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Mother's curse: the effect of mtDNA on individual fitness and population viability

Neil J. Gemmell[1], Victoria J. Metcalf[1] and Fred W. Allendorf[2]

[1]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
[2]Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

The mitochondrial genome is considered generally to be an innocent bystander in adaptive evolution; however, there is increasing evidence that mitochondrial DNA (mtDNA) is an important contributor to viability and fecundity. Some of this evidence is now well documented, with mtDNA mutations having been shown to play a causal role in degenerative diseases, ageing, and cancer. However, most research on mtDNA has ignored the possibility that other instances exist where mtDNA mutations could have profound fitness consequences. Recent work in humans and other species now indicates that mtDNA mutations play an important role in sperm function, male fertility, and male fitness. Ironically, deleterious mtDNA mutations that affect only males, such as those that impair sperm function, will not be subject to natural selection because mitochondria are generally maternally inherited and could reach high frequencies in populations if the mutations are not disadvantageous in females. Here, we review how such mtDNA mutations might affect the viability of natural populations. We consider factors that increase or decrease the strength of the effect of mtDNA mutations on population viability and discuss what mechanisms exist to mitigate deleterious mtDNA effects.

The mitochondrial genome is one of the cornerstones of modern evolutionary genetics and, for two decades, it has been widely used to reconstruct genealogies and describe population genetic structure [1]. Selective neutrality was assumed initially for mitochondrial DNA (mtDNA), but it is now clear that selection acts upon it at multiple levels [2]. Mitochondrial DNA encodes 13 out of ~85 components of the oxidative phosphorylation (OXPHOS) system crucial for aerobic respiration [3]. In humans, mutations in mtDNA have been implicated in several degenerative diseases, ageing, cancer [3], and, more recently, infertility [4,5], which raises the important question of whether pervasive, but subtle, mtDNA fitness variation is common within natural populations.

Comparative analyses of mtDNA sequence data for several species have found that the ratio of non-silent to silent polymorphisms within species is significantly greater than the ratio of non-silent to silent fixed differences between species [6–8]. This suggests that a substantial proportion of intraspecific diversity in mtDNA results from slightly deleterious mutations that become fixed within a species only rarely [6–8]. The high frequency of deleterious mtDNA alleles within species is thought to result from four primary characteristics of the mitochondrial genome: (i) high mutation rate; (ii) lack of recombination; (iii) reduced effective population size; and (iv) maternal transmission.

Mitochondrial DNA mutation rates are high, probably as a consequence of the deleterious effects of oxidative stress and inefficient DNA repair. Thus, new mtDNA alleles are being generated continuously [2]. These alleles persist, even if they are slightly deleterious, because the lack of recombination in mtDNA leads to an inevitable accumulation of LINKAGE DISEQUILIBRIUM (See Glossary), which partially conceals genetic variation for fitness at individual loci, reducing the efficacy of natural selection [9]. Further, the EFFECTIVE POPULATION SIZE of the

Glossary

Effective population size (N_e): size of an ideal population that would lose heterozygosity at a rate equal to that of the observed population.
Genetic load: the reduction in mean fitness of members of a population owing to deleterious genes, or gene combinations, in the population.
Genetic rescue: the restoration of genetic variation by migration that leads to a reduction in inbreeding depression.
Heteroplasmy: having more than one type of mtDNA molecule present in a cell.
Heterogametic: the sex having two different sex chromosomes (e.g. XY in mammals, ZW in birds).
Linkage disequilibrium: nonrandom association between genes.
Muller's ratchet: irreversible accumulation of deleterious mutations in small populations, resulting in an increase of the mutational load, owing to a ratchetlike loss of the least mutated class as a consequence of genetic drift.
Mutational meltdown: the decline in population size, spiraling downwards to zero, because of chance fixation of deleterious mutations.
Population viability: the probability that a sufficient number of individuals will survive to reproductive age to ensure continued existence of a group of organisms.
Sexually antagonistic fitness: the situation wherein a gene has a strong positive fitness effect in one sex, while having demonstrably negative fitness effects in the other sex.
Sperm competition: in species where females mate with more than one male, competition occurs between ejaculates within the female reproductive tract or between sperm of different males in external fertilizing species. Specific behaviours and morphological structures have evolved to improve a male's chances in this sperm competition.
Sperm motility: the proportion of sperm that are motile in an ejaculate.
Threshold effect: a certain level of mutant mitochondrial genomes required in a particular tissue for disease to be evident according to oxidative requirements.

