Beyond the "selfish mitochondrion" theory of uniparental inheritance

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Abstract

"Selfish" gene theories have offered invaluable insight into eukaryotic genome evolution, but they can also be misleading. The "selfish mitochondrion" hypothesis, developed in the 90s explained uniparental organelle inheritance as a mechanism of conflict resolution, improving cooperation between genetically distinct compartments of the cell. But modern population genetic models provided a more general explanation for uniparental inheritance based on mutational variance redistribution, modulating the efficiency of both purifying and adaptive selection. Nevertheless, "selfish" conflict theories still dominate the literature. While these hypotheses are rich in metaphor and highly intuitive, selective focus on only one type of mitochondrial mutation limits the generality of our understanding and hinders progress in mito-nuclear evolution theory. Recognizing that uniparental inheritance may have evolved – and is maintained across the eukaryotic tree of life – because of its influence on mutational variance and improved selection will only increase the generality of our evolutionary reasoning, retaining "selfish" conflict explanations as a special case of a much broader theory.

Introduction

Mitochondria power cellular metabolism of eukaryotic life.[1] Products of an ancient endosymbiosis, mitochondria retain their own DNA (mtDNA) of bacterial origin, and, in tandem with nuclear genes, their tiny DNA regulates mitochondrial respiration and energy production. Because of their central role in eukaryotic metabolism, deleterious mutational diversity in mitochondrial genes causes devastating disorders in humans[2] and it has equally severe fitness consequences in other eukaryotes.[3] In parallel, mitochondria interact with the external environment; mitochondrial genetic variants perform depending on the prevailing environmental and thermal conditions,[4,5] and mtDNA haplotype diversity is dictated by local climatic conditions.[6-9] We may therefore expect that natural selection should favour the

evolution of eukaryotic traits that influence this mutational diversity in positive ways, maintaining functional respiration and facilitating environmental adaptation.

One of the most puzzling aspects of eukaryotic evolution is their capacity to reproduce sexually, which involves producing haploid sex cells (gametes) that fuse to form diploid zygotes and exchange genetic material through random chromosome assortment and meiotic recombination. Indeed, sex is nearly universal across eukaryotes, and genes inducing various aspects of sex – from gamete fusion to recombination – have been found in all eukaryotic supergroups.[10-12] While evolutionary theorists traditionally approach the problem of eukaryotic sex and its potential evolutionary benefits from the perspective of nuclear genetics,[13,14] the mitochondrial point of view is equally captivating.

The general rule across eukaryotes is that in sexual reproduction mtDNA is inherited uniparentally, usually from the maternal gamete, while paternal mitochondria are excluded. Diverse examples from all branches of the eukaryotic tree of life illustrate this general pattern. In mammals, sperm mitochondria enter the egg at fertilization, but the zygotic ubiquitin-mediated proteolysis and autophagy machinery removes paternal mitochondria shortly after syngamy.[15,16] Basidiomycete yeast *Cryptococcus neoformans*, producing morphologically identical gametes of two mating types (*a* or *α*), eliminates mitochondrial nucleoids of the *α* parent post-fertilization, and then degrades the remaining *α*-mitochondrial structures through autophagy,[17] and there is similar nuclease-dependent mtDNA degradation in slime mould *Physarum polycephalum*.[18] Male fruit flies remove their mitochondria from maturing sperm before fertilization.[19] Fission yeast *Saccharomyces pombe* segregates the two parental mitochondrial types into different meiotic spores formed from the zygote.[20] Using a similar segregational strategy, bivalve mussels retain separate female and male mtDNA populations through selective mitochondrial partitioning into somatic tissues and the germline.[21]

