes, the greater the phenotypic divergence in male longevity and rate of senescence. This correlation is consistent with the idea that the male-biased mitochondrial effects were not underpinned by only one or a few single nucleotide polymorphisms (SNPs), but rather by numerous SNPs of minor affect [97]. Thus, the effects of the male-specific mitochondrial mutation load might be pervasive in males — affecting core indicators of health in reproduction and aging.

The results of the *Drosophila* studies present the first direct experimental support for the sex-specific selective sieve process. They are proof-of principle, and will now need to be validated across a range of different nuclear genetic backgrounds, and importantly tested for generality across taxa — specifically mammalian models. This pursuit is salient, and some findings would indicate the male-biased sieve could indeed be manifested in mammals. Monitoring of a pedigree from a captive colony of European brown hares indicated that males carrying one divergent mitochondrial haplotype, which originated from a disjunct location in northern Italy, were associated with lowered reproductive success. Females carrying this haplotype exhibited normal reproductive function [113]. Whether or not the sex-specific selective sieve is an evolutionary force that is relevant to influencing the dynamics of mitochondrial disease expression in humans is a question that deserves careful consideration. Clinical evidence is at least to some degree consistent with the evolutionary theory. For instance, Leber's hereditary optic neuropathy affects mainly male subjects [114,115], and increasing evidence suggests that specific mtDNA mutations [17], as well as particular mtDNA subhaplogroups [116] and haplogroups [117], are associated with clinical cases of male infertility without having any known detrimental effects on females.

4. Context dependence of mitochondrial genetic effects

One of the realizations to emerge from evolutionary research into mitochondrial genetic effects, is that these effects typically appear to be highly context dependent [11]. In particular, mitochondrial allelic effects on the phenotype are often contingent on the nuclear background in which the mitochondrial alleles are expressed, but can also vary according to the abiotic environment in which they find themselves. This results in inherent unpredictability in associating the mitochondrial genotype with phenotype. It also implies that assessment of a particular mtDNA variant as 'pathogenic' or 'normal' will not be 'black and white'. Studies show that some mitochondrial haplotypes appear perfectly normal when co-expressed alongside certain nuclear backgrounds, but confer complete male sterility when expressed alongside others. Currently within the biomedical literature, the idea that human mtDNA variants might exhibit context-dependent pathogenesis is rarely acknowledged (but see [118–122]), and for the most part empirically untested. Establishing the degree to which human mtDNA variants are context dependent in expression could prove critically important for understanding the dynamics of mitochondrial disease. In the following section, I review the empirical evidence for context-dependent mitochondrial genetic effects.

4.1. Mitochondrial–nuclear interactions: mitochondrial function relies on genes dispersed across two genomes

With the exception of complex II, succinate dehydrogenase, which consists entirely of nuclear-encoded subunits, the other four complexes of the mitochondrial respiratory chain comprise a mix of mtDNA-encoded and nuclear-encoded polypeptides [11,123]. The implication of this is clear — at its most fundamental level, mitochondrial functionality hinges on the coordinated interaction, hence the tight co-evolution, between genes that are dispersed across two obligate genomes — mitochondrial and nuclear. Thus, to understand the biology of mitochondrial function, we need to understand the evolutionary dynamics of mito-nuclear genetic interactions. One could argue that these interactions are indeed fundamental to life itself, given that the metabolic processes that take place within the mitochondria will underpin the expression of core phenotypes [11] — both in terms of natural variation in the expression of health traits such as activity levels, fertility, fecundity, and life expectancies [11], and in terms of the expression of debilitating metabolic disorders. The significance of this point is reinforced by the knowledge that the dynamics of mito-nuclear interactions might embroil hundreds more genes than the 80 odd that encode the respiratory chain subunits. Over 1000 nuclear genes have been identified as having mitochondrial-related function [124–126], and are therefore putative candidates for involvement in mito-nuclear interactions. These nuclear genes contribute to numerous processes, including the regulation of mtDNA replication, transcription and translation, respiratory chain assembly, apoptosis, calcium storage [127], mitochondrial–nuclear signaling [128,129], and general mitochondrial homeostasis [127]. Hence, the evolutionary trajectory of literally thousands of alleles, spanning hundreds of nuclear genes, might be entwined with the evolutionary processes that shape allele frequency changes in the mitochondrial genome. And of course, vice versa, evolutionary trajectories of alleles within the mitochondrial genome might depend on the presence of particular alleles within the broader nuclear genome.