Corresponding author: Neil J. Gemmell (neil.gemmell@canterbury.ac.nz).

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mitochondrial genome is generally only a quarter that of the nuclear genome because of haploidy and maternal transmission. Therefore, mtDNA mutations are much more sensitive to genetic drift and population bottlenecks than are nuclear mutations [10]. In addition, changes in allele frequency are determined primarily by genetic drift rather than by natural selection when the product of the effective population size and the selection coefficient is less than one [11] because chance effects outweigh those of selection. Therefore, natural selection will be much less effective at removing deleterious mutations in the mitochondrial genome than it is in the nuclear one.

The mitochondrial genome is transmitted only through the maternal lineage in most species, which creates a male-female asymmetry in the expected severity of mitochondrial mutations [12]. Deleterious mtDNA mutations that affect only males will not be subject to natural selection. Therefore, regardless of the selective pressure in males, mtDNA mutations that are neutral or nearly neutral in females can reach high frequencies in populations [12].

The accumulation of mildly deleterious mutations is a major extinction risk for asexually reproducing entities, such as mtDNA [9]. In the absence of recombination, there will be a progressive loss of fitness (MULLER'S RATCHET) that can be avoided only if back or compensatory mutations arise at a rate that balances or offsets the cumulative effects of the deleterious mutations. The continued fixation of deleterious mutations is expected to lead to MUTATIONAL MELTDOWN, wherein the reproductive rate of a population will decline, eventually spiraling downwards to zero. The accumulation of deleterious mutations in mtDNA is not expected to increase the probability of species extinction, except over very long periods of time when the effective population size exceeds several thousands of individuals [9]. However, this argument fails to consider how mtDNA mutations with asymmetrical fitness effects, such as those that alter male but not female fertility, affect POPULATION VIABILITY.

Human fertility researchers provided the first definitive evidence that small mutations in mtDNA could reduce both sperm function and male fertility parameters, while having apparently little or no effect on females [4]. That mtDNA might have a different fitness effect in males versus females is also supported by recent work that identified SEXUALLY ANTAGONISTIC cytoplasmic nuclear interactions in *Drosophila melanogaster*, where the cytoplasmic mtDNAs that were 'good' in females were 'bad' in males [13,14]. Together, these results present the possibility that the viability of small populations might be reduced significantly by increases in the frequency of mtDNA genotypes that lower male fertility and, consequently, fitness [15]. Here, we evaluate the possible effects of mutations on the viability of populations. We review new data on male fertility and SPERM COMPETITION that strengthen our original suggestion that mtDNA mutations can decrease population viability [15].

The role of mtDNA mutations in human male fertility

There is increasing evidence for an association between mtDNA mutations and male infertility. These mutations range from rare, massive 4.9 Kb and 7.4 Kb deletions [16–18], through to frequent, point mutations in OXPHOS genes [5,16,18,19]. St John *et al.* observed that men suffering from sperm abnormalities harboured high numbers of mtDNA deletions in their spermatozoa [16]. Likewise, in comparisons to the Cambridge mtDNA reference sequence [20], Holyoake *et al.* observed a threefold increase (range = 0–10) in nucleotide substitutions in subfertile versus fertile men for a 7 Kb region of the mtDNA that encodes key elements of the OXPHOS pathway [5].

The wealth of data gathered from work on human infertility supports the idea that sperm dysfunction might arise through mtDNA mutations that result in the formation of an incomplete electron transport chain in the OXPHOS pathway [18,21]. Collectively, such studies suggest: (i) infertility arises most frequently through the gradual accumulation of point mutations in mtDNA (Muller's ratchet), rather than through a single specific point mutation [5,16]; and (ii) infertility is not generally observed unless the mutant mtDNA is the most common haplotype present in the gametic tissue of the individual [18,22] because of the THRESHOLD EFFECT [3] (Figure 1, Box 1).

Studies of human infertility have implicated the mitochondrion and its genome regularly as a contributor to low sperm counts and MOTILITY [18,21]. However, no study of human male infertility has been able to assign a fitness component to the level of reproductive impairment observed with a given mtDNA mutation. Fortunately, there is considerable literature on sperm competition in animal models, which provides some useful insights into the factors that contribute to the differences in male reproductive success [23–28] and how this might be affected by mtDNA mutations.

