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Evolutionary codependency: insights into the mitonuclear interaction landscape from experimental and wild Caenorhabditis nematodes

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Aided by new technologies, the upsurgence of research into mitochondrial genome biology during the past 15 years suggests that we have misunderstood, and perhaps dramatically underestimated, the ongoing biological and evolutionary significance of our long-time symbiotic partner. While we have begun to scratch the surface of several topics, many questions regarding the nature of mutation and selection in the mitochondrial genome, and the nature of its relationship to the nuclear genome, remain unanswered. Although best known for their contributions to studies of developmental and aging biology, Caenorhabditis nematodes are increasingly recognized as excellent model systems to advance understanding in these areas. We review recent discoveries with relevance to mitonuclear coevolution and conflict and offer several fertile areas for future work.

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Introduction

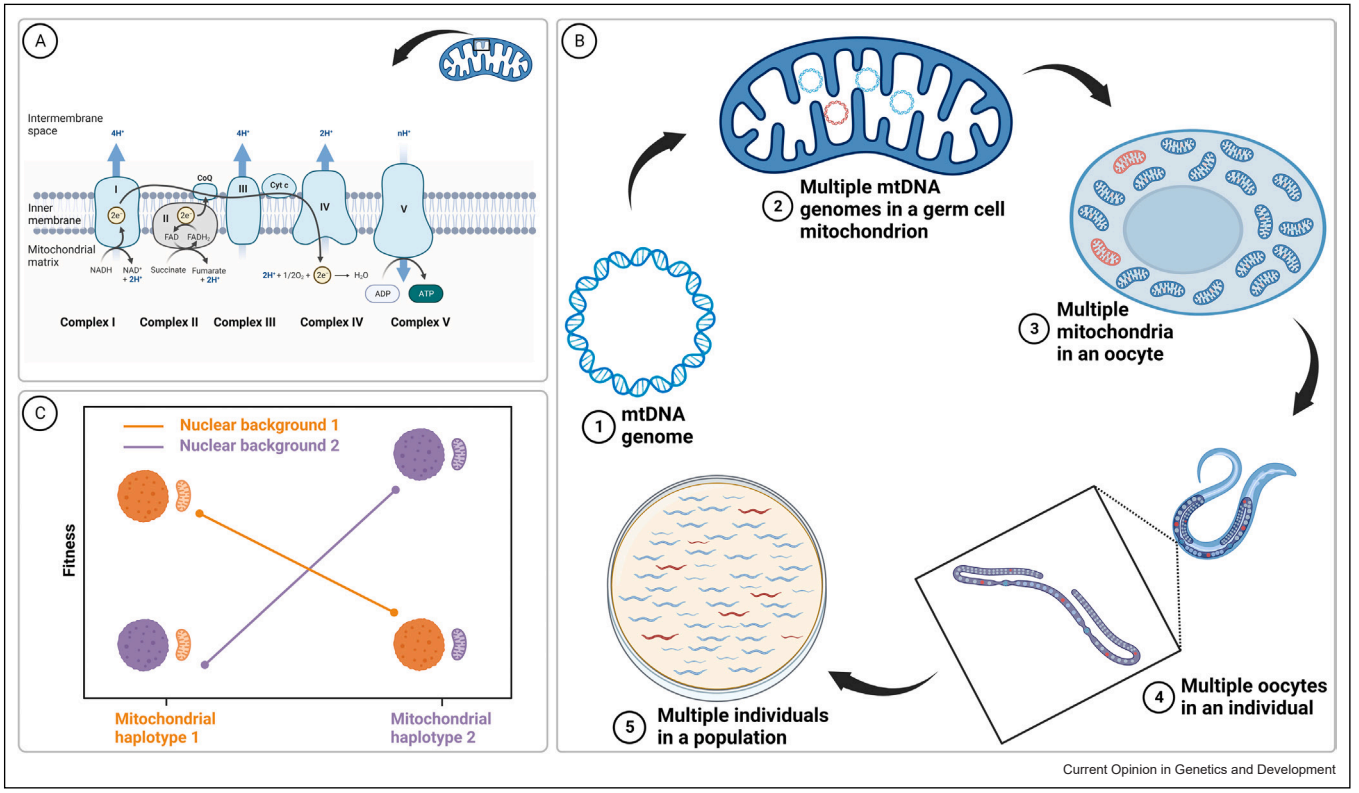
A historically consequential mutualism

In eukaryotes, mitochondrial function depends on the coordinated expression of thousands of nuclear genes and relatively few genes located within the mitochondrial genome (mtDNA), which have been coevolving for approximately 1.5 billion years. During this time, natural selection for functional mitochondria has resulted in an exquisite example of intergenomic coadaptation, with molecular machinery dual-encoded by mtDNA and nuclear DNA (nDNA) genomes being necessary to maintain metabolic function [7]. Despite their diminished genomes, the central and indispensable role of mitochondria in oxidative phosphorylation (OXPHOS) and ATP production (Figure 1a) influences numerous physiological and behavioral traits. Understanding the basic