COMPUTATIONAL AND EXPERIMENTAL ANALYSIS ON THE CONSEQUENCES OF SEXUALLY ASYMMETRIC TRANSMISSION WITH MITOCHONDRIAL-NUCLEAR INTERACTIONS

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COMPUTATIONAL AND EXPERIMENTAL ANALYSIS ON THE CONSEQUENCES OF SEXUALLY ASYMMETRIC TRANSMISSION WITH MITOCHONDRIAL-NUCLEAR INTERACTIONS

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Cellular function requires the coordinated transcription, translocation, and assembly of mitochondrial proteins which are encoded by both the mitochondrial and nuclear genomes. Sexual asymmetry in the transmission of autosomes, sex chromosomes, and the mitochondrial genome complicates predicting the nature of mitochondrial-nuclear interactions. Mitochondrial DNA is present in all offspring but is maternally transmitted, which means selection only acts in females. This allows for the accumulation of sexually antagonistic mitochondrial mutations that are neutral or advantageous in females even if they are deleterious in males. Coined Mother's Curse, this phenomenon introduces selective pressure for nuclear variants that compensate for this reduction in male fitness, generating a specific subset of mitochondrial- nuclear interactions. While analytical population genetic theory and experimental studies support the existence of these interactions, the factors that influence invasion conditions, their prevalence in natural populations, and the phenotypic consequences of these interactions remain incompletely characterized. Crucially, the chromosomal position of nuclear restorers and the influence their transmission pattern plays on these interactions have not been fully investigated.

We expand foundational theory by simulating the dispersal of sexually antagonistic mitochondrial-nuclear interactions dependent on a variety of factors including selection coefficients, genomic location, and sex-determination system to elucidate their evolution over time and their influence on population divergence. In **Chapter 2,** we start by examining differences within a single population for the dynamics of a single mitochondrial-nuclear

interaction dependent on the chromosomal location of nuclear restorers – either autosomal, X-linked, or Y-linked. We find that, in this scenario, Y-linked restorers outperform others in terms of rapidly spreading through a population quickly offsetting the male-harming consequences of Mother's Curse variants. However, in **Chapter 3**, when we expand this scenario to not only include multiple mitochondrial-nuclear interactions but also migration between populations, we find that the transmission pattern of Y-linked restorers hinders a population's ability to rescue male fitness in the face of Mother's Curse. To further explore mitochondrial-nuclear epistasis, with a focus on mitochondrial-Y interactions, we leverage 36 otherwise isogenic *Drosophila melanogaster* strains differing only in the geographical origin of their mitochondrial genome and Y chromosome in **Chapter 4**.

BIOGRAPHICAL SKETCH

Manisha Munasinghe was born in Milwaukee, Wisconsin in 1994. Her family moved to Michigan soon after she was born, and she would spend the rest of her childhood there. While she broadly enjoyed the sciences, she did not know what field she wished to commit herself to. She decided to follow in the footsteps of her parents and enrolled as a pre-medical major at Lyman Briggs College at Michigan State University. While there, she had the opportunity to serve as an undergraduate teaching assistant for Calculus I, II, and III, and she very quickly realized her love of both teaching and mathematics. She switched to a mathematics major, but she continued to take several courses in biology and genetics.

During this time, Dr. Robert Bell, Hanni Nichols, and Dr. Russell Schwab not only played crucial roles in advancing her research and teaching skills but were also incredible mentors and people. Notably, Dr. Schwab while advising Manisha's senior capstone on applied functional analysis would encourage her to consider pursuing a Ph.D. in computational biology. Once, she decided to do so, Dr. Schwab spent significant time helping her hone her application, investigate programs, and evaluate offers. Manisha ultimately decided to commit to the Ph.D. program in computational biology at Cornell University.

One of the first courses Manisha took at Cornell was Human Genomics, which was taught by Dr. Andrew Clark. Manisha would go to his office hours every week with a list of questions, and their conversations quickly became a highlight of her semester. She would go on to join his lab to pursue a series of computational and experimental projects regarding mitochondrial-nuclear interactions, all the while continuing to expand her teaching and mentoring skills.

Long-term, Manisha is passionate about pursuing a career in research and academia, where she can not only continue her work but also teach and mentor future generations. She is thankful for her experience at Cornell and for the many wonderful mentors and friends she made throughout her time here.

To everyone who lifted me up and believed in me during this difficult journey,

especially to my family who has been doing so since the beginning

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CHAPTER 1

INTRODUCTION

Biological Motivations

A longstanding question in evolutionary biology is what factors contribute to the astounding levels of observed and estimated species diversity on this planet (May 1988, May and Beverton 1990, Stork 1993, Hammond 1995, Stork 1999). Intimately tied to this question is both how new species form and how they remain distinct. The biological species concept links this process to the establishment of reproductive barriers. Under this framework, species are groups of interbreeding natural populations that are substantially but not necessarily completely reproductively isolated from other groups (Ernst 1942, Coyne and Orr 2004). Reproductive isolation is established as isolating barriers accumulate impeding gene flow between groups ((Dobzhansky 1937, Dobzhansky 1950). Exploring the influence and evolution of such isolating barriers is therefore key to understanding the mechanics of speciation.

Hybrid incompatibility, which occurs when hybrids between two species are inviable, sterile, or simply less fit than the parental populations, is an intrinsic post-zygotic reproductive barrier that, unlike others, is irreversible once complete (Muller 1942). Chromosomal rearrangements (Baker and Bickham 1986, Noor, Grams et al. 2001, Rieseberg 2001, Brown and O'Neill 2010), polyploid formation (Ramsey and Schemske 1998, Rieseberg and Willis 2007), and uncontrolled transposable element proliferation (Sawamura and Yamamoto 1997, Ferree and Barbash 2009, Michalak 2009, Brown and O'Neill 2010) can all generate hybrid incompatibility, but the most common cause is the accumulation of incompatible genetic interactions (Presgraves

2010, Maheshwari and Barbash 2011). Dobzhansky (Dobzhansky 1950) and Muller (Muller 1942) independently showed that hybridization between allopatric populations brings together untested genetic combinations that, on average, will be less fit than genetic combinations within a single population as selection has had time to remove deleterious combinations. These epistatic interactions, dubbed Dobzhansky-Muller incompatibilities, may theoretically manifest between any genetic elements.

An additional layer of complexity contributing to this process is the differential fitness optima between sexes for several key phenotypes such as morphology, reproduction, and parental behavior (Darwin 1871, Chapman 2006). Sexual conflict emerges as the interests of males and females diverge (Parker 1979, Arnqvist and Rowe 2005). This manifests as either intralocus conflict when the optima for a trait differs between the sexes or interlocus conflict when there is discord over the outcome of a male-female interaction (Chapman, Arnqvist et al. 2003). Initially confirmed in *Drosophila* and water striders (Chippindale, Gibson et al. 2001, Arnqvist and Rowe 2002), modern advances in sequencing technology have provided novel ways to address this phenomenon highlighting the often polygenic nature of sexual conflict (Kasimatis, Nelson et al. 2017, Mank 2017).

Dobzhansky-Muller incompatibilities may theoretically manifest between any two genetic elements, but interactions between mitochondrial and nuclear genes create a unique opportunity. Differential transmission from parent to offspring of autosomes, sex chromosomes, and the mitochondrial genome generate a specific form of sexual conflict which often leads to hybrid incompatibility.

Mitochondrial-Nuclear Interactions

Mitochondria are eukaryotic double membrane-bound organelles primarily responsible for energy production via oxidative phosphorylation (OxPhos), the metabolic pathway used to generate adenosine triphosphate (ATP) from glucose molecules. In addition to their role in energy production, mitochondria also play a role in calcium signaling (Rizzuto, De Stefani et al. 2012), apoptosis (Tait and Green 2010), and innate immunity (Cloonan and Choi 2013), making them critical for cellular function. However, unlike most other eukaryotic organelles, mitochondria contain their own distinct genome as a consequence of their origin from free-living α-proteobacteria that became endosymbionts within a proto-eukaryotic host cell (Sagan 1967, Margulis 1970, Gray, Burger et al. 1999). Over time, many of the original mitochondrial genes functionally transferred to the nuclear genome, became replaced by preexisting nuclear genes, or were simply lost (Adams and Palmer 2003).

In mammals, mitochondrial gene content is highly conserved, with mitochondrial DNA (mtDNA) encoding the ribosomal and transfer RNA components of the mitochondrial translation system and 13 protein subunits, a small but essential fraction of the electron transport chain and ATP synthase (Calvo and Mootha 2010). The remaining subunits, estimated to be between 1100 and 1400 distinct proteins, originate from the nuclear genome and must be actively imported, sorted, and assembled into the macromolecular complexes of OxPhos ((Neupert and Herrmann 2007, Gershoni, Templeton et al. 2009, Calvo and Mootha 2010, Schmidt, Pfanner et al. 2010). Unlike the nuclear genome, mtDNA copy number ranges from hundreds to thousands of copies per cell depending on the cell's energetic needs. The partitioning of the mitochondrial proteome across two distinct genomes requires coordinated evolution between them to maintain mitochondrial function and organismal fitness. Subsequently, any mutation that alters the

structural and biochemical properties of a subunit may require a corresponding change in the others to maintain the functionality of the complex.

Significant efforts to characterize mitochondrial-nuclear epistasis show that these interactions can greatly impact phenotype. Foundational theoretical analyses indicate that mtDNA polymorphisms and mitochondrial-nuclear interactions can only be maintained within populations under certain circumstances, such as frequency-dependent selection, sex-specific selection, or sex-linkage of nuclear compensators (Clark 1984, Gregorius and Ross 1984, Babcock and Asmussen 1996, Rand, Clark et al. 2001). Empirical studies that leverage the construction of hybrid lines to disrupt coadaptation have confirmed the existence and impact of mitochondrial-nuclear interactions. Studies done using *Drosophila* validated initial theory regarding mitochondrial-nuclear interactions, and there is an expanding field of work focusing on mitochondrial-nuclear incompatibilities with *Drosophila* (Clark 1985, Clark and Lyckegaard 1988). Göran Arnqvist's group (Uppsala University) used this technique in *Callosobruchus maculatus*, a polygamous seed beetle, to show that mitochondrial-nuclear interactions influence sperm variability and length (Dowling, Nowostawski et al. 2007), developmental time (Dowling, Abiega et al. 2007), and aging (Immonen, Collet et al. 2016). In the marine copepod *Tigriopus californicus*, Ronald Burton's group (UC San Diego) showed that mitochondrial-nuclear interactions play a dominant role in F2 hybrid breakdown which supports the role of intergenomic incompatibilities in speciation (Burton, Ellison et al. 2006, Ellison and Burton 2008, Burton and Barreto 2012, Barreto, Watson et al. 2018, Lima, Burton et al. 2019, Healy and Burton 2020).

While mitochondrial-nuclear interactions have been investigated in several species, *Drosophila melanogaster* remains an ideal model organism for studying these interactions. In

addition to the suite of biochemical, genetic, and bioinformatics tools developed to study *D. melanogaster,* the Drosophila and human mitochondrial genome are similar in regards to gene density, structure, and content. Furthermore, there is a high degree of conservation for nuclear-encoded mitochondrial proteins between *D. melanogaster* and humans, with significant efforts being taken to characterize genes that encode the mitochondrial proteome in both species (Gibson 2005, Tripoli, D'Elia et al. 2005, Calvo and Mootha 2010, Calvo, Clauser et al. 2016). The similarity of the mitochondrial genome and proteome between Drosophila and humans makes it a powerful tool for studying mitochondrial-nuclear interactions and evolution (Fernández-Moreno, Farr et al. 2007). David Rand's group (Brown University) has constructed strains of hybrid Drosophila carrying within and between-species mitochondrial-nuclear combinations to show that these interactions are common and environmentally-dependent for a variety of phenotypes including response to hypoxia (Mossman, Tross et al. 2017), longevity (Zhu, Ingelmo et al. 2014), developmental time (Mossman, Biancani et al. 2016), and gene expression (Mossman, Biancani et al. 2019).