Evidence from other species

Sperm motility in many species is correlated with mitochondrial numbers and metabolic function [28–30]. For instance, sperm midpiece volume (an indicator of mitochondrial numbers) is significantly greater in primate species in which sperm competition is predicted [28]. However, it is only recently that significant attention has focused on whether mtDNA mutations underlie the differences in sperm size, motility, and fertility observed in animals.

Several studies have lent support to the notion that maternal factors, such as mtDNA, underlie the phenotypic differences observed in sperm. For example, sperm size in the black field cricket, *Gryllus bimaculatus* [26] and yellow dung fly, *Scatophaga stercoraria* [27] responded to selection only when the maternal lineage was selected. Both studies concluded that the genes responsible for the variation in sperm size resided probably on the maternally inherited X chromosome, although a role for mtDNA could not be excluded [26,27]. Although neither study demonstrated that the phenotypic differences observed had any detectable bearing on male fertilization success or fitness, both showed that it is possible for maternal genes to have a strong bearing on male reproductive traits.

More compelling evidence that mtDNA might influence sperm function and, consequently, male fitness comes from research on sperm competition in domestic fowl,

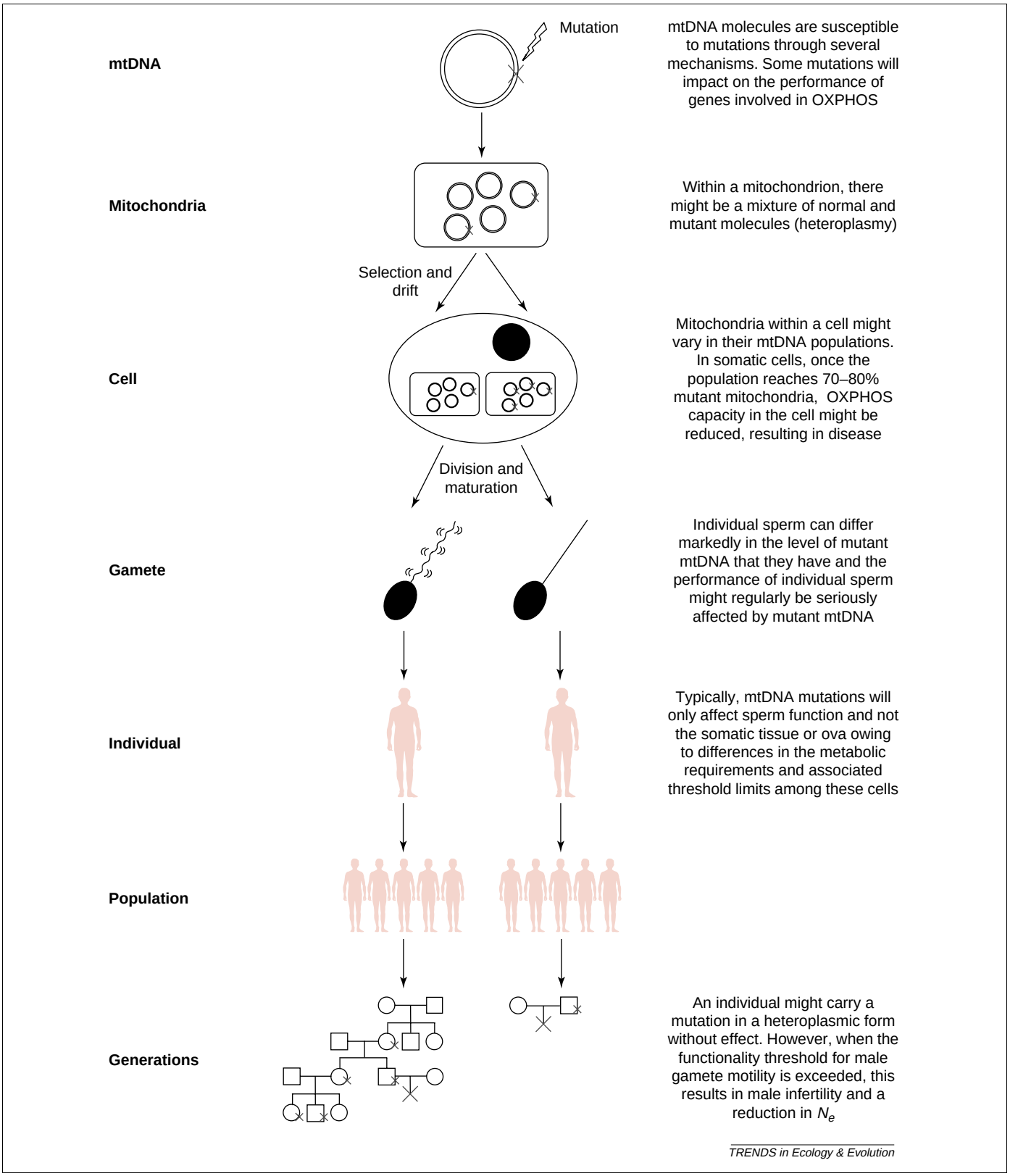

Figure 1. The nested hierarchy of mtDNA. Mitochondrial DNA (mtDNA) exists in a nested hierarchy of populations within an individual, which offers some protection from deleterious mtDNA mutations. When mtDNA mutations occur, they are generally heteroplasmic within a mitochondrion. Drift can alter the proportion of mutant and normal mtDNA in different mitochondria, even within the same cell. Sperm, which have very low numbers of mitochondria (*c.* 10–20) and very high metabolic demands, will suffer greatly from the random sorting of mutant and normal mtDNA. By contrast, the oocyte and somatic cells have lower energy requirements and higher numbers of mtDNA than sperm. Consequently, the selective disadvantage of mtDNA mutations might be much greater for males than for females. A male carrying mutant mtDNA will suffer lowered fertility and might sire few or no offspring. A female carrying mutant mtDNA will be unaffected and will continue to pass the mutation on to future generations, affecting the fertility and viability of her male offspring.

Box 1. The threshold effect and sperm dysfunction