This extraordinary diversity of mechanisms regulating mtDNA inheritance is an evolutionary mystery in its own right. Unlike the molecular machinery of sexual cell fusion, reciprocal recombination and meiosis, the mechanisms of uniparental inheritance are not evolutionarily conserved; variations on a theme of paternal mtDNA exclusion seem to either have evolved multiple times in different eukaryotic groups, or have repeatedly replaced their ancestral versions in different lineages.[22] Because of this turnover of mechanisms, the evolutionary history of mitochondrial inheritance strategies is notoriously difficult to disentangle, and it is

currently impossible to determine if the last eukaryotic common ancestor already had mechanisms of asymmetric mitochondrial transmission. And yet, the apparent evolutionary success of various uniparental inheritance strategies suggests that mitochondrial mixing at fertilization is detrimental and that natural selection favours mechanisms restricting mtDNA mixing and reducing heteroplasmy (the coexistence of divergent haplotypes within the same cytoplasm).

The evolutionary implications of asymmetric mtDNA transmission systems are far-reaching. One theory states that male and female sexes evolved driven by the need to regulate uniparental inheritance,[23] as genes modulating mitochondrial transmission are often associated with sex-determination or mating-type loci that determine gamete compatibility. Basidiomycete fungus *Ustilago maydis,* for instance, regulates its mitochondrial inheritance using genes *lga2* and *rga2* located within its mating type locus.[24] Another pathogenic fungus, *Cryptococus amylolentus*, uses its pheromone-receptor loci, also linked to its mating type, to regulate mitochondrial transmission.[25] If the evolution of mating types really is driven by selection for mitochondrial inheritance, then understanding selection for uniparental transmission will also explain the rare cases where more than two mating types are present.[26-28]

Moreover, biased mtDNA inheritance imposes asymmetric constraints on female and male germline evolution and their sexually dimorphic traits.[29] With uniparental inheritance, genetic linkage (the probability that two alleles will be transmitted together) between mitochondrial genes and female-specific nuclear loci is much stronger relative to male-specific loci that experience a new mitochondrial background every sexual generation. Theoretically, there is therefore a possibility that some mitochondrial variants will be influencing male and female fitness differently through their interactions with sex-specific traits.[30-33] Sexually antagonistic mitochondrial fitness effects have been observed experimentally, with some mtDNA mutations having detrimental effects specific to males[34-36] and contributing to sex-specific expression of mitochondrial disorders.[32,37] Similar naturally occurring mutations may have strong implications for the evolution of sexual dimorphism and sex ratios in natural populations. Relaxed selection for uniparental inheritance, resulting in paternal mtDNA leakage, would reduce the severity of these sexually antagonistic fitness effects.

Altogether, our understanding of mating type evolution, sexual dimorphism, sexual antagonism, and mitochondrial disease dynamics across sexes rests on our capacity to explain selective pressures that favour uniparental transmission strategies. When should eukaryotic populations evolve strict and stable uniparental inheritance? When should we expect eukaryotes to tolerate some paternal input or even fully symmetric biparental transmission?

These questions fall within the realm of theoretical evolutionary population genetics, and multiple hypotheses have been developed over the years. While recent mathematical advances established clear links between mitochondrial transmission strategies, genetic variance and selection strength, and offered a well-supported theoretical explanation, the literature is overwhelmingly dominated by so-called "selfish mitochondrion" theories. In the following sections I will briefly review the state of the art in mitochondrial inheritance theory, and I will discuss possible reasons for the apparent supremacy of "selfish" explanations. I will then place these hypotheses within the broader context as one special case of a much more general theory based on redistribution of mutational diversity, modulating the strength of selection across levels of hierarchical organisation.

"Selfish mitochondrion" hypotheses

The first evolutionary explanation of uniparental inheritance was the "selfish mitochondrion" hypothesis, formally developed in the 90s, although verbal arguments date back to late 70s.[38,39] According to the hypothesis, selfish competition for resources of the host cell creates a conflict of interest between genetically distinct compartments of the cell.[39-41] This conflict can manifest as increased replication rates of one mitochondrial variant, active elimination of the alternative type, or through competition for transmission into the germline.[40] In all cases, with biparental cytoplasmic inheritance from both mating partners, the "selfish" organelle can spread through the population as a sexually transmitted parasite (Fig. 1a), impairing metabolic activity of the host (Fig. 1b). Uniparental inheritance will then evolve to curtail their selfish spread and to promote cooperation among genetically distinct compartments of the eukaryotic cell.