Evidence is accumulating to suggest that genetic variation found across mtDNA haplotypes routinely modifies nuclear genome-wide patterns of gene expression [100,130,131], methylation [130,132], and fine-scale dynamics of mitochondrial metabolism [133]. Such studies implicate mtDNA haplotypes in mediating the dynamics of mito-nuclear cross-talk. Other studies have placed mtDNA haplotypes of certain species into mtDNA-less cell lines of other species. These studies have generally found that the greater the evolutionary divergence separating mtDNA and nuclear genome between species, the greater the likelihood of mitochondrial dysfunction [134–137]. In primates, mtDNA genomes from chimpanzees and gorillas are able to restore OXPHOS function in mtDNA-less human cell lines, but mtDNA from more distantly related primates is unable to do so [134,135]. Similarly rat mtDNA inside a mouse cell line results in mitochondrial dysfunction [137]. Although such crosses involve inter-species, evolutionary distant, mismatching between mtDNA and nuclear background, they are nonetheless concordant with the premise that mito-nuclear interactions will play a key role even within species and within populations, in determining the expression patterns of key phenotypes and penetrance of mitochondrial diseases.

If mito-nuclear genetic interactions affect the phenotype, this means that the phenotype associated with a particular mtDNA genotype will be contingent upon the particular nuclear genetic background with which it is co-expressed. This is inter-genomic epistasis, where some mtDNA genotypes might perform well when co-expressed alongside certain nuclear genotypes, but poorly alongside others. There is much empirical evidence for the importance of mito-nuclear interactions underlying the expression of key health related traits [90,91,98,101,138–141], and in several documented cases additive mitochondrial genetic effects on phenotype seem to be weak or absent [90,98,140,141]. Most of the evidence comes from studies that used various techniques of mitochondrial replacement to 'mix-and-match' the mitochondrial and nuclear genotypes of distinct populations within the same species, and then documented ensuing effects on patterns of phenotypic expression. Other evidence links mito-nuclear epistasis as an important facet of the genetic architecture of fitness at the within-population level as well [140]. This means that balancing selection might act to uphold the genetic polymorphisms segregating within the mitochondrial genome within populations, via mito-nuclear interactions [139,142]. The results of several studies even suggest that rank orders of naturally-occurring mitochondrial genotypes (from best- to worst-performing) can change dramatically across different naturally-occurring nuclear backgrounds [90,91,140]. Finally, the results of several studies in insects have now suggested that the trait values associated with particular mito-nuclear genetic combinations might themselves be contingent on the environment in which the study subjects are reared [90,91,140]. That is, phenotypic expression is not only partly underpinned by inter-genomic gene-by-gene interactions, but by more complex gene-by-gene-by-environment interactions. In particular, results in seed beetles *Callosobruchus maculatus* suggest that mito-nuclear interactions for juvenile fitness and metabolic performance might be thermally-sensitive [90,91].

While more studies, and higher replication, are required to confirm the above patterns for generality, the results to date are at least highly suggestive that mitochondrial genetic effects on the phenotype can be highly context-dependent, and depend on the nuclear background in which the mitochondria are co-expressed with, and the abiotic environment in which the mitochondria find themselves. Given these results, the mitochondria would thus be expected to co-adapt tightly to both the nuclear genomic and thermal environment. The signature of tight mito-nuclear coadaptation could then be revealed by experimentally disrupting coevolved mito-nuclear gene complexes, placing mitochondrial haplotypes alongside evolutionary novel nuclear genomes with which they share no direct co-evolutionary history, and then observing the ensuing effects on biochemical and phenotypic function. The logical prediction is that mitochondrial haplotypes should always perform better when co-expressed alongside their co-evolved nuclear counterpart, and in their co-evolved abiotic environment.

