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Maternal inheritance of mitochondria: implications for male fertility?

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Abstract

Evolutionary theory predicts maternal inheritance of the mitochondria will lead to the accumulation of mutations in the mitochondrial DNA (mtDNA) that impair male fertility, but leave females unaffected. The hypothesis has been referred to as 'Mother's Curse'. There are many examples of mtDNA mutations or haplotypes, in humans and other metazoans, associated with decreases in sperm performance, but seemingly few reports of associations involving female reproductive traits; an observation that has been used to support the Mother's Curse hypothesis. However, it is unclear whether apparent signatures of male bias in mitochondrial genetic effects on fertility reflect an underlying biological bias or a technical bias resulting from a lack of studies to have screened for female effects. Here, we conduct a systematic literature search of studies reporting mitochondrial genetic effects on fertility-related traits in gonochoristic metazoans (animals with two distinct sexes). Studies of female reproductive outcomes were sparse, reflecting a large technical sex bias across the literature. We were only able to make a valid assessment of sex specificity of mitochondrial genetic effects in 30% of cases. However, in most of these cases, the effects were male biased, including examples of male bias associated with mtDNA mutations in humans. These results are therefore consistent with the hypothesis that maternal inheritance has enriched mtDNA sequences with mutations that specifically impair male fertility. However, future research that redresses the technical imbalance in studies conducted per sex will be key to enabling researchers to fully assess the wider implications of the Mother's Curse hypothesis to male reproductive biology.

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Introduction

Mitochondria are thought to have evolved from an ancient union between an α-proteobacterium and archaean eukaryote ancestor (Gray *et al*. 1999); an endosymbiotic event that enabled the ancestral single-cell eukaryote to harness an extremely efficient means of energy conversion via oxidative phosphorylation (OXPHOS). Indeed, the mitochondria are intriguing from an evolutionary standpoint, because they have retained their own genome, which has been vastly streamlined throughout the course of eukaryote evolution via translocation of hundreds of genes required for mitochondrial functionality across to the nuclear genome. What remains in the mitochondrial DNA (mtDNA) of metazoans is a 15–20 kilobase sequence of 37 genes, of which just 13 encode proteins. The evolutionary implication is that the production of ATP, the key energy currency on which the integrity of eukaryotic life relies, depends on a coordinated set of interactions between genes that reside across two obligate genomes (Fig. 1) – mitochondrial and nuclear (Rand *et al*. 2004, Wolff *et al*. 2014).

The mitochondrial genotype–phenotype linkage

It was long-assumed by evolutionary and population geneticists that any genetic polymorphisms segregating within the mtDNA sequence would have negligible, if any, effect on organismal phenotypes, and result in no modifications to traits such as development rate, fertility or life expectancy (Ballard & Kreitman 1995, Galtier 2009). This notion was based on the assumption that the products of the mitochondrial genome (polypeptide subunits directly involved in OXPHOS function) are so integral to sustaining the viability of eukaryotic life, that natural selection would act swiftly to purge any *de novo* mutations appearing in the mtDNA sequence; leaving only a pool of residual genetic variation in the genome that was 'neutral' to selection, and of no consequences to health (Avise *et al*. 1987, Dowling *et al*. 2008).

In recent years, however, numerous studies have emerged that used experimental approaches to experimentally uncouple mitochondrial from nuclear genetic contributions to the expression of organismal phenotypes, in a range of invertebrate and vertebrate metazoans. These studies have routinely linked the

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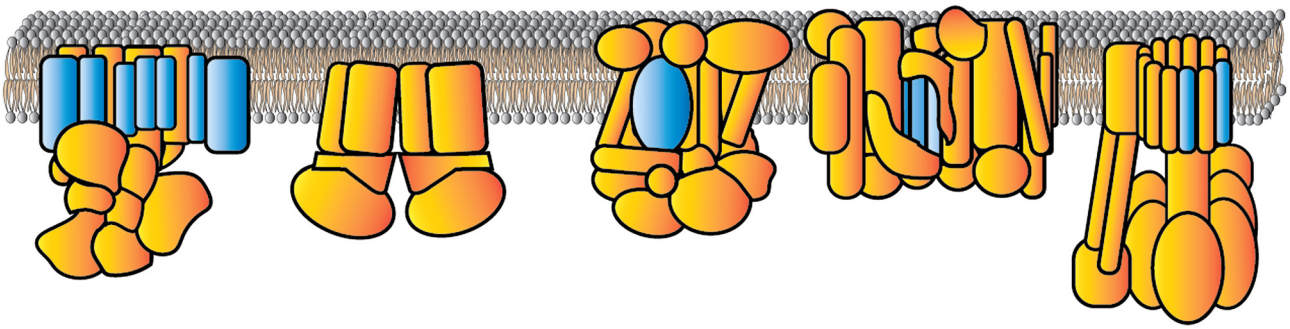

