Review

Evolutionary implications of non-neutral mitochondrial genetic variation

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Sequence variation in mitochondrial DNA (mtDNA) was traditionally considered to be selectively neutral. However, an accumulating body of evidence indicates that this assumption is invalid. Furthermore, recent advances indicate that mtDNA polymorphism can be maintained within populations via selection on the joint mitochondrial-nuclear genotype. Here, we review the latest findings that show mitochondrial and cytoplasmic genetic variation for life-history traits and fitness. We highlight the key importance of the mitochondrial-nuclear interaction as a unit of selection and discuss the consequences of mitochondrially encoded fitness effects on several key evolutionary processes. Our goal is to draw attention to the profound, yet neglected, influence of the mitochondrial genome on the fields of ecology and evolution.

Non-neutral mitochondrial genetic variation

The integration of the mitochondrial and nuclear genomes represents one of the most remarkable and fundamental symbioses in the history of life. Mitochondrial and nuclear genes interact intricately to form several of the enzyme complexes of the oxidative phosphorylation (OXPHOS) (see Glossary) system of eukaryotes (Box 1), which are responsible for almost all of our energy production. In effect, the products that the mitochondrial genome encodes are involved in some of the most vital processes performed by living organisms.

Traditionally, it has been assumed that genetic variation within the mitochondrial genome was selectively neutral [1]. Under this assumption of selective neutrality – and armed with the knowledge that the mitochondrial genome exhibits a generally high mutation rate (although the rate of evolution differs across regions within the genome [2]), strict maternal inheritance and no recombination in most species – biologists quickly adopted mitochondrial DNA (mtDNA) as the quintessential molecular tool with which to explore population structures and infer evolutionary histories.

In recent years, however, several lines of evidence have emerged that undermine the assumption of the selective neutrality of mtDNA [1,3–7]. First, at the molecular level, some data indicate a possible role for positive selection in shaping the mitochondrial genome [3,7–12] (Box 2). Second, several experimental studies have documented fitness consequences that arise from the mitochondrial genetic variation that exists naturally within [13,14] and between (see, for example, Refs [15–18]) populations. Third, theory has identified a plausible mechanism via which sequence variation in mtDNA could be maintained within populations – namely, selection on the joint mitochondrial-nuclear (mito-nuclear) genotype [13] – with empirical evidence corroborating this mechanism [13,14].

Glossary

Effective population size: the number of individuals (or in the context used here, the number of haploid genomes) in a theoretically ideal population that would give the same rate of random genetic drift as in the actual population in question.
Epistatic: refers to epistasis, which occurs when the expression of a particular phenotype is contingent on an interaction between alleles at two or more loci.
Genetic drift: the random change in allele frequency that is caused by random variation in individual reproduction.
Heteroplasmy: the presence of more than one variant of organelle genotype (in this case, mtDNA) within a cell or individual.
Hill-Robertson effects: genomic regions with low or no recombination exhibit reduced efficacy of selection and, consequently, elevated levels of genetic drift because of simultaneous selection at closely linked sites. Such effects encompass selective sweeps or hitchhiking with a beneficial mutation becoming fixed by positive selection, background selection (reduced variation owing to a linked region under purifying selection), the stochastic accumulation of deleterious mutations (Muller's Rachet) and mutual interference between mutations under weak selection.
Mutation rate: the chance of a mutation occurring in an organism or gene in each generation. In practice, because most mutations are detrimental and, thus, removed by selection before they are ever actually observed, the mutation rate can only be estimated by the level of polymorphism within or substitution (fixation events) between species [77].
Oxidative phosphorylation (OXPHOS): the metabolic pathway that uses energy that is released by the oxidation of nutrients to produce adenosine triphosphate (ATP).
Positive selection:: selection for beneficial mutations.
Promoter:: a region of DNA that is responsible for the regulation of gene transcription.
Purifying selection: selection against deleterious mutations. Also referred to as stabilizing or negative selection.
Reactive oxygen species (ROS): free radicals, oxygen ions and peroxides that are produced by the mitochondria and result from aerobic respiration; these cause cumulative oxidative damage to DNA, RNA and proteins within the cell.
Sexually antagonistic: refers to a conflict between the sexes over the optimum trait value. For instance, particular combinations of cytoplasmic and nuclear genotypes might confer high fitness when expressed in males but low fitness in females.
Transcription: the first step in the expression of a gene. It is the process via which DNA is enzymatically copied, by RNA polymerase, to produce a complementary RNA strand.

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Box 1. Mito-nuclear enzyme complexes and oxidative phosphorylation

Mitochondria produce most of the energy in eukaryotic cells by a process called OXPHOS [3]. Five multi-subunit enzyme complexes carry out this enzymatic pathway: NADH-dehydrogenase, succinate dehydrogenase, cytochrome bc_1, cytochrome *c* oxidase and ATP synthase. Complex I–IV (the electron transport system) first transports electrons from NADH or succinate to molecular oxygen to create a proton (H^+) gradient across the inner mitochondrial membrane. Complex V then uses this energy gradient to synthesize ATP, the main energy-carrying molecule of the cell.