4.2. Compensatory mito-nuclear co-adaptation

Given the propensity for non-adaptive mutation accumulation within mitochondrial genomes, it has been proposed that a mode of compensatory mito-nuclear co-adaptation will be critically important for sustaining the stability of eukaryote life. Such a model of co-adaptation would predict that deleterious allelic variants would accumulate within the mitochondrial genome, thus placing strong selection on the nuclear genome for compensatory mutations that offset any deficit to fitness. The trajectories of mito-nuclear coadaptation would be population-specific, and reciprocal mix-and-matching of the mitochondrial and nuclear genomes from any two disjunct populations would be predicted to confer marked hybrid breakdown [11,123].

The results of several studies, from yeast to invertebrates to mice, lend support to a compensatory model of mito-nuclear co-adaptation [143–150]. Some of the best evidence comes from the intertidal copepod (*Tigriopus californicus*). Populations of this species persist on rocky outcrops along the Californian coastline. Despite the fact that they are free-swimming, neighboring populations exhibit strong but fine-scale genetic differentiation, stable across space and time. Disruption of inter-genomic mito-nuclear gene complexes between populations of the copepods results in marked hybrid breakdown in bio-enzymatic [151] and phenotypic function [143], and function is only restored when populations with mismatched mito-nuclear complexes are back-crossed to their maternal populations, hence rematching the co-adapted mito-nuclear combinations [152]. Intriguingly, parallels are seen in the vertebrate model for biomedical research — the mouse. Mito-nuclear mismatches created upon introgressive back-crossing between divergent inbred strains resulted in mice exhibiting reductions in performance [144], learning and exploratory capacity [145].

Above, I documented evidence for the sex-specific selective sieve/Mother's curse hypothesis (Section 3.3), by outlining results that support the existence of a special pool of mtDNA mutations that are male-biased in effect and that accumulate under maternal inheritance of the mitochondria. If males are prone to inadvertently accumulate a set of sex-specific mutations that selection is unable to directly target, and which affect core pillars of health such as reproduction and aging, then this should likewise place strong counter-selection on the nuclear genome to evolve co-adapted compensatory nuclear allelic modifiers. If so, then the dynamics of compensatory mito-nuclear coevolution should proceed along two lines. First, via selection for nuclear modifiers that restore mildly pathogenic mtDNA mutations affecting both of the sexes [11,123], and second, overlayed by selection for nuclear modifiers that mitigate the effects of the pool of male-specific mitochondrial mutations [110]. If this is the case, then we can expect that inter-population crosses that result in mismatches in mito-nuclear genotypes will incur reduced viability and health breakdown in both sexes, but that the magnitude of the reduction will be greater for males.

Tentative evidence for this prediction comes from a study of *Drosophila* that investigated effects of mito-nuclear mis-matching between congeneric species [153], and from a study providing some evidence for hybrid breakdown in competitive male fertility upon inter-population mis-matching of mito-nuclear genotypes within *D. melanogaster* [110].

4.3. Mitochondrial alleles that are adaptive in one locality might be maladaptive elsewhere

As a whole, the biomedical research community has arguably been slow on the uptake of the idea that mtDNA effects on disease expression might be characterized by considerable context dependency. Indeed, the idea is rarely discussed, and for the most part empirically untested, thus preventing definitive conclusions. That said, there have been some

strong proponents for the idea [118–121,154,155], and some emerging striking results. Above, Section 3.2, I discussed evidence for climatic-adaptation of human mtDNA sequences. The authors of these studies explicitly noted the potential for these historical patterns of climatic adaptation to impact on contemporary human health [83,84,118,155]. For instance, two core functions of mitochondria in mammals are energy (ATP) production and thermogenesis. The balance between these two functions is determined by efficiency of OXPHOS function, which is partly under control of the *ATP6* mtDNA gene. Highly efficient OXPHOS generates more ATP and less heat, and less efficient OXPHOS functionality does the opposite. The latter scenario should be non-adaptive in the tropics, but adaptive at higher latitudes — principally the Arctic. The authors of the climate-driven human mtDNA papers described in Section 3.2, contended that certain polymorphisms were key to allowing humans to adapt to the extreme cold in the Arctic, noting the hyper-variability in the *ATP6* gene in haplogroups evolved in these geographic regions [83,156]. These polymorphisms persist in the mtDNA sequences of human populations, but increased gene flow (immigration and emigration) would place individuals with certain mtDNA sequences adaptive for one environment (e.g. associated with low ATP in the arctic) into novel environments in which they might be mildly pathogenic and maladaptive [84,119,156].