biology of mitochondria and mitonuclear interactions, which play out in several molecular arenas [7,8], is therefore of major importance for numerous questions in evolutionary biology and medicine. MtDNA variation has been implicated in fundamental evolutionary processes from speciation and the evolution of sex, to adaptation and lifespan [9–18]. Furthermore, with their high mutation rate and apparent lack of recombination, animal mtDNA genomes in particular should be vulnerable to deleterious mutation accumulation and Muller's ratchet [19]. However, many questions regarding the mitigation of deleterious mutations in the mtDNA and the evolutionary fate of mitochondria remain open. Are mitochondria 'ticking time bombs' destined for extinction due to genetic erosion, or can a combination of intracellular quality control, compensatory or beneficial mutations, and selection against deleterious mutations arrest mtDNA's slide into obsolescence [20,21]?

The promise of Caenorhabditis for understanding mitonuclear relationships

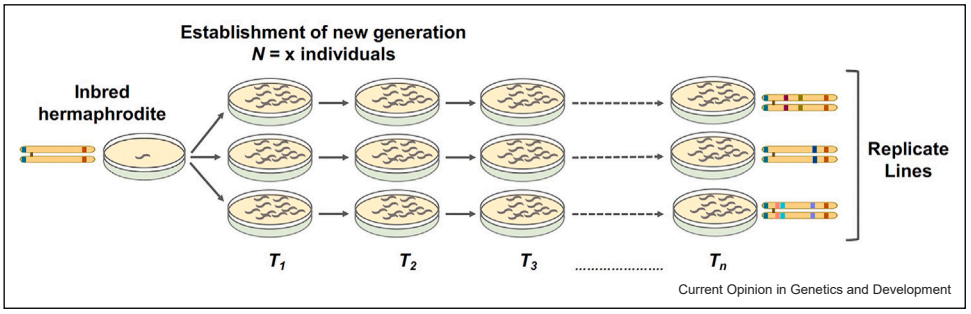
Discoveries in *Caenorhabditis* nematodes, particularly *C. elegans* and *C. briggsae*, have already substantially contributed to our understanding of the evolution of animal mitochondrial genomes and, more recently, the evolutionary patterns, phenotypic consequences, and genomic signatures of mitonuclear coevolution. We point the reader to [22–24] for overviews of their advantages for these and other studies. These advantages include their amenability to experimental evolution (Figure 2) and high-throughput technologies, *in vivo* mitochondrial morphology and physiology studies, RNAi by feeding, and treatment with mitotoxicants; the availability of vast libraries of mutant strains, wild isolates, and recombinant lines; and the ability to genetically manipulate mating systems. Below, we provide the necessary background and discuss several areas of recent progress and possible future work.