Notably, the OXPHOS enzyme complexes (with the exception of complex II) consist of both mitochondrial and nuclear polypeptides, which are intricately linked to enable the enzymatic reactions. For example, complex IV (cytochrome *c* oxidase), which oxidizes the nuclear protein cytochrome *c* to pass electrons to molecular oxygen, consists of ten nuclear and three mitochondrial subunits. In the copepod *Tigriopus californicus*, single amino acid substitutions in cytochrome *c* have detrimental effects on complex IV activity that lead to hybrid breakdown [53]. Given the fundamental importance of mitochondrial function and energy production to life, strong selection is predicted for the optimal co-function of mitochondrial and nuclear gene products.

These lines of evidence raise the exciting prospect that the mitochondrial genome is not the innocent bystander in adaptive evolution that it was once taken for. But if this genome is truly a profound player in the evolutionary dynamics of populations, then the existence of non-neutral genetic variation in mtDNA must be a common phenomenon. Most of the non-neutral variation detected in mtDNA has been at the between-population level [15–18] (see examples that follow later). This is not surprising because the pool of mutations that enter a population and are subsequently fixed by selection or by the stochastic process of genetic drift will differ across populations.

Box 2. Positive selection and adaptive evolution of mtDNA

How strong is the evidence for positive selection on mtDNA? An increasing number of authors have suggested that positive selection has been important in shaping the evolution of mtDNA [3,7,8,11,12]. One way to assess the nature of selection on mtDNA is to study the characteristics of mutational changes by evaluating the ratio of nonsynonymous to synonymous fixations between species (d_N/d_S). Ratios above 1 indicate positive selection, ratios below 1 indicate purifying selection, and a d_N/d_S ratio of 1 implies strict neutrality [3,7]. Although some studies have shown d_N/d_S ratios greater than 1 and, thus, provided evidence for positive selection on mtDNA (in particular those regions in tight interaction with nuclear DNA residues [11]), other studies have simply shown increases in nonsynonymous substitution rates for mitochondrially encoded proteins [12]. The latter results do not provide compelling evidence for positive selection because accelerated evolution could simply arise through relaxed functional constraint.

A recent study [8] indicated that the recurrent fixation of advantageous mutations could explain the homogenous pattern of mtDNA diversity that is observed across animal taxa. The findings ran counter to the prediction, which arose from neutral theory, that mtDNA diversity will co-vary positively with population size and, thus, they have attracted substantial attention (see, for example, Ref. [7]). However, it is worth noting that these findings were not replicable when considering only mammalian mtDNA diversity [78]. Currently, the significance of positive selection in shaping mtDNA evolution remains a contentious issue, and further research clearly is needed to carefully disentangle positive from relaxed selection as the cause of the observed patterns [7].

On the face of it, there would seem to be less scope for standing non-neutral mtDNA variation within populations, given that purifying selection seems to be the predominant selective force that moulds mtDNA evolution [4,5,7,19,20] and this will act to reduce genetic variation. Furthermore, given that the mitochondrial genome is haploid, with all alleles continually exposed to selection (i.e. with no heterozygosity to mask recessives), it is easy to envisage that any new mutations will be either swiftly purged or fixed. However, the mutation rate of mtDNA is thought to be generally high [21], and it is typically argued that the efficiency of natural selection acting on the mitochondrial genome is reduced relative to the nuclear genome [21], which will increase the scope for standing genetic variation for fitness in mtDNA. The mitochondrial genome is generally considered to have a reduced efficacy of selection because the effective population size, *Ne*, is thought to be a quarter of the size of the nuclear genome, owing to its haploidy and maternal inheritance [21]. The generality of this assumption has, however, recently been called into question given that higher male-to-female variance in reproductive success, which is a characteristic of polygamous mating systems, should lower the *Ne* of the nuclear compared to the mitochondrial genome [5]. Nonetheless, regardless of whether this fourfold reduction is an accurate estimate, the *Ne* of the mitochondrial genome will undoubtedly be substantially reduced by the absence of recombination in mtDNA because of the associated Hill-Robertson effects that will result [22].

Thus, there indeed seems to be scope for non-neutral variation in mtDNA to accumulate within populations. Furthermore, once polymorphism in mtDNA exists in a population, be it through selection or drift, mito-nuclear genetic interactions for fitness might have a role in maintaining this polymorphism [13,14]. In this review, we outline the latest evidence for mitochondrial genetic effects on fitness. In particular, we highlight the importance of the mito-nuclear interaction as a key unit of selection and discuss the potential for genetic variation in mtDNA to fuel mito-nuclear co-evolution. We then discuss the likely implications of mito-nuclear co-evolution for a range of fundamental evolutionary processes.