Other molecular evolutionary studies have examined the possible functionality and putative pathogenicity of recurrent mutations that occur at the stems of unrelated nodes of the human mtDNA phylogenetic tree [157,158]. Some of these mutations have similar characteristics to disease-causing mutations but have been maintained within the mtDNA phylogeny despite selection [157,158], and one of these studies suggests that this is potentially because they were historically shaped by positive selection and adaptive in the local environment under which they evolved, but pathogenic in their contemporary environment [158].

4.4. Putative healthy mtDNA variants and pathogenesis

In Section 3.3, I discussed research from *D. melanogaster* supporting the sex-specific selective sieve/Mother's curse phenomenon in mitochondrial genome evolution [97,100]. Several naturally-occurring and putatively healthy mitochondrial haplotypes were placed alongside an isogenic nuclear background, and phenotypes and patterns of gene expression scored. One of the mitochondrial haplotypes, derived from Texas USA, was completely male sterile, but functioned normally in females, in this isogenic nuclear background [98–100,110]. The male sterility can be traced to a single SNP within the cytochrome b subunit of respiratory complex III. Testes from the sterile males do not form individualized spermatids [99]. Yet, when the sterility-conferring haplotype is co-expressed alongside its native coevolved nuclear background (from Texas USA), the haplotype is male-fertile, and when expressed alongside another distinct foreign nuclear genotype the haplotype is sub-fertile in males but not sterile [110]. The sterility phenotype is completely contingent on the nuclear genetic background.

Further studies are required to home in on the prevalence of such mitochondrial mutations, which might appear normal in one context but confer stark pathogenesis in another. Meiklejohn et al. [150] provide one such example, albeit drawn from mix-and-matching mitochondrial and nuclear alleles from congeneric *Drosophila* species. Using this inter-species model, they described an amino acid polymorphism in the nuclear encoded tyrosyl-tRNA synthetase of *D. melanogaster*, which interacts with a polymorphism in the mitochondrially-encoded $tRNA^{Tyr}$ of *Drosophila simulans*, to interfere negatively with development, bristle formation and fecundity. The study is important in that it traced the negative epistatic polymorphisms to the nucleotide level, and the authors note that their results are informative to medical researchers, as a plausible mechanism to explain the variable penetrance and genetic complexity of mitochondrial disease expression [150]. This serves as an excellent proof-of-concept, and should motivate researchers to trace such inter-genomic incompatibilities to the levels of the interacting nucleotides at the 'within-species' level that is relevant to evolutionary and medical studies. Such identification of mito-nuclear incompatibilities to the exact nucleotide pairs constitutes an advance with capacity to greatly augment understanding of the dynamics of mitochondrial disease expression.

Finally, I return to the issue of heteroplasmy in mitochondrial disease expression. Research into mtDNA heteroplasmy has centered squarely on the issue of inter-generational shifts in the frequency of a known pathogenic mtDNA mutant relative to a wild type mtDNA molecule, and relative to the critical threshold for mitochondrial disease expression. These studies have previously not found evidence that heteroplasmy per se is disadvantageous to the individual [53–55,58–60]. However, experimental findings presented by Sharpley et al. in 2012 [159] have changed the dynamics of this debate by presenting evidence, contrary to earlier reports [54], that heteroplasmy can be pathogenic in itself — regardless of the mtDNA molecules involved. The authors admixed two putatively normal mtDNA variants, in heteroplasmy in approximately equal proportions in individual mice, coexpressed alongside a standard homozygous nuclear background. They reported two key findings; first, a consistent reduction in the frequency of one of the two mtDNA variants over one to two generations, a hallmark of purifying selection against this particular variant. While this result is superficially consistent with the Fan et al. [58] and Stewart et al. [60] studies discussed in Section 2.1, in those cases the mutations targeted by selection were debilitating, while in the current case, the mtDNA variants were both putatively normal, but the trans-generational shifts in the frequency of each variant indicated that they were under selection. Second, the authors reported that heteroplasmic mice experienced deficiencies in a range of functions (reduction in activity, food intake and the respiratory exchange ratio, increased stress response and harmful cognitive effects) relative to lines in which each of the mtDNA variants were expressed in homoplasmy alongside the standard nuclear background. Thus, the study provides an intriguing indication that heteroplasmy – even when comprising healthy mtDNA variants – can be genetically unstable and confer a reduction in individual performance relative to the homoplasmic state.

