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Review Article

Evolving mtDNA populations within cells

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Mitochondrial DNA (mtDNA) encodes vital respiratory machinery. Populations of mtDNA molecules exist in most eukaryotic cells, subject to replication, degradation, mutation, and other population processes. These processes affect the genetic makeup of cellular mtDNA populations, changing cell-to-cell distributions, means, and variances of mutant mtDNA load over time. As mtDNA mutant load has nonlinear effects on cell functionality, and cell functionality has nonlinear effects on tissue performance, these statistics of cellular mtDNA populations play vital roles in health, disease, and inheritance. This mini review will describe some of the better-known ways in which these populations change over time in different organisms, highlighting the importance of quantitatively understanding both mutant load mean and variance. Due to length constraints, we cannot attempt to be comprehensive but hope to provide useful links to some of the many excellent studies on these topics.

Introduction

Mitochondria are endosymbiotic organelles that facilitated and continue to support all complex life. Due to their evolutionary history, mitochondria in present-day eukaryotic cells retain highly reduced genomes, which encode genes vital for cellular bioenergetics. Eukaryotic cells may contain hundreds or thousands of mitochondrial DNA (mtDNA) molecules. This mini review will discuss how these cellular mtDNA populations evolve over time, particularly focussing on populations involving a mixture of mtDNA types.

The gene content of mtDNA varies dramatically across life [1,2]. Parasitic organisms typically have highly reduced genomes; some have lost mtDNA altogether, retaining highly reduced mitochondrion-related organelles or MROs [3,4] (which may sometimes retain aerobic capacity [5]). Many bilaterians have similar mtDNA complements, although some diversity in gene content and genome structure certainly exists, and in non-bilaterian animals, this diversity expands [6]. Plants often retain more genes and can have huge mtDNA genomes largely filled with non-coding content [7,8]. The highest mtDNA gene counts yet found are retained in some protists [9]. The reasons for this diversity in gene content remain debated but may involve species- and environment-specific resolutions to an evolutionary tension [7,10] between retaining genes for local convenience [11–13] and transferring them to the nucleus for genetic robustness [14–16].

In addition to this diversity in gene content, mtDNA sequences vary within cells and populations. MtDNA is subject to mutation [17–20]. In animals, mtDNA sequence mutation rates are higher than nuclear mutation rates [17,18]. Plant mtDNA, by contrast, has a lower sequence mutation rate than the nucleus [21]. However, the rate of structural mutation (reorganisation of mtDNA molecules) is high in plants [21–25], while animal mtDNA structure is relatively stable [10,19]. Fungi differ again: while mtDNA recombination is common [26,27] and structural variants frequently arise (including the well-known 'petite' mutant, with large deletions and an inability to respire [28]), mtDNA mutation rates remain high relative to the nucleus [17,18].

Given this potential for sequence changes, population histories lead to, for example, geographical variation in mtDNA types. In humans, this variation is used to track population histories [29,30] and is a potentially important source of stratification in personalised medicine [31].

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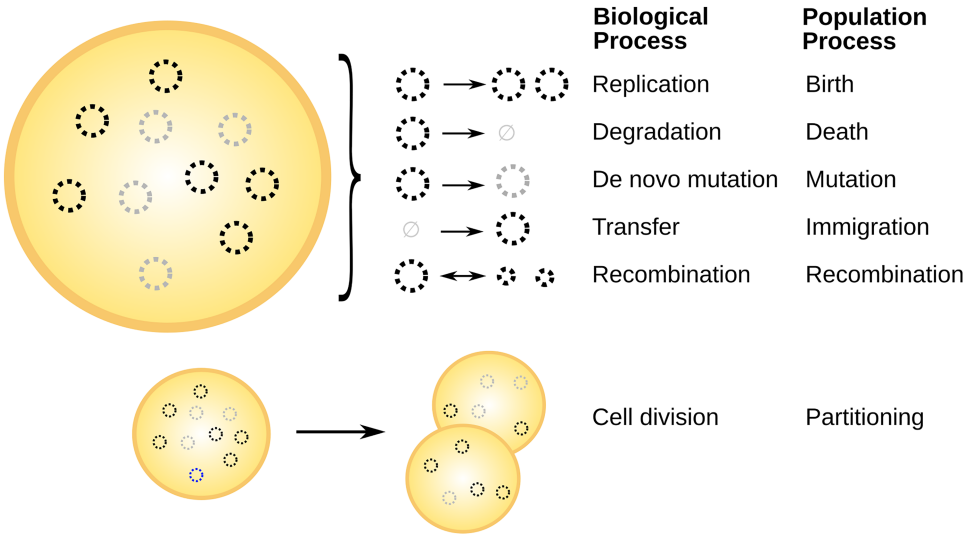

Figure 1. Processes influencing evolving mtDNA populations.

Within a heteroplasmic cell containing different types of mtDNA molecule (left), different processes (right) can change the structure of the cellular mtDNA population. These include replication, degradation, *de novo* mutation, intercellular transfer, and recombination. Cell divisions, where mtDNA molecules may be partitioned between daughter cells according to several possible mechanisms, also influence mtDNA statistics. The rates of these processes depend on organism-, sequence-, tissue-, and time-dependent factors. Several correspond directly to processes from the theory of stochastic population processes [57,58].

MtDNA is physically contained within mitochondria. Animal and fungal mitochondria have physically flexible forms, undergoing fusion into reticulated networks and fission into smaller fragments, and with each organelle typically containing several copies of mtDNA [32]. These molecules are packaged in nucleoids, the size of which is debated [33–35] but which recent evidence suggests usually contain under two mtDNA molecules [36] and are internally genetically homogeneous [37]. Plant mitochondria usually (with some exceptions [38]) remain more as discrete, dynamic organelles [39–43] and often contain no mtDNA [44].

Within cells, different processes act to dynamically change the structure of mtDNA populations (Figure 1). Across species, mtDNA replication and degradation changes the makeup of the cellular population over time. This is often pictured as 'relaxed replication' [45], (replication co-ordinated with, but not directly linked to, the cell cycle [46]), and under nuclear control [47–50]. In animals, mtDNA is largely asexual and exists in reasonably consistent circular forms (with some exceptions, including mtDNA networks in the human heart [51]). In fungi and plants, active recombination mixes and reforms mtDNA content [27,52,53]. This may result in a large variety of branched and linear forms containing different gene content [7,54,55]. The susceptibility of mtDNA to processes including degradation and recombination depends on the physical dynamics of mitochondria, coupling the physical and genetic structure of the mitochondrial population [7,50,56]. *De novo* mutation, and mtDNA transfer between cells, also influence the makeup of mtDNA populations.

The reader will notice the analogy with ecology. Individual mtDNAs exist in cellular 'ecosystems', replicating and degrading, mutating, potentially moving between ecosystems, occupying new ground when cells divide, and in some systems also undergoing recombination. The natural question emerges — how heterogeneous are these populations [50]?

Heteroplasmy

While several mechanisms exist to keep cellular mtDNA populations homogeneous ([59]; see below), sequence and structural differences between mtDNA molecules can result in so-called heteroplasmy, a mixture of different mtDNA variants in the same cell [35,60]. These variants may involve single nucleotide polymorphisms or more dramatic structural changes. Heteroplasmy may emerge from *de novo* mutation, intercellular transfer, recombination, inheritance of different mtDNA types, or synthetically via gene therapies.

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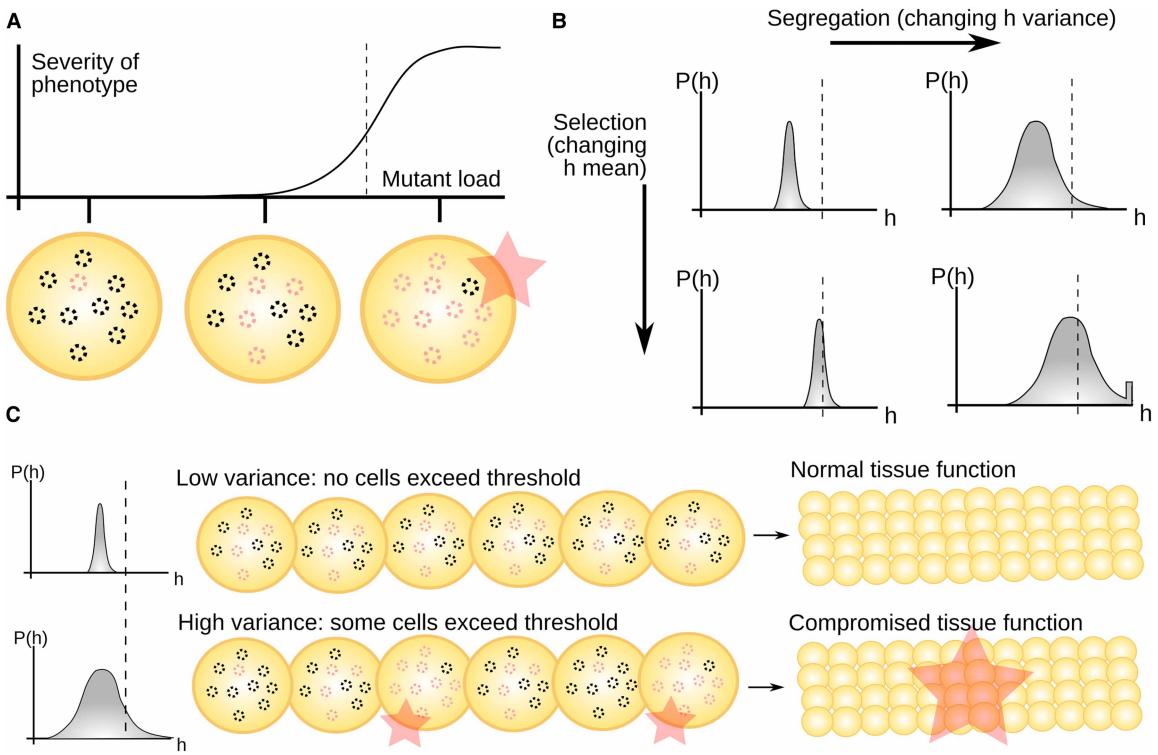

Figure 2. Thresholds in mtDNA mutant load.

(**A**) The severity of symptoms associated with a pathogenic mtDNA type (red) is low or negligible until a mutant load threshold value (dashed line) is crossed, whereupon the disease severity increases dramatically [73]. (**B**) Influence of cellular processes on cell-to-cell mutant load distributions ($P(h)$ is the probability of a cell having a given mutant load h). Segregation widens mutant load distributions; selection shifts their mean. Wider distributions have more probability of crossing mutant load thresholds (dashed lines). (**C**) Even a small number of high-mutant-load, dysfunctional cells can compromise organ-wide functionality [74,75]. If the cell-to-cell variance of heteroplasmy is low, few cells will cross the threshold and the organ can function as normal. If the cell-to-cell variance is high, even with a low mean mutant load, more cells will cross the mutant load threshold and disease will be manifest. We therefore need to understand (at least) both the mean and the cell-to-cell variance of heteroplasmic cells.

Naturally occurring mtDNA heteroplasmy is common across life [61]. Early examples of heteroplasmy were reported in organisms as diverse as fly [62], seaweed [63], fungi [64], maize [65], brittle stars [66], flatfish [67], and vegetatively propagated olive trees [68]. Low-level heteroplasmy is ubiquitous in humans [69] and more broadly across vertebrates [70].

Some sequence variants may compromise bioenergetic functionality. However, because of the many copies of mtDNA in each cell and some redundancy, these variants typically need to be present above a certain mutant load in order to have a detrimental effect (Figure 2A) [71,72]. This is the so-called threshold effect in mitochondrial disease [73].