Hastings[42] developed a formal mathematical model of this type of evolutionary conflict. He modelled the invasion of a selfish mitochondrion that replicates faster but makes a smaller contribution to the cell's energetic budget relative to the wild-type organelle. In populations without sexes or mating types, he found that selfish organelles could easily spread through

the population, in particular when sex was frequent, reducing mean population fitness. The nuclear allele *U* restricting mitochondrial transmission from one of the fusing gametes then reduced the spread of non-cooperative organelles, even when the uniparental inheritance was costly. *U* itself invaded the population to moderate frequencies, resulting in some – but never all – gamete unions being uniparental. Hurst and Hamilton[43] modelled the spread of the "destroyer" organelle capable of eliminating its more cooperative competitor. They found that a nuclear "suppressor" gene inducing uniparental inheritance can evolve to supress the destructive spread of the parasitic organelle, alongside gamete self-incompatibility system akin to sexes or protist mating types. Along the similar lines of thinking, Law and Hutson[23,44] considered proliferation of an intracellular symbiont, again reducing host cell fitness, and found that selection can then favour nuclear alleles eliminating male cytoplasmic elements.
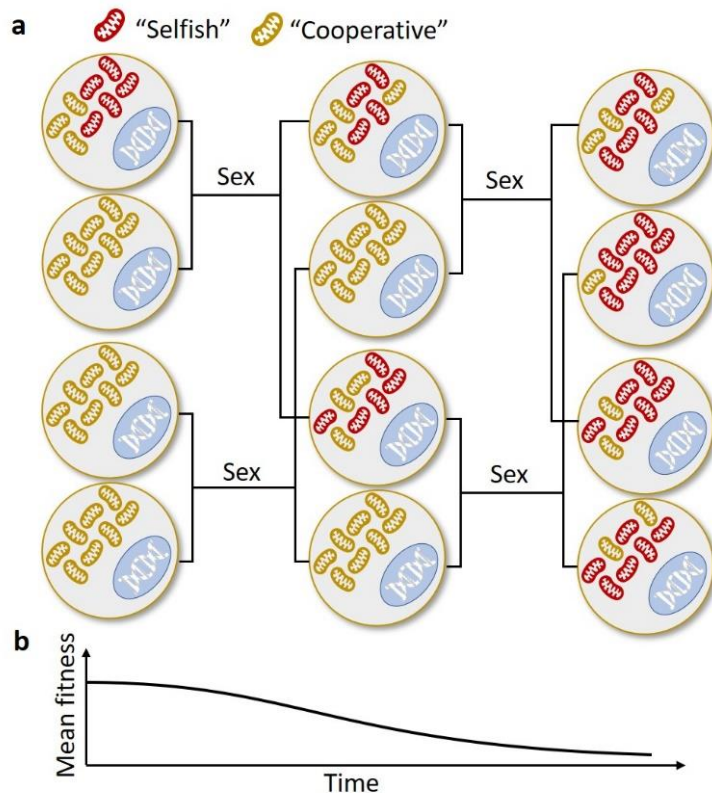
FIGURE 1. "Selfish" mitochondrial proliferation with biparental cytoplasmic inheritance. Initially, "selfish" mitochondrial variants (red) are present in only a small number of individuals (a). As gametes fuse to form zygotes, "selfish" mitochondria spread at the expense of wild-type cooperative organelles (yellow). With biparental inheritance, both sexually produced daughter cells will contain the parasitic organelle, which can spread through an entire population in subsequent rounds of sexual reproduction. Because "selfish" mitochondria impair cellular metabolism, the mean fitness of the population decreases as the parasitic variant spreads (b).