Figure 1



Mitochondrial MRC, levels of organization, and interaction with nuclei. **(a)** MRC protein complexes I, III, IV, and V (blue) are all dual-encoded, containing both mtDNA- and nDNA-encoded subunits, while complex II (gray) is entirely nDNA-encoded. In reality, *C. elegans* MRC protein complexes exist and function within supercomplexes [1] believed to increase MRC efficiency and lessen reactive oxygen species production [2]. **(b)** Levels of mtDNA evolution. The hierarchical biological levels of organization are depicted from 1) single mtDNA molecules, to 2) mtDNAs within germ cells, 3) mitochondria within oocytes, 4) germ cells within individuals, and 5) laboratory nematode populations. Heteroplasmy — the presence of both wild-type and mutant mtDNA genomes, organelles, germline tissues, or nematode populations (red) — is shown in levels 2–5. Note that *C. elegans* are estimated to contain 3.5 ± 0.12 mtDNA genomes per germ cell mitochondrion and 71.2 ± 6.5 genomes per germ cell [3]. Konrad et al. [4] estimated the mitochondrial effective population size per nematode generation to be approximately 62, exceeding estimates for other eukaryotes and suggesting that mtDNA bottlenecks and associated genetic drift may not be particularly severe in this species. **(c)** Mitonuclear epistasis. Orange and blue organelles and nuclei illustrate the concept of matched and mismatched pairings between mitochondria and nuclei associated with higher and lower fitness, respectively. Negative epistases between mtDNA and nDNA alleles evolving in allopatric populations have been hypothesized to disproportionately underlie Dobzhansky–Muller incompatibilities contributing to incipient speciation [5]. Created with BioRender.com. Redrawn from Ref. [6].

Figure 2



Nematode experimental evolution. Schematic depicting experimental evolution setup with multiple, independently evolving lineages of wild-type or mutant nematode strains. Many replicate isogenic lines can be initiated from the offspring of a single hermaphrodite and maintained across generations by transferring a predetermined number of offspring to fresh media. *T* indicates the generational time point and *N* the number of individual offspring transferred. Transfers of $N = 1$ (or $N = 2$ for obligately outcrossing strains or species) will maximize genetic drift and minimize selection (i.e. a standard MA experiment), while large transfer population sizes will achieve the opposite effect (i.e. a recovery or laboratory adaptation experiment). While experiments can also be initiated with genetically variable populations, we depict a diploid chromosome devoid of genetic variation in the ancestor that independently acquires and fixes different mutations within each replicate line. A cryopreserved ancestor and/or control (not shown) is usually genotyped and phenotyped alongside the evolved lines.
Adapted from Refs. [25,26].

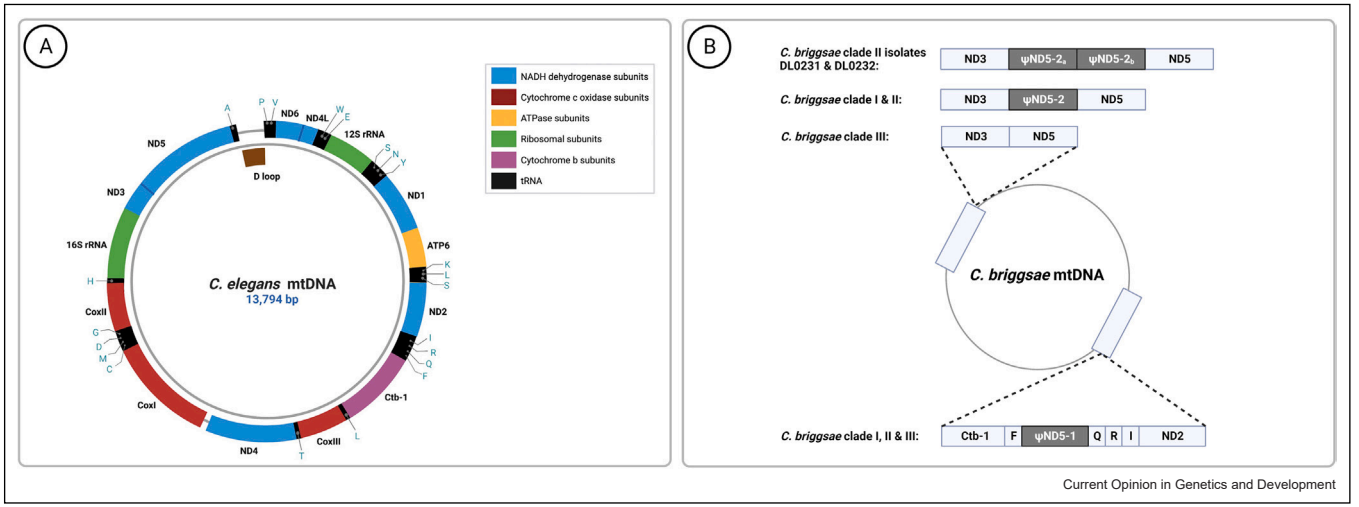
Caenorhabditis mtDNA genomes and mutation processes