Mito-nuclear fitness interactions

In light of the coordination that is required between mitochondrial and nuclear genes for respiration [3] (Box 1), the joint mito-nuclear genotype will clearly be an important unit for selection to act on. Sequence polymorphism among mito-nuclear genotypes might correspond to variation in metabolic performance, which should then have concomitant effects on a wide range of life-history traits and fitness *per se*, given the likely link between such traits and the metabolic rate (see, for example, Ref. [23]). Any selection on mito-nuclear genotypes (Figure 1) could plausibly result in mito-nuclear co-evolution.

Knowing that deleterious mutations in mtDNA can accumulate within populations because of genetic drift [21], there certainly seems to be scope for mito-nuclear co-evolution to proceed via a 'compensatory' model. Under this model, deleterious mutations accumulate in the mitochondrial genome, with selection then favouring an adap-

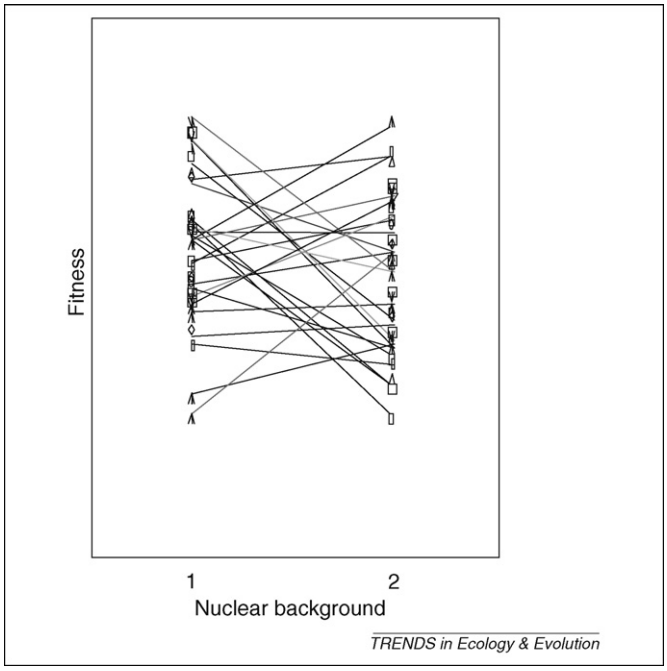

Figure 1. Hypothetical example of a mito-nuclear interaction for fitness. In this interaction plot, each line denotes the fitness of a given mitochondrial variant when expressed in each of two different nuclear backgrounds. The substantial crossing over of reaction norms indicates the non-additive nature of the effects. That is, the relative fitness of a given mitochondrial variant is contingent on the nuclear background with which it is expressed.

tive response in the nuclear genome to restore any compromised metabolic function [24]. In effect, mtDNA mutations will act as the drivers of adaptive evolution in nuclear genes. This scenario is not unlikely, given that more than 1000 nuclear-encoded proteins, which are essential for metabolism, are transported into the mitochondrion [25].

Additionally, given that at least some mtDNA polymorphism might have been shaped via positive selection [7,8], scope might also exist for mito-nuclear co-evolution to proceed via a model in which adaptive mutations in one genome select for a response in the other (Box 2). This likelihood might well be increased if environmental (e.g. thermal) selection is driving this co-evolution, as discussed later.

mtDNA effects on fitness

Here, we showcase the most recent literature that demonstrates mitochondrially encoded (or cytoplasmically encoded; see Box 3) fitness effects at both the between- and within-population levels in animals, focusing in particular on the many recent studies that have demonstrated mito-nuclear effects.

Between-population effects and mito-nuclear co-adaptation

At the between-population level, the evidence in support of mtDNA sequence polymorphism affecting phenotypic variation in metabolism, life-history traits and fitness is compelling. Much of this work has been conducted on just a few model species, such as fruit flies (*Drosophila melanogaster* and *Drosophila simulans*), the intertidal copepod *Tigriopus californicus*, and mice (*Mus musculus*), and has been reviewed previously [3–6,24,26,27]. Such studies represent classic examples of mitochondrial genetic effects on fitness, with effects that were typically manifested via epistatic mito-nuclear fitness interactions [13,15,28]. The results of other studies that utilized non-model organisms have confirmed the likely ubiquity of mito-nuclear interactions for fitness [29,30]. In general, studies that have sought to detect mito-nuclear fitness effects at the between-population level have found such effects. In the following paragraphs, we highlight the most recent examples of mtDNA-induced fitness effects.