Figure 1 Schematic depiction of the enzyme complexes of the oxidative phosphorylation (OXPHOS) chain located within the mitochondrial inner membrane. Subunits in yellow are encoded by the nuclear genome, subunits in blue encoded by the mitochondrial genome. ATP production thus relies on the function of five enzymes, four of which depend on coordinated interactions involving both nuclear- and mitochondrial-encoded subunits.

genetic variation that delineates naturally occurring 'mtDNA haplotypes' to modifications in a range of phenotypes related to health, such as metabolism, growth and longevity, in both invertebrate (Rand 2001, Meikeljohn *et al*. 2007, Camus *et al*. 2012, 2015, Yee *et al*. 2013) and vertebrate metazoans (Fontanillas *et al*. 2005, Smith *et al*. 2010, Boratyński *et al*. 2016). Many of these papers have been reviewed elsewhere (Ballard & Kreitman 1995, Blier 2001, Ballard & Whitlock 2004, Ballard & Rand 2005, Dowling *et al*. 2008, Ballard & Pichaud 2014, Dobler *et al*. 2014, Dowling 2014) and will not be discussed here. Coupled with these findings, it has become increasingly clear that disease-causing mtDNA mutations might commonly exist at appreciable frequencies within human populations (Chinnery *et al*. 2000, Taylor & Turnbull 2005, Reeve *et al*. 2008), with around one in 200 people estimated to carry a pathogenic mtDNA mutation (Elliott *et al*. 2008), and around one in 5000 ultimately expressing a mitochondrial disease phenotype at some stage of their lives (Thorburn 2004). Furthermore, different mtDNA variants have recently been implicated in the penetrance or severity of a range of late-onset human diseases that were previously thought to be unrelated to mitochondrial function, from schizophrenia, to multiple sclerosis and Parkinson's disease (Hudson *et al*. 2014). Thus, it is becoming apparent that the genetic variation that accumulates within the mtDNA sequences of natural populations of metazoans does play an important role in determining health outcomes, by contributing to metabolic health, development trajectories, lifespan and the risk of disease progression (Wallace 2005, Dowling *et al*. 2008, Reinhardt *et al*. 2013, Dowling 2014).

Theory linking the mitochondria to male fertility

In 1996, Frank and Hurst formulated an evolutionary hypothesis based on a simple population genetic model that predicts that mitochondrial genomes will be enriched for mutations that impair male fertility. Gemmell *et al*. (2004) called the hypothesis '*Mother's Curse*'; a name we adopt here given it has since been used by the majority of studies that address this hypothesis (Wade & Brandvain 2009, Smith *et al*. 2010, Hedrick 2011, Zhang *et al*. 2012, Wade 2014). The mitochondria are strictly maternally inherited in metazoans, with males rarely, if ever, transmitting their mtDNA to their offspring. Thus, males are effectively evolutionary dead-ends when it comes to mtDNA transmission, and as a result 'natural selection' should only be effective at shaping evolutionary changes within the mitochondrial genome through females (i.e. when the genome is carried inside of females). That is, if a mutation arises in the mtDNA sequence that is relatively neutral in its effects on females, this mutation will evade selection and could accumulate within populations, even if the effects of the very same mutation are negative for males (Frank & Hurst 1996, Innocenti *et al*. 2011, Frank 2012, Beekman *et al*. 2014). In theory, mtDNA mutations might even arise that augment female function, but decrease male function. In such cases, these 'male-harming' mtDNA mutations would be expected to increase in their frequency within a population under positive Darwinian selection, given that evolutionary adaptation of the mtDNA sequence proceeds through females (Beekman *et al*. 2014).