C. elegans' mitochondrial genome is fairly typical of animal mitochondria with respect to size (13 794 bp) and gene content [27,28]. It comprises 36 genes encoding 12 subunits of the mitochondrial respiratory chain (MRC), two rRNAs and 22 tRNAs; and two AT-rich noncoding regions (NCRs) (Figure 3a). The absence of OXPHOS gene duplicates, as well as the near-absence of nDNA of mitochondrial origin (NUMTs) [33], simplifies molecular analysis of mtDNA variation. *C. elegans*' mtDNA replication appears to proceed by a rolling-circle mechanism with multiple replication cycles on a single template, which generates multimeric mtDNA chromosomes that are later resolved into monomers [30]. In contrast to what is observed in many metazoans, *C. elegans* mitochondrial genes are all transcribed in the same direction [27].

The spontaneous mutation rate of *C. elegans* mtDNA has been estimated at $1–2 \times 10^{-7}$ mutations/site/generation in two independent mutation-accumulation (MA) experiments (Figure 2) — two orders of magnitude greater than that of the nuclear genome [4,34]. This rate may be underestimated due to within- and among-individual selection in such experiments [35]. Indeed, a recent study by Ref. [33] that applied duplex sequencing to mix-staged *C. elegans* populations — wherein mutations should experience less selection bias than in MA studies — found an order-of-magnitude more low-frequency mtDNA variants than previously reported.

As in many other metazoans, *C. elegans*' mitochondrial genome is highly AT-biased (75%) and the mutation spectrum is characterized by a strong bias toward increased AT content that should push the AT content even higher in the absence of natural selection [4,33]. Waneka et al. [33] found that *de novo* mtDNA mutations in *C. elegans* were characterized by a high proportion of CG→AT transversions compared with other metazoans as well as significant strand asymmetry. C→T and G→T changes were also more pronounced on the forward (coding) strand relative to the template strand, albeit to a lesser extent than observed for other animals [33]. While roles for mtDNA replication- or transcription-associated errors or deficiencies in repair machinery have not been fully ruled out as contributors to *C. elegans* mtDNA mutational spectra, these observations may suggest that oxidative damage is an especially important source of new mtDNA mutations in nematodes. Overall similar average rates and broad patterns of mtDNA mutation may exist for *C. briggsae* [36] and other species [37]. However, with the caveat that several relevant studies suffer from low mutation sample sizes, occasionally extreme among-line or among-isolate/clade differences in mtDNA mutation characteristics have been observed. For example, Wagner et al. [38] discovered that single-nucleotide mtDNA variants were fixed only within *C. briggsae* MA lines containing a *nduo-5* deletion (Figure 3b), indicating a role for genomic context. Perhaps, we should embrace more fully the possibility that no single mtDNA mutation rate, spectrum, or genomic distribution exists — a necessary avenue of future study (Table 1). Variation in aspects of mtDNA genome dynamics has also been observed. We found that normalized mtDNA copy number increased under drift conditions for *C. elegans* [39] but decreased under the same conditions for *C. briggsae* [38]. Understanding the patterns and mechanisms of mtDNA mutation and copy number change is critical as they have the potential to set in motion mitonuclear coevolutionary dynamics with far-reaching consequences for evolution and disease.

Figure 3



C. elegans and C. briggsae mtDNA genomes. **(a)** *C. elegans* mitochondrial genome. The molecule comprises genes for 12 MRC protein subunits (blue = NADH dehydrogenase, red = cytochrome-c oxidase, and violet = ATPase subunits), and two rRNAs (green) and 22 tRNAs (black) [27]. The larger of two NCRs (466 bp) is located between *nduo-5* and *nduo-6* (ND5 and ND6 in the figure), and is presumed to contain a replication origin akin to the D-loop (brown) [27,28]. A smaller NCR, a 109-nt hairpin forming sequence between *nduo-4* and *cox-1*, has been hypothesized to contain a second replication origin [29,30] Compared with human, *C. elegans* mtDNA genomes lack *atp-8*, which encodes an ATP synthase (MRC complex V) subunit. **(b)** Position of pseudogenes in *C. briggsae* mitochondrial genome. The mtDNA genome of congener, *C. briggsae*, is broadly similar to that of *C. elegans* apart from the presence of 1–2 pseudogenes derived from partial duplications of the *nduo-5* gene, in certain wild isolates [31]. Genes are indicated by white rectangles (single-letter abbreviation used for tRNA genes), and gray boxes show pseudogenes. The intraspecific groups in which each arrangement is observed are indicated on the left of each displayed mtDNA region. See Table 1 in Ref. [32] for genome sizes and other details of mtDNA genomes in a variety of Caenorhabditis species. Created with BioRender.com.
Adapted from [77].