The threshold effect means that cell-to-cell differences in mtDNA mutant load are important. Imagine that an organism carries a mutant at an average 50% mutant load. If all cells are identical, none will pass a 60% threshold for the disease. However, if substantial cell-to-cell variability exists, some cells may exceed the disease threshold (Figure 2B). This potential for threshold crossing is important because of another nonlinearity. Some tissues require the concerted functionality of many cells working together. Just a small number of compromised cells can then lead to a pathology (Figure 2C). The presence of mitochondrially compromised cells has been shown to cause pathologies including arrhythmias in the heart [74] and damage in muscle fibres [75].

As this mini review will argue, these nonlinearities mean that it is important to study at least both the mean and the cell-to-cell variance of mutant statistics in mtDNA populations (and ideally the full distributions [58,76,77]). Changes to either can lead to pathological situations (Figure 2B) and both have consequences in

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the fundamental biology of inheritance and evolution [27,35,60]. In this mini review, we will highlight some of the several classes of biological process that alter these statistics of cellular mtDNA populations over time. We will focus on selection and segregation, respectively, changing the mean and variance of mutant mtDNA statistics. Due to length constraints, we can only briefly mention recombination, *de novo* mutations, and intercellular mitochondrial transfer, other processes which impact mtDNA populations in cells.

Within this scope, the big questions are: under what circumstances (organism-tissue-mtDNA sequence-time) does mtDNA selection pressure act? And how is cell-to-cell variability generated in mtDNA populations?

Changing mean heteroplasmy

Perhaps the most dramatic process influencing cellular mtDNA populations in many sexually reproducing organisms is the clearing of mtDNA from one parent (usually paternal). This clearance strongly diminishes or removes mtDNA content from one parent around fertilisation, avoiding admixtures of maternal and paternal mtDNA. Postulated reasons for this clearance include the general exclusion of any foreign DNA from the fertilised oocyte, the avoidance of nuclear-mtDNA or mtDNA-mtDNA incompatibilities, and the mitigation of selfish mtDNA behaviour (reviewed in [59,78]). However, the search for a universal explanation is complicated by the diverse modes of mtDNA inheritance across life [27,61,79–82]. While maternal mtDNA inheritance is common, some organisms display parental or doubly unipaternal inheritance (DUI), and rare mtDNA 'leakage' (for example, rare retention of limited paternal mtDNA) can retain some heteroplasmy.

Animal mtDNA inheritance is usually maternal [27,61], with some exceptions including bivalves adopting DUI [83,84], and paternal leakage sometimes reported (and highly debated) in humans [85,86]. Plants usually inherit mitochondria maternally, with exceptions including paternal leakage [87], inheritance, or DUI in some species [88–91]. Fungal mtDNA inheritance is more complex and different species may undergo uniparental inheritance and/or DUI [26,27] and can also involve the inheritance of mitochondrial 'plasmids' [92].

If heteroplasmy exists after fertilisation (for example, due to leakage or mutation), cellular processes may change heteroplasmy statistics over time (Figure 1). In developing animals, heteroplasmy changes in a tissue-, sequence-, and time-dependent way [93]. Animal models have allowed increasingly detailed insight into these dynamics. Typically, a model is constructed or acquired harbouring an admixture of two mtDNA types, and techniques including pyrosequencing, qPCR, and dPCR are used to compare heteroplasmy in aged organisms against some reference. Mouse models have been particularly well explored here, including a widely used pairing of C57BL/6 or BALB and NZB [94–96], other pairings [97,98], and the more recent construction of admixtures with a range of genetic distances between the interrogated haplotypes [99,100]. Fly [101,102] and livestock [103,104] models have also been investigated. Recent advances in these model systems have included minipigs [105], and an elegant system in *Drosophila* allowing different modes of mtDNA selection to be characterised [106].

The ongoing development of diverse models has underlined that selection, leading to systematic changes in mean heteroplasmy, is common among mtDNA pairings. Within a pairing, one type may experience an advantage in some tissues and a disadvantage in others. Selective differences are often particularly pronounced in liver, spleen, kidney, and blood (observed in most references above), but are manifest in many tissues, including post-mitotic tissues including brain, heart, and muscle [99,105]. For some pairings, we have found that selective differences depend on time and developmental stage [99]. The expansion of mouse models has suggested that the magnitude of these selective differences may be related to the genetic diversity of the mtDNA pairing, with more diverse pairs showing stronger differences [99] and similar pairs showing little difference [100]. However, *in vitro* results from human oocytes and oocyte-derived material have challenged this picture, showing little relationship between heteroplasmy shift and genetic diversity [107,108] (see corrected data [109] for Ref. [108]). The mapping between these *in vitro* results, with associated passage protocols, culture conditions, etc., and natural development is not yet completely straightforward. However, a potential reconciliation of all approaches involves viewing selective differences as resulting from a combination of genetic features; more diverse molecules have a higher probability, but not a necessity, of differing at these features. The substantial mtDNA diversity present in human populations suggests that selective differences may be common in pairings arising from gene therapies [110].

Individual-level mtDNA selection is observed in disease-causing human mutations. MtDNA carrying the 3243A>G mutation, for example, is depleted over time in leucocytes [111]. Notably, the presence of the 3243A>G mutation affects overall cellular mtDNA copy number, perhaps via a compensatory mechanism aiming to maintain a given wild-type content [112,113].

Selection in the germline has proved more controversial, due in part to the lower magnitudes of selective difference observed. Studies on mammalian germline development have shown that the development of oocytes and development post-fertilisation can show different patterns of selection. Several studies in mice [114] and human [115,116] found random drift to explain heteroplasmy distribution in oocytes. However, selection has been observed to act on these random oocyte distributions before, or during, their development to offspring [117–119]. Selection acting on deleterious human mutations, for example, the 3243A>G mutation above, has been suggested in germline development [120,121], and a recent large-scale study has found evidence for germline selection, under nuclear control, at different mtDNA loci [122]. To dissect the dynamics of germline mtDNA selection, we recently described mtDNA dynamics during development and between generations in two mouse models with different mtDNA pairings [123]. One showed selection for mtDNA content in oocytes that was subsequently reversed in transmission to pups; the other contrasting case showed no selection apparent in oocytes, but a clear selective difference was found in pups. This work both revealed mammalian germline mtDNA selection and identified haplotype-specific timing differences in its manifestation [123].

In plants, the diversity of naturally occurring mtDNA forms supports a wider range of dynamic behaviour. Heteroplasmy in mtDNA structure, as well as sequence exists, perhaps reflecting a functional difference between large and small/absent molecules and their corresponding organelles [7,39–42,124–126]. Some plants seem to maintain a relatively simple tripartite system of one large and two smaller mtDNA molecules [125,127]. Others partition their genome into dozens of different 'chromosomes' [22,128]. Some structural variants are present at very low copy numbers, 10–1000 times lower than the dominant genomes [52], so that sometimes only a small fraction of plant cells contains these so-called 'sublimons' [129]. Of particular note is substoichiometric shifting, where sublimon mtDNA types at initially low copy number are rapidly elevated to dominate mtDNA populations [130,131]. These fast heteroplasmy shifts often have dramatic phenotypic consequences including cytoplasmic male sterility (CMS) [55,132–134], where the ability to produce functional pollen, anthers, or male gametes is compromised. CMS has been observed naturally in over 150 species [135]. This is detrimental for the plant but of profound use in crop breeding, allowing the easy construction of productive hybrids [134,136], increasing crop production in an increasingly challenged world [137].

In fungi, a history of literature has considered competition between 'petite' mutants where mtDNA suffers a deletion (ρ^-) or is absent (ρ^0) and wildtype (ρ^+) in single yeast cells [138,139]. Selfish replication is often observed, where small mtDNAs with relatively many origins of replication outcompete longer mtDNAs [140]. The magnitude of this advantage can be changed by modulating the functional challenges that the cell's mitochondria face [141].

These species-, sequence-, tissue-, and time-dependent observations mean that the circumstances under which selection acts on mtDNA populations (i.e. inducing a systematic, reproducible change in mean heteroplasmy) remain unresolved. How are these different dynamics manifest at the molecular level? Several possible mechanisms for selective differences likely compete [142]. In several systems, 'selfish' behaviour of molecules with an intrinsic replicative advantage has been found [78,143,144]. These include deletion mutants in nematodes [145], short molecules with high replication origin density in yeast [140] and plants [146], and possibly particular D-loop variants in humans [108,147,148].

Features beyond replication rate may also influence mtDNA selection. Some nuclear-encoded factors influencing segregation have been identified [149]. Mitochondrial quality control [56] acts to remove poorly performing organelles, which may have a selective effect if different mtDNA types vary in metabolic or bioenergetic function. Differences in oxidative phosphorylation exist between human haplogroups [150] and in reactive oxygen species production in mouse strains [151]. Some evidence exists for the magnitudes of selective differences being linked to the turnover rate of mtDNA in cells (or cells themselves) [99]. Environmental pressures may provide further selective pressures. Although association studies with mtDNA are challenging [152,153], evidence in fish suggests that mtDNA variants have been shaped by local climate [154], and environmental effects on human mtDNA have been reported [155] including a role for altitude [156] and temperature [157].

Changing heteroplasmy variance

In parallel with changing mean heteroplasmy, the cell-to-cell variance in heteroplasmy is also changed by several biological processes. Typically, changes in variance are harder to detect than changes in mean, and the large uncertainties involved are often ignored [158]. This is because limited sampling challenges estimates of variance, measurement noise can confound observations of variance, and averaging across cells (as in, for example, amalgamated tissue samples) loses information on cell-to-cell variance.

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In animals, a developmental 'genetic bottleneck'[1] increases cell-to-cell heteroplasmy variance from the fertilised oocyte (which, as a single cell, has zero variance) [159,160]. One purpose of this process appears to be to generate heteroplasmy variance between oocytes in the next generation. Cells carrying low levels of pathogenic mutations can then be fertilised and those carrying high levels can be discarded, overcoming Muller's ratchet [161] via cell-level selection.

The genetic bottleneck was originally found in cattle [162,163] and has since been demonstrated in animals from mice [160,164–166] and salmon [167] to humans [168–172]. The mechanism of the genetic bottleneck remains debated [160,164–166]. A physical bottleneck, involving a reduction in cellular mtDNA copy number during germline development, occurs in several animals [164,166,170,173,174]. This physical bottleneck likely plays a role both through the amplification of genetic drift and variability induced from mtDNA population processes (Figure 1) but is not equivalent to the genetic bottleneck [160,175]. Other processes generating mtDNA variability — that may be amplified by the physical bottleneck — include random turnover due to stochastic mtDNA replication and degradation [45,47,164], (related) participation of a random subset of mtDNA molecules in replication [166], and random partitioning of individual mtDNAs [164], or clusters of mtDNA molecules [165] at cell divisions. Using all available experimental data from mice, and new experiments, we used an unbiased approach to compare these mechanisms and found that random turnover and binomial partitioning (BDP or birth-death-partitioning) was the most supported mechanism [160].

In plants, germline development is complex and debated [176,177]. Different modes of inheritance are observed (paternal, maternal, biparental) for mitochondria (and plastids) in different species [87,88,91]. MtDNA variance certainly exists, with a suggestion of a 'bottleneck' in plants made after observing mean and variance changes after two generations of sexual reproduction [68]. Tissue variability in subgenomic mtDNA molecules has been reported [178] and is predicted to arise from random mtDNA dynamics [146,179].