Comparatively, little work has focused on identifying candidate mitochondrial-nuclear interactions in human populations. However, specific mtDNA variants and haplogroups have been directly linked to a growing subset of human diseases (Nunnari and Suomalainen 2012, Picard, Wallace et al. 2016). These disorders show a wide range of phenotypic and clinical variability as the impact of mtDNA mutations is not a simple presence or absence model, but instead dependent on a 'threshold-effect' where disease severity is dependent on the proportion of mutant mtDNA present (Taylor and Turnbull 2005, Parikh, Goldstein et al. 2015, Picard, Wallace et al. 2016).

Table 1.1 Common mitochondrial disorders associated with mtDNA point mutations

Mitochondrial DNA disorder	Mutation	Gene affected	Phenotype
LHON [112,113]	m.3460 G→A m.11778 G→A m.14484 T→C	*MT-ND1* *MT-ND4* *MT-ND6*	Subacute bilateral visual failure and optic atrophy
Leigh syndrome [114]	m.8993 T→C	*MT-ATP6*	Onset 4–5 months, developmental delay, psychomotor delay, pyramidal signs, dystonia, seizures, respiratory failure
NARP [115]	m.8993 T→G	*MT-ATP6*	Sensory neuropathy, cerebellar ataxia, retinitis pigmentosa, dementia, proximal weakness
MELAS [36]	m.3243 A→G m.3271 T→C	*MT-TL1* *MT-TL1*	Onset ca.10 years, stroke-like episodes before 40, seizures, dementia, lactic acidosis
MERRF [116]	m.8344 A→G m.8356 T→C	*MT-TK* *MT-TK*	Myoclonus, seizures, cerebellar ataxia, myopathy
AID [117]	m.1555 A→G	*MT-RNR1*	Aminoglycoside-induced non-syndromic deafness
MIDD [35]	m.3243A→G	*MT-TL1*	Diabetes and deafness

AID, aminoglycoside-induced deafness; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy and ragged-red fibres; MIDD, maternally-inherited diabetes and deafness; NARP, neurogenic weakness, ataxia and retinitis pigmentosa.

Note. Adapted from Greaves, L.C., Reeve, A.K., Taylor, R.W., & Turnbull, D.M. Mitochondrial DNA and disease. *The Journal of Pathology* **226**, 274-285, doi:10.1002/path.3028 (2012)

Table 1.1, adapted from a recent review by Greaves et al. highlights the most common mitochondrial disorders caused by single point mutations in mtDNA (Greaves, Reeve et al. 2012).

In addition to primary mitochondrial disorders, mitochondrial haplotypes have been shown to confer sex-specific disease susceptibility for a suite of common diseases including psychiatric disorders (Rollins, Martin et al. 2009, Sequeira, Rollins et al. 2015), cancer (Singh and Kulawiec 2009, Fang, Shen et al. 2010), and neurodegenerative disorders, such as Alzheimer and Parkinson (Gaweda-Walerych and Zekanowski 2013, Ridge, Koop et al. 2013, Liou, Chuang et al. 2016). Cytoplasmic hybrid, or 'cybrid', human cell lines have been used to show the consequences of mtDNA on cellular physiology and biochemistry linking mutations to clinical phenotypes confirming the role of mtDNA in human disease (Bunn, Wallace et al. 1974, King and Attardi 1989, Wilkins, Carl et al. 2014). However, differences between 'cybrid' cell lines and the human system limit their application for studying mitochondrial-nuclear interactions. Mitochondrial replacement therapy in humans, while nascent, can be used to generate hybrid

humans with mitochondrial genomes from a third parent potentially creating novel mitochondrial-nuclear combinations (Craven, Tuppen et al. 2010, Paull, Emmanuele et al. 2013, Hyslop, Blakeley et al. 2016). As researchers are conflicted over the potential consequences to disrupting co-evolved mitochondrial-nuclear interactions in mitochondrial-replacement therapy, understanding their evolutionary dynamics and phenotypic consequences is increasingly relevant (Innocenti and Morrow 2010, Gemmell and Wolff 2015, Eyre-Walker 2017).

Asymmetric Transmission and Mother's Curse

What is missing from much of the published work on mitochondrial-nuclear interactions is the centrality of the sex-asymmetry of mitochondrial transmission. In contrast to the biparental transmission of autosomes, mitochondrial DNA is nearly universally uniparentally, and in most cases maternally, inherited. Uniparental transmission of cytoplasmic genomes provides an adaptive benefit; it restricts the rapid spread of deleterious mutations with a replicative advantage (Hoekstra 1987, Law and Hutson 1992, Hoekstra 2000). However, it also allows female-advantageous, male-deleterious mitochondrial mutations to rise in frequency within a population. First characterized by Frank and Hurst in 1996 and coined 'Mother's Curse' by Gemmell et al. in 2004, the exclusively maternal inheritance of and lack of recombination in the mitochondrial genome leads to the accumulation of male-harming mtDNA mutations creating the opportunity for sexual conflict (Frank and Hurst 1996, Gemmell, Metcalf et al. 2004). Frank and Hurst showed that the expected equilibrium frequency of a mitochondrial mutation in a large population is approximately $q = \frac{\mu}{s_f}$ where μ is the mitochondrial mutation rate and 1 - s_f is the relative fitness of a female with the mutation (Frank and Hurst 1996). mtDNA mutations that are neutral (or adaptive) in females but deleterious in males can drift (or be selected) to high

frequency, reducing the overall fitness of the population and causing intragenomic conflict (Lewis 1941, Burt and Trivers 2006). For Mother's Curse variants, their maintenance in the population depends on their selection coefficient in females and whether their reduction in male fitness significantly reduces population-level viability (Gemmell and Allendorf 2001). In order to mitigate these fitness consequences, selection should favor variants at nuclear loci that compensate for deleterious mtDNA mutations (Rand, Haney et al. 2004, Dowling, Friberg et al. 2008).

The canonical example of Mother's Curse is cytoplasmic male sterility (CMS) which has been documented in over 150 plant species (Mackenzie, He et al. 1994). Lesions in the mitochondrial genome induce male sterility by disrupting pollen production while nuclear restorer-of-fertility genes can either suppress or counteract the phenotype (Schnable and Wise 1998, Chase 2007, Case, Finseth et al. 2016). Damian Dowling's group (Monash University) has used a similar approach to David Rand's to explore the fitness consequences for mitochondrial-nuclear incompatibility in *D. melanogaster* to show that males suffer more obviously from these consequences (Innocenti, Morrow et al. 2011, Camus, Clancy et al. 2012, Dean, Lemos et al. 2015, Yee, Rogell et al. 2015, Nagarajan-Radha, Aitkenhead et al. 2020). Patel et al. identified one of the clearest cases of Mother's Curse in *D. melanogaster* by discovering a mtDNA hypomorph of cytochrome oxidase II that causes defects in sperm development and function (Patel, Miriyala et al. 2016). Not only is the effect of this single missense mutation temperature-dependent (indicating a gene by environment interaction) but it can also be fully suppressed when placed on the Oregon R nuclear background (indicating a gene by gene interaction) highlighting the complexity of these interactions.

Disruption of interactions between Mother's Curse mitochondrial variants that reduce male fitness and nuclear restorers that mitigate this effect likely lead to hybrid incompatibility. While clear examples demonstrating this exist, there are still several unanswered questions regarding their dynamics. The traditional technique of generating hybrids to disrupt these interactions and evaluating differences in male and female fitness under specific environmental conditions, while effective, captures a relatively small portion of the diversity of these interactions. Additionally, only a few studies have been able to identify the nuclear variants within a specific background that restore organismal fitness for mtDNA variants (Meiklejohn, Holmbeck et al. 2013, Adrion, White et al. 2016). This has greatly limited our understanding of where in the genome nuclear restorers are located, including whether they are randomly distributed or are clustered on specific chromosomes

Asymmetric transmission of autosomes versus sex chromosomes further complicates this question. In species with an XY sex determination system and a 1:1 sex ratio, autosomes spend equal time in both chromosomal sexes, the X chromosome spends 2/3 of its time in females, and the Y chromosome spends all of its time in males. The amount of time spent in each of the sexes differentially influences several evolutionary processes: mutation rate is generally higher the longer a genetic element spends in males (Malcom, Wyckoff et al. 2003, Kirkpatrick and Hall 2004, Wilson Sayres and Makova 2011), the effective population size of the X and Y chromosome is reduced compared to autosomes (proportionally ¾ and ¼ respectively) magnifying the effect of drift and selection (Vicoso and Charlesworth 2006, Mank 2012), and sexual antagonism will select for specific chromosomal locations to offset the deleterious cost in one sex (Gibson, Chippindale et al. 2002, Mank 2009).

The role of asymmetric transmission as a driver of sexual conflict, in the case of maternally inherited mtDNA, and as a solution, in the case of autosomal or sex-linked nuclear restorers, is unclear. Here, we intend to directly address these questions using a combination of population genetic theory and experimental analyses. In **Chapter 2**, we use analytical models and computer simulations to comprehensively examine how transmission asymmetries of nuclear, mitochondrial, and sex chromosome linked genes differ in both causing and resolving sexual conflicts within a single population. **Chapter 3** expands this work to explore how disruption of co-evolved mitochondrial-nuclear interactions impacts population isolation and divergence under not only a variety of nuclear restorer chromosomal locations but also under several different migration schemes. We then leverage specifically constructed *Drosophila melanogaster* lines in **Chapter 4** to test for evidence of mitochondrial-Y interactions to experimentally examine the effects of the uniparentally inherited parts of the genome, as well as their interactions in males. We assay both longevity and gene expression, phenotypes previously demonstrated to be sensitive to both mitochondrial and Y haplotype.

CHAPTER 2

SEXUAL CONFLICT THROUGH MOTHER'S CURSE AND FATHER'S CURSE[1]

Abstract

In contrast with autosomes, lineages of sex chromosomes reside for different amounts of time in males and females, and this transmission asymmetry makes them hotspots for sexual conflict. Similarly, the maternal inheritance of the mitochondrial genome (mtDNA) means that mutations that are beneficial in females can spread in a population even if they are deleterious in males, a form of sexual conflict known as Mother's Curse. While both Mother's Curse and sex chromosome induced sexual conflict have been well studied on their own, the interaction between mitochondrial genes and genes on sex chromosomes is poorly understood. Here, we use analytical models and computer simulations to perform a comprehensive examination of how transmission asymmetries of nuclear, mitochondrial, and sex chromosome-linked genes may both cause and resolve sexual conflicts. For example, the accumulation of male-biased Mother's Curse mtDNA mutations will lead to selection in males for compensatory nuclear modifier loci that alleviate the effect. We show how the Y chromosome, being strictly paternally transmitted provides a particularly safe harbor for such modifiers. This analytical framework also allows us to discover a novel kind of sexual conflict, by which Y chromosome-autosome epistasis may result in the spread of male beneficial but female deleterious mutations in a population. We

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christen this phenomenon Father's Curse. Extending this analytical framework to ZW sex chromosome systems, where males are the heterogametic sex, we also show how W-autosome epistasis can lead to a novel kind of nuclear Mother's Curse. Overall, this study provides a comprehensive framework to understand how genetic transmission asymmetries may both cause and resolve sexual conflicts.