Table 1

Relevant topics of future work utilizing *Caenorhabditis.* Abbreviations: experimental evolution (EE), mitochondrial unfolded protein response (UPRmt), mutation accumulation (MA), piwi-interacting RNA (piRNA), reactive oxygen species (ROS), RNA interference (RNAi), whole-genome sequencing (WGS), wildtype (WT).

Topic	Question(s)	Worm-supported approaches	Selected helpful references
mtDNA mutation rate and spectrum	Is there variation in the rate and spectrum of mtDNA mutation? Can mitonuclear interactions affect these metrics? To what extent are mtDNA genomes experiencing ongoing deleterious MA?	Short-term MA experiments with mitonuclear and WT strains coupled with third-generation DNA sequencing and advanced phenotyping; comparisons with mtDNA substitutions obtained from mitogenomics of wild isolates. No current approach avoids bias from within-individual selection of mtDNAs.	[4,33,35,38–40]
mtDNA transmission bias	What factors, including mitonuclear interactions, influence heteroplasmy dynamics - including those of selfish mtDNA elements - within and across generations?	EE of WT and mitonuclear strains manipulating strengths or forms of selection (e.g., by varying population size or selective agent), levels of mitochondrial fission-fusion-autophagy, UPRmt, ROS levels, etc. using relevant mutant alleles, RNAi or drug treatments. Pair with WGS and genomic analysis to evaluate heteroplasmy alongside fitness and phenotypic effects.	[41-47]
mitonuclear communication	How do mtDNA mutations influence genome-wide transcription via retrograde signaling pathways? What are the impacts on phenotype and mtDNA mutational dynamics?	Identify candidate nDNA genes with altered expression profiles in mtDNA mutants compared to WT; inactivate/knock out via RNAi or CRISPR/Cas9; quantify fitness effect and whether inactivation leads to dysregulation of mtDNA mutant copy number.	[41,48-50]
mitonuclear phenotypes	To what extent do mitonuclear genotypes underly life-history traits and tradeoffs, GxE, variation in intracellular phenotypes, male fitness, sex ratios, etc.?	Experimentally test for phenotypic effects of mitonuclear variation in different environments.	[51-56]
	To what extent does mtDNA-driven adaptation or compensatory evolution happen in nature?	Compare genomic outcomes of EE, preferably using *C. briggsae* isolates or reciprocal cybrids adapting to different thermal regimes, to patterns of standing genetic variation; e.g., among wild isolates inhabiting different thermal environments. Additonally, WGS and genome analyses to identify allele frequency changes specific to one direction of interpopulation crosses to identify putative coadapted mitonuclear loci underlying thermal adaptation.	[57-60]
mitonuclear adaptation	To what extent can mtDNA genomes capacitate rapid mitonuclear adaptation?	EE of mitonuclear and WT strains under more realistic conditions than previously tested; e.g., presence of standing genetic variation. Quantify fitness and measure/explore the role of mtDNA copy number plasticity in mitonuclear adaptation.	[61,62]
	What are the structural and functional effects of compensatory or adaptive mitonuclear mutations?	EE of mitonuclear and WT strains. (1) Quantify fitness evolution; genomic analyses to evaluate whether compensatory mutations cluster within the same functional network as deleterious mutation(s) or affect the same protein complex, and to evaluate the roles of: piRNAs, accessory MRC proteins, mtDNA micropeptides or epimutations driven by small RNAs in mitonuclear adaptation. (2) Generate cryo-EM structures of *C. elegans* MRC protein complexes (preferable) or employ homology protein modeling and docking experiments to predict MRC complex functional effects and assess whether compensatory mutations tend to affect physically-interacting protein residues.	[61,63-65]
mitonuclear variation and sex	How do mating system and the presence of Mother's Curse effects influence mitonuclear	EE using mitonuclear and WT strains experiencing different mating systems (e.g.,	[7,13,14,66]