In fungi, segregation of mtDNA at cell divisions was reported in the 1970s [180], and the interplay of segregation, recombination, and uniparental inheritance in increasing or stabilising mtDNA variance has been explored since [181,182]. In yeast, tighter control (i.e. closer to perfect halving than random binomial sampling) of mtDNA partitioning has been demonstrated [183], limiting but not removing mtDNA variability.

During organismal ageing, and in somatic tissues, the variance of mtDNA populations also increases over time.[2] Re-analysis of data from Ref. [93] shows increasing variance even in tissue-averaged samples in mouse brain (slow cell turnover) and intestine (fast cell turnover) (Figure 3). Somatic, tissue-specific increases in heteroplasmy variance have been inferred during human embryogenesis using a powerful population phylogenetic approach [172]. In the mouse germline, we recently showed that cell-to-cell variance continues to increase as mothers age [123]. This observation supports theoretical modelling [48] and re-analysis of earlier results from fly [102] and mouse [166] (in Ref. [48]).

In humans, increasing heteroplasmy variance — via the mtDNA bottleneck and other processes — has the effect of complicating clinical planning for inherited diseases, because the mutant load inherited by a given child is a random variable. The increase in heteroplasmy variance between generations is different for different mtDNA mutations [184]. A striking example is the fast shifts towards homoplasmy for 8993T>C/G mutations compared with the slower increase in variance associated with 3243A>G [120,184]. In human cell lines harbouring the 3243A>G mutation, a variety of outcomes exists, reflecting either direction of drift or comparative stability, perhaps modulated by nuclear genes [185,186].

Mutations and intercellular transfer

While not a focus of this mini review, we briefly note that the appearance and physiological influence of *de novo* mutations in evolving mtDNA populations has been a matter of some debate. Redox imbalance is hypothesised to be an important source of DNA damage [187,188]. However, the link between mitochondrial redox activity and mutation is not uncontroversial. In some experiments, more severe oxidative damage did not dramatically influence mtDNA mutation rates [189], and mtDNA mutational profiles suggest that other

[1]When discussing 'the bottleneck' it is important to be clear about which process is being referred to. The 'genetic bottleneck' is a model describing how much heteroplasmy variance is generated over a given period, but it corresponds to an 'effective' quantity that does not reflect a given observable number of mtDNA molecules [48,79]. There is also a 'physical bottleneck', a specific observable depletion of mtDNA copy number, that contributes to, but is not identical to, the genetic bottleneck.

[2]Here, we avoid the term 'genetic bottleneck' because of its aforementioned potential confusion with mtDNA population size reduction.

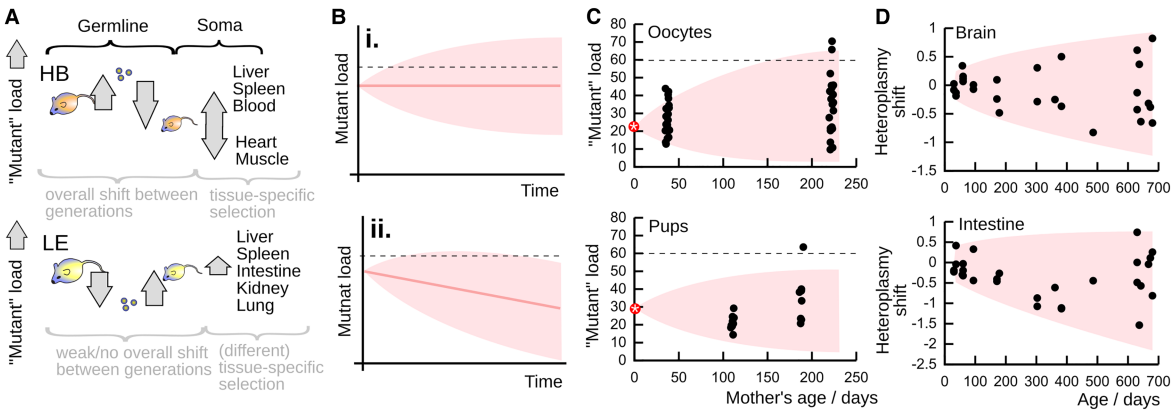

Figure 3. Changes in heteroplasmy mean and cell-to-cell variance in germline and somatic tissues.

Mouse models consisting of wild-derived mtDNA haplotypes in cellular admixture with the C57Bl/6N haplotype have recently demonstrated diverse mtDNA behaviour [93,123]. Here, HB and LE are two different mouse models, consisting of an admixture of wild-derived mtDNA (either haplotype HB or LE, referring, respectively, to Hohenberg and Lehsten, the localities where the original wild mice were captured) with C57BL/6N mtDNA. (**A**) The HB model shows an overall germline mean shift manifest by an increase in oocyte mutant load and an overcompensatory decrease in pup mutant load, followed by the somatic selection that shows tissue-specific variation in direction. The LE model, like other mouse models, shows little overall germline shift but does show an inverted decrease in oocyte mutant load matched by a compensatory increase in pup mutant load. LE also shows tissue-specific selection during development, in different patterns to HB. (**B**) We generally find an increase in cell-to-cell mutant load variance over time (i). Even when coupled with a mean decrease in mutant load (ii), an increase in variance can still lead to threshold crossing. (**C**) Increasing variance in HB oocytes and pups, from Ref. [123]. The increased spread of mutant load values over time (sketched in shaded regions) leads to oocytes and pups from older mothers (with comparable initial heteroplasmies, red stars) crossing thresholds. (**D**) Increasing mtDNA heteroplasmy variance in many-cell samples from the brain (low cell turnover) and intestine (high cell turnover) from Ref. [93].

sources like replication errors may be more important [190,191]. Regardless of generative mechanism, in humans, *de novo* point mutations are a common cause of mtDNA disease [192].

To dissect the role of mtDNA mutations in physiology, the elegant 'mutator mouse' system has been developed, where defective mtDNA polymerase leads to the accumulation of mtDNA mutations over time [193]. These mice show severe phenotypes reminiscent of ageing [193], though the inference that these phenotypes provide a causative link between mtDNA mutations and ageing has been debated [194].

Another way in which cellular mtDNA populations can change is through the 'immigration' of mitochondria from external sources. Horizontal transfer of mitochondria between cells has been reported in a variety of (often pathological) circumstances, and through a range of mechanisms including tunnelling nanotubes, extracellular vesicles, gap junctions, and cell fusion (reviewed in [195–197]). Several studies have shown that, in cells lacking mtDNA, external acquisition of mtDNA rescues depleted respiratory function and tumorigenic potential [198,199]. In accordance with stochastic theory [57,58], this external 'immigration' of mtDNA can stabilise heteroplasmy distributions that may otherwise be unstable [200].

Theory

The analogy of organisms in an ecosystem translates through to several ideas from population genetics that have been used to describe the dynamics of mtDNA populations. Approaches from statistical genetics (i.e. focussing on summary statistics of populations) [76,201,202], stochastic modelling (i.e. considering the influence of random processes on populations of molecules) [45,47,48,58,146,160,179], and simulation (i.e. computational representation of mixed or spatially distributed molecules) [203–206] have been proposed and recently reviewed in ref. [207]. A theory has been proposed describing the stochastic behaviour of general physical organelles [208] and associated steady-state [77] and time-dependent [58] distributions have been calculated. Stochastic approaches specific to mtDNA have characterised changes in heteroplasmy mean [99] and variance [123,160], identified the general prediction that heteroplasmy variance increases linearly with time and mtDNA

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turnover [48], described the capacity for cellular control on mtDNA [48,209], elucidated recombination dynamics in plants [146,179], revealed links between physical and genetic mitochondrial dynamics [203–205,207], and dissected variability arising from natural and experimental sources [158,206].

Some straightforward insights from this body of theory can help increase the power and reliability of studies on heteroplasmy. First, it must be remembered that the analysis of percentage point differences in mutant load (e.g. labelling a change of 50% to 60% as 10 percentage points) has several limitations when analysing mtDNA data. Under the same selective pressure, the mutant load will change by different amounts depending on its initial value (for example, a change of 10 percentage points from 50 to 60% is very possible, but a change from 95% to 105% is not). Heteroplasmy changes across samples with different starting values are therefore not immediately comparable. Mathematical theory motivates a simple transformation [48,99], reflecting the difference in fitness between two mtDNA types [94,101], that accounts for this and allows heteroplasmy readings at different levels to be compared:

$$\beta t = \ln\left(\frac{h(h_0 - 1)}{h_0(h - 1)}\right), \tag{1}$$

where h is an observed 'final' mutant load, h_0 is a reference 'initial' mutant load, and t (if known) is the time between these measurements. βt reflects a selective difference β acting over a time t, arising from the mathematical prediction that mean mutant load will evolve through sigmoidal dynamics according to:

$$E(h) = \frac{1}{1 + \frac{1 - h_0}{h_0} e^{-\beta t}} \tag{2}.$$

This representation fails in homoplasmic situations ($h = 0$ or $h = 1$); including homoplasmy requires a more detailed distributional picture (see below).

Predictions of heteroplasmy variance are challenging in the face of selection. For neutral mtDNA evolution and no cell divisions, a detailed, stochastic, microscopic model of mtDNA dynamics predicts that cell-to-cell mutant load variance $V(h)$ (linked to the widths of the distributions in Fig. 2) increases linearly with time t [48]:

$$V(h) \propto h_0(1 - h_0)\frac{2\nu t}{N}, \tag{3}$$

where ν is the rate of mtDNA turnover and N the size of the cellular mtDNA population. The constant of proportionality is predicted in recent work to be f, the fraction of fragmented mitochondria (i.e. those subject to degradation) [50]. Variance increase due to cell divisions [210] can also readily be included via an additional term in equation (3) [48]. This linear increase in $V(h)$ is compatible with our recent experimental observations above [123,160]. Previous work often uses expressions including $V(h) = h(1 - h_0)/N_{eff}$ or $V(h) = h(1 - h_0)(1 - b)$ to define an effective 'bottleneck parameter' b [76] or 'bottleneck size' N_{eff} (found in several studies based on a binomial sampling model of the bottleneck). Equation 3 allows us to start linking these effective quantities (which, as above, do not directly correspond to observable numbers of molecules) to real biological measurements ν, f, t, and N.

Under neutral conditions (no systematic selection), the Kimura distribution has been proposed as a model for cell-to-cell distributions of mutant load [76]. This has advantages over normal and binomial alternatives, although it must be remembered that a fit to a Kimura distribution does not necessarily provide evidence against selection: an mtDNA population where the mean heteroplasmy has changed over time may still conform to a Kimura distribution. A truncated Kimura distribution has been proposed to include one mode of selection in distributional calculations, by disallowing mutant load values above a given cutoff [121]. The full distributional solutions for mtDNA populations that may be under selection, undergo cell divisions, and systematically change population size through a physical bottleneck have been derived [160], though as these are complex, a more heuristic combination of a truncated normal distribution accounting for homoplasmy has been heuristically used [160].

Another branch of mtDNA modelling has addressed evolutionary questions, including the interplay between mtDNA and the evolution of sex [211] and uniparental inheritance [212], recombination strategies [146,179,213], and the emergence of a distinct germline [214]. We recently used a modelling approach to reveal the features governing mtDNA gene loss across life [10] and to propose a hypothesis for the differences between plant and animal mtDNA structure and dynamics based on the immobility of plants [7].

Conclusions

This mini review has argued that both mean and cell-to-cell variance, and ideally full distributions, of mtDNA mutant load are important to understand both for basic science and clinical planning. Heteroplasmy variance can lead to pathological thresholds being exceeded even for populations with lower mean mutant load and provides an important source of cell heterogeneity both within and between generations. Ongoing progress in characterising the processes that affect the cell-to-cell variance of mtDNA populations is highly desirable.