Individuals are afforded some level of protection from deleterious mtDNA mutations because mtDNA exists in a nested hierarchy (Figure 1, main text), with 10–20 mtDNAs within each mitochondrion, 10^4–10^5 mitochondria in each cell, and multiple cells in each organ [2]. Mutational pressures will introduce mtDNA variation (HETEROPLASMY), but deleterious mutations generally will not have fitness consequences unless selection and drift act to increase the frequency of the mutation across the full nested hierarchy of mtDNA [2,40]. The point at which deleterious mutations have fitness effects is known as the threshold effect. (Figure 1).

The threshold effect is an important component of mitochondrial diseases, where the disease state generally is not observed until ~80% of the 10^4–10^5 mtDNA present in each cell have the mutation. In sperm, where there might be only a few mitochondria powering the cell [18], there is considerable opportunity for a functionality threshold to be exceeded. In such circumstances, it is expected that even low proportions (10–20%) of deleterious mtDNA in the gametic tissue of an individual could have a major effect on the reproductive performance of an individual through the production of a gametic population of lower average fitness. It might also explain why the performance of sperm in a single ejaculate is so variable [25,32].

To understand how threshold effects influence an individual's sperm function, it is necessary to know more about how mtDNA sort during spermatogenesis. Compensatory mechanisms are observed frequently in mitochondrial pathology and should also be promoted strongly in spermatogenesis. Normally, spermatogenesis is associated with a reduction in mtDNA content, so that each of the ~20 mitochondria in a mature human sperm contains only one copy of mtDNA [18,41,42], although the actual copy number of mtDNA in mature spermatozoa remains equivocal [41,42]. However, because sperm maturation is an energy-intensive process, maturing sperm compensate by carrying more mitochondria forward through the maturation divisions when their mitochondria are behaving erroneously. Two independent studies support this contention. May-Panloup *et al.* used real-time PCR to show that subfertile males have 4–7 times as many mtDNA molecules per sperm than do fertile men [42], whereas Díez-Sánchez *et al.* used Southern hybridization to demonstrate that non-motile sperm contain ~2 times more mtDNA than do motile sperm [41]. Both groups concluded that this increase might be a compensatory mechanism for decreased respiratory chain activity resulting from mtDNA mutations. If this mtDNA-content upregulation is observed in other species, it might well prove to be a useful indicator of individuals within populations that are suffering the effect of mtDNA mutations.