The "selfish" mitochondrion theories rose to prominence when the focus of evolutionary theorists was rapidly shifting away from viewing organisms as well-integrated units of selection, and towards the view that individual genes represent the most consequential units of evolution.[45,46] According to this "gene's eye view" of evolution,[47,48] the mitochondrial and nuclear genomes have been involved in an evolutionary arms race, wherein fitness of one genome can sometimes be maximized only at the expense of the other.

Conceptually, however, the models developed by Hastings, Hamilton, Hurst, Hudson, and others lie closer to the multilevel selection framework of evolution that partitions the overall evolutionary change into discrete levels of hierarchical and temporal organisation.[49-52] At the lower level of hierarchical organisation (within the cell) and at shorter timescales, selection favours mitochondrial variants increasing their own replication rates. But at the higher level, and when longer timescales are considered, evolution may favour cooperative organelles, because their fitness is tightly coupled to the fitness of the eukaryotic cell as a whole. Eventually, selfish and purifying selection may reach an equilibrium where selfish spread is balanced by higher-level selection against the most severely affected cells.[53] Uniparental inheritance then evolves as a higher-level adaptation that promotes tighter integration of lower-level units.[54]

Mitochondrial mutations that appear to behave "selfishly" at the lower level of selection do exist in nature, and they are a fascinating subject of ongoing empirical investigations.[33,53,55,56] Often, "selfish" mtDNA that persists in natural populations contains a large deletion, as in "petite" mtDNA variants of yeasts.[57] *uaDf5* deletion affecting 11 genes in nematode *Caenorhabditis elegans* coexists in natural populations in a heteroplasmic state with the wild-type mtDNA, and it has been suggested that *uaDf5* can somehow escape the cellular copy-number regulation machinery,[58] although their proliferation depends on the availability of nutrients.[56] In nematode *Caenorhabditis briggsae*, *nad5Δ* deletion increases in frequency and persists in heteroplasmic organisms in different geographical regions, while in parallel reducing fertility and having other deleterious fitness effects.[59] In *Drosophila*, selfish transmission has been shown to depend on genetic variation in non-coding regions of the mtDNA that contain origins of replication,[60] with "selfish" drive of distantly related mtDNA variants eventually compromising cellular function.

Three decades after their publication, "selfish" conflict theories still dominate the literature on mitochondrial inheritance evolution. However, while it appears that "selfish" theories may

have some empirical support from the limited number of selfish mutants identified to date, we simply don't know how common these variants are in nature, nor how strong the lower-level selection for these variants is likely to be. Recent work[53] suggested that mutations affecting the stability of G-quadruplexes in the mtDNA control region may contribute to their more rapid proliferation by favouring replication over transcription, but how frequent these mutations are likely to be is unclear. The repeated evolution and continual maintenance of uniparental inheritance across the eukaryotic tree of life would require a constant pool of selfish variants circulating within the population. However, it is more likely that such mutants represent only a tiny fraction of all mitochondrial variants in natural populations, while deleterious mutations that do not increase mtDNA replication rates are more frequent. Beyond the "selfish" conflict theories, what else can explain the evolution and maintenance of uniparental organelle transmission?

Uniparental inheritance without "selfish" mutations

In the last decade, the field of mito-nuclear population genetics has seen several innovations, that together provided a more general explanation for the evolution of maternal mtDNA transmission. Focusing on "selfish" mitochondrial variants, early theories downplayed some of the most unique aspects of mtDNA population genetics that, as we now know, dictate mtDNA evolution, inheritance, and mitochondrial disease dynamics.[61] These aspects include heteroplasmy (coexistence of dissimilar mtDNA in the same cell) and its intrinsic fitness costs,[62,63] mitochondrial-nuclear interactions,[64] variable linkage between mitochondrial and nuclear genes,[22] and the interplay between mutational variance generated through segregational drift and selection strength at the level of the cell.[65] A new generation of population genetic models, while often using more sophisticated mathematical and computational methods, could for the first time track the complete distributions of mtDNA mutations within heteroplasmic cells with many copies of the mitochondrial genome as they interact with nuclear genes. Taken together, the results of these models provided the following unified picture of mitochondrial inheritance evolution.