There are caveats to the conclusion of the Sharpley et al. [159] study. First, the nuclear background used was homozygous across all lines, and this has some implications in this 'experimental evolution' framework. First, homozygosity of the nuclear background prevented the potential for any evolutionary response by the nuclear genome to the existence of the heteroplasmy. A consequence is that the results reported are specific to the nuclear background used in their study (i.e. one of the mtDNA variants performed worse than the other in this particular inbred nuclear background used), which is essentially a random genotype drawn from a near-infinitesimal pool. This is not a condemnation of the study — indeed other studies providing breakthroughs in mitochondrial biology have done so against a homozygous nuclear background [97,100]. Indeed, the study serves an important proof-of-concept of a premise that was previously unrealized — i.e. heteroplasmy in itself can cause phenotypic effects. But, the effects of heteroplasmy should now be tested for generality in other nuclear backgrounds, and in other taxa. Second, maternal inheritance of mtDNA greatly constrains the possibility for mtDNA molecules, of divergent sequences, being found within the one individual. Typical cases of heteroplasmy generally involve closely related mtDNA molecules that differ by one or a few SNPs (point heteroplasmy) [160,161], with the frequency of point mutations increasing with age. In such cases, pathogenicity tied to heteroplasmy would clearly be expected to be driven by the relative frequency per tissue of the mutant SNP(s) rather than the heteroplasmy per se. Ultimately, the general relevance of the Sharpley et al. [159] study to our understanding of mitochondrial disease might hinge on the prevalence by which paternal mtDNA bypasses the process of ubiquitination [162] and becomes paternally inherited. Several such cases have now been reported in humans [163–166].

5. Conclusion

This review has highlighted the extraordinary genetic complexity that underpins the expression of mitochondrial disease. By outlining major advances in mitochondrial biology to come from two divergent scientific fields, I contend that tangible insights into understanding the genetics of this disease can be achieved. Understanding the contributions of genetic drift, selection, maternal inheritance, and mutation–selection balance to shaping the genetic architecture of mitochondrial genomes remains very much an open question in biology. It is a question that is grounded in a fundamental evolutionary conceptual basis, but it is a question whose resolution has clear and tangible cross-over to the biomedical sciences in terms of understanding the genetics of human mitochondrial disease. A roadmap for further work should draw on the dual insights from each field, and foster greater levels of cooperation and exchange of ideas between biomedical researchers and evolutionary biologists. While the principal motivations of researchers working within each field might differ, the same question ultimately lies at the heart of each field — what mechanisms explain the existence of, and effects linked to, function-changing mitochondrial mutations within populations.

Here, I have purposely not discussed the proximate mechanisms that may mediate the mitochondrial genetic effects outlined within this review. While these mechanisms remain largely elusive, insights and hypotheses are nonetheless quickly emerging on this front [167], and these have the potential to change the way we think about the mitochondria in systems biology. Indeed, retrograde signaling from mitochondria to nuclear genome might be so pervasive that the mitochondria be likened to the conductor of an orchestra, regulating widespread control of nuclear genome expression and functionality.

Finally, many of the concepts and results that I discuss here, could also prove informative in a predictive capacity — for instance in the context of prospective clinical IVF-based mitochondrial gene therapies, which seek to replace the defective mtDNA from the germ line of prospective mothers who suffer from mitochondrial disease. Biological conception under sexual reproduction invariably pairs the mother's mitochondrial genotype with a haploid copy of the mother's nuclear genes, thus perpetually preserving any tightly coevolved mito-nuclear allelic combinations that are maintained under strong selection. On the contrary, IVF-based techniques that harness mitochondrial replacement, and draw on a set of healthy mtDNA molecules sourced from an unrelated donor female [168,169], may inadvertently create evolutionary novel combinations of mito-nuclear genotypes in the offspring, and this might have unintended and hitherto overlooked phenotypic consequences [170] (see Section 4.2).

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