Table 1 (*continued*)

Topic	Question(s)	Worm-supported approaches	Selected helpful references
	adaptation? Is there definitive evidence for the mitonuclear sex hypothesis?	using *xol-1* and *fog-2* mutant backgrounds) and controlling for starting fitness; evaluate evolution of outcrossing in facultatively-outcrossing strains (mitonuclear sex hypothesis), and sex-specific fitness effects of identified mtDNA mutations (Mother's Curse). Because *C. elegans* have an X0 sex chromosome system, follow-on hypotheses regarding the genomics of sexual conflict are out of reach.	
cytonuclear incompatibility and speciation	To what extent do mitonuclear interacting genotypes contribute to incipient speciation?	Genetic crosses between divergent natural isolates within species and among closely-related species to test for contributions of mitonuclear variation to hybrid breakdown.	[15,67-71]

Hierarchies of mtDNA selection and presence of selfish elements

Like other multicellular eukaryotes, Caenorhabditis mtDNA genomes exist in nested hierarchies of populations (Figure 1b). Mutations initially give rise to single-copy mtDNA heteroplasmies, which are subject to competition, natural selection, and genetic drift operating at any level of the hierarchy [72,73]. Selection can also sometimes favor mtDNA variants within cells that are detrimental at the organismal level, creating a conflict between different levels of selection and between the nuclear and mitochondrial genomes [74]. This is exemplified by deletions of the mtDNA nadh-dehydrogenase-5 (*nduo-5*) gene present in certain wild isolates of *C. briggsae* [31]. These deletions conform to the definition of selfish or cheater elements in that they exhibit a strong transmission bias across generations (under drift conditions) despite deleterious impacts on organismal fitness at high individual heteroplasmic frequency [52,75,76]. Interestingly, the deletions are associated with an additional NCR — a pseudogene derived from a partial duplication of *nduo-5* — located directly upstream of *nduo-5* ([31,77]; Figure 3b). This arrangement makes the *C. briggsae* mtDNA more prone to acquiring deletions between homologous sequences in *nduo-5* and the upstream NCR, suggesting that the presence of the NCR is by itself strongly deleterious [31,78]. MtDNA haplotypes in which the NCR sequence was slightly less identical to *nduo-5* due to the presence of base substitutions had lower heteroplasmic deletion frequency, suggesting that the substitutions were compensatory mutations, that is, they reduced the deleterious, deletion-generating ability of the NCR [31,43]. These findings raise the question of why this pseudogene has not been eliminated considering the increased mutational load associated with its presence. It has been hypothesized that NCRs such as this one serve unknown functions with regard to mtDNA replication and gene expression [32]. Finally, because nuclear genetic background appears to have an outsized role in determining mitochondrial physiological traits in *C. briggsae* [52], there is almost certainly a role for mitonuclear interaction in shaping mtDNA deletion levels.

Compensating for a friend turned foe

Discovery of the mechanisms that either promote or prevent proliferation of strongly deleterious mtDNA mutations is important for several reasons. For example, whether and to what extent nDNA mutations arise to suppress deleterious mitochondrial mutations has implications for speciation since such mutations could contribute to Dobzhansky–Muller incompatibilities with mtDNA alleles present in different populations [71] (Figure 1c). Several mechanisms can hypothetically favor the replication and transmission of deleterious mitochondrial genomes, including (i) faster replication of smaller, deletion-bearing mtDNA molecules, (ii) an increase in the number of replication origins, (iii) conflict between transcription and mtDNA replication that favors replication of mutant mtDNA, and (iv) increased replication or survival of mutant mtDNA resulting from underperforming mitochondria [74,79–81]. Recently, great strides have been made in our understanding of cellular pathways in *C. elegans* that result in biased replication of mtDNA deletion mutations (ΔmtDNA) in particular. As exemplified in the preceding section, deleterious ΔmtDNAs occur readily in laboratory populations of *C. elegans* and *C. briggsae* [4,36,38,44,76,78]. So far, studies lend overwhelming support to hypothesis (iv) and a role for mitonuclear interaction, that is, that nuclear-encoded mechanisms compensating for poor mitochondrial performance can, in some instances, increase the percentage of defective mtDNA molecules in cells.