Gallus gallus domesticus [24,25,31]. Key among these studies, Froman *et al.* showed that sperm motility, a trait known to strongly determine fertilization success [31], was influenced strongly by maternal genes [25]. Maternal influence on sperm traits had been suggested previously to act either through the X chromosome [26,27] or through mtDNA [4,17]. However, the female is the HETEROGAMETIC sex in birds. Therefore, the only molecule that could be specifically transmitted from mother to son is the mtDNA, implying strongly a role for mtDNA in sperm function and male fitness in birds.

The evolutionary implications of mtDNA control over male reproductive traits are significant. The inability for selection to eliminate maternally inherited mtDNA haplotypes that affect male, but not female, fitness [12] can also explain why high variability in sperm morphology and performance can be maintained in populations despite strong, consistent, directional selection [25,32].

mtDNA and population viability

The potential relationship between male infertility and sperm competition is significant for ecology and evolution. The cellular connection between mitochondrial mutations and reduced male fertility is now established clearly [4,5,16,18]. However, the implications of this association for evolution, population biology, and conservation remain largely unexplored. Here, we explore what life-history characteristics make species more or less vulnerable to mtDNA mutations that affect male reproductive fitness and what mechanisms, if any, exist to circumvent the deleterious effect of these mutations.

mtDNA mutations detrimental to male fitness can reach high frequencies

Genetic drift and inbreeding depression in small populations can result in reduced population viability [33,34]. The observed reductions in survival, fertility, and physiological vigor associated with inbred populations are attributed usually to increased homozygosity of deleterious, recessive alleles in the diploid nuclear genome. However, the link between mtDNA and infertility establishes firmly a mechanism through which the haploid mtDNA can play a role in this effect [15].

There is compelling evidence to support the notion that mtDNA mutations that are potentially detrimental to male fitness can reach high frequencies. For example, human mtDNA haplogroup T [20] is observed at a frequency of 20% in some European populations, despite exhibiting a 20% reduction in mitochondrial metabolic performance and a 27% reduction in sperm mobility when compared with the most common and best performing human mtDNA haplogroup H [4].

The significance of mtDNA mutations that affect male fertility in natural populations is predicted to depend upon the frequency of mutations in which selection pressure on males is greater than that on females. Detrimental mtDNA mutations can be sustained at a high frequency in a population only if their effects in females are, at most, mild, and it is now clear that mtDNA content in the oocyte might affect female fertility and fitness [35]. However, we expect that selection asymmetry for mtDNA will occur between males and females in many taxa because (i) oocytes have many mitochondria per cell and higher levels of gamete quality control, and (ii) female gametes and gametogenesis have very low energetic requirements when compared with their male counterparts.

mtDNA mutations detrimental to male fitness can reduce population viability

The importance of mtDNA mutations that affect male fertility will depend ultimately on whether the reduction in male fertility and fitness they confer reduces the viability of the population (Box 2). The strength of this effect will depend on the mating system and reproductive biology of the particular population, but it seems probable that a reduction in male fertility will lower the number of progeny produced under a wide array of circumstances. At a minimum, the presence of mtDNA genotypes that reduce the fertility of some males would increase the variability in male reproductive success and thereby decrease effective

Box 2. mtDNA and the inbreeding effect

Inbreeding depression is the reduction in fitness of individuals resulting from matings between related individuals. Mating between related individuals will occur even in randomly mating small populations simply because all or most individuals within a small population will be related. This has been called the 'inbreeding effect' of small populations and sometimes contributes to reduced viability of small populations [43].

The viability of small populations could also be affected by mtDNA. However, the effects of mtDNA on the viability on small populations cannot result from inbreeding because mtDNA is haploid. Instead, genetic drift in small populations might cause the increase in frequency or fixation of mtDNA molecules that contain deleterious mutations. These mutations could have phenotypic effects (including impaired male fertility) that result in a genetic load, wherein a population has reduced fitness relative to the population from which it originated.

It is crucial to determine how much of the genetic load in small populations is due to mtDNA because different management actions are needed to restore fitness for mtDNA versus nuclear-based genetic load. The reduction in nuclear heterozygosity caused by inbreeding can be restored easily by even modest amounts of male or female gene flow, which can lead to increased heterozygosity and result in GENETIC RESCUE [43]. However, the introduction of males into a small population will have no effect on any genetic load resulting from mtDNA. Restoring the genetic load due to mtDNA will require the actual replacement of the mtDNA causing reduced fitness, which will require a greater amount of gene flow and will be much slower because of the lower variance in reproductive success of females.