The expansion of available animal models, in conjunction with developing theory, is increasing our knowledge of the diverse ways that mtDNA populations change over time. One recent example is coupled experimental evidence [123] and theoretical support [48,50] for a linear increase in heteroplasmy variance over time during ageing.

An expansion of theory that is able to describe the mean and variance (and distributional details) of mtDNA populations under selection will improve our ability to characterise mtDNA populations. Currently, several common analytical approaches are not robust to even small selective differences. The field will in future benefit from an expansion of the available mtDNA pairings that can be considered in biological models, which will increase our ability to identify and verify the genetic features governing these biologically and medically important shifts in mtDNA population structure.

Perspectives

- Evolving mtDNA populations within cells are vital across eukaryotic life, from plants and fungi to humans. How they change with time underlies fundamental biology and translational bioenergetics, from inherited diseases to crop sterility. Nonlinear links between mutant load and cellular phenotype mean that it is important to understand both the cell-to-cell mean and variance (and ideally the full distributions) of mtDNA populations.
- Model organisms and increasingly high-resolution technology provide valuable insight into the dynamics of mtDNA populations, but many mechanistic questions remain. This is particularly true in non-mammalian organisms, where mtDNA dynamics can be much more complex (for example, mtDNA recombination in plants). The organism-, tissue-, sequence-, and time-dependent features that cause changes in mtDNA population structure remain poorly understood.
- More diverse biological models, in tandem with more developed quantitative theory, will in future help to reveal the mechanisms shaping these essential populations.

Abbreviations

CMS, cytoplasmic male sterility; DUI, doubly-unipaternal inheritance; mtDNA, mitochondrial DNA.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript

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https://doi.org/10.1042/BST20190238

References

1 Smith, D.R. and Keeling, P.J. (2015) Mitochondrial and plastid genome architecture: reoccurring themes, but significant differences at the extremes. *Proc. Natl Acad. Sci. U.S.A.* **112**, 10177–10184 https://doi.org/10.1073/pnas.1422049112

2 Blanchard, J.L. and Lynch, M. (2000) Organellar genes: why do they end up in the nucleus? *Trends. Genet.* **16**, 315–320 https://doi.org/10.1016/S0168-9525(00)02053-9

3 Hjort, K., Goldberg, A.V., Tsaousis, A.D., Hirt, R.P. and Embley, T.M. (2010) Diversity and reductive evolution of mitochondria among microbial eukaryotes. *Phil. Trans. R. Soc. B* **365**, 713–727 https://doi.org/10.1098/rstb.2009.0224

4 Makiuchi, T. and Nozaki, T. (2014) Highly divergent mitochondrion-related organelles in anaerobic parasitic protozoa. *Biochimie* **100**, 3–17 https://doi.org/10.1016/j.biochi.2013.11.018

5 John, U., Lu, Y., Wohlrab, S., Groth, M., Janouškovec, J., Kohli, G.S. et al. (2019) An aerobic eukaryotic parasite with functional mitochondria that likely lacks a mitochondrial genome. *Sci. Adv.* **5**, eaav1110 (https://doi.org/10.1126/sciadv.aav1110

6 Lavrov, D.V. and Pett, W. (2016) Animal mitochondrial DNA as we do not know it: mt-genome organization and evolution in nonbilaterian lineages. *Genome. Biol. Evol.* **8**, 2896–2913 https://doi.org/10.1093/gbe/evw195

7 Johnston, I.G. (2018) Tension and resolution: dynamic, evolving populations of organelle genomes within plant cells. *Mol. Plant* **12**, 764–783 https://doi.org/10.1016/j.molp.2018.11.002

8 Sloan, D.B., Alverson, A.J., Chuckalovcak, J.P., Wu, M., McCauley, D.E., Palmer, J.D. et al. (2012) Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. *PLoS Biol.* **10**, e1001241 https://doi.org/10.1371/journal.pbio.1001241

9 Burger, G., Gray, M.W., Forget, L. and Lang, B.F. (2013) Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. *Genome Biol. Evol.* **5**, 418–438 https://doi.org/10.1093/gbe/evt008

10 Johnston, I.G. and Williams, B.P. (2016) Evolutionary inference across eukaryotes identifies specific pressures favoring mitochondrial gene retention. *Cell Syst.* **2**, 101–111 https://doi.org/10.1016/j.cels.2016.01.013

11 von Heijne, G. (1986) Why mitochondria need a genome. *FEBS Lett.* **198**, 1–4 https://doi.org/10.1016/0014-5793(86)81172-3

12 Björkholm, P., Harish, A., Hagström, E. , Ernst, A.M. and Andersson, S.G.E. (2015) Mitochondrial genomes are retained by selective constraints on protein targeting. *Proc. Natl Acad. Sci. U.S.A.* **112**, 10154–10161 https://doi.org/10.1073/pnas.1421372112

13 Allen, J.F. (2015) Why chloroplasts and mitochondria retain their own genomes and genetic systems: colocation for redox regulation of gene expression. *Proc. Natl Acad. Sci. U.S.A.* **112**, 10231–10238 https://doi.org/10.1073/pnas.1500012112

14 Saccone, C., Gissi, C., Lanave, C., Larizza, A., Pesole, G. and Reyes, A. (2000) Evolution of the mitochondrial genetic system: an overview. *Gene* **261**, 153–159 https://doi.org/10.1016/S0378-1119(00)00484-4

15 Allen, J.F. and Raven, J.A. (1996) Free-radical-induced mutation vs redox regulation: costs and benefits of genes in organelles. *J. Mol. Evol.* **42**, 482–492 https://doi.org/10.1007/BF02352278

16 Adams, K.L. and Palmer, J.D. (2003) Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* **29**, 380–395 https://doi.org/10.1016/S1055-7903(03)00194-5

17 Lynch, M. (1997) Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. *Mol. Biol. Evol.* **14**, 914–925 https://doi.org/10.1093/oxfordjournals.molbev.a025834

18 Lynch, M. and Blanchard, J.L. (1998) Deleterious mutation accumulation in organelle genomes. *Genetica* **102**, 29–39 https://doi.org/10.1023/A:1017022522486

19 Lynch, M., Koskella, B. and Schaack, S. (2006) Mutation pressure and the evolution of organelle genomic architecture. *Science* **311**, 1727–1730 https://doi.org/10.1126/science.1118884

20 Neiman, M. and Taylor, D.R. (2009) The causes of mutation accumulation in mitochondrial genomes. *Proc. R. Soc. B* **276**, 1201–1209 https://doi.org/10.1098/rspb.2008.1758

21 Palmer, J.D. and Herbon, L.A. (1988) Plant mitochondrial DNA evolved rapidly in structure, but slowly in sequence. *J. Mol. Evol.* **28**, 87–97 https://doi.org/10.1007/BF02143500

22 Wu, Z., Cuthbert, J.M., Taylor, D.R. and Sloan, D.B. (2015) The massive mitochondrial genome of the angiosperm *Silene noctiflora* is evolving by gain or loss of entire chromosomes. *Proc. Natl Acad. Sci. U.S.A.* **112**, 10185–10191 https://doi.org/10.1073/pnas.1421397112

23 Palmer, J.D., Adams, K.L., Cho, Y., Parkinson, C.L., Qiu, Y.-L. and Song, K. (2000) Dynamic evolution of plant mitochondrial genomes: mobile genes and introns and highly variable mutation rates. *Proc. Natl Acad. Sci. U.S.A.* **97**, 6960–6966 https://doi.org/10.1073/pnas.97.13.6960

24 Adams, K.L., Qiu, Y.-L., Stoutemyer, M. and Palmer, J.D. (2002) Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proc. Natl Acad. Sci. U.S.A.* **99**, 9905–9912 https://doi.org/10.1073/pnas.042694899

25 Christensen, A.C. (2014) Genes and junk in plant mitochondria – repair mechanisms and selection. *Genome Biol. Evol.* **6**, 1448–1453 https://doi.org/10.1093/gbe/evu115

26 Taylor, J.W. (1986) Fungal evolutionary biology and mitochondrial DNA. *Exp. Mycol.* **10**, 259–269 https://doi.org/10.1016/0147-5975(86)90011-3

27 Barr, C.M., Neiman, M. and Taylor, D.R. (2005) Inheritance and recombination of mitochondrial genomes in plants, fungi and animals. *New Phytologist* **168**, 39–50 https://doi.org/10.1111/j.1469-8137.2005.01492.x

28 Williamson, D. (2002) The curious history of yeast mitochondrial DNA. *Nat. Rev. Genet.* **3**, 475–481 https://doi.org/10.1038/nrg814

29 Behar, D.M., Rosset, S., Blue-Smith, J., Balanovsky, O., Tzur, S., Comas, D. et al. (2007) The Genographic Project public participation mitochondrial DNA database. *PLoS Genet.* **3**, e104 https://doi.org/10.1371/journal.pgen.0030104

30 Ruiz-Pesini, E., Lott, M.T., Procaccio, V., Poole, J.C., Brandon, M.C., Mishmar, D. et al. (2006) An enhanced MITOMAP with a global mtDNA mutational phylogeny. *Nucleic Acids. Res.* **35**(suppl_1), D823–D828 https://doi.org/10.1093/nar/gkl927

31 Colijn, C., Jones, N., Johnston, I.G., Yaliraki, S. and Barahona, M. (2017) Toward precision healthcare: context and mathematical challenges. *Front. Physiol.* **8**, 136 https://doi.org/10.3389/fphys.2017.00136

32 Okamoto, K. and Shaw, J.M. (2005) Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu. Rev. Genet.* **39**, 503–536 https://doi.org/10.1146/annurev.genet.38.072902.093019

33 Bogenhagen, D.F. (2012) Mitochondrial DNA nucleoid structure. *Biochim. Biophys. Acta* **1819**, 914–920 https://doi.org/10.1016/j.bbagrm.2011.11.005