In agreement with findings from other organisms (e.g. [82]), a compensatory increase in mtDNA copy number, necessitating coordination with the nucleus, is commonly observed in *C. elegans* harboring ΔmtDNA [41,44]. This suggests that increases in absolute mtDNA copy number aimed at sustaining mitochondrial OXPHOS

is one mechanism of mutant mtDNA genome proliferation. A second homeostatic mechanism, the mitochondrial unfolded protein response (UPR^{MT}), paradoxically facilitates the proliferation of *uaDf5* [41], a selfish 3.1-kb ΔmtDNA derived from an ethyl methanesulfonate (EMS) mutagenesis screen [83]. Deletion or knockdown of *atfs-1*, a nuclear-encoded transcription factor central to UPR^{MT} induction, was found to reduce *uaDf5* heteroplasmy levels, and suggested that the UPR^{MT} response protects deletion-bearing genomes from removal by mitophagy [41]. UPR^{MT} activation also promotes the accumulation of ΔmtDNA independent of mitophagy by promoting mtDNA replication and mitochondrial biogenesis [84,85]. Additionally, Ref. [46] found that mutations affecting the mitochondrial fission and fusion processes strongly reduce *uaDf5* levels across generations. Still other discoveries utilizing *uaDf5 C. elegans* include that ΔmtDNA proliferation also depends on nutritional status, which is required for mtDNA biogenesis, as well as the stress-response transcription factor FoxO/DAF-16, which suppresses fitness costs associated with *uaDf5* mtDNA independently of mitophagy, fission, or apoptosis [42]. Last, inhibition of nDNA-encoded mtDNA polymerase, POLG, was found to reduce *uaDf5* levels, highlighting the importance of DNA replication in the proliferation of ΔmtDNA [84,85]. Taken together, these findings demonstrate that nDNA-encoded mechanisms that preserve mitochondrial quality can both compensate for the deleterious consequences of ΔmtDNA and contribute to their proliferation — examples of what has been called 'the tragedy of the cytoplasmic commons' where bad behavior, that is, poorly functioning mitochondrial genomes, is rewarded [81].

Intergenomic coadaptation revealed through mitonuclear discord

Evidence of mitonuclear coevolution is often revealed by cases of hybrid breakdown resulting from unfavorable mitonuclear genome combinations (Figure 1c). Mitonuclear mismatch has been discovered in hybridization studies with nematode species, including *C. briggsae*, *C. elegans*, and *C. nouraguensis*, the latter of which exhibits a lethal cytoplasmic–nuclear incompatibility thought to result from mitochondrial dysfunction [68]. In *C. briggsae*, typically far milder mitonuclear incompatibilities have been discovered in hybrids generated from multiple pairs of phylogeographically diverse wild isolates [15,55,69,86,87]. These experiments have also revealed strong mitonuclear interactions involving the X chromosome [69], some of which were temperature-dependent [55]. Interestingly, we found that certain intra- or interclade crosses involving genetically divergent isolates from Japan revealed evidence for symmetrical paternal mitochondrial transmission [87]. Like *C. elegans*, *C. briggsae* is androdioecious and predominantly self-fertilizing, meaning that its nuclear genome exhibits high homozygosity. Together with its abundant mitochondrial genetic variation, phylogeographic population structure [88], and variation in mitochondrial form and function [52,53], this feature will make the species a useful model for future studies of incipient allopatric speciation and environmental adaptation (c.f. [89]).