First, this new generation of population genetic models revealed a stark contrast in mtDNA mutational diversity dynamics in populations using uniparental and biparental strategies of cytoplasmic transmission. With recurrent mtDNA mutations, which need not be selfish, mitochondrial mixing associated with biparental transmission reduces variability in the extent

of heteroplasmy between cells.[65,66] If two gametes containing, say, 0 and 10 copies of a specific mtDNA mutation fuse, the daughter cells produced after the zygote splits will each contain closer to 5 mutations, considerably reducing the cell-level variance in their phenotypic effects. Uniparental inheritance has the opposite effect: it prevents heteroplasmy and increases dispersion in aggregate cell-level phenotypic effects of heteroplasmy, the effect similar to genetic bottlenecking[67] (Fig. 2). This increased variance aids purifying selection against mitochondria carrying deleterious mutations.
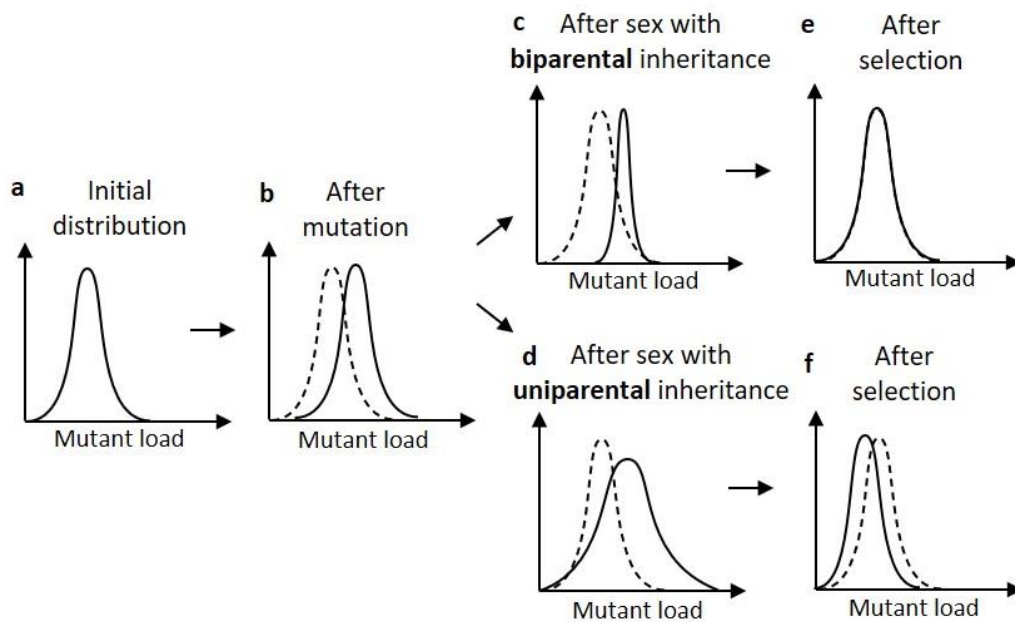
FIGURE 2. Mitochondrial mutational distributions in a hypothetical lifecycle of a unicellular organism with biparental and uniparental strategies of mtDNA transmission. The population is characterized by equilibrium heteroplasmy distributions, wherein some individuals have more mtDNA mutations, and others have fewer (a). New deleterious mutations accumulate each generation, shifting the distribution to the right (b). The dotted line represents the initial distribution as seen in (a). Sexual reproduction with biparental inheritance reduces variance in mtDNA mutational distributions because of random mitochondrial mixing (c), but with uniparental inheritance, variance increases due to random binomial segregation of mtDNA variants (d). Purifying selection at the level of the cell is more efficient when mutational variance is greater, reducing the mean mutant load with uniparental mitochondrial transmission (f) relative to biparental inheritance (e). Similar arguments apply to advantageous mitochondrial mutations and adaptive selection.