34 Kukat, C. and Larsson, N.-G. (2013) mtDNA makes a U-turn for the mitochondrial nucleoid. *Trends Cell Biol.* **23**, 457–463 https://doi.org/10.1016/j.tcb.2013.04.009
35 Wallace, D.C. and Chalkia, D. (2013) Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harb. Perspect. Biol.* **5**, a021220 https://doi.org/10.1101/cshperspect.a021220
36 Jakobs, S. and Wurm, C.A. (2014) Super-resolution microscopy of mitochondria. *Curr. Opin. Chem. Biol.* **20**, 9–15 https://doi.org/10.1016/j.cbpa.2014.03.019
37 Poe, B.G., Duffy, C.F., Greminger, M.A., Nelson, B.J. and Arriaga, E.A. (2010) Detection of heteroplasmy in individual mitochondrial particles. *Anal. Bioanal. Chem.* **397**, 3397–3407 https://doi.org/10.1007/s00216-010-3751-3
38 Seguí-Simarro, J.M., Coronado, M.J. and Staehelin, L.A. (2008) The mitochondrial cycle of Arabidopsis shoot apical meristem and leaf primordium meristematic cells is defined by a perinuclear tentaculate/cage-like mitochondrion. *Plant Physiol.* **148**, 1380–1393 https://doi.org/10.1104/pp.108.126953
39 Logan, D.C. (2010) The dynamic plant chondriome. *Semin. Cell Dev. Biol.* **21**, 550–557. Elsevier
40 Logan, D.C. (2010) Mitochondrial fusion, division and positioning in plants. *Biochem. Soc. Trans.* **38**, 789–795 https://doi.org/10.1042/BST0380789
41 Logan, D.C. and Leaver, C.J. (2000) Mitochondria-targeted GFP highlights the heterogeneity of mitochondrial shape, size and movement within living plant cells. *J. Exp. Bot.* **51**, 865–871 https://doi.org/10.1093/jexbot/51.346.865
42 Logan, D.C. (2006) The mitochondrial compartment. *J. Exp. Bot.* **57**, 1225–1243 https://doi.org/10.1093/jxb/erj151
43 Arimura, S-i. (2017 Fission and fusion of plant mitochondria, and genome maintenance. *Plant Physiol.* **176**, 152–161 https://doi.org/10.1104/pp.17.01025
44 Preuten, T., Cincu, E., Fuchs, J., Zoschke, R., Liere, K. and Börner, T. (2010) Fewer genes than organelles: extremely low and variable gene copy numbers in mitochondria of somatic plant cells. *Plant J.* **64**, 948–959 https://doi.org/10.1111/tpj.2010.64.issue-6
45 Chinnery, P.F. and Samuels, D.C. (1999) Relaxed replication of mtDNA: a model with implications for the expression of disease. *Am. J. Hum. Genet.* **64**, 1158–1165 https://doi.org/10.1086/302311
46 Chatre, L. and Ricchetti, M. (2013) Prevalent coordination of mitochondrial DNA transcription and initiation of replication with the cell cycle. *Nucleic Acids Res.* **41**, 3068–3078 https://doi.org/10.1093/nar/gkt015
47 Capps, G.J., Samuels, D.C. and Chinnery, P.F. (2003) A model of the nuclear control of mitochondrial DNA replication. *J. Theor. Biol.* **221**, 565–583 https://doi.org/10.1006/jtbi.2003.3207
48 Johnston, I.G. and Jones, N.S. (2016) Evolution of cell-to-cell variability in stochastic, controlled, heteroplasmic mtDNA populations. *Am. J. Hum. Genet.* **99**, 1150–1162 https://doi.org/10.1016/j.ajhg.2016.09.016
49 Hoitzing, H., Gammage, P.A., Haute, L.V., Minczuk, M., Johnston, I.G., Jones, N.S. et al. (2019) Energetic costs of cellular and therapeutic control of stochastic mtDNA populations. *PLoS Comput. Biol.* **15**, e1007023 https://doi.org/10.1371/journal.pcbi.1007023
50 Aryaman, J., Johnston, I.G. and Jones, N.S. (2018) Mitochondrial heterogeneity. *Front. Genet.* **9**, 718 https://doi.org/10.3389/fgene.2018.00718
51 Pohjoismäki, J.L.O. , Goffart, S., Tyynismaa, H., Willcox, S., Ide, T., Kang, D. et al. (2009) Human heart mitochondrial DNA is organized in complex catenated networks containing abundant four-way junctions and replication forks. *J. Biol. Chem.* **284**, 21446–21457 https://doi.org/10.1074/jbc.M109.016600
52 Woloszynska, M. (2009) Heteroplasmy and stoichiometric complexity of plant mitochondrial genomes-though this be madness, yet there's method in't. *J. Exp. Bot.* **61**, 657–671 https://doi.org/10.1093/jxb/erp361
53 Arrieta-Montiel, M.P. and Mackenzie, S.A. 2011 Plant mitochondrial genomes and recombination. In *Plant Mitochondria* (F. Kempken, ed.), pp. 65–82. Springer, New York
54 Arimura, S-i. (2018) Fission and fusion of plant mitochondria, and genome maintenance. *Plant Physiol.* **176**, 152–161 https://doi.org/10.1104/pp.17.01025
55 Chen, Z., Zhao, N., Li, S., Grover, C.E., Nie, H., Wendel, J.F. et al. (2017) Plant mitochondrial genome evolution and cytoplasmic male sterility. *Crit. Rev. Plant Sci.* **36**, 55–69 https://doi.org/10.1080/07352689.2017.1327762
56 Twig, G., Hyde, B. and Shirihai, O.S. (2008) Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. *Biochim. Biophys. Acta* **1777**, 1092–1097 https://doi.org/10.1016/j.bbabio.2008.05.001
57 Renshaw, E. (2015) *Stochastic Population Processes: Analysis, Approximations, Simulations*, Oxford University Press, Oxford
58 Johnston, I.G. and Jones, N.S. (2015) Closed-form stochastic solutions for non-equilibrium dynamics and inheritance of cellular components over many cell divisions. *Proc. R. Soc. A* **471**, 20150050 https://doi.org/10.1098/rspa.2015.0050
59 Lane, N. (2012) The problem with mixing mitochondria. *Cell* **151**, 246–248 https://doi.org/10.1016/j.cell.2012.09.028
60 Stewart, J.B. and Chinnery, P.F. (2015) The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nat. Rev. Genet.* **16**, 530–542 https://doi.org/10.1038/nrg3966
61 White, D.J., Wolff, J.N., Pierson, M. and Gemmell, N.J. (2008) Revealing the hidden complexities of mtDNA inheritance. *Mol. Ecol.* **17**, 4925–4942 https://doi.org/10.1111/mec.2008.17.issue-23
62 Hale, L.R. and Singh, R.S. (1986) Extensive variation and heteroplasmy in size of mitochondrial DNA among geographic populations of *Drosophila melanogaster*. *Proc. Natl Acad. Sci. U.S.A.* **83**, 8813–8817 https://doi.org/10.1073/pnas.83.22.8813
63 Coyer, J.A., Hoarau, G., Stam, W.T. and Olsen, J.L. (2004) Geographically specific heteroplasmy of mitochondrial DNA in the seaweed, *Fucus serratus* (Heterokontophyta: Phaeophyceae, Fucales). *Mol. Ecol.* **13**, 1323–1326 https://doi.org/10.1111/mec.2004.13.issue-5
64 Barroso, G. and Labarere, J. (1997) Genetic evidence for nonrandom sorting of mitochondria in the basidiomycete *Agrocybe aegerita*. *Appl. Environ. Microbiol.* **63**, 4686–4691
65 Yamato, K.T. and Newton, K.J. (1999) Heteroplasmy and homoplasmy for maize mitochondrial mutants: a rare homoplasmic nad4 deletion mutant plant. *J. Hered.* **90**, 369–373 https://doi.org/10.1093/jhered/90.3.369
66 Steel, D.J., Trewick, S.A. and Wallis, G.P. (2000) Brief communication. Heteroplasmy of mitochondrial DNA in the iphiuroid *Astrobrachion constrictum*. *J. Hered.* **91**, 146–149 https://doi.org/10.1093/jhered/91.2.146
67 Hoarau, G., Holla, S., Lescasse, R., Stam, W.T. and Olsen, J.L. (2002) Heteroplasmy and evidence for recombination in the mitochondrial control region of the flatfish *Platichthys flesus*. *Mol. Biol. Evol.* **19**, 2261–2264 https://doi.org/10.1093/oxfordjournals.molbev.a004049

68 García-Díaz, A., Oya, R., Sánchez, A. and Luque, F. (2003) Effect of prolonged vegetative reproduction of olive tree cultivars (*Olea europaea* L.) in mitochondrial homoplasmy and heteroplasmy. *Genome* **46**, 377–381 https://doi.org/10.1139/g03-017
69 Payne, B.A.I., Wilson, I.J., Yu-Wai-Man, P., Coxhead, J., Deehan, D., Horvath, R. et al. (2012) Universal heteroplasmy of human mitochondrial DNA. *Hum. Mol. Genet.* **22**, 384–390 https://doi.org/10.1093/hmg/dds435
70 Rensch, T., Villar, D., Horvath, J., Odom, D.T. and Flicek, P. (2016) Mitochondrial heteroplasmy in vertebrates using ChIP-sequencing data. *Genome Biol.* **17**, 139 https://doi.org/10.1186/s13059-016-0996-y
71 Rossignol, R., Malgat, M., Mazat, J.-P. and Letellier, T. (1999) Threshold effect and tissue specificity implication for mitochondrial cytopathies. *J. Biol. Chem.* **274**, 33426–33432 https://doi.org/10.1074/jbc.274.47.33426
72 Rocher, C., Taanman, J.-W., Pierron, D., Faustin, B., Benard, G., Rossignol, R. et al. (2008) Influence of mitochondrial DNA level on cellular energy metabolism: implications for mitochondrial diseases. *J. Bioenerg. Biomembr.* **40**, 59–67 https://doi.org/10.1007/s10863-008-9130-5
73 Rossignol, R., Faustin, B., Rocher, C., Malgat, M., Mazat, J.-P. and Letellier, T. (2003) Mitochondrial threshold effects. *Biochem. J.* **370**, 751–762 https://doi.org/10.1042/bj20021594
74 Baris, O.R., Ederer, S., Neuhaus, J.F.G., von Kleist-Retzow, J.-C., Wunderlich, C.M., Pal, M. et al. (2015) Mosaic deficiency in mitochondrial oxidative metabolism promotes cardiac arrhythmia during aging. *Cell Metab.* **21**, 667–677 https://doi.org/10.1016/j.cmet.2015.04.005
75 Wanagat, J., Cao, Z., Pathare, P. and Aiken, J.M. (2001) Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. *FASEB J.* **15**, 322–332 https://doi.org/10.1096/fj.00-0320com
76 Wonnapinij, P., Chinnery, P.F. and Samuels, D.C. (2008) The distribution of mitochondrial DNA heteroplasmy due to random genetic drift. *Am. J. Hum. Genet.* **83**, 582–593 https://doi.org/10.1016/j.ajhg.2008.10.007
77 Craven, C.J. (2016) Evaluation of predictions of the stochastic model of organelle production based on exact distributions. *Elife* **5**, p.e10167 https://doi.org/10.7554/eLife.10167
78 Greiner, S., Sobanski, J. and Bock, R. (2015) Why are most organelle genomes transmitted maternally? *Bioessays* **37**, 80–94 https://doi.org/10.1002/bies.201400110
79 William Birky, Jr, C (2001) The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. *Annu. Rev. Genet.* **35**, 125–148 https://doi.org/10.1146/annurev.genet.35.102401.090231
80 Birky, C.W. (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc. Natl Acad. Sci. U.S.A.* **92**, 11331–11338 https://doi.org/10.1073/pnas.92.25.11331
81 Hoekstra, R.F. (2000) Evolutionary origin and consequences of uniparental mitochondrial inheritance. *Human Reprod.* **15**(suppl_1), 102–111 https://doi.org/10.1093/humrep/15.suppl_2.102
82 Xu, J. (2005) The inheritance of organelle genes and genomes: patterns and mechanisms. *Genome* **48**, 951–958 https://doi.org/10.1139/g05-082
83 Zouros, E., Freeman, K.R., Ball, A.O. and Pogson, G.H. (1992) Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel Mytilus. *Nature* **359**, 412–414 https://doi.org/10.1038/359412a0
84 Passamonti, M., Boore, J.L. and Scali, V. (2003) Molecular evolution and recombination in gender-associated mitochondrial DNAs of the Manila clam Tapes philippinarum. *Genetics* **164**, 603–611
85 Luo, S., Valencia, C.A., Zhang, J., Lee, N.-C., Slone, J., Gui, B. et al. (2018) Biparental inheritance of mitochondrial DNA in humans. *Proc. Natl Acad. Sci. U.S.A.* **115**, 13039–13044 https://doi.org/10.1073/pnas.1810946115
86 Lutz-Bonengel, S. and Parson, W. (2019) No further evidence for paternal leakage of mitochondrial DNA in humans yet. *Proc. Natl Acad. Sci. U.S.A.* **116**, 1821–1822 https://doi.org/10.1073/pnas.1820533116
87 McCauley, D.E. (2013) Paternal leakage, heteroplasmy, and the evolution of plant mitochondrial genomes. *New Phytol.* **200**, 966–977 https://doi.org/10.1111/nph.12431
88 Mogensen, H.L. (1996) The hows and whys of cytoplasmic inheritance in seed plants. *Am. J. Bot.* **83**, 383–404 https://doi.org/10.1002/j.1537-2197.1996.tb12718.x
89 Erickson, L. and Kemble, R. (1990) Paternal inheritance of mitochondria in rapeseed (*Brassica napus*). *Mol. Gen. Genet.* **222**, 135–139 https://doi.org/10.1007/bf00283034
90 Fauré, S., Noyer, J.-L., Carreel, F., Horry, J.-P., Bakry, F. and Lanaud, C. (1994) Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in bananas (*Musa acuminata*). *Curr. Genet.* **25**, 265–269 https://doi.org/10.1007/BF00357172
91 Nagata, N. (2010) Mechanisms for independent cytoplasmic inheritance of mitochondria and plastids in angiosperms. *J. Plant Res.* **123**, 193–199 https://doi.org/10.1007/s10265-009-0293-x
92 Yang, X. and Griffiths, A.J. (1993) Male transmission of linear plasmids and mitochondrial DNA in the fungus Neurospora. *Genetics* **134**, 1055–1062
93 Burgstaller, J.P., Johnston, I.G. and Poulton, J. (2015) Mitochondrial DNA disease and developmental implications for reproductive strategies. *Mol. Hum. Reprod.* **21**, 11–22 https://doi.org/10.1093/molehr/gau090
94 Jenuth, J.P., Peterson, A.C. and Shoubridge, E.A. (1997) Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nat. Genet.* **16**, 93–95 https://doi.org/10.1038/ng0597-93
95 Acton, B.M., Lai, I., Shang, X., Jurisicova, A. and Casper, R.F. (2007) Neutral mitochondrial heteroplasmy alters physiological function in mice. *Biol. Reprod.* **77**, 569–576 https://doi.org/10.1095/biolreprod.107.060806
96 Sharpley, M.S., Marciniak, C., Eckel-Mahan, K., McManus, M., Crimi, M., Waymire, K. et al. (2012) Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. *Cell* **151**, 333–343 https://doi.org/10.1016/j.cell.2012.09.004
97 Takeda, K., Takahashi, S., Onishi, A., Hanada, H. and Imai, H. (2000) Replicative advantage and tissue-specific segregation of RR mitochondrial DNA between C57BL/6 and RR heteroplasmic mice. *Genetics* **155**, 777–783
98 Inoue, K., Ogonuki, N., Yamamoto, Y., Takano, K., Miki, H., Mochida, K. et al. (2004) Tissue-specific distribution of donor mitochondrial DNA in cloned mice produced by somatic cell nuclear transfer. *genesis* **39**, 79–83 https://doi.org/10.1002/(ISSN)1526-968X
99 Burgstaller, J.P., Johnston, I.G., Jones, N.S., Albrechtova, J., Kolbe, T., Vogl, C. et al. (2014) MtDNA segregation in heteroplasmic tissues is common in vivo and modulated by haplotype differences and developmental stage. *Cell Rep.* **7**, 2031–2041 https://doi.org/10.1016/j.celrep.2014.05.020
100 Pan, J., Wang, L., Lu, C., Zhu, Y., Min, Z., Dong, X. et al. (2019) Matching mitochondrial DNA haplotypes for circumventing tissue-specific segregation bias. *iScience* **13**, 371–379 https://doi.org/10.1016/j.isci.2019.03.002