While the Mother's Curse hypothesis is theoretically sound, its biological relevance necessarily rests on an assumption that mutations that confer sex-specific effects can and do accumulate within the mtDNA sequence. Below, we address the capacity for such sex specificity. The genetic correlation between the sexes is expected to be strongly positive for most traits (Bonduriansky & Chenoweth 2009). This is because the sexes share the same genomes, both nuclear and mitochondrial (Pennell & Morrow 2013), and the genes encoding trait function are likely to have much the same role in each of the sexes. Thus, if a mutation was to arise in a mitochondrial gene that encodes a critical element of OXPHOS function and has a negative effect on males, then it is reasonable to assume that under most scenarios, this same mutation would exert a similarly negative effect on females. Selection would then operate on females

to remove this mtDNA mutation from the population (i.e., females that carry it would be less likely to pass on their mtDNA), and males of future generations would salvage the benefits of this female-mediated optimisation of the mtDNA sequence. Accordingly, when it comes to the optimisation of mitochondrial function, males should generally be able to depend on natural selection operating through females, to meet their metabolic requirements.

Biological research, however, has provided many examples of sexual dimorphism in trait expression (Karp *et al*. 2017). Indeed, there are no traits that exhibit greater differences between the sexes than those tied directly to reproduction – the gonads and gametes. The male homologs of these traits (the testes and sperm) exhibit high metabolic activity (Ewing *et al*. 1966, Short 1997), while their female counterparts (ovaries and ova) are relatively quiescent (Short 1997). Indeed, in vertebrates, the capacity of sperm to fertilise the ova depends primarily on their motility, powered by a small number of mitochondria packed within the sperm mid-piece. It seems plausible to assume that the metabolic requirements of the testes and sperm must to some degree depend on products of the mtDNA. Yet, paradoxically, when it comes to mtDNA-mediated optimisation of gonad and gamete function (via natural selection), this will in theory proceed through selection of mtDNA sequence variants that maximise performance in the ovaries and ova; and this could lead to the accumulation of mtDNA variants that while optimised for function in the female gonads and gametes, confer suboptimal function in the male homologs.

Furthermore, even in the absence of mtDNA mutations that directly encode sex-specific reproductive effects, mtDNA-mediated sex specificity in gamete function could plausibly arise through a second mechanism. The capacity for sex-general mtDNA mutations (mutations that exert similar pathogenicity in both females and males) to accumulate to appreciably high frequencies in the gametes is greater in males than in females, due to vast discrepancies in the mitochondrial content of the sperm and ova. The mature oocyte may contain hundreds of thousands of mitochondria in humans (Shoubridge & Wai 2007), whereas the mature sperm carries less than 100 (Ankel-Simmons & Cummins 1996). Mutations in the mtDNA will generally only confer pathogenicity when their frequency within a cell lineage surpasses a biochemical threshold of mutant-to-wild type molecules. This threshold is typically around 70–80%, although varies across tissue types (Wallace 1999). Such thresholds for a mutant mtDNA molecule within the ovum might be extremely difficult to surpass by stochastic processes alone (the random segregation of mutant-to-wild-type mtDNA molecules) given the high copy number of mtDNA in this cell, but might be more routinely reached in the sperm in which there are very few copies of mtDNA (Gemmell *et al*. 2004).

Accordingly, the male gamete is predicted to be much more sensitive to the effects of mtDNA mutations than its female counterpart. In fact, in theory, the accumulation of male fertility-impairing mutations in the mtDNA sequence should in itself select for a variety of evolutionary mechanisms that offset the negative effects to males. Such mechanisms might include genetic adaptations that occasionally permit paternal leakage in transmission of mtDNA at conception, thereby enabling male-harming haplotypes associated with particular ancestral matrilines to be replaced (Kuijper *et al*. 2015, Radzvilavicius *et al*. 2017). Alternatively, the presence of male fertility-impairing mutations in the mtDNA should invoke selection on the nuclear genome for compensatory counter-adaptations able to restore male fertility (Beekman *et al*. 2014, Connallon *et al*. 2017). However, while instances of paternal transmission have been previously observed in metazoans, these are typically only observed at low rates (Bromham *et al*. 2003, White *et al*. 2008, Wolff *et al*. 2013, Dokianakis & Ladoukakis 2014) or when involving reproductive events between very distantly related genetic lineages. Similarly, when it comes to evidence for compensatory capacities of nuclear genotypes that have coevolved to offset male-harming mutations, empirical evidence for such counter-adaptations remains scarce in metazoans (Yee *et al*. 2013, Wolff *et al*. 2017).