One study [25] comprehensively sequenced mitochondrial and nuclear genes associated with the cytochrome *c* oxidase (complex IV) of the OXPHOS system and then conducted several bioenergetic and fitness assays in *D. simulans*. The results indicated that mitochondrial genetic variation was linked to cytochrome *c* oxidase activity (COX, an enzyme that is partially encoded by mtDNA and essential for cellular respiration), egg size and fecundity. Other

Box 3. Mitochondrial effects – causal or correlated?

Studies that seek to demonstrate the fitness consequences of mitochondrial genetic variation are complicated by the inherent difficulty of disentangling the effects of mitochondrial genes from those of other genomes. At the very least, it is essential to control for the nuclear background. One simple way to screen for potential mitochondrial genetic effects is to conduct reciprocal crosses between two populations. The resulting daughters of each cross will then share an identical set of possible nuclear backgrounds but will have mtDNA that is derived from the maternal parental genotype and, thus, differences between the two crosses could be indicative of mtDNA fitness effects (see, for example, Ref. [33]). However, a problem associated with this method is the difficulty in separating mitochondrial-linked from direct maternal effects; also, the daughters might be differentially imprinted. Alternatively, techniques of forced chromosome replacement, using balancer chromosomes that are available in *D. melanogaster*, also enable mitochondrial haplotypes to be placed alongside specific chromosomes or nuclear backgrounds, with which mito-nuclear interactions can then be tested via line crosses [13,15,28].

One useful technique that can largely negate the confounding effects of nuclear genes is that of introgressive backcrossing, whereby mitochondrial haplotypes are introgressed into controlled or randomized nuclear backgrounds over successive generations [14,16,17]. However, it must be noted that even the most rigorous backcrossing programs might fail to break up some of the most tightly co-evolved mito-nuclear gene complexes [17]. It is even more complicated to eliminate the confounding effects of other cytoplasmic genetic elements because these are also maternally inherited and will co-segregate with mtDNA. A range of cytoplasmically transmitted bacteria, such as *Wolbachia* and *Rickettsia*, infect insects, other arthropods and nematodes and have known fitness effects [79]. Moreover, the prevalence of *Wolbachia* infection across common laboratory model organisms, such as *Drosophila*, is very high [79]. Diagnostic methods are available to detect some of these bacteria, and they can be eliminated through the antibiotic treatment of the stock populations in focus. Less is known about the prevalence and fitness effects of other cellular endosymbionts, such as protists and viruses, which might have biased maternal transmission with potential fitness effects, although the effects of certain such viruses seem to be minimal (see, for example, Ref. [80]). We suggest that as long as careful precaution is taken to eliminate the presence of intracellular bacteria, then in cases where mitochondrial genetic variation is first established, the best explanation available for subsequent associations between cytoplasmic genetic variation and fitness is that a causal effect of mtDNA variation exists.

recent work has identified a range of mitochondrial bioenergetic differences across two distinct mtDNA haplotypes in *D. simulans* [31,32].

Recently, mitochondrial genetic effects on lifespan and patterns of ageing were found in lines of *D. elanogaster* harbouring either a *D. simulans* or a *D. melanogaster* mtDNA haplotype [33]. Specifically, mtDNA–nuclear epistasis made a significant contribution to lifespan, and the magnitude of the epistatic effects increased with the degree of mtDNA divergence between populations or species being crossed (i.e. it increased from intrapopulation, to interpopulation, to interspecies crosses). This pattern of an increasing effect size with increasing genetic dissimilarity is mildly consistent with a scenario of mito-nuclear co-adaptation. It is usually predicted that in this scenario, co-evolution will occur between the mitochondrial and nuclear genomes within a given population, such that if these coevolved mito-nuclear gene complexes are disrupted by hybridization between populations, then some sort of functional breakdown should result [27]. However, the direction of effects in the study reported here did not follow the typical pattern of breakdown upon the disruption of the co-adapted mito-nuclear complexes. Consequently, the authors suggested that the prediction of functional breakdown is oversimplified and that cellular states or patterns of gene expression upon the disruption of co-adapted gene complexes might be just as likely to result in increased lifespans [33].

Despite this suggestion, three recent examples (albeit two at the between-species level) have shown functional breakdown upon the disruption of mito-nuclear complexes, with effects that can be attributed to the mitochondrial genome. First, in the copepod *T. californicus*, the low fitness of F_3 hybrids that result from population crosses is completely restored by backcrossing to the maternal, but not paternal, populations (i.e. by restoring the original mitochondrial genetic background) [34]. Second, the hybridization of the wasps *Nasonia giraulti* and *Nasonia vitripennis* results in increased mortality that is attributable to mito-nuclear genetic incompatibilities [35]. Third, in reciprocal crosses between species of freshwater fish that belong to the Centrarchidae, the maternal parent exhibiting faster mitochondrial evolution tends to produce less-viable F_1 offspring. This result is consistent with the prediction that the probability of mito-nuclear incompatibilities occurring upon hybridization will increase in cases where the maternal species has a faster rate of mtDNA evolution [36].