Introduction

Males and females differ in a wide variety of phenotypic traits, including reproduction, behavior, and morphology (Darwin 1871). Because the sexes largely share the same genome, an allele that moves a trait toward the optimal value for one sex may move it away from the optimal value of the other, a phenomenon known as intralocus sexual conflict (Arnqvist and Rowe 2005, Pennell and Morrow 2013). Loci underlying the sexually antagonistic trait may also differ between the sexes, resulting in so-called interlocus sexual conflict (Trivers 1972, Parker 1979). Phenotypic assays in *Drosophila (Chippindale, Gibson et al. 2001)* and water striders (Arnqvist and Rowe 2002) provided early direct empirical support for the occurrence and dynamic nature of both intralocus and interlocus sexual conflicts respectively. More recently, rapid advancements in sequencing technology have provided the means to tackle these questions molecularly, leading to a revived interest in the topic (reviewed in e.g. (Wright and Mank 2013, Kasimatis, Nelson et al. 2017, Mank 2017)). Several studies have examined the genomics of sexual conflict (Innocenti and Morrow 2010, Cheng and Kirkpatrick 2016, Wright, Fumagalli et al. 2018), however, the exact nature of these signatures is still poorly understood.

The genetics of sexual conflict is further complicated by the parts of the genome not equally shared by males and females. In contrast with autosomes, lineages of sex chromosomes

spend different amounts of time in males and females, and this transmission asymmetry makes them hotspots for sexual conflict (Jaenike 2001, van Doorn and Kirkpatrick 2007, Mank, Hosken et al. 2014). For example, in species with an XY sex chromosome system and a 50:50 sex ratio, a given X chromosome spends 2/3 of its time in females. This has led to the predication that the X chromosome should become 'feminized' or 'demasculinzed', which may occur through inter-chromosomal translocations, gene duplications, or alterations in sex-specific gene expression (Connallon and Clark 2011, Gallach and Betrán 2011). Consistent with this, an overrepresentation of female ovary-specific, and an under-representation of male testis-specific genes on the X has been reported in several *Drosophila* species (Sturgill, Zhang et al. 2007, Meisel, Malone et al. 2012, Allen, Bonduriansky et al. 2013), though not in, for example, humans or mice (Lercher, Urrutia et al. 2003, Yang, Schadt et al. 2006). Analogously, in a ZW sex chromosome system, where females are the heterogametic sex, we would then expect to see a masculinization of the Z chromosome (Rice 1984). In line with this predication, a series of gene expression studies of the Z have reported a trend of male-biased expression (Kaiser and Ellegren 2006, Storchová and Divina 2006, Mank and Ellegren 2009).

By similar logic, the maternal inheritance of the mitochondrial genome (mtDNA) means that mutations that are beneficial in females can spread in a population even if they are deleterious in males (Lewis 1941, Charlesworth and Charlesworth 1978, Frank 1989, Frank and Hurst 1996, Gemmell, Metcalf et al. 2004, Vaught and Dowling 2018). This has been particularly well studied in flowering plants, where mitochondrial mutations that prevent pollen production in otherwise hermaphroditic plants are widespread, a phenomenon known as cytoplasmic male sterility (Lewis 1941, Budar, Touzet et al. 2003, Case, Finseth et al. 2016). Selection on male function, so-called nuclear restorers of fertility, has then led to a co-

evolutionary arms race, which may result in the reproductive isolation of diverged populations (reviewed in (Rieseberg and Blackman 2010, Ågren 2013, Case, Finseth et al. 2016).

Similar examples from animal systems were long-missing. However, during the last few years, several studies have provided evidence of the occurrence of Mother's Curse, as this form of sexual conflict is typically referred to in animals (Gemmell, Metcalf et al. 2004). Experimental work in *Drosophila melanogaster* has demonstrated that males are more sensitive to mitochondrial genetic variation than females, which has been interpreted as evidence of Mother's Curse (Camus, Clancy et al. 2012). Furthermore, in a technical *tour de force* with *D. melanogaster*, Patel et al. (2016) identified $COII^{G177S}$, a mitochondrial hypomorph of cytochrome oxidase II, as male-harming locus causing a reduction in male fertility through a disruption of sperm development, but without any negative effects in females. It is worth noting that the male-sterile effect of $COII^{G177S}$ is dependent on the nuclear background. Such epistasis is in line with earlier suggestions that the accumulation of male-harming mitochondrial mutations should, just like in the plant cytoplasmic male sterility example, result in nuclear-encoded restorers of male fitness (Rand, Haney et al. 2004, Dowling, Friberg et al. 2008). Finally, evidence of Mother's Curse has also recently emerged in humans with the example of the mitochondrial mutation resulting in Leber's hereditary optic neuropathy, the degradation of retinal ganglion cells, resulting in loss of vision, which predominantly affects males (Milot, Moreau et al. 2017).

Thus, mitochondrial, X and Y, and Z and W genes have all been shown to be involved in sexual conflicts. However, while these examples have received both theoretical and empirical attention on their own, we lack a clear picture of how mitochondrial, nuclear, and sex-linked genes may interact in sexually antagonistic ways. Here, we develop a comprehensive theoretical

framework to examine the role of genetic transmission asymmetries in sexual conflicts. We consider the sexually antagonistic consequences of mitochondrial-autosome, mitochondrial-sex chromosome, and autosome-sex chromosome interactions in XY and ZW sex chromosome systems. These models show how the fate of a nuclear allele restoring male fitness in the face of Mother's Curse depends on whether it is located on autosomes, X, Y, or Z. Our models also allow us to discover a novel kind of sexual conflict by which Y chromosome-autosome, or Y-X, epistasis may result in the spread of male beneficial but female deleterious mutations in a population. We name this phenomenon Father's Curse. Analogously, we find that W-autosome, or W-Z interactions, may also result in a nuclear version of the Mother's Curse. Taken together, our results extend previous theoretical work on how genetic transmission asymmetries may lead to sexually antagonistic selection, but also on occasion reduce sexual conflict.

Material and Methods

Modeling Framework

We consider 9 two-locus two-allele models to investigate the fitness consequences for males and females of epistatic interactions between mitochondria and autosomes, mitochondria and sex chromosomes, and between sex chromosomes and autosomes (**Fig 2.1)**. We perform this analysis in both XY and ZW sex chromosomes systems. For each of the nine models below, we first discuss previous theoretical work and the available empirical data, and then outline our model and results. Genotypes and fitnesses for males and females in all models are summarized in **Table 2.1**.

Most of these models feature a biallelic locus that generates the sexual conflict and another biallelic locus that acts to restore fitness to the disadvantaged sex. With the resulting four

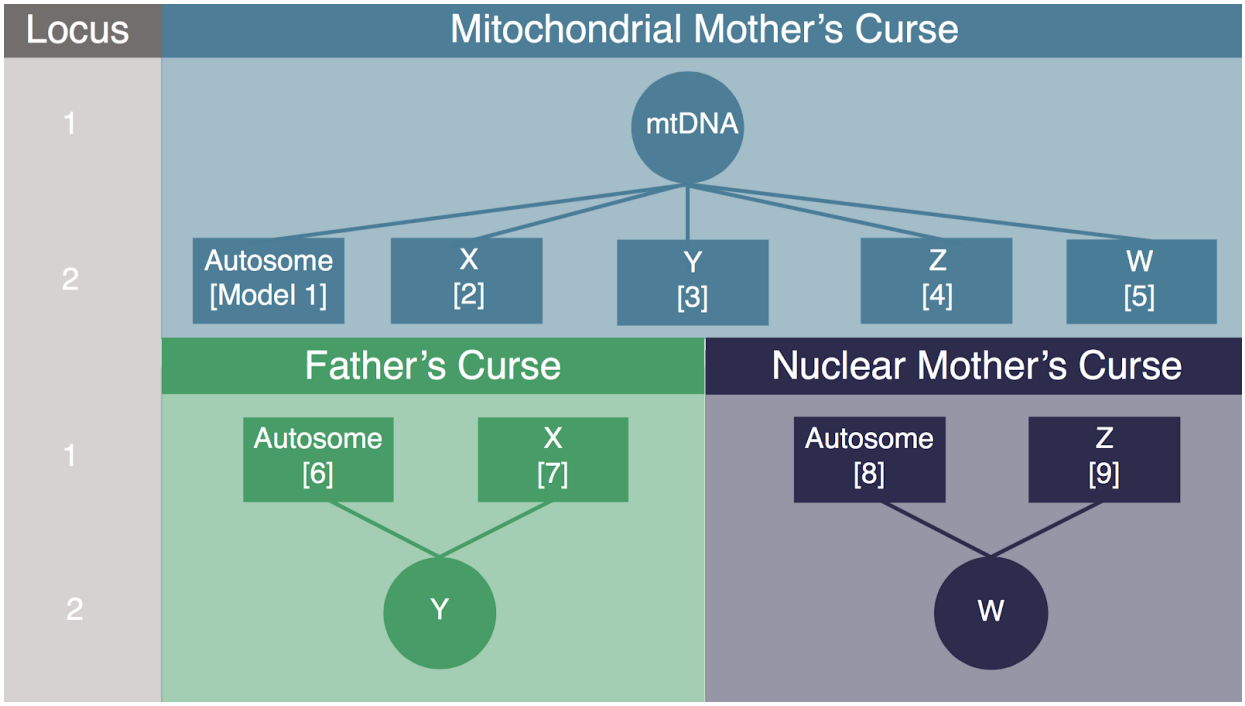

Figure 2.1. Graphical Representation of Models Considered. 9 two-locus two-allele models are considered. Models 1-5 capture the dynamics of a mitochondrial Mother's Curse mutation and a nuclear restorer located on an autosome or sex chromosome, respectively. In each model, Locus 1 is the primary sex-asymmetric locus, and Locus 2 harbors alleles that act to restore the fitness of the disadvantaged sex, except Model 5, where it further contributes to the advantaged sex. In Models 6-9, we model how selection acting on a mutation on the uniparentally inherited sex chromosome (Y, Models 6-7; W, Models 8-9) can lead to the spread of a mutation that is beneficial in the heterogametic sex but deleterious in the homogametic sex, Father's Curse and Nuclear Mother's Curse respectively.

gametic types, the models typically have three degrees of freedom and can be described by allele frequencies at the two loci, plus a linkage-disequilibrium-like term (Clark 1984, Asmussen, Arnold et al. 1987). None of these models harbors a stable joint polymorphism at both loci, so our only interest is in the stability of the fixation states, where the disequilibrium terms collapse to zero. For simplicity, we consider the two-dimensional systems characterized by the allele frequencies at the two loci, and we determine local stability from the leading eigenvalue of the linearized recursion.

To further confirm the analytical results, we modeled selection using forward simulations that incorporated selection as a deterministic process with initial zygote frequencies, followed by selection which acts as weights on the frequencies of the zygotes, and then random mating and gamete formation following Mendelian rules. In each model, the simulations track all possible genotypes in each sex (which are denoted for each model in **Table 2.1)**.