Similar studies in the less genetically diverse congener, *C. elegans*, have utilized a wild isolate, CB4856 from Hawaii [90], containing a *cox-1* mtDNA variant affecting the MRC complex-IV catalytic core [91]. This gene encodes the main subunit of cytochrome-c oxidase, deficiency of which is causal in numerous inherited human metabolic disorders [92,93] and hybrid incompatibilities [94,95] resulting from breakup of coadapted genes involving this enzyme in other species. Panels of recombinant inbred advanced intercrossed lines from crosses between CB4856 and standard wild-type Bristol N2 have found evidence for widespread associations between mitonuclear genome compatibility and several quantitative traits [64,96,97]. Assays of cybrid strains containing CB4856 mtDNA against a wild-type nDNA background showed that this polymorphism reduced lifespan, motility, spermatogenesis, and fertilization rates, while increasing age-correlated reactive oxygen species content and altering the expression profiles of > 900 piRNAs predicted to affect nDNA genes involved in reproduction, development, and aging [64,65]. This suggests that the *cox-1* polymorphism may have exerted these phenotypic effects through its impact on piRNA biosynthesis, which is known to be modulated by mitochondrial function [98]. A follow-on study by Ref. [99] found evidence for retrograde signaling such that the CB4856 mtDNA genotype significantly regulated expression of several nuclear genes, including a downregulation of *dct-15*, which was strongly associated with the reduced lifespan and other phenotypes. Better understanding how mtDNA variation may translate into quantitative trait variation, particularly through an influence on the nuclear transcriptome, is a ripe area for further research [56].

An unsung heroine of rapid evolution?: direct evidence of mtDNA-driven mitonuclear adaptation

By revealing mitonuclear discord, these hybrid studies have demonstrated wild isolate-specific coevolutionary relationships between the two genomes. This dynamic is thought to be maintained via compensatory coevolution wherein the effects of a deleterious mutation acquired by one genome (or an allele that becomes deleterious in a new environment) will be ameliorated by a secondary mutation acquired by the other genome [7,100]. The nuclear genome was originally hypothesized to be the primary driver of mitonuclear coevolution, that is, the nuclear compensation hypothesis (reviewed in Ref. [8]), in essence tidying up after the ongoing decay believed to be experienced by the mitochondrial genome [19]. Inspired by [101] whose phylogenetic study suggested that adaptive evolution in nDNA-encoded primate MRC

genes had been accelerated to compensate for degradation of their mtDNA counterparts, we conducted the first direct, nonretrospective study of adaptive evolution of the mitochondrial MRC using a laboratory adaptation study (Figure 2) with a nuclear-encoded MRC mutant strain of *C. elegans* [61]. The results were indicative of rapid adaptation that was at least partially driven by fixation of compensatory mitochondrial mutations affecting the same MRC complex (but not physically adjacent residues). In other words, mitonuclear cooperation was partially or fully restored by new mutations fixed in the evolved lines (Figure 1c). The results also highlighted the importance of independent assortment of nuclear and mitochondrial information — that occurs even under self-fertilization — in adaptive evolution. We also found evidence of considerable parallel evolution with two of these mutations discovered in 2–3 independently evolved lines, and hypothesized that they partially ameliorated the reduced complex-I activity caused by the ancestral nuclear mutation — perhaps by improving ubiquinone binding. This study also found that lineages tended to achieve greater fitness gains when they also evolved high male frequency and rates of outcrossing in alignment with evolutionary theory [102,103]. Our follow-on study [66], which utilized a variety of both nuclear- and mitochondrial-encoded MRC mutants experiencing evolution within three different mating systems (selfing, facultatively outcrossing, and outcrossing), provided some additional support, suggesting that mitonuclear mismatch may occasionally favor outcrossing as predicted by mitonuclear sex hypothesis [13].

Although many features of mitochondrial biology suggest that their genomes should be subject to genetic drift and deleterious mutational erosion [104], many others indicate capacity for repair and positive selection (e.g. [20]; reviewed in Ref. [105]). The studies highlighted above demonstrate that mitogenome variation functionally influences quantitative traits and identifies specific mitonuclear interactions with nuclear-genome-wide impacts that contribute to such variation. They also suggest that mitochondrial genomes may hold great capacity for driving rapid adaptation that may in some cases be enhanced by outcrossing.

Concluding remarks

While studies utilizing *Caenorhabditis* nematodes have begun to elucidate patterns, mechanisms, and evolutionary consequences of mitonuclear interrelationships, we contend that they hold immense promise to significantly further our understanding of this remarkable symbiosis. While far from exhaustive, Table 1 provides a listing of many careers' worth of outstanding questions, and ideas for nematode-enabled approaches to their study.

Data Availability

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

Declaration of Competing Interest

The authors declare no conflict of interest.

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- • of special interest
- •• of outstanding interest.

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