Indeed, over the past two decades, several studies have reported links between mitochondrial genetic variation and negative male reproductive outcomes (Gemmell *et al*. 2004), suggesting that the evolution of compensatory mechanisms is, at best, only partly effective in offsetting the effects of male-harming mtDNA mutations. Moreover, there appears to be a sex bias emerging in the reporting of such effects, with numerous studies suggesting that different mtDNA variants affect components of male fertility (Folgerø *et al*. 1993, Kao *et al*. 1998, Ruiz-Pesini *et al*. 2000*a*,*b*, Froman & Kirby 2005, Montiel-Sosa *et al*. 2006, Colagar *et al*. 2013, Yee *et al*. 2013, Tourmente *et al*. 2017), but seemingly less reporting similar effects in females (Nakada *et al*. 2006, Xu *et al*. 2008, Dowling *et al*. 2009, Smith *et al*. 2010, Patel *et al*. 2016). These studies have therefore generated attention because they appear consistent with the predictions of the Mother's Curse hypothesis. However, it is critical to note that although such sex biases might reflect true biological differences between the sexes in the fertility effects conferred by different mtDNA variants, they could alternatively reflect a broader sex bias in the design and implementation of animal experiments. Indeed, there has been a historical trend for researchers in the animal sciences to focus on male subjects in their scientific research (Zucker & Beery 2010, Karp *et al*. 2017). One of the implications of this bias in the use of male subjects is that it could then create an illusion of biological sex bias and lead to misinterpretations of biological processes, given the

sex biases might be based on an absence of female data, rather than an absence of effects *per se* in females.

Therefore, the primary goal of this study was to provide an up-to-date, systematic review of the effects of mtDNA variation on components of reproduction in gonochoristic metazoans (animals with two distinct sexes), to enable us to appraise two questions; firstly, whether previously reported male biases in mitochondrial genetic effects on fertility are pervasive across metazoans (from invertebrates to humans), and secondly whether any such biases reflect true sex biases in nature or a sampling (technical) bias by animal researchers. Evidence for the former requires that fertility effects of particular mtDNA variants or haplotypes in males are corroborated by parallel evidence of null effects in females.

Evidence for sex-specific mitochondrial fertility effects

We conducted a comprehensive literature search of papers reporting mitochondrial and cytoplasmic effects on components of reproduction in animals, using the *Web of Science*, and by screening reference lists of key review articles in the subject area. The final Web of Science search was adapted from Dobler *et al*. (2014) and contained the terms for the field 'topic' as follows:

((cytop* OR mitoch* OR cytoty* OR mitoty* OR mtDNA* OR cybrid* OR 'mtDNA mutation*' OR 'mtDNA varia*' OR 'mtDNA deletion*' OR 'mitochondrial deletion*' OR 'mitochondrial mutation*' OR 'mitochondrial genom*' OR 'mtDNA polymorphi*' OR 'mtDNA snp*' OR 'mitochondrial polymorphi*' OR 'mitochondrial snp*' OR 'mitochondrial DNA*' OR 'mitochondrial DNA hypomorph*' OR 'mtDNA hypomorph*' OR haplotype* OR haplogroup* OR 'Mother's Curse' OR 'Frank and Hurst') AND (varia* OR effect* OR affect* OR interaction) NOT cance* NOT neuro* NOT phylogeo* NOT bacter*) AND (reprod* OR fertility* OR fecund* OR sperm* OR infertil* OR fertil* OR oligozoosperm* OR asthenozoosperm* OR 'reproductive senescence' OR 'reproductive ag*' OR ejacul* OR testes* OR semen* OR semin* OR 'mating* rate').

This search was further limited by including only primary literature and by excluding irrelevant fields. The fields included in the search were limited to the following: genetics, heredity, evolutionary biology, reproductive biology, ecology, biochemistry, molecular biology, cell biology, zoology, biology, developmental biology, multidisciplinary sciences, andrology, entomology, agriculture, dairy animal science, marine biology and freshwater biology.