Co-adaptation to the local environment?

Several authors have advocated the idea that mtDNA, and mito-nuclear gene complexes, might evolve adaptively to selection imposed from the prevailing thermal environment [5,37,38]. The logic here is that mtDNA encodes multiple subunits in four of the five respiratory enzyme complexes [3,24] and that enzymatic processes are temperature sensitive. Thus, adaptation to a novel thermal environment might result in selection for gene products with different optimized thermal properties [5]. This concept is supported by several studies that demonstrate thermal selection on joint mito-nuclear genotypes. For example, some studies have observed temperature effects on mitochondrial function (see, for example, Ref. [39]) and experiments in which mtDNA heteroplasmy (i.e. multiple mtDNA variants) has been induced in *Drosophila* have revealed temperature-dependent biases in the transmission of certain mtDNA variants [40]. In *D. simulans*, there are three main mtDNA haplotypes (*si*I–*si*III). In this species, it seems that mtDNA variation might be linked to the environment; flies that harbour the *si*II haplotype recover faster from cold coma than flies with the *si*III haplotype, and flies that harbour *si*III are more resistant to starvation than flies with *si*II [25]. Temperature has dramatic effects on both COX and the nuclear-encoded cytochrome *c* (CYC) activity [41], and on epistatic interactions between CYC alleles and mtDNA variation [42] in *T. californicus*. In the seed beetle *Callosobruchus maculatus*, certain mitochondrial lineages result in faster egg-to-adult development when expressed in particular nuclear backgrounds, with the outcomes of these mito-nuclear interactions dependent on the temperature in which the beetles are reared [16].

The obvious question, then, is whether mitochondrial genes can generally respond adaptively to this selection. Here, the evidence is more contentious. Although some authors have linked the adaptive evolution of human mtDNA to different climates [10,43], this idea has been heavily criticized by others who suggest that the observed patterns are best explained by random drift combined with purifying selection (see, for example, Refs [44,45]). The accurate identification of the historical processes that were responsible for shaping the genetic variation that we see today is a pursuit fraught with problems. We believe that one fruitful avenue in resolving the above contentions will be to focus on the future, rather than the past, by exploring whether mtDNA can, in fact, respond adaptively to thermal selection, by using protocols of laboratory-based experimental evolution in model species, such as *D. melanogaster* or *T. californicus*.

Of course, temperature is not the only extrinsic factor that could affect the dynamics of mito-nuclear co-evolution. Other abiotic drivers of such co-evolution might include salinity (via effects on the osmotic properties of the mitochondrial membrane), sulphide concentration (sulphide is a pollutant that might inhibit COX activity given that sulphide oxidation takes place within mitochondria) and oxygen concentration (which might affect the rate of metabolism).

Within-population effects

Another controversial question in the field is whether mitochondrial genetic variation for fitness might exist at the within-population level. Over two decades, theoreticians have evaluated whether mtDNA polymorphism could be maintained via mito-nuclear interactions on fitness [46–49]. Earlier studies indicated that the conditions that are necessary to maintain a polymorphism in mtDNA were very restricted, and the initial empirical studies [15,28] supported these conclusions. However, these studies focused exclusively on mito-nuclear interactions involving autosomal loci. Recently, this question was re-evaluated [13], motivated by the idea that the dynamics of mito-

nuclear fitness interactions might differ if the nuclear genes involved in the interactions are located on the sex chromosomes, which have a different pattern of inheritance (Box 4). Accordingly, new theoretical models confirmed the potential for multiple mtDNA haplotypes to be maintained through mito–X chromosome interactions within a population, with empirical evidence verifying the presence of sexually antagonistic mito–X chromosome interactions for juvenile fitness (offspring viability) in *D. melanogaster* [13] (Box 4). That is, particular genetic combinations of mtDNA haplotypes and X chromosomes often resulted in high juvenile fitness when expressed in females but low juvenile fitness when expressed in males, and vice versa. The sexual antagonism that existed between these particular genetic combinations seems to be tightly linked with the fact that the nuclear polymorphism involved in these mito-nuclear interactions was located on the X chromosome, a known hot spot for sexually antagonistic fitness variation [50].