101 de Stordeur, E., Solignac, M., Monnerot, M. and Mounolou, J.-C. (1989) The generation of transplasmic *Drosophila simulans* by cytoplasmic injection effects of segregation and selection on the perpetuation of mitochondrial DNA heteroplasmy. *Mol. Gen. Genet.* **220**, 127–132 https://doi.org/10.1007/BF00260866
102 Solignac, M., Génermont, J., Monnerot, M. and Mounolou, J.-C. (1987) *Drosophila* mitochondrial genetics: evolution of heteroplasmy through germ line cell divisions. *Genetics* **117**, 687–696
103 Ferreira, C.R., Burgstaller, J.P., Perecin, F., Garcia, J.M., Chiaratti, M.R., Méo, S.C. et al. (2010) Pronounced segregation of donor mitochondria introduced by bovine ooplasmic transfer to the female germ-line. *Biol. Reprod.* **82**, 563–571 https://doi.org/10.1095/biolreprod.109.080564
104 Takeda, K., Tasai, M., Iwamoto, M., Akita, T., Tagami, T., Nirasawa, K. et al. (2006) Transmission of mitochondrial DNA in pigs and progeny derived from nuclear transfer of Meishan pig fibroblast cells. *Mol. Reprod. Dev.* **73**, 306–312 https://doi.org/10.1002/(ISSN)1098-2795
105 Cagnone, G., Tsai, T.-S., Srirattana, K., Rossello, F., Powell, D.R., Rohrer, G. et al. (2016) Segregation of naturally occurring mitochondrial DNA variants in a mini-pig model. *Genetics* **202**, 931–944 https://doi.org/10.1534/genetics.115.181321
106 Ma, H. and O'Farrell, P.H. (2016) Selfish drive can trump function when animal mitochondrial genomes compete. *Nat. Genet.* **48**, 798–802 https://doi.org/10.1038/ng.3587
107 Hyslop, L.A., Blakeley, P., Craven, L., Richardson, J., Fogarty, N.M.E., Fragouli, E. et al. (2016) Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. *Nature* **534**, 383–386 https://doi.org/10.1038/nature18303
108 Kang, E., Wu, J., Gutierrez, N.M., Koski, A., Tippner-Hedges, R., Agaronyan, K. et al. (2016) Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. *Nature* **540**, 270–275 https://doi.org/10.1038/nature20592
109 Kang, E., Wu, J., Gutierrez, N.M., Koski, A., Tippner-Hedges, R., Agaronyan, K. et al. (2019) Author Correction: Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. *Nature* **567**, E5–E9 https://doi.org/10.1038/s41586-019-0876-1
110 Røyrvik, E.C., Burgstaller, J.P. and Johnston, I.G. (2016) mtDNA diversity in human populations highlights the merit of haplotype matching in gene therapies. *Mol. Hum. Reprod.* **22**, 809–817 https://doi.org/10.1093/molehr/gaw062
111 Pyle, A., Taylor, R.W., Durham, S.E., Deschauer, M., Schaefer, A.M., Samuels, D.C. et al. (2007) Depletion of mitochondrial DNA in leucocytes harbouring the 3243A>G mtDNA mutation. *J. Med. Genet.* **44**, 69–74 https://doi.org/10.1136/jmg.2006.043109
112 Durham, S.E., Samuels, D.C., Cree, L.M. and Chinnery, P.F. (2007) Normal levels of wild-type mitochondrial DNA maintain cytochrome c oxidase activity for two pathogenic mitochondrial DNA mutations but not for m. 3243A>G. *Am. J. Hum. Genet.* **81**, 189–195 https://doi.org/10.1086/518901
113 Monnot, S., Samuels, D.C., Hesters, L., Frydman, N., Gigarel, N., Burlet, P. et al. (2013) Mutation dependance of the mitochondrial DNA copy number in the first stages of human embryogenesis. *Hum. Mol. Genet.* **22**, 1867–1872 https://doi.org/10.1093/hmg/ddt040
114 Jenuth, J.P., Peterson, A.C., Fu, K. and Shoubridge, E.A. (1996) Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat. Genet.* **14**, 146–151 https://doi.org/10.1038/ng1096-146
115 Brown, D.T., Samuels, D.C., Michael, E.M., Turnbull, D.M. and Chinnery, P.F. (2001) Random genetic drift determines the level of mutant mtDNA in human primary oocytes. *Am. J. Hum. Genet.* **68**, 533–536 https://doi.org/10.1086/318190
116 Chinnery, P.F., Thorburn, D.R., Samuels, D.C., White, S.L., Dahl, H.-H.M., Turnbull, D.M. et al. (2000) The inheritance of mitochondrial DNA heteroplasmy: random drift, selection or both? *Trends Genet.* **16**, 500–505 https://doi.org/10.1016/S0168-9525(00)02120-X
117 Freyer, C., Cree, L.M., Mourier, A., Stewart, J.B., Koolmeister, C., Milenkovic, D. et al. (2012) Variation in germline mtDNA heteroplasmy is determined prenatally but modified during subsequent transmission. *Nat. Genet.* **44**, 1282–1285 https://doi.org/10.1038/ng.2427
118 Stewart, J.B., Freyer, C., Elson, J.L., Wredenberg, A., Cansu, Z., Trifunovic, A. et al. (2008) Strong purifying selection in transmission of mammalian mitochondrial DNA. *PLoS Biol.* **6**, e10 https://doi.org/10.1371/journal.pbio.0060010
119 Fan, W., Waymire, K.G., Narula, N., Li, P., Rocher, C., Coskun, P.E. et al. (2008) A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science* **319**, 958–962 https://doi.org/10.1126/science.1147786
120 Monnot, S., Gigarel, N., Samuels, D.C., Burlet, P., Hesters, L., Frydman, N. et al. (2011) Segregation of mtDNA throughout human embryo fetal development: m. 3243A>G as a model system. *Hum. Mutat.* **32**, 116–125 https://doi.org/10.1002/humu.v32.1
121 Otten, A.B.C., Sallevelt, S.C.E.H., Carling, P.J., Dreesen, J.C.F.M., Drüsedau, M., Spierts, S. et al. (2018) Mutation-specific effects in germline transmission of pathogenic mtDNA variants. *Human Reprod.* **33**, 1331–1341 https://doi.org/10.1093/humrep/dey114
122 Wei, W., Tuna, S., Keogh, M.J., Smith, K.R., Aitman, T.J., Beales, P.L. et al. (2019) Germline selection shapes human mitochondrial DNA diversity. *Science* **364**, eaau6520 https://doi.org/10.1126/science.aau6520
123 Burgstaller, J.P., Kolbe, T., Havlicek, V., Hembach, S., Poulton, J., Piálek, J. et al. (2018) Large-scale genetic analysis reveals mammalian mtDNA heteroplasmy dynamics and variance increase through lifetimes and generations. *Nat. Commun.* **9**, 2488 (https://doi.org/10.1038/s41467-018-04797-2
124 Lonsdale, D.M., Hodge, T.P. and Fauron, C.M.-R. (1984) The physical map and organisation of the mitochondrial genome from the fertile cytoplasm of maize. *Nucleic Acids Res.* **12**, 9249–9261 https://doi.org/10.1093/nar/12.24.9249
125 Palmer, J.D. and Shields, C.R. (1984) Tripartite structure of the *Brassica campestris* mitochondrial genome. *Nature* **307**, 437–440 https://doi.org/10.1038/307437a0
126 Sloan, D.B. (2013) One ring to rule them all? Genome sequencing provides new insights into the 'master circle' model of plant mitochondrial DNA structure. *New Phytol.* **200**, 978–985 https://doi.org/10.1111/nph.12395
127 Chen, J., Guan, R., Chang, S., Du, T., Zhang, H. and Xing, H. (2011) Substoichiometrically different mitotypes coexist in mitochondrial genomes of Brassica napus L. *PLoS ONE* **6**, e17662 https://doi.org/10.1371/journal.pone.0017662
128 Gualberto, J.M. and Newton, K.J. (2017) Plant mitochondrial genomes: dynamics and mechanisms of mutation. *Annu. Rev. Plant Biol.* **68**, 225–252 https://doi.org/10.1146/annurev-arplant-043015-112232
129 Arrieta-Montiel, M., Lyznik, A., Woloszynska, M., Janska, H., Tohme, J. and Mackenzie, S. (2001) Tracing evolutionary and developmental implications of mitochondrial stoichiometric shifting in the common bean. *Genetics* **158**, 851–864
130 Abdelnoor, R.V., Yule, R., Elo, A., Christensen, A.C., Meyer-Gauen, G. and Mackenzie, S.A. (2003) Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. *Proc. Natl Acad. Sci. U.S.A.* **100**, 5968–5973 https://doi.org/10.1073/pnas.1037651100
131 Janska, H., Sarria, R., Woloszynska, M., Arrieta-Montiel, M. and Mackenzie, S.A. (1998) Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. *Plant Cell* **10**, 1163–1180 https://doi.org/10.1105/tpc.10.7.1163