The literature search returned 4175 results in total, which were individually screened for content by RCV. Of the 53 papers assessed as relevant to our study, 38 comprised studies of individual mutations or sets of mutations (category 1: mutation studies), in which researchers screened for associations between known mtDNA mutations and traits associated with fertility, such as metrics of sperm performance or reproductive outcomes *per se*. For each of these mtDNA mutations, or set of mutations examined, we could assess whether associated fertility effects had been tested in each of the sexes (i.e., not just in males), and if so, whether the reported effects of the mutations on the reproductive phenotypes were negative, positive or neutral. Another 15 studies utilised 'panels' of mtDNA haplotypes (category 2: panel studies) to screen for effects of natural variation across mtDNA haplotypes on fertility-related traits (sperm or reproductive traits). These studies come in two types. One subset was screened for associations between mtDNA haplotypes and sperm or reproductive traits, but was not controlled for segregating variation in the nuclear background. These are by definition association studies. The other subset utilised experimental approaches to place a defined set of naturally occurring mtDNA haplotypes alongside a standardised nuclear genetic background, thus allowing effects of mitochondrial genetic variation to be directly mapped to the reproductive traits involved. In studies of panels, it is generally not possible to ascribe directionality to the effects of the mtDNA haplotypes on the fertility-related traits, because each study tests the effects of many different mtDNA haplotypes on the traits rather than individual candidate mutations. Rather, here we simply record whether the studies found associations (Yes/No) between mitochondrial haplotype variation and fertility-related traits in either sex.

We focus on the identity of the mtDNA mutation or mutation set in category 1 studies, and the identity of the panel in category 2 studies, as our units of inference rather than the individual study/paper. This is because some mutations/panels have been the focus of multiple studies. This is essential to avoid issues of pseudoreplication (i.e., numerous studies of the same mutation/set of mutations/panel) from confounding our interpretations. We have collated the data on phenotypic effects associated with these mutations/panels on traits associated with male fertility, female fertility or on other non-reproductive traits (e.g. mitochondrial disease phenotypes, respiratory capacity, longevity, etc.) (Supplementary Table 1, see section on Supplementary data given at the end of this article). In total, we collated phenotypic effects associated with 30 different mutations/sets, and 10 different panels, spanning 10 different species. Of these, only 30% of mutations/mutation sets/panels contained data for both sexes (7 of 30 for mutations/sets, and 5 of 10 for panels). Of the mutations/mutation sets/panels that contained data for both males and females (i.e., of the cases that were not affected by a technical sex bias), 10 of 12 exhibited signatures of male bias in the presence and/or severity of the reported effects (6/7 for mutations/sets and 4/5 for panels).

From these results, it is clear that a large technical sex bias exists within the literature. Most mutations/mutation sets/panels contain data for only one sex, usually males. Furthermore, of the 28 mutations/mutation sets/panels exhibiting a technical sex bias, only 5 of these had been created and studied with the explicit goal of testing the Mother's Curse hypothesis. Thus, the technical bias persists because of other factors, such as the ease by which male reproductive traits, such as sperm parameters, can be measured relative to their female counterparts (ova quality), the latter of which might be more routinely monitored through non-invasive and indirect screens of hormone levels and ovulation. Moreover, we note that it is possible that other studies that tested effects of mtDNA mutations on components of reproduction in males or females might have withheld results from publication, if their results were in line with the null hypothesis (a phenomenon known as the 'file drawer' problem). Such withholding of null results from the literature could in theory influence interpretations of the dataset that we have collated. Our study is the first to systematically quantify a glaring technical sex bias when it comes to mitochondrial genetic effects on components of reproduction.

Notwithstanding this large technical bias, we observed a clear signature of biological male bias in effects for 10 of the 12 mutations/mutations sets/panels in which data existed for both males and female components of reproduction. This result is consistent with the evolutionary hypothesis of Mother's Curse and reinforces the importance of future studies redressing the technical bias identified above, to enable a full appreciation of the generality by which mutations in the mtDNA might confer sex differences in reproductive outcomes across metazoans. Currently, most of the data for Mother's Curse effects on reproduction comes from humans and two well-studied model organisms (*Drosophila* and *Mus*), and we specifically discuss these case studies below.