Table 2.1. Genotypes and Fitnesses in Models 1 - 9

Model	**Category**	**Males**		**Females**	
		Genotype	Fitness	Genotype	Fitness
1 mtDNA-Autosome	Mitochondrial Mother's Curse	*M-AA* *M-Aa* *M-aa* m-AA *m-Aa* *m-aa*	1 1 1 1-s_m 1-s_m+$\frac{s_a}{2}$ 1-s_m+s_a	*M-AA* *M-Aa* *M-aa* m-AA *m-Aa* *m-aa*	1 1 1 1+s_f 1+s_f 1+s_f
2 mtDNA-X	Mitochondrial Mother's Curse	*M-X* *M-x* *m-X* *m-x*	1 1 1-s_m 1-s_m+s_x	M-XX M-Xx M-xx m-XX m-Xx m-xx	1 1 1 1+s_f 1+s_f 1+s_f
3 mtDNA-Y	Mitochondrial Mother's Curse	M-Y M-y m-Y m-y	1 1 1-s_m 1-s_m+s_y	M m	1 1+s_f
4 mtDNA-Z	Mitochondrial Mother's Curse	M-ZZ M-Zz M-zz m-ZZ m-Zz m-zz	1 1 1 1-s_m 1-s_m+$\frac{s_z}{2}$ 1-s_m+sz	M-ZW M-zW m-ZW m-zW	1 1 1+s_f 1+s_f
5 mtDNA-W	Mitochondrial Mother's Curse	M-ZZ m-ZZ	1 1-s_m	M-ZW m-ZW M-Ztt	1 1+s_f 1 1+s_f

6 Autosome-Y	Father's Curse	AA-Y Aa-Y aa-Y AA-y Aa-y aa-y	1 1 1 1 $1+\frac{s_m}{2}$ $1+s_m$	AA Aa aa	1 $1-\frac{s_f}{2}$ $1-s_f$
7 X-Y	Father's Curse	XY xY Xy xy	1 1 1 $1+s_m$	XX Xx xx	1 $1-\frac{s_f}{2}$ $1-s_f$
8 Autosome-W	Nuclear Mother's Curse	AA Aa aa	1 $1-\frac{s_m}{2}$ $1-s_m$	AA-W Aa-W aa-W AA-w Aa-w aa-w	1 1 1 1 $1+\frac{s_f}{2}$ $1+s_f$
9 Z-W	Nuclear Mother's Curse	ZZ Zz zz	1 $1-\frac{s_m}{2}$ $1-s_m$	ZW Zw zW zw	1 1 1 $1+s_f$

Mitochondrial Mother's Curse simulations (Models 1-5) track the frequency and time to fixation of the mutant alleles for a given parameter set of $s_f, s_m, s_x,$ and s_z for an arbitrary 5,000 generations. Similarly, Father's Curse and nuclear Mother's Curse track the frequency and time to fixation of the mutant alleles for a given parameter set of s_f and s_m for 10,000 generations. All simulations were run in R v.3.3.1, and scripts are on GitHub (https://github.com/mam737/ParentalCurseScripts).

Results

Models – Mitochondrial Mother's Curse

Model 1 Mitochondrial Mother's Curse and Autosomal Restoration

Early theoretical work on mitochondrial-autosome interactions was done in the 1970s (Charlesworth and Charlesworth 1978, Charlesworth and Ganders 1979) and 1980s (Charlesworth 1981, Clark 1984, Asmussen, Arnold et al. 1987, Arnold, Asmussen et al. 1988). Models of cytoplasmic male sterility as an evolutionary route from hermaphroditism to dioecy, via gynodioecy (the presence of hermaphrodites and females) has also received extensive attention in flowering plants, and these models have since accumulated abundant empirical attention (Saur Jacobs and Wade 2003, Delph, Touzet et al. 2007, Charlesworth, Qiu et al. 2010). Furthermore, the importance of mito-nuclear compatibility in flowering plants is also revealed by the large number of cases of mito-nuclear induced reproductive isolation reported (Bomblies 2010, Rieseberg and Blackman 2010). More recently, several theoretical models of male fitness restoration in response to Mother's Curse have been developed (Unckless and Herren 2009, Wade and Brandvain 2009, Wade and Drown 2016, Connallon, Camus et al. 2018). This upswing in interest is partially due to an increased general appreciation of the importance of mitochondrial variation on fitness (Camus, Clancy et al. 2012, Mossman, Tross et al. 2016, Baris, Wagner et al. 2017), as well as direct test of the Mother's Curse (Patel, Miriyala et al. 2016, Milot, Moreau et al. 2017). Thus, the basic population dynamics of mito-autosome interactions are well worked out, so only for completeness do we lay out the model here.

There are two mitochondrial types (*M* and *m*) such that the ancestral type (*M*) has fitness 1 and the derived type (*m*) has an advantage in females and disadvantage in males. There is one autosomal locus that can serve as a restorer of fitness in males, so each sex has six cytogenotypes (*M-AA*, *M-Aa*, *M-aa*, *m-AA*, *m-Aa* and *m-aa*; **Table 2.1**). The fitness of all females with the *M* mitotype are 1 regardless of the autosomal locus, and those with the *m* mitotype have fitness $1+s_f$. Male genotypes with the *M* mitotype also all have fitness 1, and those with the *m*

mitochondria have fitnesses (in the order as above: 1, 1-s_m+s_a/2, and 1-s_m+s_a), where s_m is the deleterious male effect of the new mitochondrial type and s_a is the effect of the restorer (which is assumed to be additive). By normalizing frequencies within each sex, we are implicitly assuming that males can inseminate all reproductive females, and that a skewed sex ratio plays no role in the dynamics of either locus.

Whenever $s_f > 0$, the $m = 0$ equilibrium is unstable, and the Mother's Curse mitochondrial type increases in frequency all the way to fixation, regardless of the fitnesses of the male genotypes. The conditions for invasion of the compensatory *a* allele depend on the frequency of *m*, but once the *m* allele has gone to fixation, the condition for invasion is simply $s_a > s_m$ (**Fig. 2.2**). Connallon et al. (2018) consider the male genetic load in a similar model, noting that the load depends on the arrival interval of both mitochondrial and autosomal mutations with appropriate fitness effects, as well as the distribution of fitness effects of those mutations. This suggests that the interesting empirical question that remains is to better quantify these mutation rates and distributions of fitness effects.

Model 1 as stated has highly constrained fitnesses, and one might ask how the dynamics change when, for example, different mito-autosomal combinations fix in different populations such that the *M-AA* and *m-aa* female genotypes both have the highest fitnesses. Clark (1984) showed that, in a one-sex model with autosomal and mitochondrial loci, regardless of the configuration of the six fitnesses, there is no admissible stable polymorphism for both loci.

Model 2 Mitochondrial Mother's Curse and X-linked Restoration

The co-evolution between mitochondrial and X-linked genes has been modelled by Rand et al. (2001), as well as by Wade and Drown (2016). These models highlight how mito-X

epistasis can maintain mito-nuclear polymorphisms, and because the X spends 2/3 of its time in females it can exacerbate the spread of male-harming mitochondrial mutations. Rand et al. (2001) also used experiments in *Drosophila melanogaster* to provide direct empirical support for the importance of mito-X interactions. Further indirect evidence of mito-X interactions come from studies of the genomic distribution of nuclear genes with mitochondrial functions, which are assumed to have migrated from the mitochondrial to the nuclear genome. Early evidence suggested a general pattern by which such genes were under-represented on the X across multiple taxa (Drown, Preuss et al. 2012). However, later work using phylogenetic independent methods demonstrated that this pattern was restricted to therian mammals and *Caenorhabditis elegans* (Dean, Zimmer et al. 2014). All other animal species studied show no chromosomal bias in their distribution. The same lack of biased distribution was also seen in a dioecious plant with sex chromosomes (Hough, Ågren et al. 2014).

Again, we have mitochondrial alleles (*M* and *m*) and now a pair of X chromosomal alleles (*X* and *x*). Females are of six genotypes (*M-XX*, *M-Xx*, *M-xx*, *m-XX*, *m-Xx*, and *m-xx*), and males are of four genotypes (*M-XY*, *M-xY*, *m-XY*, and *m-xY*). In males, the *m* mitochondrial allele comes with a fitness cost, but only with the ancestral X chromosome, and the *x* allele provides fitness compensation of s_x. Thus, the fitnesses of females (in the order above) are (1,1,1, $1+s_f$, $1+s_f$, $1+s_f$) and the male fitness costs (in the order above) are (1, $1-s_m$, 1, $1-s_m+s_x$).

As in Model 1, whenever $s_f > 0$, the *m* mitochondrial type invades when it is initially rare, and increases in frequency all the way to fixation, regardless of male fitness. In this simplified parameter space, whenever $s_x > s_m$, the X-linked restorer will invade and go to fixation. The interesting contrast to Model 1 lies in the comparison of rates of increase of the Mother's Curse mitochondria and of the restorer allele, and these questions are explored numerically below (**Fig.**

2.2). Allowing a more generalized assignment of fitnesses readily produces quite complex behavior, including protected polymorphism where neither the Mother's Curse allele nor the restorer go to fixation but instead both cycle in frequency indefinitely (Rand, Clark et al. 2001)

Model 3 Mitochondrial Mother's Curse and Y-linked restoration

The Y chromosome, being strictly paternally inherited, is a promising candidate location for restorers of male fitness in the face of the Mother's Curse. However, the dynamics of this interaction has never, as far as we are aware, been formally modeled. Some empirical evidence suggests that mito-Y interactions may be an important determinant of male fitness. Comparing nuclear autosomal genes whose expression has been shown to be sensitive to either mitochondrial variation (Innocenti, Morrow et al. 2011) or Y-linked variation (Lemos, Araripe et al. 2008) reveals considerable overlap (Rogell, Dean et al. 2014). Direct tests of mito-Y interactions have come from experiments using crosses between mitochondrial and Y chromosome replacement lines in *Drosophila melanogaster* (Dean, Lemos et al. 2015, Yee, Rogell et al. 2015). These studies find extensive mito-Y epistasis, which at least partly appears to be environmentally sensitive, but to date there are no reports of the presence of Y-linked restorers of male fitness from the Mother's Curse.

We consider a pair of mitochondrial alleles (*M* and *m*) and a pair of Y chromosomes (*Y* and *y*). Females are of two genotypes (*M-XX* and *m-XX*). Males are of four genotypes (*M-XY, m-XY, M-Xy*, and *m-Xy*). We imagine the mitochondrial mutation as having an advantage in females, so the fitness of females (*M* and *m*) are (1 and $1 + s_f$) respectively. In males, the *m* mitochondrial allele comes at a fitness cost of s_m, but only with the ancestral Y chromosome,

and the *y* allele provides fitness compensation of s_y. Thus, the four male genotypes (in the order above) have respective fitnesses (1, 1-s_m, 1, and 1-s_m+s_y). Because of the simple transmission rules of mtDNA and the Y chromosome, the recursion for the frequency of the *m* mitochondrial type (whose frequency is p_m) is:

$$p_m' = p_m(1+s_f)/[(1-p_m) + (p_m)(1+s_f)]$$

From the initial condition of $p_m = 0$, the invasion condition for the novel Mother's Curse mitochondria to have the eigenvalue of the linearized system exceed 1, and this occurs whenever $s_f > 0$. Thus, whenever there is any advantage to the daughters, the new mitochondrial type invades and goes to fixation, regardless of its effect in males. The recursion for the male-fitness restoring *y* allele is:

$$p_y' = [p_y(1-s_m+s_y)] / [(1-p_y)(1-s_m) + (p_y)(1-s_m+s_y)]$$

Here the fixation of $p_y = 0$ is unstable, and both invasion and fixation of the restorer Y chromosomes is guaranteed when $s_y > s_m$. There are no stable polymorphic states, and in fact this model has very rapid and simple dynamics, so the proportion of time the population is in transient polymorphism would be small. The fate of the population seems to reside in a mutation-limited state, where the waiting time to the next Mother's Curse and next Y restorer will determine the balance of male and female fitness.