But can this influence on variance in mutant load and its phenotypic effects drive the evolution of nuclear alleles that encode uniparental inheritance mechanisms? After all, increased genetic variance is not always beneficial despite facilitating selection in the long term; depending on the peculiarities of epistatic interactions, equilibrium genotype

distributions, and the strength of linkage to the nuclear modifier gene, increased variance can be selected against.[68] Recent mito-nuclear population genetic analyses revealed a complex evolutionary picture, because the fitness effects of individual mtDNA mutations are not additive and generally show negative epistatic interactions (Fig. 3a). These nonlinear gene-gene interactions mean that the overall fitness effect of a single mutation increases with the total mitochondrial mutant load, resulting in co-called mitochondrial threshold effects[69,70] wherein cell's metabolism is impaired only when heteroplasmy reaches a certain threshold value, typically in the range of 60-80%.[71]

Because of these nonlinearities in fitness effects, mitochondrial mixing can have a short-term positive fitness effect, as, on the average, biparentally produced offspring will be less likely to contain a high, above-threshold mtDNA mutant load (Fig. 3b). Nuclear alleles that are weakly linked to mitochondrial populations and therefore cannot respond to long-term effects of reduced efficiency of selection, can evolve to benefit from this short-term masking of mitochondrial mutations.[65] Nevertheless, if nuclear loci are co-inherited with mitochondria more often – as is the case for female sex chromosomes – the models show that uniparental inheritance will evolve, because it increases between-cell diversity and aids purifying selection in the longer term.[22,72,73]

Second, new experiments have shown that heteroplasmy in itself has negative fitness consequences, even when the mtDNA variants are neutral when cells are homoplasmic.[62,63] While the exact molecular mechanisms behind these negative fitness effects of heteroplasmy are not known, it is possible that heteroplasmy disrupts intracellular signalling and increases production of reactive oxygen species or causes detrimental interactions between membrane protein subunits from different mitochondria. Regardless of these specifics, population genetic analyses show that selection against detrimental heteroplasmy strongly favours the evolution of uniparental inheritance,[74] even when the mtDNA mutation rates generating heteroplasmy are slow, as is the case in plants and basal metazoans.
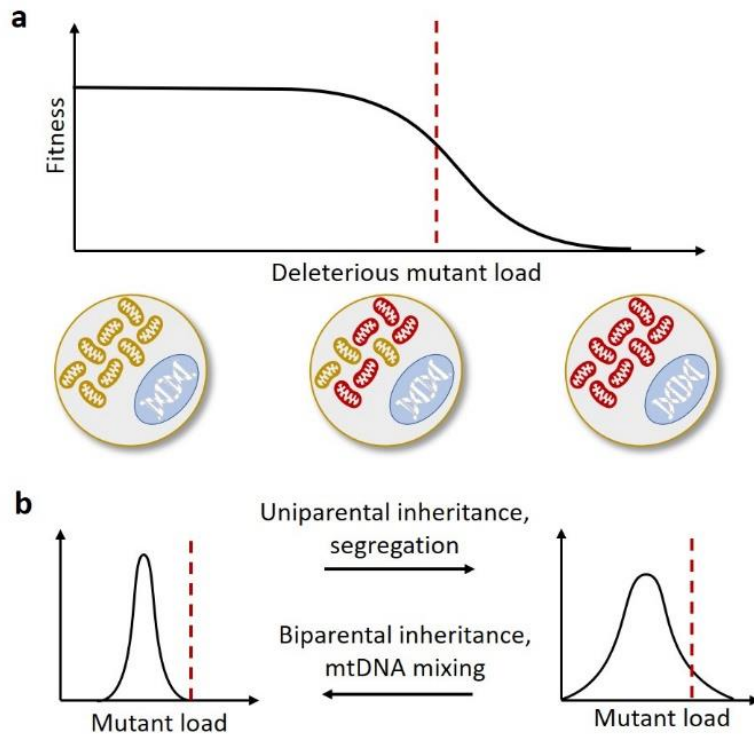
FIGURE 3. Threshold effects in mitochondrial mutant load and eukaryotic fitness. Detrimental fitness effects are negligible when the frequency of mtDNA mutations within the cell is low (a), because the remaining metabolically functional mitochondria can compensate for the reduced energetic activity of compromised organelles. Cellular function is compromised only when deleterious mutant load crosses a threshold value (dashed line). Although uniparental transmission of mitochondria increases variance in mutant load (b), this may reduce mean fitness in the short term, because cells will be more likely to contain a high above-threshold number of mtDNA mutations (to the right of the dashed line), while biparental inheritance has the opposite effect. Theory predicts that uniparental inheritance will evolve only when the long-term evolutionary advantage of increased efficacy of selection with higher variance overwhelms this short-term fitness cost, and this depends on how strong the mito-nuclear genetic linkage is.[22]