The previous study [13] identified mito-nuclear interactions on juvenile fitness. This is striking, considering that adult fitness accounts for much more of the nuclear genetic variance in total fitness than juvenile fitness does in *D. melanogaster* and that virtually all sexual intralocus conflict is found at the adult, and not juvenile, stage [51]. Indeed, it would indicate that mito-nuclear interactions might well be considerably more ubiquitous and sizeable than has been previously considered. To assess this idea, a recent study screened for cyto-nuclear interactions on adult female fitness within a separate population of *D. melanogaster* [14]. Sizeable cyto-nuclear fitness interactions were detected that accounted for at least 5% of the observed phenotypic variance in adult female fitness. Having eliminated the potential for cytoplasmically inherited bacteria, such as *Wolbachia*, to confound the results, the authors argued that these cytoplasmic genetic effects probably reflected mitochondrial genetic variation [14]. Additive cytoplasmic genetic effects (again, presumably mtDNA in origin) were detected for female lifespan and patterns of ageing within the same population of flies [52].

How much variation is enough?

An important question that stems from the preceding section is 'How much sequence variation in mtDNA is necessary to produce a phenotypic effect?' Although some of the model species previously discussed exhibit substantial mtDNA polymorphism across populations (e.g. more than 18% divergence in the COXI gene in *T. californicus* [27]), pronounced mtDNA-induced fitness effects have also been found in *D. simulans*, in which there is only 3% divergence between populations [17,25,31,32].

D. melanogaster typically has been considered a poor model with which to address the fitness effects of mtDNA because it has much lower levels of mtDNA variation than, for example, the other species mentioned previously. However, as described previously, evidence squarely points to there being adequate mitochondrial genetic variation within a single population of *D. melanogaster* to fuel mito-nuclear fitness interactions [13,14]. Whether slight variations in mtDNA sequence can commonly result in striking alterations to the phenotype remains to be seen through further experimentation. We do note, however, that a timely study on *T. californicus* has demonstrated effects of single amino acid substitutions in the CYC protein on the functional interaction with another OXPHOS protein, COX, which is partly encoded by

Box 4. Sex chromosomes and opportunity for mito-nuclear co-evolution

For more than two decades, theory reinforced by empirical evaluation indicated that selection on the joint mito-nuclear genotype was unlikely to contribute to the maintenance of genetic polymorphism in mtDNA. These studies were all based on the scenario that the nuclear genes that were involved in any mito-nuclear fitness interactions were located on the autosomes. However, because the transmission patterns of the sex chromosomes differ to those of the autosomes, the patterns of co-transmission between the sex chromosomes and mitochondrial genomes will also invariably differ. In species in which females are the homogametic sex, X chromosomes will co-segregate with the cytoplasm in two-thirds of cases, compared to only half of cases for the autosomes (Figure Ia). Furthermore, X chromosomes spend two-thirds of their time in females, which makes them a hot spot for sexually antagonistic fitness variation [50]. This altered rate of co-transmission between the X chromosome and the mitochondrial genome, in relation to the autosomes and mitochondrial genome, recently motivated a re-evaluation of whether mito-nuclear fitness interactions could uphold mtDNA polymorphism within populations [13]. Theoretical and empirical analyses indicated that such a polymorphism could indeed be maintained when the nuclear genes involved in the mito-nuclear interaction were X-linked. In support of the idea that mito–X chromosome interactions are important in OXPHOS function, we note that although most nuclear-encoded mutations that cause mitochondrial OXPHOS dysfunction in humans are autosomal, some X-linked mutations have, indeed, been identified [81].

Apart from this breakthrough, the role of the sex chromosomes in mito-nuclear co-evolution remains largely unexplored territory. For instance, it will be worth assessing whether the potential for dynamic mito-nuclear co-evolution is increased in species that harbour larger X chromosomes. Additionally, one might contemplate the likelihood for mito-nuclear co-evolution in taxa that exhibit the common variant to the XY system – the ZW system – that is found in birds, lepidopterans, some lizards and fish. In these species, the heterogametic sex is reversed, with females carrying one copy of the Z and W chromosomes and males carrying two copies of the Z chromosome. In these cases, the Z chromosome is co-transmitted in only one-third of cases alongside the mitochondrial genome, whereas the W chromosome is invariably co-transmitted with the mitochondrial genome in all cases (Figure Ib). The significance of these altered rates of mitochondrial-sex chromosome co-transmission for mito-nuclear co-evolution is unclear, but we can envisage Hill-Robertson effects having a negative impact on the potential for mito-nuclear co-evolution here because of the perfect linkage between mitochondrial and W-linked genes, which will reduce the efficacy of selection on both mtDNA and the W chromosome.

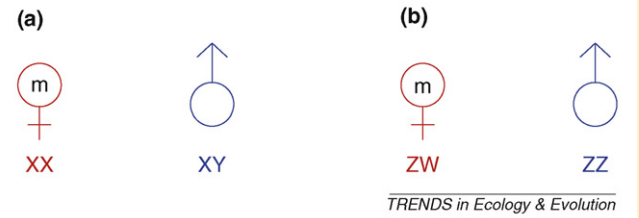

Figure I. **(a)** In an XY sex-determining system, mtDNA (m) is co-transmitted to the next generation with the X chromosome in two out of three cases and never co-transmitted with the Y chromosome. **(b)** In a ZW system, mtDNA is always transmitted with the W chromosome and is co-transmitted with the Z chromosome in one out of three cases.

mtDNA. Remarkably, in that study, interpopulation hybrid breakdown in COX activity could be attributed to a single naturally occurring amino acid substitution in CYC [53]. It seems entirely reasonable to expect the same sorts of effects on the interaction between CYC and COX via single amino acid substitutions encoded by the mitochondrially-encoded COX subunits.