The question remains as to how much of the increased genetic load in small populations can be readily attributed to mtDNA versus nuclear mutations. We are unaware of any available data to determine how much of the genetic load of small populations results from mtDNA. However, there are several experimental approaches that could be taken to answer this question. In some model species, it has been shown that the introduction of a few migrants into a small population can have a rescue effect [44]. The results of such controlled introductions could be compared for males and females to determine the contribution of mtDNA to genetic load. In some species, it is also possible to create groups of individuals that have identical nuclear genotypes but different mtDNA genotypes. Fitness measurements of lines of individuals with the same nuclear background and different mtDNA types would provide information about the role of mtDNA in fitness reductions.

population size. This would increase the rate of loss of heterozygosity, augmenting the other effects of inbreeding depression that might reduce population viability [33,34]. In addition, there might be some selective removal of mtDNA genotypes by population selection if populations in which poorly functioning mtDNA genotypes reach high frequencies are more susceptible to extinction.

Data from sperm competition research provide additional support for this theory. In a series of artificial insemination experiments in which all variables apart from sperm motility were controlled, sperm motility was shown to be the primary determinant of fertilization success in the domestic fowl under conditions of sperm competition [31]. Given that sperm motility is influenced strongly by maternal genes [25], most probably mtDNA [4], these data suggest that mtDNA mutations could result in reductions in male fertilization success, which would lower population viability if a large percentage of the population harboured these mutations.

The inability for selection to eliminate maternally inherited mtDNA haplotypes that only impair sperm motility should lead to observations of high variability in sperm morphology and performance among individuals in most natural populations. Such variability in male reproductive traits will lead to a direct demographic effect on female reproductive performance in populations that are polymorphic for mutations that reduce male fertility. There will also be an indirect genetic effect on effective population size caused by the increase in the variance in male reproductive success. How then do organisms cope with the constant threat of nonreproduction owing to mtDNA mutations that affect male fertility?

Counteracting the effects of mtDNA mutations detrimental to male fitness

Mating with multiple males and the ensuing sperm competition would be one approach that females could utilize to insure against the detrimental effects of mating with an infertile male, although such tactics come at considerable cost, such as increased risk of mate abandonment and increased risk of disease [23]. In promiscuous species, female reproductive success will not be affected strongly because females are likely to mate with both fertile and subfertile males, but the increased variance in male reproductive success in these species will accelerate the rate of genetic drift and the loss of genetic variation [36].

By contrast, we predict that monogamous species will be affected strongly by the demographic effect of mitochondrial mutations that reduce male fertility. A female who pairs and mates with a subfertile male will produce many fewer progeny throughout her lifetime, with an obvious effect on her individual fitness and on the average growth of the population. However, the effect of mtDNA mutations on the genetic variability of monogamous species is expected to be modest because the reproductive success of both females and males will be affected similarly.

If the fitness effects of mitochondrial mutations are as widespread as comparative studies predict [2,7,8,37], the obvious question is why has mtDNA not been implicated in population or species extinction before now? One answer is that we might expect few species to be imperiled by this process on timescales of less than one million years, owing to modest mutation rates and large population size [9]. Nevertheless, we might expect to see evidence of this phenomenon in populations maintained at a small size over many generations. That we have not observed such evidence is probably more a reflection of our collective ignorance of this possibility, rather than of its nonexistence.

An alternative, but not incompatible, explanation is that there are compensatory mechanisms that might buffer populations from this peril (Box 3). Female gametic selection (atresia) might eliminate the worst mtDNA mutations [38]. Paternal inheritance (leakage) of mtDNA might also mitigate the effect of mtDNA by providing an opportunity for natural selection to act directly upon mtDNAs that impair male fitness. Recombination should also help purge deleterious alleles and, although the occurrence of recombination in animal mtDNA remains hotly debated, evidence is accumulating that mtDNA recombination can occur [39]. The final identifiable mechanism through which organisms might avoid the

Box 3. Protecting against the deleterious effects of mtDNA

If no mechanisms existed to counter the deleterious effects of mtDNA mutations that affect male, but not female, fitness, then these mutations should continue to accumulate over time leading to ever-higher levels of nonreproduction and inevitably extinction. Because most populations remain viable over significant timescales, there must be a way to overcome this problem.