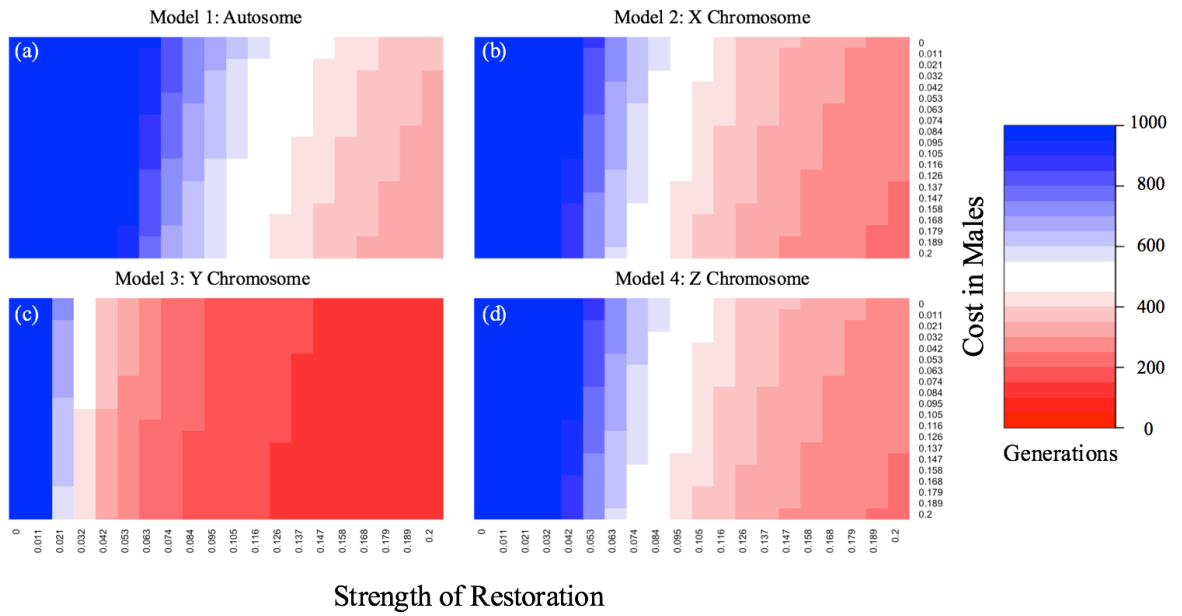

Figure 2.2. Fixation Time for Mother's Curse Compensators. The rate of fixation is shown for a compensator located on an autosome (a; Model 1), X chromosome (b; Model 2), Y chromosome (c; Model 3), or Z chromosome (d; Model 4) for a given female benefit (s_f= 0.105; simulations were run for 19 different values evenly spaced between 0.02 and 0.4). Colors show the number of generations (up to 1000) required for a nuclear compensator that restores male fitness to fix. The *x*-axis is the strength of restoration of the compensator (s_a, s_x, s_y, and s_z respectively).

Model 4 Mitochondrial Mother's Curse and Z-linked restoration

In a ZW system, the Z chromosome spends ⅔ of its time in males and only ⅓ of its time in females. As a consequence, the masculinized Z (Wright, Moghadam et al. 2012) may be expected to harbor male-biased restorers of Mother's Curse (Wade and Drown 2016). A recent study of the chromosomal location of nuclear genes with mitochondrial function, however, did not find any overrepresentation of such genes on the Z (Dean, Zimmer et al. 2014). Nevertheless, as this model shows, there remains good motivation to perform a screen for Z-linked variants that may have Mother's Curse restorer function.

Following the nomenclature that is familiar by now, females will have four genotypes (*M-ZW*, *M-zW*, *m-ZW*, and *m-zW*). Males are of six genotypes (*M-ZZ*, *M-Zz*, *M-zz*, *m-ZZ*, *m-Zz*, and *m-zz*). The fitnesses of the females (in the order above) are (1, 1, 1+s_f, 1+s_f) and the males are (1, 1, 1, 1-s_m, 1-s_m+s_z/2, 1-s_m+s_z). Whenever $s_f > 0$ the derived mitochondrial mutation *m* confers an advantage to females, and this comes at a cost to males of s_m. If males acquire the

derived z allele, their fitness is restored by $\frac{s_z}{2}$ in Zz heterozygotes and by s_z in zz homozygotes. The recursions for the allele frequency dynamics for both mitochondrial type that causes the female-favoring male-disfavoring effects and the Z-linked allele that restores male fitness are presented in the **Supplement**, along with the stability conditions of the fixation point where the frequencies of these alleles (p_m,p_z) are (0,0). Even in the absence of the (Z,z) polymorphism, the m mitochondrial type invades if s_f>0, and the z allele accelerates fixation of the m allele (**Fig. 2.2**).

Model 5 Mitochondrial Mother's Curse and W-linked restoration

The mitochondrial genome and the W chromosome are always co-transmitted and any mito-W combination that improves female fitness should spread. An explicit screen of mito-W interactions did detect some W-linked mito-nuclear genes in the blood fluke *Schistosoma mansoni*, but found no overrepresentation of these genes on the W (Dean, Zimmer et al. 2014). In addition, the low levels of mitochondrial genetic variation in birds compared to mammals has been suggested to be due to Hill-Robertson effects on the W (Berlin, Tomaras et al. 2007).

We consider a pair of mitochondrial alleles (M and m) and a pair of W-linked alleles (W and w). In this case, we assume no segregating variation on the Z. Females can be of four genotypes (*M-ZW*, *m-ZW*, *M-Zw*, and *m-Zw*). Males are two genotypes (*M-ZZ* and *m-ZZ*). Because the males always transmit the Z chromosome, and they do not transmit their mtDNA, the males essentially become irrelevant in the model, apart from assuming that even with an extremely biased sex ratio, all females can be fertilized. To be consistent with the XY Mother's curse, the fitnesses of the *M-ZW* and *M-Zw* females are both 1, and the *m-ZW* and *m-Zw* females have fitness $1+s_f$. The male fitnesses are (in the order above) 1 and $1 - s_m$.

This case behaves essentially like clonal evolution, as each female transmits the mitochondrion and W chromosome to all of her female offspring. Thus, trivially, whichever female genotype has the highest fitness always goes to fixation. The W chromosome and mitochondrion are inseparable with respect to sex-specific functional mutations that they may harbor, and each can drive the other's selective sweeps. This observation raises the empirical question of whether there are W-mtDNA epistatic interactions, regardless of whether they cause or resolve sexual conflict.

Models - Father's Curse

Model 6 Father's Curse with Y variation driving a female deleterious autosomal allele

It has long been recognized that the differential transmission of autosomes and sex chromosomes will influence their evolutionary trajectories (Charlesworth, Coyne et al. 1987). The transmission asymmetry will also affect the epistatic interactions between genes located on autosomes and sex chromosomes modeled the conditions for the evolution of X chromosome-autosome incompatibilities, a phenomenon that has been suggested as an explanation for Haldane's Rule (see (Coyne 2018) for a recent discussion of this possibility).

Here, we are introducing a novel kind of sexual conflict, which we call Father's Curse. This curse arises when a derived Y-linked allele impacts expression of an autosomal locus such that one allele that favors males over females. A potential biological example is the high heterochromatin content of the Y chromosome of *Drosophila*, which has long been known to influence the chromatin state of the $white^{mottled4}$ allele and suppress position effect variegation (reviewed in (Elgin and Reuter 2013)). Subsequently, it has also been demonstrated that

differences in heterochromatin composition of the Y can impact gene expression and chromatin state of many regions of the genome (Lemos, Araripe et al. 2008, Jiang, Hartl et al. 2010, Lemos, Branco et al. 2010, Silkaitis and Lemos 2014), consistent with the occurrence of Y chromosome driven sexual conflict.

Consider Y-linked alleles (*Y* and *y*) and autosomal alleles (*A* and *a*). Females are of only three genotypes (*AA*, *Aa* and *aa*), and males are of six genotypes (*AA*-*Y*, *Aa*-*Y*, *aa*-*Y*, *AA*-*y*, *Aa*-*y*, and *aa*-*y*). Female fitnesses are (1, 1-s_f/2, and 1-s_f) and male fitnesses are (1, 1, 1, 1, 1+s_m/2, 1+s_m). These conditions allow the *y* allele to provide a selective advantage for *aa* males that may be big enough to increase the *a* allele frequency even when it is disfavorable in females.

The dynamics of the Y chromosome are simple, owing to its haploid transmission. If the mean fitness of the bearers of the *y* allele exceeds the mean fitness of the bearers of the *Y* allele, then the *y* allele will increase in frequency. As the *y* allele increases in frequency, the shifting proportions of *y* and *Y* alleles may alter the marginal fitnesses of the autosomal genotypes, but given the constraints on the fitnesses, the *y* allele will always fix if it can invade. While on this trajectory toward fixation, the *y* allele can result in driving up the frequency of an autosomal allele in both sexes, even if that allele is disfavorable in females. Hence the name Father's Curse.

One interesting behavior occurs when s_f is sufficiently large compared to s_m, and the autosomal allele is lost. The Y alleles may then continue to segregate in the population, but they become effectively neutral. This underscores how Father's Curse requires an autosomal locus that harbors the alleles with the sexual conflict, and under some circumstances the Y variant can serve to drive the male-favoring (and female-disfavoring) allele to fixation (**Fig. 2.3**). For further details of the dynamics of this model, see the **Supplement**.

Model 7 Father's Curse with Y variation driving a female deleterious X-linked allele

The effect of the Y chromosome on nuclear gene expression is not restricted to autosomes. Experimental work in *D. melanogaster* has shown that Y-linked genes can alter the expression of genes on the X chromosome (Jiang, Hartl et al. 2010). In these studies, genes on the X that were located close to the euchromatin–heterochromatin boundary were particularly sensitive. Here, we consider the case in which a novel variant of the Y chromosome impacts expression of an allele at an X-linked locus that favors males over females. Co-evolution of the X and the Y results in an increase in frequency of the male-favoring X at the cost of displacing females from their fitness optimum.

Consider two Y alleles (*Y* and *y*) and two X-linked alleles (*X* and *x*). Females are of only three genotypes (*XX*, *Xx* and *xx*), and males are of four genotypes (*XY, Xy, xY,* and *xy*). Female fitnesses are (1, 1-s_f/2, and 1-s_f) and male fitnesses are (1, 1, 1, 1+s_m). So the *y* allele has an advantage for *xy* males, that may be big enough to drive the X-locus to an allele frequency that is disfavorable in females. Together with the autosome-Y example described above (Model 5) this provides another example of how a father's alleles serve to favor sons at the expense of the daughter's fitness, and thus we have another example of Father's Curse.

The **Supplement** establishes the recursions for this system, which we linearize to determine the conditions for invasion of the *y* allele. Because the initial Y chromosome allele dynamics depend only on the males, if $s_m > 1$, the *y* allele can invade, and this is consistent with the numerical results. The Y chromosome allele dynamics are very rapid because of its haploid-like transmission, and as it invades it drags the female-deleterious *x* allele with it (**Fig. 2.3**).