Fruit fly case studies

All of the fruit fly examples come from studies of *D. melanogaster*, and many of these have utilised the same panel of naturally occurring mtDNA haplotypes (Clancy *et al*. 2008, 2011, Innocenti *et al*. 2011, Yee *et al*. 2013, Dowling *et al*. 2015, Wolff *et al*. 2016*a*,*b*, 2017), which were originally sourced from different global localities and which exhibit generally low levels of sequence divergence (~0.4%); a level of divergence paralleling that seen between major human mtDNA haplogroups (Morrow *et al*. 2015). Replicate genetic strains have been created for each of these haplotypes, with each haplotype placed alongside a standardised isogenic background (i.e. a nuclear genetic background devoid of segregating allelic variation), which allowed direct estimation of the magnitude of the mitochondrial haplotype effect on a range of phenotypes. One haplotype in this panel, originally sourced from a wild population in Brownsville, Texas, USA (Rand *et al*. 1994), is associated with reduced male fertility, and its protein-coding sequence differs from the other haplotypes in the panel by a unique SNP within the mitochondrial cytochrome b gene of respiratory complex III, which results in an amino acid transition (*mt:CytB*^{A278T}). Thus, this is the candidate SNP causing the sterility, although it is possible that other currently cryptic variation in the D-loop is driving the effects (Wolff *et al*. 2017). These sub-fertility effects associated with this haplotype have been confirmed across all nuclear genetic and environmental contexts in which males harbouring this haplotype have been tested (Clancy *et al*. 2008, Yee *et al*. 2013, Dowling *et al*. 2015, Wolff *et al*. 2016*a*,*b*, 2017). Indeed, in one nuclear background in which the haplotype has been tested, the spermatids of these males fail to individualise and degrade within the testes (Clancy *et al*. 2011), leaving the males completely infertile. In contrast, females that carry this Brownsville haplotype, and its candidate male-sterilising *mt:CytB* SNP, enjoy high reproductive success typical of females carrying other wild-type haplotypes (Camus & Dowling 2017). Thus, this Brownsville haplotype specifically depresses male, but not female, fertility. Intriguingly, the most recent study on this panel of haplotypes documented a pattern of sexual antagonism in reproductive outcomes; those haplotypes associated with the lowest reproductive success in males were the ones that conferred highest reproductive success in females. This pattern supports the notion that mitochondrial genomes accumulate mutations that confer beneficial effects in females, but negative effects on males, with these mutations accumulating under positive selection as a result of mitochondrial maternal inheritance (Unckless & Herren 2009, Innocenti *et al*. 2011, Beekman *et al*. 2014).

Patel *et al*. (2016) reported another mtDNA mutation rendering male sub-fertility in *D. melanogaster*, which was found to be naturally segregating in the laboratory population of a common fruit fly strain known as w^{1118}. The mutation is a non-synonymous SNP, resulting in a glycine to serine substitution at position 177 in subunit II of the cytochrome c oxidase (*mt:COII*^{G177S}) gene of respiratory complex IV. The glycine variant at position 177 is conserved across eukaryotes. The serine variant conferred a reduction in male, but not female fertility, with the magnitude of the reduced fertility in males increasing with advancing age and also greatly exacerbated at higher temperatures.

While the former examples involve genetic polymorphisms that evolve and segregate naturally in wild or laboratory populations, further evidence for male infertility-impairing mtDNA mutations in *D. melanogaster* comes from an earlier study which genetically-engineered mutant mtDNA variants. Xu and coworkers (Xu *et al*. 2008) used targeted restriction

enzymes to select for germ-line mtDNA mutations in subunit I of the mitochondrial cytochrome c oxidase gene. Using this approach, they identified three sequence variants, one of which possessed a normal phenotype (*mt:CoI*^{A302T}, normal for fertility, cytochrome c oxidase activity and ATP production), while the other two conferred male sterility. More specifically, males carrying *mt:CoI*^{R301Q} were sterile, producing low numbers of motile sperm, but were otherwise healthy, exhibiting normal cytochrome c oxidase activity and ATP levels. A fertility effect on females was not reported, but presumed absent. Males carrying *mt:CoI*^{R301S} were also completely sterile, lacking mature sperm; while females carrying this variant also experienced a large reduction in fertility relative to their wild-type counterparts (producing 20% the offspring of wild-types). *mt:CoI*^{R301S} flies also experienced greatly reduced cytochrome c oxidase activity and ATP levels (Xu *et al*. 2008).

In sum, in *D. melanogaster*, mutations that compromise male, but not female, fertility appear to be a feature of the mutational landscape of mitochondrial genomes, with at least three independent mutations, spanning two genes, now identified (Xu *et al*. 2008, Clancy *et al*. 2011, Dowling *et al*. 2015, Patel *et al*. 2016), and accumulating evidence of numerous other mutations of smaller effect that depress male but increase female fertility (Camus & Dowling 2017). Evidence therefore exists in *D. melanogaster* that male biases in these reported cases reflect true sex biases in the manifestation of these mtDNA-mediated fertility effects and are not technical artefacts of a sex bias in design and implementation of experiments.