132 Hu, J., Huang, W., Huang, Q., Qin, X., Yu, C., Wang, L. et al. (2014) Mitochondria and cytoplasmic male sterility in plants. *Mitochondrion* **19**, 282–288 https://doi.org/10.1016/j.mito.2014.02.008
133 Touzet, P. and Meyer, E.H. (2014) Cytoplasmic male sterility and mitochondrial metabolism in plants. *Mitochondrion* **19**, 166–171 https://doi.org/10.1016/j.mito.2014.04.009
134 Chen, L. and Liu, Y.-G. (2014) Male sterility and fertility restoration in crops. *Annu. Rev. Plant Biol.* **65**, 579–606 https://doi.org/10.1146/annurev-arplant-050213-040119
135 Carlsson, J., Leino, M., Sohlberg, J., Sundström, J.F. and Glimelius, K. (2008) Mitochondrial regulation of flower development. *Mitochondrion* **8**, 74–86 https://doi.org/10.1016/j.mito.2007.09.006
136 Bohra, A., Jha, U.C., Adhimoolam, P., Bisht, D. and Singh, N.P. (2016) Cytoplasmic male sterility (CMS) in hybrid breeding in field crops. *Plant Cell Rep.* **35**, 967–993 https://doi.org/10.1007/s00299-016-1949-3
137 Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science* **327**, 818–822 https://doi.org/10.1126/science.1183700
138 Contamine, V. and Picard, M. (2000) Maintenance and integrity of the mitochondrial genome: a plethora of nuclear genes in the budding yeast. *Microbiol. Mol. Biol. Rev.* **64**, 281–315 https://doi.org/10.1128/MMBR.64.2.281-315.2000
139 Thrailkill, K.M., Birky, C.W., Lückemann, G. and Wolf, K. (1980) Intracellular population genetics: evidence for random drift of mitochondrial allele frequencies in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Genetics* **96**, 237–262
140 Lorimer, H.E., Brewer, B.J. and Fangman, W.L. (1995) A test of the transcription model for biased inheritance of yeast mitochondrial DNA. *Mol. Cell. Biol.* **15**, 4803–4809 https://doi.org/10.1128/MCB.15.9.4803
141 Karavaeva, I.E., Golyshev, S.A., Smirnova, E.A., Sokolov, S.S., Severin, F.F. and Knorre, D.A. (2017) Mitochondrial depolarization in yeast zygotes inhibits clonal expansion of selfish mtDNA. *J. Cell Sci.* **130**, 1274–1284 https://doi.org/10.1242/jcs.197269
142 Klucnika, A. and Ma, H. (2019) A battle for transmission: the cooperative and selfish animal mitochondrial genomes. *Open Biol.* **9**, 180267 https://doi.org/10.1098/rsob.180267
143 Doolittle, W.F. and Sapienza, C. (1980) Selfish genes, the phenotype paradigm and genome evolution. *Nature* **284**, 601–603 https://doi.org/10.1038/284601a0
144 Gemmell, N.J., Metcalf, V.J. and Allendorf, F.W. (2004) Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends Ecol. Evol.* **19**, 238–244 https://doi.org/10.1016/j.tree.2004.02.002
145 Clark, K.A., Howe, D.K., Gafner, K., Kusuma, D., Ping, S., Estes, S. et al. (2012) Selfish little circles: transmission bias and evolution of large deletion-bearing mitochondrial DNA in *Caenorhabditis briggsae* nematodes. *PLoS ONE* **7**, e41433 https://doi.org/10.1371/journal.pone.0041433
146 Albert, B., Godelle, B., Atlan, A., De Paepe, R. and Gouyon, P.H. (1996) Dynamics of plant mitochondrial genome: model of a three-level selection process. *Genetics* **144**, 369–382
147 Li, M., Schönberg, A., Schaefer, M., Schroeder, R., Nasidze, I. and Stoneking, M. (2010) Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. *Am. J. Hum. Genet.* **87**, 237–249 https://doi.org/10.1016/j.ajhg.2010.07.014
148 Samuels, D.C., Li, C., Li, B., Song, Z., Torstenson, E., Clay, H.B. et al. (2013) Recurrent tissue-specific mtDNA mutations are common in humans. *PLoS Genet.* **9**, e1003929 https://doi.org/10.1371/journal.pgen.1003929
149 Jokinen, R., Marttinen, P., Sandell, H.K., Manninen, T., Teerenhovi, H., Wai, T. et al. (2010) Gimap3 regulates tissue-specific mitochondrial DNA segregation. *PLoS Genet.* **6**, e1001161 https://doi.org/10.1371/journal.pgen.1001161
150 Gómez-Durán, A., Pacheu-Grau, D., López-Gallardo, E., Díez-Sánchez, C., Montoya, J., López-Pérez, M.J. et al. (2010) Unmasking the causes of multifactorial disorders: OXPHOS differences between mitochondrial haplogroups. *Hum. Mol. Genet.* **19**, 3343–3353 https://doi.org/10.1093/hmg/ddq246
151 Moreno-Loshuertos, R., Acín-Pérez, R., Fernández-Silva, P., Movilla, N., Pérez-Martos, A., de Cordoba, S.R. et al. (2006) Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants. *Nat. Genet.* **38**, 1261–1268 https://doi.org/10.1038/ng1897
152 Samuels, D.C., Carothers, A.D., Horton, R. and Chinnery, P.F. (2006) The power to detect disease associations with mitochondrial DNA haplogroups. *Am. J. Hum. Genet.* **78**, 713–720 https://doi.org/10.1086/502682
153 Johnston, I.G. (2016) Multiple hypothesis correction is vital and undermines reported mtDNA links to diseases including AIDS, cancer, and Huntingdon's. *Mitochondrial DNA A* **27**, 3423–3427 https://doi.org/10.3109/19401736.2015.1022732
154 Blier, P.U., Dufresne, F. and Burton, R.S. (2001) Natural selection and the evolution of mtDNA-encoded peptides: evidence for intergenomic co-adaptation. *Trends Genet.* **17**, 400–406 https://doi.org/10.1016/S0168-9525(01)02338-1
155 Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V. and Wallace, D.C. (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* **303**, 223–226 https://doi.org/10.1126/science.1088434
156 Luo, Y., Yang, X. and Gao, Y. (2013) Mitochondrial DNA response to high altitude: a new perspective on high-altitude adaptation. *Mitochondrial DNA* **24**, 313–319 https://doi.org/10.3109/19401736.2012.760558
157 Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A.G., Hosseini, S. et al. (2003) Natural selection shaped regional mtDNA variation in humans. *Proc. Natl Acad. Sci. U.S.A.* **100**, 171–176 https://doi.org/10.1073/pnas.0136972100
158 Wonnapinij, P., Chinnery, P.F. and Samuels, D.C. (2010) Previous estimates of mitochondrial DNA mutation level variance did not account for sampling error: comparing the mtDNA genetic bottleneck in mice and humans. *Am. J. Hum. Genet.* **86**, 540–550 https://doi.org/10.1016/j.ajhg.2010.02.023
159 Zhang, H., Burr, S.P. and Chinnery, P.F. (2018) The mitochondrial DNA genetic bottleneck: inheritance and beyond. *Essays Biochem.* **62**, 225–234 https://doi.org/10.1042/EBC20170096
160 Johnston, I.G., Burgstaller, J.P., Havlicek, V., Kolbe, T., Rülicke, T., Brem, G. et al. (2015) Stochastic modelling, Bayesian inference, and new in vivo measurements elucidate the debated mtDNA bottleneck mechanism. *eLife* **4**, e07464 (https://doi.org/10.7554/eLife.07464
161 Muller, H.J. (1964) The relation of recombination to mutational advance. *Mutat. Res.* **106**, 2–9 https://doi.org/10.1016/0027-5107(64)90047-8
162 Hauswirth, W.W. and Laipis, P.J. (1982) Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. *Proc. Natl Acad. Sci. U.S.A.* **79**, 4686–4690 https://doi.org/10.1073/pnas.79.15.4686
163 Ashley, M.V., Laipis, P.J. and Hauswirth, W.W. (1989) Rapid segregation of heteroplasmic bovine mitochondria. *Nucleic Acids Res.* **17**, 7325–7331 https://doi.org/10.1093/nar/17.18.7325