Models - Nuclear Mother's Curse

Model 8 Nuclear Mother's Curse with W Variation Driving a Male Deleterious Autosomal Allele

Analogously to Model 6, where the Y chromosome-autosome epistasis causes the spread of a female-harming autosomal mutation, the W chromosome may drive the invasion of a male-harming autosomal mutation. This is a form of Mother's Curse, but unlike previously described examples, it is not due to a cytoplasmic (mitochondrial) mutation. We call this a nuclear Mother's Curse. We have yet to find a study that sought to detect this effect in a natural population, and such a study would appear to be well motivated.

Consider two W alleles (*W* and *w*) and autosomal alleles (*A* and *a*). Males have three genotypes (*AA*, *Aa*, and *aa*), females are of six genotypes (*AA*-*W*, *Aa*-*W*, *aa*-*W*, *AA*-*w*, *Aa*-*w*, and *aa*-*w*). Male fitnesses are (1, 1-s_m/2, and 1-s_m) and female fitnesses are (1, 1, 1, 1, 1+s_f/2, 1+s_f). The *w* allele provides an advantage for the *Aa* and *aa* females, which may be big enough to make a spread despite its fitness cost in males. Contrasting this model with Model 6 (Y-autosome Father's Curse) is instructive. Examination of the fitnesses in **Table 2.1** reveals that the two models are equivalent, except for the sex labels, and this is consistent with the recursions and the appearance of the simulation results in **Figure 2.3**. Of course, biologically these two situation may not be expected to be so analogous, given the large difference in the variance in offspring numbers of males and females, the expected greater impact of sexual selection in males than females in most species, and the unknown degree to which the W chromosome impacts chromatin state and gene expression genome-wide.

Model 9: Nuclear Mother's Curse with W variation driving a male deleterious Z-linked allele

In this model, variation on the W chromosome interacts with a Z-linked locus to favor alleles that are female-advantageous and male disadvantageous to cause a second kind of nuclear Mother's Curse.

Consider W alleles (*W* and *w*) and Z-linked alleles (*Z* and *z*). Males are of only three genotypes (*ZZ*, *Zz*, and *zz*). Females are of four genotypes (*ZW*, *zW*, *Zw*, and *zw*). Males fitnesses are 1, 1-s_m/2, and 1-s_m and female fitnesses are 1, 1, 1, and 1+s_f respectively. Inspection of these fitness parameters shows that it is the sex-flipped version of Model 7 (X-Y Father's curse), with

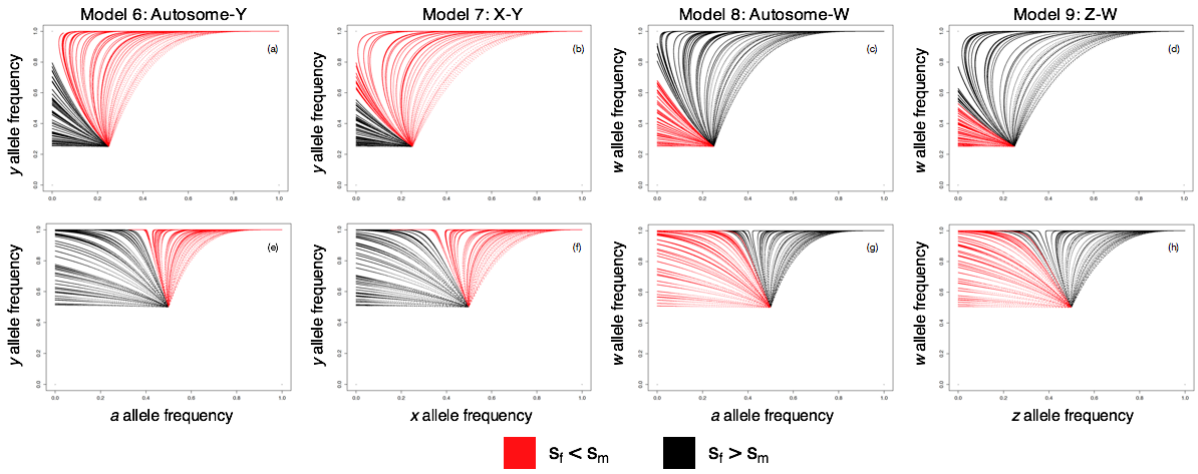

Figure 2.3. Invasion Conditions for Father's Curse and Nuclear Mother's Curse. In Father's Curse (Models 6 and 7) and Nuclear Mother's Curse (Models 8 and 9), the uniparentally inherited sex chromosome (Y or W) can drive the fixation of an allele that is deleterious in the homogametic sex. Analytical work examines invasion of the sex-specific alleles (Y and W) and the sexually antagonistic alleles (X, Z, or autosomal) from the lower left corner, but higher frequency starting points better illustrate the dynamical behavior. These figures clearly demonstrate that both variants will fix as long the selective cost in the 'cursed' sex is smaller than the selective advantage in the benefitting sex. For Models 6-9, both alleles start with an initial frequency of either 0.25 (a-d) or 0.5 (e-h), and the change in allele frequency at every generation is tracked for a total of either 5000 (a,e,c,g) or 10,000 (b,f,d,h) generations. Simulations were run for 100 randomly selected combinations of s_f and s_m for each model. Trajectories where the absolute value of the selection coefficient in females (s_f) is less than that in males (s_m) are shown in red, and the opposite in black.

the same parameterization. As mentioned above for the contrast of Models 6 and 8, aspects of the biology will likely prevent Models 7 and 9 from being biologically equivalent, but with respect to the models presented here, the stability conditions and dynamics will be the same (see **Fig. 2.3**). This means that the dynamics of the W chromosome will be more rapid than the Z, and the

conditions for the initial invasion of the *w* allele depend on polymorphism at the Z locus. Further details can be found in the **Supplement**.

Discussion

In their highly cited letter to *Nature*, Frank and Hurst (1996) describe the logic of the population genetics argument for what we now call Mother's Curse as "indisputable." In this paper, we have extended this logic to consider the full spectrum of parental curses caused by the uniparental inheritance of genes. Using a combination of analytical models and computer simulations, we determine the fitness consequences of transmission asymmetries of nuclear, mitochondrial, and sex chromosome-linked genes and how they may both cause and resolve sexual conflicts. Contrary to recent arguments that the evolution of nuclear restorer genes is an unlikely route to reverse the mother's curse (e.g. (Wade 2014)), we show how restorers can readily evolve, but the rate depends on the exact transmission pattern of the chromosome harboring the restorer locus. We also demonstrate a novel way by which interactions between uniparentally and biparentally inherited genes can lead to sexual conflict: Father's Curse and nuclear Mother's Curse. Here, both variants increase in frequency, and often reach fixation, as long as the selective advantage in the benefitting sex is larger than the selective cost in the cursed sex.

The general framework developed here opens up many avenues for informative extensions. In a recent paper, Connallon et al. (2018) developed a quantitative measure of male mitochondrial load–the reduction in fitness due to the accumulation of mitochondrial mutations–in the context of Mother's Curse. This effort is well motivated by the observation that the dynamics of the model are relatively simple, and so an important feature is the steady state

frequency of curse alleles in transition to fixation or loss. It also motivates a contrast of the levels of load across the three classes of parental curse models outlined here, including mitochondrial Mother's Curse, nuclear Mother's Curse, and Father's Curse.

Demographic processes may alter some of the trajectories described here. Our models assume random mating, but Wade and Brandvain (2009) and Unckless and Herren (2009) have both showed how inbreeding, mating between close relatives, will limit the spread of mitochondrial Mother's Curse variants because it links a mother's fitness with that of her sons. Similarly, if males affect the fitness of their sisters, kin selection may have the same effect (Wade and Brandvain 2009). In addition to altering the trajectory of the Mother's Curse allele, we also expect the spread of male restorers to be affected by these processes.

In all our models we have assumed that male gametes are never limiting, which is formally equivalent to assuming that the sex ratio remains 50:50 throughout. Hamilton (1967) showed how a driving Y chromosome can result in a male biased ("extraordinary") sex ratio, which may eventually drive the population extinct. Since then, several sex chromosome drivers have been identified (reviewed in e.g. (Bravo Núñez, Nuckolls et al. 2018)). Maternally inherited mitochondrial mutations should yield a female-biased sex ratio, which has also been suggested as an alternative explanation to Haldane's Rule (Hurst and Pomiankowski 1991). A biased sex ratio will likely affect the dynamics of our models, and relaxing the 50:50 assumption would allow us to incorporate another kind of genetic conflict.

In Models 1-5 we are modeling the fate of a nuclear mutation restoring male fitness, and we assume the restoration is associated with no cost. However, this assumption is questionable. The cost of nuclear restorers has been best explored in the context of the evolution of gynodioecy, the presence of females and hermaphrodites in a population (Bailey and Delph

2007, Delph, Touzet et al. 2007). Studies in a variety of plant systems have characterized the molecular mechanism and fitness cost of nuclear restorers of male fertility in hermaphrodites. A recent example is the study of the cytoplasmic male sterility system in *Brassica napus* by Montgomery et al. (2014), which demonstrated not only the presence of costs but also showed how the fitness cost differed between two different nuclear restorers. Without such costs, theory predicts that restorers will always fix and they are therefore crucial to the maintenance of gynodioecy (Delph, Touzet et al. 2007). As we learn more about the fitness consequences of mitochondrial mutations, including in animals, and how they depend on the nuclear background, incorporating the cost of nuclear restorers could be a productive focus of future modeling efforts.

As is typical for these kinds of models, we assume no paternal leakage (inheritance from the father) of the mitochondrial genome. While maternal inheritance of the mitochondrial genome is shared across the tree of life, exceptions exist. Molluscan bivalves, for example, have doubly uniparental inheritance, whereby females pass on their mitochondrial genomes to both sons and daughters, whereas males only transmit to sons (Breton, Beaupré et al. 2007). In some plants, including cucumber (Havey 1997) and many conifers (Worth, Yokogawa et al. 2014), uniparental paternal inheritance appears to be the norm. Lastly, paternal leakage has also been reported in variety of systems, including but not limited to humans (Schwartz and Vissing 2002), fruit flies (Wolff, Nafisinia et al. 2013), sheep (Zhao, Li et al. 2004), and chicken (Alexander, Ho et al. 2015). Paternal leakage will introduce direct purifying selection on the male deleterious mitochondrial mutations, thereby limiting the spread such mutations. Paternal leakage, however, appears to be rare (an early estimate in mice put it at 10^{-4}; (Gyllensten, Wharton et al. 1991) and

it is generally considered too weak of a force to reverse the Mother's Curse (Engelstädter and Charlat 2006, Wade and Brandvain 2009, Connallon, Camus et al. 2018).

Our models also have implications for the potential role of transmission asymmetries in generating reproductive isolation through Bateson-Dobzhansky-Muller incompatibilities. The Bateson-Dobzhansky-Muller model (Bateson 1909, Dobzhansky 1937, Muller 1942) characterizes the emergence of incompatibilities between two allopatric populations due to the fixation of variants within one population that, while neutral in their original background, are deleterious in the genetic background of the other. (Burton and Barreto 2012) highlight numerous such incompatibilities between mitochondrial and nuclear genomes that result in hybrid breakdown, and the evolution of pairwise incompatibilities among mitochondrial, autosomal, and X-linked genes in parapatric populations has also been further explored through analytical models (Höllinger and Hermisson 2017).