Mouse case studies

Studies of mice have also provided evidence that mtDNA mutations exist that confer negative effects on male, but not female, fertility. Nakada *et al*. (2006) studied mice strains that were heteroplasmic for a large deletion from position 7759 from the *mt:tRNA*^{Lys} gene to position 12,454 of the *mt:ND5* gene. The authors reported various pathologies, the expression of which depended on the proportion of the mutant mtDNA molecules (level of heteroplasmy) per individual mouse. Males carrying less than 68% of the mutant mtDNA were healthy and fertile. Males carrying between 70 and 80% of the mutant mtDNA were also mostly healthy, exhibiting only slight respiratory deficiencies. However, although these mice produced copulation plugs, they produced fewer, less motile sperm, and consequently experienced a drastic reduction in fertility compared to control mice (as gauged by an approximate 60% decrease in the number of eggs, submitted to IVF with the experimental males' sperm, reaching the two-cell embryonic stage). Males carrying more than 80% of the mutant mtDNA type were completely infertile, failed to produce copulation plugs and produced only very few immotile sperm. These males with the highest mutant loads also exhibited widespread respiratory deficiencies, and other pathologies associated with mitochondrial disease (myopathy, lactic acidosis, renal failure, deafness). Although male fertility was particularly sensitive to the presence of this mtDNA deletion, the authors noted that females carrying more than 70% of the mutant mtDNA type still produced normal numbers of progeny, at least up until six months of age when they died of mitochondrial pathologies associated with the mutation (Inoue *et al*. 2000, Nakada *et al*. 2004).

The approach of Nakada and colleagues is complemented by two other studies in mice. Firstly, Trifunovic and colleagues (Trifunovic *et al*. 2004) generated mouse strains carrying mtDNA polymerase with a mutation in the catalytic *PolgA* subunit, rendering it proofreading deficient, and subsequently resulting in mice exhibiting a threefold to fivefold increase in the level of point mutations accumulating in their mtDNA sequences. These 'mtDNA-mutator' mice expressed normal phenotypes until around 25 weeks of age. While mtDNA-mutator mice of both sexes exhibited fertility reductions relative to wild-type mice, the magnitude of reduction was again male biased. Almost all mtDNA-mutator females produced one or two litters of normal size up until the age of 20 weeks, at which point they were no longer reproductive. Mutator males, however, were mostly infertile, with only one of eight males tested producing a litter; and mtDNA-mutator males possessed testes of much smaller size relative to wild-type males, even early in adult life (at 12 weeks). Secondly, Ma *et al*. (2016) created reciprocal (conplastic) combinations of mitochondrial and nuclear genotypes in the zygotes of two divergent mice strains; *Mus m. domesticus* (*B6* strain) and *Mus m. musculus* (*PWD* strain) mice. Male offspring carrying PWD mtDNA in a B6 nuclear autosomal background exhibited reduced fertility, as gauged by number of litters produced, but females were unaffected. This male infertility effect is likely attributable to mutations in the mtDNA sequence of the PWD mice, which are normally rescued by modifier mutations that lie within the PWD nuclear background, but which were not present in the experimental mice carrying the B6 background (Ma *et al*. 2016).

In sum, there have been three studies in mice that have examined effects of mtDNA variation on reproductive outcomes of both sexes (Inoue *et al*. 2000, Nakada *et al*. 2006, Ma *et al*. 2016). Each of these studies has documented male biases in fertility reductions that map to mutations in the mitochondrial genome. Again, consistent with the *Drosophila* case studies reviewed above, the studies on mice provide empirical support for the tenet that mtDNA sequences are enriched for mutations that specifically depress male components of reproduction.

Human case studies

Unlike the *Drosophila* and mouse studies, which are able to leverage experimental approaches to partition mitochondrial genetic, from nuclear genetic and environmental effects on fertility outcomes, human studies must rely principally on inferences drawn from correlations between mtDNA variants and fertility outcomes. Various associations have been reported between naturally occurring mtDNA haplogroups and incidences of male infertility. Ruiz-Pesini *et al*. (2000*a*,*b*) reported that the human T haplogroup is overrepresented among males with low sperm motility, while the H haplogroup is overrepresented among males with high motility. Montiel-Sosa *et al*. (2006) reported associations between distinct sub-haplogroups within the U haplogroup and variation in sperm motility and attempted to map these patterns to particular polymorphisms that distinguish the sub-haplogroups. Various other studies linked particular point mutations or deletions in human mtDNA to sperm dysfunction (Folgerø *et al*. 1993, Huang *et al*. 1994, Kao *et al*. 1995, Fadic *et al*. 1997, Lestienne *et al*. 1997, Kao *et al*. 1998, Holyoake *et al*. 2001, St John *et al*. 2001, Spiropoulos *et al*. 2002, Thangaraj *et al*. 2003, Kumar *et al*. 2009, Baklouti-Gargouri *et al*. 2013*a*,*b*, Colagar *et al*. 2013, Chari *et al*. 2015, Lu *et al*. 2015). Yet, some caution must be applied to interpreting correlations of the type presented in these studies, since the patterns could be mediated by other confounding factors, such as differences in nuclear genetic structure between groups of individuals possessing the different mtDNA haplotypes. Furthermore, it remains unknown whether similar correlations exist between these human haplotypes and candidate mutations to patterns of female fertility.