Paternal leakage could be one means by which the effects of the accumulation of deleterious mtDNA mutations could be eased because it would provide an opportunity for the male-specific fitness effects of mtDNA to be exposed directly to selection. Paternal leakage remains a contentious issue, but it is clear that it can and does occur in many species [45,46]. However, the significance of paternal transmission in the biology of natural populations remains unclear because most evidence for paternal transmission of mtDNA is derived from interspecific crosses, which, by definition, are uncommon in nature [47]. Backcrossing studies in mouse hybrids showed 'leakage' of one paternal mtDNA for every 10^3–10^4 maternal mtDNAs [48], similar to the ratio of sperm to oocyte mtDNA seen at fertilization [49]. The key question for our theory is whether paternal leakage at 1 in 10 000 molecules [48] is enough to alleviate the effect of mtDNA mutations that have strong effects in males, but weak effects in females.

A second molecular safeguard might be mitochondrial recombination. However, to buffer populations from the deleterious effects of mtDNA mutation, mitochondrial populations would need to comprise more than one type of mtDNA (i.e. be heteroplasmic) and recombination would need to be a common event if new haplotypes were to be produced. Recent work on paternal leakage [45,46], together with a large body of anecdotal evidence [2], shows that heteroplasmy does occur and is probably widespread. However, whether recombination *per se* is adequate to avoid the otherwise inexorable reduction in fitness expected in natural populations harbouring high numbers of deleterious mtDNA mutations remains unknown for all cases but cytoplasmic male sterility (CMS) in plants. CMS is a maternally inherited condition where affected plants are unable to produce functional pollen [50]. Mitochondrial recombination has been observed to restore fertility in CMS plants [50].

Several nuclear genes have now been characterized that function to suppress or eliminate the deleterious effects of CMS-associated mitochondrial abnormalities [50]. The existence of these restorer genes suggests that similar restoration mechanisms might also exist in animals.

detrimental effects of deleterious mtDNAs might be restorer genes (Box 3).

Conclusions

It is now well established that mtDNA is important for organismal viability and fecundity, with clear linkages established between mtDNA mutations and infertility in humans, animals, and plants. What is yet to be determined is how common mtDNA mutations in natural populations are that affect male but not female fitness and whether these mutations actually impact on the viability of those populations (Box 4). Human fertility research suggests that such mutations are common and that they can have negative effects on sperm motility [4,5], and work on sperm competition demonstrates that poor sperm motility reduces male reproductive success significantly [31]. Collectively, these data suggest that mtDNA mutations that decrease male, but not female, fitness will impact on the reproductive output of a population, contributing significantly to the GENETIC LOAD of small populations and potentially playing a major role in the 'inbreeding effect' [15]. It is vital that we develop a better understanding of the role of mtDNA mutations in population viability. Once mtDNA effects are recognized and quantified, our current population viability models will be more realistic and better able to predict the threats to a given species. If mtDNA mutations do reduce population viability significantly, then actions taken to reduce the effects of inbreeding depression at nuclear genes might not necessarily reduce the genetic load resulting from mtDNA [15].

Box 4. Outstanding questions and challenges

Substantial evidence has accumulated in support of our original assertion that mtDNA mutations affect population viability [15]. However, numerous questions remain about the molecular, population, and evolutionary aspects of this theory. We need to know how mtDNA mutations are generated, how often they have male-specific phenotypic consequences, and what compensatory mechanisms might exist to help organisms avoid the costs of such mutations. We also need to determine how widespread such mutations are in nature, identify populations affected by mtDNA mutations, and gauge the sex-specific fitness consequences of these mutations.

At present, the best indicator of mtDNA mutations that might affect population viability is low sperm motility correlated with specific mtDNA polymorphisms. Such data are only available for humans and the search is currently on to identify a system more amenable to experimental manipulation along with screening techniques that will enable the rapid detection of individuals affected by mtDNA fertility problems. Once an experimental system is identified, it should be possible to determine rapidly the consequences of mtDNA mutations predicted to affect population viability, greatly advancing our working knowledge of this phenomenon.

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