While our models only consider a single population, the potential rapid fixation of both alleles in every model highlights the potential accumulation of two variants that in combination are either neutral or advantageous. Mating between two previously isolated populations could disrupt these interactions leading to hybrid incompatibilities. Extensions of our models that incorporate multiple populations with migration among them may therefore provide further insight into how asymmetrically inherited genomic components contribute to the genetics of reproductive isolation.

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Author Contributions

J.A.Å and A.G.C conceived the study. J.A.Å, A.G.C, and M.M. designed the theoretical models. A.G.C calculated the analytical results, while M.M coded the simulations to confirm them. J.A.Å wrote the first draft and all authors contributed to the writing of the manuscript. All authors approved the final version of the manuscript. A.G.C supervised the project.

Data Accessibility

The scripts for all simulations can be found on GitHub: https://github.com/mam737/ParentalCurseScripts

Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S2.A - Stability conditions for Models 4, and 6–9.

CHAPTER 3

MIGRATION RESTORES HYBRID INCOMPATIBILITY DRIVEN BY NUCLEAR-MITOCHONDRIAL SEXUAL CONFLICT[2]

Abstract

Cellular function requires the coordinated transcription, translation, and assembly of mitochondrial proteins encoded by both the mitochondrial and nuclear genomes. Sexual asymmetry in mitochondrial-genome transmission favors mutations that are advantageous in females even if they are deleterious in males. Coined Mother's Curse, this phenomenon induces a selective pressure for nuclear variants that compensate for this reduction in male fitness, generating a specific subset of mitochondrial-nuclear interactions. Previous work has demonstrated not only the existence of these interactions but also their potential for generating hybrid incompatibility between populations. While it is easy to see how Mother's Curse mtDNA variants and their nuclear compensators could act as Dobzhansky-Muller loci, it is not clear how readily it would give rise to and sustain hybrid incompatibilities. Here, we apply computer simulations using SLiM3 to expand analytical theory to investigate the consequences of sexually antagonistic mitochondrial-nuclear interactions in a subdivided population. We consider both the chromosomal location, and consequently the transmission pattern, of nuclear restorers and distinct migration schemes, such as continuous symmetric migration, 1 generation of symmetric migration, continuous asymmetric migration, and continuous sex-specific symmetric migration.

[2] Manuscript in preparation: Munasinghe M., B. Haller, and A.G. Clark. Migration Restores Hybrid Incompatibility Driven By Nuclear-Mitochondrial Sexual Conflict.

We find that disrupting these co-evolved interactions results in less-fit males consequently skewing the sex-ratio towards females. Restoration of male fitness depends on both the chromosomal location of nuclear restorers and the migration scheme. Our results clearly show that these interactions do act as Dobzhansky-Muller incompatibilities, but the strength of these interactions is not enough to drive population isolation on their own. Combined these models show the varied ways in which populations respond to the disruption of co-evolved mitochondrial-nuclear interactions via migration.

Introduction

A fundamental question in evolutionary genetics and biology more broadly is how new species form and remain distinct (Mayr 1995, Coyne and Orr 2004). Dobzhansky (Dobzhansky 1935) and Mayr (Mayr 1942) argued that this process hinges on the evolution of reproductive isolation, which acts to limit gene flow between species. The 'biological species concept' formalized this idea and explicitly defined species as groups of interbreeding natural populations that are substantially but not necessarily completely reproductively isolated from other groups (Mayr 1942, Coyne and Orr 2004). Reproductive isolation is established as isolating barriers accumulate. These barriers may prevent members of different species from mating or forming zygotes (prezygotic) or may act after fertilization if hybrids are incompatible (postzygotic) (Dobzhansky 1937, Dobzhansky 1951). Understanding the genetic basis of hybrid incompatibility, which encompasses hybrid inviability, sterility, or simply reduced fitness of hybrids when compared to the parental populations, consequently allows us to better understand the mechanics of speciation.

Bateson (Bateson 1909), Dobzhansky (Dobzhansky 1934), and Muller (Muller 1939, Muller 1940, Muller 1942) first detailed how hybrid incompatibility could emerge between two allopatric populations. Populations acquire unique mutations while separated, and selection acts to remove any mutation that is incompatible with its genetic background. However, when populations reunite and hybridize, untested interactions between mutations are exposed and may result in reduced hybrid fitness. We now have several examples of negative epistatic interactions, dubbed Dobzhansky-Muller incompatibilities, that generate hybrid incompatibility and, consequently, contribute to reproductive isolation (Sweigart, Fishman et al. 2006, Lowry, Modliszewski et al. 2008, Presgraves 2010). In spite of this, it remains unclear whether and which specific genetic interactions may become Dobzhansky-Muller incompatibilities.

Interactions between the mitochondrial and nuclear genomes have been put forward as promising candidates for generating these epistatic interactions (Burton and Barreto 2012, Jy and Jy 2015, Hénault and Landry 2017). This stems from the unique function and transmission of mitochondrial DNA. The mitochondrial genome encodes the ribosomal and transfer RNA components of the mitochondrial translation system and 13 protein subunits, a small but essential fraction of the electron transport chain and ATP synthase (Calvo and Mootha 2010). Approximately 1,500 nuclear genes must be actively imported, sorted, and assembled in order to interact with the mitochondrial genome to ensure proper energy production (Neupert and Herrmann 2007, Gershoni, Templeton et al. 2009, Calvo and Mootha 2010, Schmidt, Pfanner et al. 2010). Coordination between these genomes is essential, as improper mitochondrial function is associated with a wide variety of pathogenic phenotypes (Cohen and Gold 2001, Duchen 2004, Pieczenik and Neustadt 2007, McFarland, Taylor et al. 2010, Gorman, Chinnery et al. 2016).

The different inheritance modes between the mitochondrial genome and the nuclear genome however naturally result in intergenomic conflict. The exclusively maternal transmission of mtDNA means selection only ever acts in females. Consequently, sexually antagonistic mutations that are neutral or advantageous in females but deleterious in males can easily spread through a population (Lewis 1941, Charlesworth and Charlesworth 1978, Frank 1989, Frank and Hurst 1996, Gemmell, Metcalf et al. 2004, Vaught and Dowling 2018). Coined Mother's Curse by Gemmell et al. (2014), these sexually antagonistic mitochondrial mutations are best studied in plants where they prevent pollen production in otherwise hermaphroditic species (Lewis 1941, Kaul 1988, Budar and Pelletier 2001, Budar, Touzet et al. 2003, Case, Finseth et al. 2016). The accumulation of male-harming mutations that cannot be removed places selective pressure on the nuclear genome to evolve variants that restore male fitness, essentially counteracting the cost of these Mother's Curse variants, and this response has been documented.

The ultimate dynamics of these interactions depends on the chromosomal position of nuclear restorers. In an XY sex-determining system with an equivalent sex ratio, autosomes spend equal time in both sexes, the X chromosome spends ⅔ of its time in females, and the Y chromosome spends all of its time in males. This difference influences several evolutionary processes. The mutation rate is generally higher in genetic elements the longer it spends in males (Malcom, Wyckoff et al. 2003, Kirkpatrick and Hall 2004, Wilson Sayres and Makova 2011), and the effective population size of the X and Y chromosome is reduced compared to autosomes (proportionally ¾ and ¼ respectively) which magnifies the effect of drift and selection (Vicoso and Charlesworth 2006, Mank 2012). Finally, sexual antagonism will select for specific chromosomal locations to minimize the deleterious cost of specific variants in one sex (Gibson, Chippindale et al. 2002, Mank 2009).

Exploration into the chromosomal placement of nuclear genes that interact with the mitochondrial genome shows a complex landscape. Combinations that are co-adaptive will likely result in nuclear restorers on the X chromosome, while combinations that mitigate sexual conflict, like those involved with mitochondrial Mother's Curse variants, are likely to move off of the X chromosome (Drown, Preuss et al. 2012). Direct theoretical comparisons between autosomes, the X chromosome, and the Y chromosome suggest that nuclear restorers on the Y chromosome most rapidly spread and fix within a single population (Ågren, Munasinghe et al. 2019). While the Y chromosome is gene poor, which suggests that nuclear restorers may appear on the Y chromosome with less frequency than on autosomes, it is worth noting that genes exhibiting sex-specific sensitivity to mtDNA are overrepresented among genes known to be sensitive to Y-chromosomal variation suggesting that the Y chromosome may play a regulatory role (Lemos, Araripe et al. 2008, Rogell, Dean et al. 2014).

Direct identification of Mother's Curse nuclear restorers, which may provide insight into the chromosomal placement of nuclear restorers, is limited. This is driven in part by the experimental difficulty of identifying these interactions. Empirical studies that attempt this often rely on the construction of hybrid lines which purposefully disrupt co-evolved mitochondrial and nuclear interactions. Furthermore, these interactions are highly sensitive to environmental conditions meaning they may often be overlooked (Dowling, Friberg et al. 2008, Arnqvist, Dowling et al. 2010, Case, Finseth et al. 2016, Patel, Miriyala et al. 2016). These studies do, however, highlight the potential strength of these interactions as Dobzhansky-Muller incompatibilities. Despite this, theoretical work has mostly focused on the evolution of these interactions within a single-population (Gregorius and Ross 1984, Clark 1985, Babcock and

Asmussen 1996, Rand, Clark et al. 2001, Unckless and Herren 2009, Unckless and Orr 2009, Wade and Brandvain 2009).

Here, we construct a theoretical framework for exploring the consequences of disrupting co-evolved mitochondrial-nuclear interactions and track their dynamics over time using computer simulations. We limit ourselves to two allopatric populations of equivalent size each fixed for a unique set of mitochondrial Mother's Curse variants and corresponding nuclear restorers. We consider three distinct chromosomal locations for these nuclear restorers: autosomal, X-linked, or Y-linked. At the beginning of the simulation, we implement one of four distinct migration schemes: continuous symmetric migration, 1 generation of continuous migration, continuous asymmetric migration, and continuous sex-specific migration. We find that disrupting co-evolved mitochondrial-nuclear interactions results in less-fit male hybrids, which consequently skews the sex ratio towards the female sex. Restoration of male-fitness and return to an equivalent sex ratio depends both on the migration scheme and the location of nuclear restorers. In total, our results highlight the nuanced role of mitochondrial-nuclear interactions on hybrid incompatibility and population divergence.

Material and Methods

Model Design

We consider two diploid, dioecious sexual populations, p_1 and p_2. We start by defining two genomic elements: mitochondrial and nuclear. The mitochondrial genome is exclusively maternally inherited and considered homoplasmic in all individuals, which allows us to treat it as haploid. The nuclear genomic element may represent either an autosome, X chromosome, or Y chromosome with the affiliated transmission patterns and ploidy. We consider only biallelic

mitochondrial Mother's Curse variants, where the wild-type variant is neutral in both sexes and the mutant variant is advantageous in females but deleterious in males. For each mitochondrial Mother's Curse locus, there is a corresponding biallelic restorer locus in the nuclear genome that fully restores fitness for any male carrying the mutant Mother's Curse variant (see **Table 3.1** for full list of possible genotypes and fitnesses for one interaction). An individual's final fitness is calculated multiplicatively across all interactions.