Third, the redistribution of mutational diversity modulates the strength of organism-level selection for positive mtDNA mutations and their combinations, and it reduces genetic "hitchhiking" of deleterious substitutions during selective sweeps.[75] Recent mathematical models and simulations revealed that selective sweeps of beneficial mtDNA variants can drive the evolution of uniparental inheritance, facilitating adaptative evolution.[75,76] There is strong empirical support for adaptive mitochondrial evolution in natural populations,[77-79] and, given that mitochondria interface with their external environments and their energy-production performance depends on thermal conditions,[4,80] this positive effect of uniparental transmission can explain rapid eukaryotic adaptation to shifting environments.[81]

What do we miss when we focus on "selfish" mutants?

The "selfish" conflict theories of mitochondrial inheritance have dominated the evolutionary thinking of recent decades, and, despite the greater generality of the more recent explanations, are still the most commonly invoked evolutionary explanation of maternal mtDNA transmission. However, focusing on only one type of mitochondrial mutant, these theories overlook the complexities associated with mitochondrial mutational variance redistribution, threshold effects, and nuclear modulation of cell-level selection. This has often led to verbal hypotheses that contradicted both empirical observations and predictions made by formal mito-nuclear coevolution models, obscuring the more general evolutionary picture.

Authors that explain uniparental inheritance evolution solely by invoking selfish conflict arguments tend to miss the improved purifying selection effect, discussing largely "haploid and asexual"[82] mtDNA population genetics. In their review on organelle inheritance strategies, Greiner et al.[83] argued that maternal transmission stops the spread of parasitic organelles, but that it also prevents mtDNA recombination and leads to irreversible accumulation of mildly deleterious mutations known as Muller's Ratchet.[84] This then requires eukaryotic lineages occasionally reverting to biparental strategy of organelle exchange and more frequent mtDNA recombination. Earlier, similar views have been presented by Hoekstra[82] and Aanen et al.[85] In the light of the latest models, however, we know that maternal inheritance improves selection against deleterious mutations, and therefore it will also mitigate mitochondrial mutational ratchet, while biparental inheritance will have the opposite effect of weaker selection and faster accumulation of mutations.[67] Indeed, a recent study showed that increased efficacy of purifying selection with uniparental transmission reduces the rate of mtDNA mutational erosion, even relative to biparental inheritance with frequent homologous recombination.[86]