164 Cree, L.M., Samuels, D.C., de Sousa Lopes, S.C., Rajasimha, H.K., Wonnapinij, P., Mann, J.R. et al. (2008) A reduction of mitochondrial DNA molecules during embryogenesis explains the rapid segregation of genotypes. *Nat. Genet.* **40**, 249–254 https://doi.org/10.1038/ng.2007.63
165 Cao, L., Shitara, H., Horii, T., Nagao, Y., Imai, H., Abe, K. et al. (2007) The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells. *Nat. Genet.* **39**, 386–390 https://doi.org/10.1038/ng1970
166 Wai, T., Teoli, D. and Shoubridge, E.A. (2008) The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. *Nat. Genet.* **40**, 1484–1488 https://doi.org/10.1038/ng.258
167 Wolff, J.N., White, D.J., Woodhams, M., White, H.E. and Gemmell, N.J. (2011) The strength and timing of the mitochondrial bottleneck in salmon suggests a conserved mechanism in vertebrates. *PLoS ONE* **6**, e20522 https://doi.org/10.1371/journal.pone.0020522
168 Marchington, D.R., Hartshorne, G.M., Barlow, D. and Poulton, J. (1997) Homopolymeric tract heteroplasmy in mtDNA from tissues and single oocytes: support for a genetic bottleneck. *Am. J. Hum. Genet.* **60**, 408–416
169 Li, M., Rothwell, R., Vermaat, M., Wachsmuth, M., Schröder, R., Laros, J.F.J. et al. (2016) Transmission of human mtDNA heteroplasmy in the Genome of the Netherlands families: support for a variable-size bottleneck. *Genome Res.* **26**, 417–426 https://doi.org/10.1101/gr.203216.115
170 Floros, V.I., Pyle, A., Dietmann, S., Wei, W., Tang, W.W.C., Irie, N. et al. (2018) Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos. *Nat. Cell Biol.* **20**, 144–151 https://doi.org/10.1038/s41556-017-0017-8
171 Rebolledo-Jaramillo, B., Su, M.S.-W., Stoler, N., McElhoe, J.A., Dickins, B., Blankenberg, D. et al. (2014) Maternal age effect and severe germ-line bottleneck in the inheritance of human mitochondrial DNA. *Proc. Natl Acad. Sci. U.S.A.* **111**, 15474–15479 https://doi.org/10.1073/pnas.1409328111
172 Wilton, P.R., Zaidi, A., Makova, K. and Nielsen, R. (2018) A population phylogenetic view of mitochondrial heteroplasmy. *Genetics* **208**, 1261–1274 https://doi.org/10.1534/genetics.118.300711
173 Otten, A.B.C., Theunissen, T.E.J. , Derhaag, J.G., Lambrichs, E.H., Boesten, I.B.W., Winandy, M. et al. (2016) Differences in strength and timing of the mtDNA bottleneck between zebrafish germline and non-germline cells. *Cell Rep.* **16**, 622–630 https://doi.org/10.1016/j.celrep.2016.06.023
174 Cotterill, M., Harris, S.E., Fernandez, E.C., Lu, J., Huntriss, J.D., Campbell, B.K. et al. (2013) The activity and copy number of mitochondrial DNA in ovine oocytes throughout oogenesis in vivo and during oocyte maturation in vitro. *Mol. Hum. Reprod.* **19**, 444–450 https://doi.org/10.1093/molehr/gat013
175 Jokinen, R. and Battersby, B.J. (2013) Insight into mammalian mitochondrial DNA segregation. *Ann. Med.* **45**, 149–155 https://doi.org/10.3109/07853890.2012.693190
176 Lanfear, R. (2018) Do plants have a segregated germline? *PLoS Biol.* **16**, e2005439 https://doi.org/10.1371/journal.pbio.2005439
177 Schmidt, A., Schmid, M.W. and Grossniklaus, U. (2015) Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. *Development* **142**, 229–241 https://doi.org/10.1242/dev.102103
178 Suzuki, T., Kawano, S., Sakai, A., Hirai, A. and Kuroiwa, T. (1996) Variability of mitochondrial subgenomic molecules in the meristematic cells of higher plants. *Genes Genet. Syst.* **71**, 329–333 https://doi.org/10.1266/ggs.71.329
179 Atlan, A. and Couvet, D. (1993) A model simulating the dynamics of plant mitochondrial genomes. *Genetics* **135**, 213–222
180 Birky, C.W., Strausberg, R.L., Forster, J.L. and Perlman, P.S. (1978) Vegetative segregation of mitochondria in yeast: estimating parameters using a random model. *Mol. Gen. Genet.* **158**, 251–261 https://doi.org/10.1007/BF00267196
181 Birky, C.W. (1983) The partitioning of cytoplasmic organelles at cell division. *Int. Rev. Cytol. Suppl.* **15**, 49–89
182 Basse, C.W. (2010) Mitochondrial inheritance in fungi. *Curr. Opin. Microbiol.* **13**, 712–719 https://doi.org/10.1016/j.mib.2010.09.003
183 Jajoo, R., Jung, Y., Huh, D., Viana, M.P., Rafelski, S.M., Springer, M. et al. (2016) Accurate concentration control of mitochondria and nucleoids. *Science* **351**, 169–172 https://doi.org/10.1126/science.aaa8714
184 Wilson, I.J., Carling, P.J., Alston, C.L., Floros, V.I., Pyle, A., Hudson, G. et al. (2016) Mitochondrial DNA sequence characteristics modulate the size of the genetic bottleneck. *Hum. Mol. Genet.* **25**, 1031–1041 https://doi.org/10.1093/hmg/ddv626
185 Lehtinen, S.K., Hance, N., El Meziane, A., Juhola, M.K., Juhola, K.M.I., Karhu, R. et al. (2000) Genotypic stability, segregation and selection in heteroplasmic human cell lines containing np 3243 mutant mtDNA. *Genetics* **154**, 363–380
186 Raap, A.K., Tafrechi, R.S.J., van de Rijke, F.M., Pyle, A., Wählby, C., Szuhai, K. et al. (2012) Non-random mtDNA segregation patterns indicate a metastable heteroplasmic segregation unit in m. 3243A>G cybrid cells. *PLoS ONE* **7**, e52080 https://doi.org/10.1371/journal.pone.0052080
187 Friedberg, E.C., Walker, G.C., Siede, W. and Wood, R.D. (2005) *DNA Repair and Mutagenesis*, American Society for Microbiology Press, Washington
188 Wright, A.F., Murphy, M.P. and Turnbull, D.M. (2009) Do organellar genomes function as long-term redox damage sensors?. *Trends Genet.* **25**, 253–261 https://doi.org/10.1016/j.tig.2009.04.006
189 Itsara, L.S., Kennedy, S.R., Fox, E.J., Yu, S., Hewitt, J.J., Sanchez-Contreras, M. et al. (2014) Oxidative stress is not a major contributor to somatic mitochondrial DNA mutations. *PLoS Genet.* **10**, e1003974 https://doi.org/10.1371/journal.pgen.1003974
190 Kennedy, S.R., Salk, J.J., Schmitt, M.W. and Loeb, L.A. (2013) Ultra-sensitive sequencing reveals an age-related increase in somatic mitochondrial mutations that are inconsistent with oxidative damage. *PLoS Genet.* **9**, e1003794 https://doi.org/10.1371/journal.pgen.1003794
191 Ameur, A., Stewart, J.B., Freyer, C., Hagström, E., Ingman, M., Larsson, N.-G. et al. (2011) Ultra-deep sequencing of mouse mitochondrial DNA: mutational patterns and their origins. *PLoS Genet.* **7**, e1002028 https://doi.org/10.1371/journal.pgen.1002028
192 Sallevelt, S.C.E.H., de Die-Smulders, C.E.M., Hendrickx, A.T.M., Hellebrekers, D.M.E.I., de Coo, I.F.M., Alston, C.L. et al. (2017) De novo mtDNA point mutations are common and have a low recurrence risk. *J. Med. Genet.* **54**, 73–83 https://doi.org/10.1136/jmedgenet-2016-103876
193 Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E. et al. (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**, 417–423 https://doi.org/10.1038/nature02517
194 Khrapko, K., Kraytsberg, Y., De Grey, A.D., Vijg, J. and Schon, E.A. (2006) Does premature aging of the mtDNA mutator mouse prove that mtDNA mutations are involved in natural aging? *Aging Cell* **5**, 279–282 https://doi.org/10.1111/ace.2006.5.issue-3
195 Berridge, M.V., McConnell, M.J., Grasso, C., Bajzikova, M., Kovarova, J. and Neuzil, J. (2016) Horizontal transfer of mitochondria between mammalian cells: beyond co-culture approaches. *Curr. Opin. Genet. Dev.* **38**, 75–82 https://doi.org/10.1016/j.gde.2016.04.003
196 Sinha, P., Islam, M.N., Bhattacharya, S. and Bhattacharya, J. (2016) Intercellular mitochondrial transfer: bioenergetic crosstalk between cells. *Curr. Opin. Genet. Dev.* **38**, 97–101 https://doi.org/10.1016/j.gde.2016.05.002
197 Torralba, D., Baixauli, F. and Sánchez-Madrid, F. (2016) Mitochondria know no boundaries: mechanisms and functions of intercellular mitochondrial transfer. *Front. Cell. Dev. Biol.* **4**, 107 https://doi.org/10.3389/fcell.2016.00107

Biochemical Society Transactions (2019) **47** 1367–1382
https://doi.org/10.1042/BST20190238

198 Tan, A.S., Baty, J.W., DongL.-F., Bezawork-Geleta, A., Endaya, B., Goodwin, J. et al. (2015) Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell. Metab.* **21**, 81–94 https://doi.org/10.1016/j.cmet.2014.12.003
199 Spees, J.L., Olson, S.D., Whitney, M.J. and Prockop, D.J. (2006) Mitochondrial transfer between cells can rescue aerobic respiration. *Proc. Natl Acad. Sci. U.S.A.* **103**, 1283–1288 https://doi.org/10.1073/pnas.0510511103
200 Jayaprakash, A.D., Benson, E.K., Gone, S., Liang, R., Shim, J., Lambertini, L. et al. (2015) Stable heteroplasmy at the single-cell level is facilitated by intercellular exchange of mtDNA. *Nucleic Acids. Res.* **43**, 2177–2187 https://doi.org/10.1093/nar/gkv052
201 Kimura, M. (1955) Solution of a process of random genetic drift with a continuous model. *Proc. Natl Acad. Sci. U.S.A.* **41**, 144–150 https://doi.org/10.1073/pnas.41.3.144
202 Wright, S. (1942) Statistical genetics and evolution. *Bull. Am. Math. Soc.* **48**, 223–247 https://doi.org/10.1090/S0002-9904-1942-07641-5
203 Tam, Z.Y., Gruber, J., Halliwell, B. and Gunawan, R. (2013) Mathematical modeling of the role of mitochondrial fusion and fission in mitochondrial DNA maintenance. *PLoS ONE* **8**, e76230 https://doi.org/10.1371/journal.pone.0076230
204 Tam, Z.Y., Gruber, J., Halliwell, B. and Gunawan, R. (2015) Context-dependent role of mitochondrial fusion-fission in clonal expansion of mtDNA mutations. *PLoS Comput. Biol.* **11**, e1004183 https://doi.org/10.1371/journal.pcbi.1004183
205 Mouli, P.K., Twig, G. and Shirihai, O.S. (2009) Frequency and selectivity of mitochondrial fusion are key to its quality maintenance function. *Biophys. J.* **96**, 3509–3518 https://doi.org/10.1016/j.bpj.2008.12.3959
206 Poovathingal, S.K., Gruber, J., Halliwell, B. and Gunawan, R. (2009) Stochastic drift in mitochondrial DNA point mutations: a novel perspective ex silico. *PLoS Comput. Biol.* **5**, e1000572 https://doi.org/10.1371/journal.pcbi.1000572
207 Hoitzing, H., Johnston, I.G. and Jones, N.S. (2017) Stochastic models for evolving cellular populations of mitochondria: disease, development, and ageing. In *Stochastic Processes, Multiscale Modeling, and Numerical Methods for Computational Cellular Biology* (D. Holcman, ed.), pp. 287–314. Springer, Cham
208 Mukherji, S. and O'Shea, E.K. (2014) Mechanisms of organelle biogenesis govern stochastic fluctuations in organelle abundance. *Elife* **3**, p.e02678 https://doi.org/10.7554/eLife.02678
209 Marshall, W.F. (2007) Stability and robustness of an organelle number control system: modeling and measuring homeostatic regulation of centriole abundance. *Biophys. J.* **93**, 1818–1833 https://doi.org/10.1529/biophysj.107.107052
210 Huh, D. and Paulsson, J. (2011) Non-genetic heterogeneity from stochastic partitioning at cell division. *Nature Genet.* **43**, 95–100 https://doi.org/10.1038/ng.729
211 Radzvilavicius, A.L. and Blackstone, N.W. (2015) Conflict and cooperation in eukaryogenesis: implications for the timing of endosymbiosis and the evolution of sex. *J. R. Soc. Interface* **12**, 20150584 https://doi.org/10.1098/rsif.2015.0584
212 Hadjivasiliou, Z., Pomiankowski, A., Seymour, R.M. and Lane, N. (2012) Selection for mitonuclear co-adaptation could favour the evolution of two sexes. *Proc. R. Soc. B* **279**, 1865–1872 https://doi.org/10.1098/rspb.2011.1871
213 Radzvilavicius, A.L., Kokko, H. and Christie, J.R. (2017) Mitigating mitochondrial genome erosion without recombination. *Genetics* **207**, 1079–1088 https://doi.org/10.1534/genetics.117.300273
214 Radzvilavicius, A.L., Hadjivasiliou, Z., Pomiankowski, A. and Lane, N. (2016) Selection for mitochondrial quality drives evolution of the germline. *PLoS Biol.* **14**, e2000410 https://doi.org/10.1371/journal.pbio.2000410