Finally, we point out that genetic variation within the protein-coding regions of the mitochondrial genome is certainly no prerequisite for the genome to have a profound impact on fitness variation. Variation in transcriptional promoter sites in mtDNA could well correspond with variation in the levels of gene expression, with subsequent fitness effects. Furthermore, the RNA polymerase that is responsible for the transcription of mtDNA is encoded by nuclear DNA, as are some of the transcription factors involved. Thus, the transcription of mtDNA entails a mito-nuclear interaction [27].

General implications

The research that we have highlighted evokes broad and exciting consequences for several prominent evolutionary theories. We have presented evidence that mito-nuclear interactions can make a sizeable contribution to individual fitness and that such interactions are likely to be common across taxa, perhaps even at a within-population level. We now outline some of the potential consequences of these interactions.

Speciation

There is good reason to believe that the accumulation of non-neutral mitochondrial genetic variation within populations might play a role in speciation. As we have detailed previously, hybrid breakdown can sometimes be directly attributed to the disruption of population-specific, co-adapted mito-nuclear gene complexes. Such hybrids are at a selective disadvantage, and selection for reproductive isolation based on negative mito-nuclear interactions might, thus, drive the populations towards speciation. Despite the above findings, the contribution of mito-nuclear genetic incompatibilities to speciation theory has received scant attention among animal biologists (however, see Ref. [54] for a discussion of this idea in plants). Meanwhile, researchers in the fields of phylogeography and DNA barcoding continue to provide numerous examples of large sequence divergence in mtDNA between populations. We suggest that the mtDNA polymorphism that accumulates between populations might ultimately act as an 'engine of speciation' by driving differentiation in associated parts of the nuclear genome. Although we acknowledge the speculative nature of these suggestions, we think it is worth noting that phylogeographic breaks in mtDNA could, theoretically, originate intrinsically (i.e. in the absence of a geographic barrier to gene flow) [55] and, thus, the possibility exists that mito-nuclear incompatibilities might even initiate episodes of sympatric speciation.

Life history

Life-history theory centres on the assumption that trade-offs exist between fitness-related traits. These trade-offs traditionally have been explained in the context of resource limitation, with energy as the typical currency. This neglects the fact that the proximate mechanisms underlying such trade-offs are, in most cases, unknown [56]. Notably, variation in metabolic rates might well be a general mediator in life-history evolution [57]. In addition, a mounting body of evidence indicates a role for reactive oxygen species (ROS) in the evolution of key life-history traits such as the cost of reproduction (see, for example, Ref. [58]) and ageing [59]. Given the strong conceptual underpinnings of the mitochondria in metabolism and ROS production, there seems to be ample opportunity for mtDNA variation to play a non-trivial role in shaping life-history evolution.

Ageing

The evolution of ageing is incompletely understood. The mitochondrial theory of ageing [59] posits that mitochondria produce more ROS as they age. This promotes a vicious cycle of ROS production and subsequent damage to mtDNA, which causes senescence. Although well developed, the empirical data to support this concept is contentious (see, for example, Refs [60,61]). In *Drosophila*, the available evidence indicates that mtDNA does have a profound role in the ageing process [17,33,52]. Although the proximate mechanisms that underlie these effects remain unresolved and controversial, if such patterns hold true across taxa, then the mitochondria might have a profound part to play in furthering our understanding of the ageing process.

Sperm competition

The strict maternal transmission of mtDNA could enable mtDNA mutations to accumulate that are harmful when expressed in males but neutral in females because such mutations will remain hidden from selection [62]. Sperm seem to be ideal candidates to accumulate these sorts of mutations. First, sperm motility is purportedly achieved using energy produced from the mitochondria in the sperm midpiece or tail (see examples in Refs [63,64]). Second, the production of vast numbers of high-quality sperm is energetically very demanding (see examples in Refs [63,64]) and sensitive to ROS production [65], and both of these factors are likely to be dependent on adequate mitochondrial (and mtDNA) function.

Accordingly, certain mtDNA deletions or haplotypes in humans have been linked to reduced sperm performance (see, for example, Ref. [43]), and sperm traits in birds also have been associated with mitochondrial genetic variation [66,67]. These correlations were backed up recently by an experimental study that utilized introgressive backcrossing between seed beetle *C. maculatus* populations to detect cytoplasmic genetic effects (which were presumably mitochondrial in origin) and cyto-nuclear effects on sperm viability and sperm length [64].