Likewise, it has been argued that while uniparental inheritance protects lineages from parasitic mitochondrial mutants and endosymbionts, the resultant asexuality of the mtDNA transmission should make natural selection less effective, making mitochondria unable to adapt to changing environmental conditions.[87] Empirical evidence, however, points to the contrary and consistently shows that uniparentally inherited mitochondrial genomes readily undergo adaptive evolution, especially in animals.[77-79,88,89] The observations are consistent with modern population genetic descriptions of mitochondrial mutational variance evolution

with uniparental inheritance, and its effects on cell-level selection strength. These theoretical studies revealed not only that maternal transmission aids selection for positive mutations, reduces interference and promotes adaptive evolution,[76] but also that nuclear modifiers inducing uniparental transmission invade as adaptive mtDNA variants sweep through the population.[75] So long as there is enough mutational variance supplied either through *de novo* substitutions or exploiting standing genetic variation, uniparental transmission can be seen as an adaptive strategy eukaryotes use to respond to environmental shifts.[81]

Altogether, selective focus on "selfish" theories of mitochondrial inheritance evolution obscures the advantageous influence of uniparental inheritance on maintaining mitochondrial quality, reducing heteroplasmy, and improving adaptation to changing climates. While there is no doubt that the selfish conflict models and their mathematical formalism are themselves correct, the disproportionate focus on one type of mtDNA variant can have unintended consequences hindering our understanding of the mito-nuclear evolutionary dynamics. Because naïve intuition can be misleading, verbal arguments stemming from "selfish" hypotheses should always be tested through formal mathematical modelling.

More generally, selfish mtDNA variants represent only a subset of all mitochondrial mutations that can have both positive and negative fitness effects across the two levels of selection (individual mitochondrion and the whole cell).[90] Recent models that expanded their scope beyond the "selfish" mtDNA variants have indeed shown that regardless of the nature of mitochondrial variants, the causal mechanism responsible for the evolution of uniparental strategies is the same: mutational variance redistribution.[66,72,90] This work has increased the generality of our understanding, and generated predictions that are far more consistent with empirical observations, including some of the more puzzling aspects of mtDNA transmission related to paternally-regulated restriction of mtDNA transmission ("killing one's own cytoplasm").[22] The "selfish" conflict between genetically distinct compartments of the eukaryotic cell may play important roles in eukaryotic evolution but given the greater generality and explanatory power of the variance-based arguments, we are better off viewing "selfish mitochondrion" hypotheses as one part of a much more general theory.

Conclusions

The "selfish mitochondrion" theories of uniparental inheritance evolution have clear parallels to the evolutionary conflict and cooperation literature and the multilevel selection theory. The evolution of life's complexity can be conceptualized as a series of transitions in hierarchical organisation.[91] Evolutionary conflicts among lower-level units have to be resolved for the higher level to emerge and attain evolutionary stability.[52,54,92,93] In this context, Haig likened mitochondrial populations to herds, that have to be managed by nuclear genes to resolve the intracellular public goods dilemma,[94,95] conflicts suppressed by top-down sanctions akin to higher-level institutions in human societies.[96]

Evolutionary conflicts of interest have undoubtedly played important roles in eukaryotic evolution, and social interactions across levels of selection contributed to the evolution of biological complexity, in particular in chimeric eukaryotic cells.[93,97] However, evolutionary conflict theories were never meant to encompass every aspect of life's evolving complexity. In the case of mitochondrial inheritance strategies, the ease of understanding through intuition and metaphor so abundant in selfish theories comes at a cost of sidelining the more general, biologically realistic – even if mathematically complex – theory, based on mitochondrial mutational diversity redistribution that modulates the efficacy of selection.

The evolutionary theory literature disproportionately focusing on "selfish" variants misses the overwhelming majority of clinically relevant mitochondrial mutational variants that can be deleterious without being selfish, it overlooks the influence of uniparental inheritance on improving purifying selection, facilitating adaptive evolution, and reducing detrimental heteroplasmies. Ultimately, this bias in theory may be counterproductive, and it may prevent further exploration and slow down the progress in both theoretical and experimental mitochondrial biology. Accepting that uniparental inheritance may have evolved because it modulates variance in heteroplasmy and its phenotypic effects, and because it improves purifying and adaptive selection will only increase the generality of our evolutionary understanding, retaining "selfish conflict" theories as a special case of a much broader theory.

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