However, in a recent development, Martikainen and colleagues (Martikainen *et al*. 2017) analysed the reproductive success of males drawn from a database of British patients suffering mitochondrial disease and reported that reproductive success of these males was only 65% of that of males from the general population. The magnitude of the effect on reproductive success increased with the severity of the mitochondrial disease symptoms in these males. The effects on fertility were equally evident among the cohort of males whose disease symptoms were caused by pathogenic mutations in the mtDNA or mutations in nuclear genes that encode mitochondrial function. Many of the patients suffering from mtDNA-mediated mitochondrial disease in this study carried the m.3243A>G mutation, which is associated with a large range of mitochondrial disease symptoms (Brown & Wallace 1994) and has been previously associated with low sperm motility (Spiropoulos *et al*. 2002). Remarkably, these patterns linking mtDNA mutations to reduced reproductive success were not found in female patients of the same database carrying pathogenic mtDNA mutations (Gorman *et al*. 2015). Generally, little is known about putative reproductive effects in females carrying the m.3243A>G mutation, other than an investigation of a single Chinese family carrying this mutation, some of the females of which experienced frequent miscarriages (Huang *et al*. 1994).

In sum, the recent evidence in humans is consistent with the evolutionary hypothesis prediction that mitochondrial genomes will harbour mutations that confer male biases in reproductive outcomes and aligns with the experimental evidence acquired from the study of *Drosophila* and mice. Future association studies in humans should, however, focus on the possible effects of the mutations under study on components of female reproductive success, to redress what appears to be a sex bias in the study of human mtDNA variation and its effects on fertility.

Conclusion

Evolutionary theory predicts that maternal inheritance of the mitochondria will lead to the accumulation of mtDNA mutations that confer male-specific effects on fertility (Frank & Hurst 1996). Yet, until now, a systematic investigation of Mother's Curse effects across metazoans has not been conducted, and evidence for Mother's Curse has largely remained theoretical (Frank & Hurst 1996, Gemmell *et al*. 2004) or limited to a few prominent empirical examples (Smith *et al*. 2010, Patel *et al*. 2016). Our literature search revealed a striking technical sex bias in research examining effects of mtDNA mutations on reproductive outcomes. However, even when excluding case studies affected by technical bias from further consideration, the majority of the remaining cases (over 80%) exhibited male biases in the magnitude of mitochondrial genetic effects on fertility, suggestive of Mother's Curse effects. We have focused our discussion on robust case studies from *Drosophila*, mice and humans, because these encompass the vast majority of studies conducted to date and are underscored by reproductive data collected from both sexes. We note, however, that these case studies are supported in several cases by intriguing patterns of male bias in mitochondrial effects on fertility in non-model species (Smith *et al*. 2010). Taken together, these findings suggest that mitochondrial genomes might be enriched for male fertility-impairing mutations across metazoans.

Yet, challenges lie ahead before such a suggestion can be confirmed, and the most obvious is the need to redress the large technical sex bias in research linking the mtDNA to fertility outcomes. For example, almost all of the association studies conducted in humans, which have studied the effects of particular mtDNA haplotypes or mutations on reproductive performance, focused exclusively on males. As such, while several cases of

male-specific mtDNA-mediated infertility have now been confirmed across model species, it remains unclear whether these cases represent anomalies specific to a few mtDNA mutations appearing in one or a few animal species or whether they represent a small fraction of the total male-biased mtDNA variants that might be segregating in animal mitochondrial genomes. We believe the coming years will provide crucial resolution of these questions, inspired by emerging research that suggests a role for the Mother's Curse hypothesis in human reproductive biology (Dowling 2014, Milot *et al*. 2017).

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/REP-17-0600.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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