Several authors have suggested that this sort of mitochondrial genetic variation for sperm quality might have profound implications for sperm competition theory [68,69]. Counter to this suggestion, the only tests of this idea to date were unable to detect any cytoplasmic genetic variation on the outcomes of sperm competition [63,70]. However, further testing is certainly warranted in other

species to establish whether mtDNA effects on sperm function could ever be large enough to translate into meaningful effects on overall male fitness *per se*.

Given the potential for male-expression-specific deleterious mtDNA mutations to accumulate through genetic drift and for mtDNA mutations with sexually antagonistic effects to be fixed by positive selection in females, there should be intense selection on compensatory mutations in nuclear genes to restore any compromised function to male fertility traits. Support for this idea comes from studies that show the existence of testes-specific isoforms of both the nuclear-encoded COX VIb subunit [71] and the cytochrome *c* subunit [72] in mammals. Such isoforms might have evolved to accommodate the high energy production that is required for high-quality sperm production and to alleviate ailing function resulting from mutations in the mitochondrially encoded subunits of the respiratory enzyme complexes. Convincing evidence for the presence of such compensatory nuclear genes would be provided by examples in which the disruption of coevolved mito-nuclear gene complexes leads to depressed sperm function or depressed male fertility. Currently, no such examples exist.

Lastly, the doubly uniparental inheritance of bivalve mtDNA offers an ideal opportunity to examine the adaptive evolution of mtDNA in the context of sperm competition. In these systems, male-transmitted mtDNA is expressed primarily within the testes and, thus, can respond to selection pressures that are imposed via sperm competition. An interesting twist to these systems is that mtDNA that is characterized by a female-type coding sequence has occasionally invaded the male route of inheritance and, thus, has been transmitted as a new male type (masculinised mtDNA) (reviewed in Ref. [73]). Accordingly, two studies have now examined whether sperm motility differs between sperm carrying the standard male type (which has had many generations to adapt to selection that is imposed via sperm competition) and the recently masculinised mtDNA (which has had far fewer generations to adapt). Counter to expectation, one study found no difference in motility across the two mtDNA types (reviewed in Ref. [73]), and the other found that the masculinised mtDNA was associated with faster sperm [74]. Despite these odd returns, it certainly will be worthwhile to test the performance of other sperm traits (e.g. viability) and the outcomes of sperm competition *per se* across these two mtDNA types, to shed more light on the scope for the adaptive evolution of mtDNA.

General evolutionary dynamics and the maintenance of genetic variation

The role of mito-nuclear fitness interactions on the general dynamics of adaptive evolution deserves further attention. Previously, we reviewed evidence that indicates that epistatic interactions between mitochondrial and nuclear genes, within populations, can account for a significant amount of variation in fitness [13,14] and could be ubiquitous. How genetic variation can be maintained within populations in the face of directional selection represents a general problem in evolutionary biology [75]. The existence of mito-nuclear fitness interactions might contribute to the maintenance of this genetic variation. Furthermore, recent experimental studies have detected mtDNA–nuclear-DNA–environment interactions for fitness [14,16], which indicates that the fitness of any given mito-nuclear genotype is contingent on the environment. Spatial environmental heterogeneity is one factor that can contribute to the maintenance of genetic polymorphism [76]. Whether environmental heterogeneity might have a role in upholding variation in the mitochondrial genome within populations, via environmental selection on the mito-nuclear interaction, deserves theoretical attention.

Finally, we suggest that the opportunity for mito-nuclear co-evolution might be greater in taxa other than arthropods, which harbour a high prevalence of maternally-inherited cytoplasmic microorganisms, such as *Wolbachia*. Because such microorganisms are maternally inherited, there is perfect linkage between mtDNA and the microorganism. Thus, for example, when a new strain of *Wolbachia* invades a previously uninfected population, the mitochondrial genome of the founding female (which carries the *Wolbachia* infection) will probably sweep to fixation along with the *Wolbachia*. Consequently, taxa with such infections are likely to have very reduced mtDNA polymorphism, thereby constraining the potential for mito-nuclear co-evolution. In addition to this, such linkage between the mitochondrial genome and the *Wolbachia* will induce stronger Hill-Robertson effects, thereby further reducing the efficacy of selection on mtDNA.

Concluding remarks

This review highlights the expanding body of research that promotes an important role of mitochondrial genetic variation in determining fitness, with an emphasis on the mito-nuclear interaction. Its goal is to encourage evolutionary ecologists to think about mitochondria from a new angle, rather than as the passive evolutionary players that we once considered them, in the hope that an increasing number of researchers will be inspired to focus their attention on the evolutionary dynamics of these organelles.

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