Each population starts with a unique, fixed set of 20 mutant mitochondrial Mother's Curse variants with 20 corresponding fixed mutant nuclear restorers such that we are tracking 40 loci in the mitochondrial and nuclear genomic element respectively (for a total of 80 loci across both populations). We assume that in each population the remaining 20 Mother's Curse and nuclear restorer loci are fixed for the wild-type variant (**Figure 3.1a**). As there are no good estimates for the number of expected mitochondrial Mother's Curse variants or nuclear restorers, we choose 20 as it allows us to explore a substantial number of interactions without impacting the runtime of our simulations. For simplicity, we do not allow new mutations to emerge at any point. We allow recombination between autosomal and X-linked nuclear restorers and assume restorers are fully unlinked to each other. As recombination does not occur within either the mitochondrial genome or the Y chromosome, we assume no recombination in those genomic elements.

We then allow migration between the two populations, which consequently disrupts these co-evolved mitochondrial Mother's Curse and nuclear restorer interactions. We explore four different migration schemes: continuous symmetric migration, 1 generation of symmetric migration, continuous asymmetric migration, and continuous sex-symmetric migration **(Fig 3.1b**). These represent common ways that co-evolved interactions may be disrupted in natural

populations. Consequently, our models represent two allopatric populations that have evolved unique mitochondrial-nuclear interactions that are disrupted as migration allows gene flow between them. In order to simulate these models, we use SLiM3, a powerful and flexible genetic simulation framework, which is capable of incorporating all of these elements under the nonWF foundation (Haller and Messer 2019).

SLiM3 Simulation Framework

We leverage the nonWF model foundation in SLiM3 as it allows us to directly script several key facets of our models including the generation of offspring, migration events, and epistatic fitness calculations. There are two key aspects of nonWF models in SLiM3 we must stress - first how fitness is evaluated and then how populations are regulated. Fitness, in nonWF SLiM3 models, influences survival, and, consequently, fitness represents absolute fitness.

Table 3.1 Genotypes and Fitnesses of Males and Females for a Single Interaction (1 mtDNA Mother's Curse Locus: 1 Nuclear Restorer Locus) dependent on Nuclear Restorer Chromosomal Location

Nuclear Restorer Location	**Females**		**Males**	
	Genotype	Fitness	Genotype	Fitness
Autosome	*M-AA* *M-Aa* *M-aa* *m-AA* *m-Aa* *m-aa*	1 1 1 $1+s_f$ $1+s_f$ $1+s_f$	*M-AA* *M-Aa* *M-aa* *m-AA* *m-Aa* *m-aa*	1 1 1 $1-s_m$ 1-sm2 1
X	*M-XX* *M-Xx* *M-xx* *m-XX* *m-Xx* *m-xx*	1 1 1 $1+s_f$ $1+s_f$ $1+s_f$	*M-XX* *M-xY* *m-XY* *m-xY*	1 1 $1-s_m$ 1

Y	*M-XX* *m-XX*	1 $1+s_f$	*M-XY* *M-Xy* *m-XY* *m-Xy*	1 1 $1-s_m$ 1

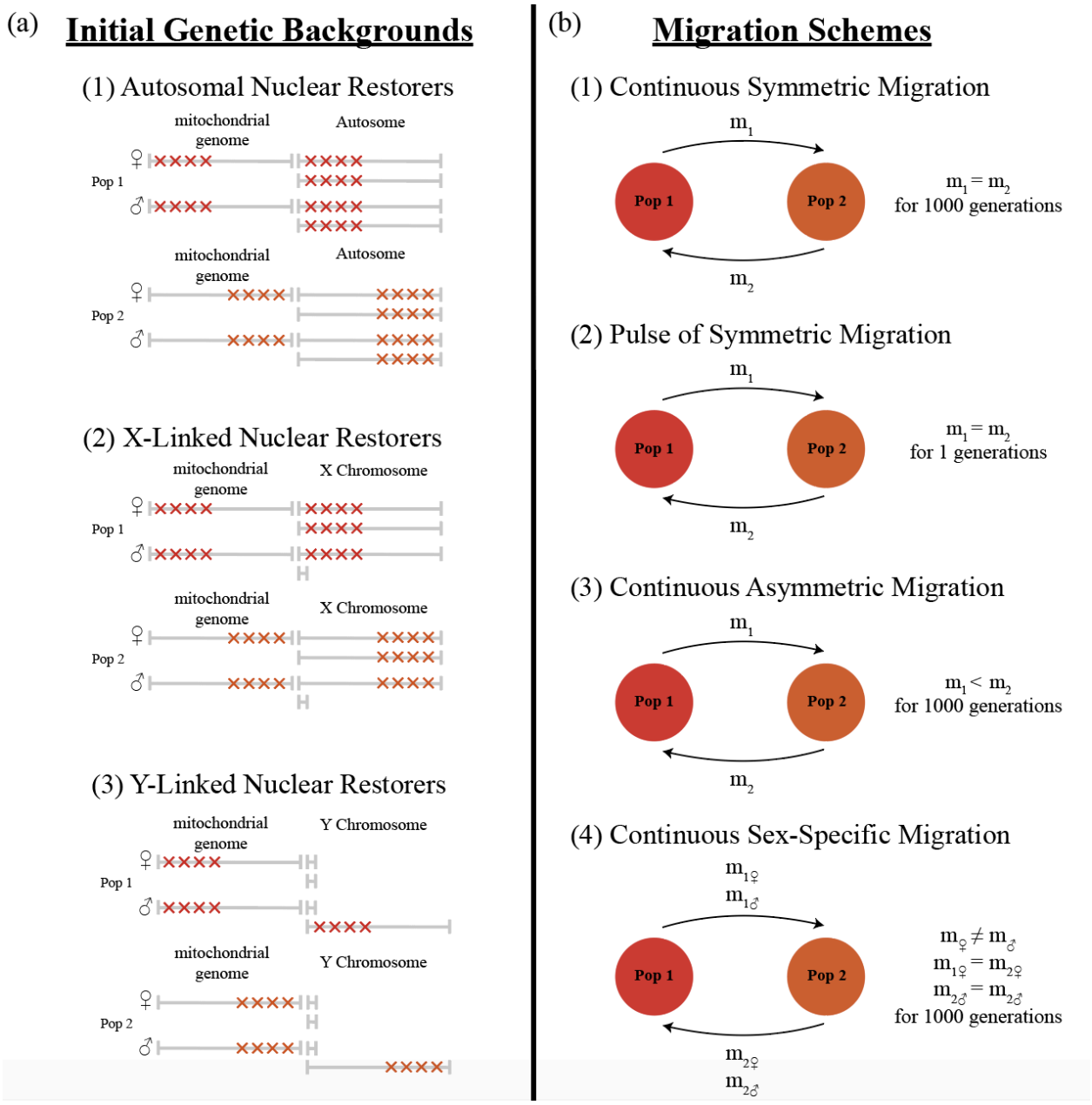

Figure 3.1. **Visual Schematic of Model Design**. Red represents population 1, while orange represents population 2. (a) Representation of Genetic Backgrounds for Females and Males in each population dependent on the chromosomal location of nuclear restorers. (b) Representation of the 4 distinct migration schemes explored.

Table 3.1 detailed the fitness of an individual for a specific interaction (1 Mother's Curse loci: 1 Nuclear Restorer loci). The final fitness of an individual is calculated multiplicatively across all mutations possessed by an individual. It is this final fitness that represents the likelihood that any given individual will survive to maturity. We enforce discrete, non-overlapping generations (an assumption not automatically made by nonWF SLiM3 models) by setting the fitness of non-newborns to 0 to ensure that these individuals are killed. Related, population regulation is not managed automatically (i.e. there is no fixed population size) in these models. Instead, population size is dependent on how many offspring are created versus how many individuals die due to selection in each generation (which as we mentioned above is determined by their final fitness). An important consequence of this is that the sex ratio may fluctuate if the fitness varies significantly between the sexes, which is expected to occur in our models. The populations can grow up to a supplied carrying capacity, but we expect there to be fluctuations in both the population size and sex ratio. Ultimately, our simulations more robustly represent the dynamics of natural populations.

We initialize our models by establishing two genomic elements: one mitochondrial and one nuclear, which can represent either an autosome, X chromosome, or Y chromosome. We set the mutation and recombination rate accordingly. We establish 4 mutation classes: mitochondrial Mother's Curse variants originating in p_1 (MC1), nuclear restorer variants originating in p_1 (NR1), mitochondrial Mother's Curse variants originating in p_2 (MC2), and nuclear restorer variants originating in p_2 (NR2). We evaluate their fitness as detailed in **Table 3.1.** We then construct two populations initially sized at 2000 (this will serve as the carrying capacity) with an equal number of males and females. We then place our mutations, such that all individuals in p_1 have 20 unique MC1 and NR1 variants and all individuals in p_2 have 20 unique MC2 and NR2

variants. Each MC and NR variant carries a specific tag such that each Mother's Curse variant has one nuclear restorer that compensates for male fitness. Once our populations are established, we start the simulation and allow migration.

Within a generation, the creation of offspring is the first step and is detailed within a *reproduction()* callback. 1000 individuals are subsampled within each population agnostic to their sex and may serve as parents. 2000 offspring are generated from this pool of parents. The mitochondrial genome contains a marker mutation (i.e. has no impact on fitness) that allows us to ensure the maternal transmission of the mitochondrial genomic element by checking for the presence of this mutation in the maternally inherited mitochondrial genomic element and its absence in the paternally inherited mitochondrial genomic element. After this check is made, we clear the paternally inherited mitochondrial genomic element of any mutations. This results in a functionally haploid mitochondrial genomic element that is inherited maternally. Similarly, for any model that uses a Y-linked nuclear restorer. We employ a similar method, but in reverse, to ensure that the Y chromosome is exclusively transmitted to males clearing both nuclear genomic elements in females.

As mentioned earlier, we do not allow any new mutations to emerge. We also do not allow any recombination within either the mitochondrial genomic element or Y nuclear genomic element. If the nuclear genomic element represents either an autosome or an X chromosome, we set the recombination rate to 0.5 such that each restorer is fully unlinked from the others. No recombination in the mitochondrial genome means that an individual will either have the 20 MC1s from p_1 or the 20 MC2s from p_2; we can consider these as two unchanging haplogroups that derive from either p_1 or p_2. Note, that under this design, female fitness is equal and constant in both populations as it depends only on the mitochondrial haplogroup present and each

haplogroup has equal fitness. Male fitness varies as it depends on the number of nuclear restorers present. Mean population fitness is then dependent on the mean fitness of males in each population and the sex ratio.

It is at this point that SLiM3 calculates the fitness of all individuals and viability selection occurs. Of the remaining individuals, we select some number to migrate to the other population dependent on the migration scheme. For continuous symmetric migration, we have one migration parameter m which defines the proportion of males and females that are moved from p_1 to p_2 and vice versa. We use the same migration parameter for a 1 generation pulse of symmetric migration, but, after 1 generation, m is set to 0 to eliminate migration. For continuous asymmetric migration, we have two migration parameters m_1 and m_2 . m_1 determines the number of males and females that are moved from p_1 to p_2, and m_2 determines the reverse. We set $m_1 < m_2$ such that p_1 receives more migrants from p_2 than p_2 receives from p_1. Finally, for continuous sex-specific migration, we assign two migration parameters m_f and m_m. m_f determines the number of females that move from one population to the other, while m_m determines the number of males that do so. Note that when $m_f = m_m$ we replicate continuous symmetric migration, so we are particularly concerned with when $m_f \neq m_m$ (see **Fig 3.1b** for visual representation of all migration schemes).

After migration, the cycle then starts again and repeats for 1000 generations. We track the mean fitness trajectories of each population, the allele frequencies of all variants, and the sex ratio every 10 generations to discern how the populations respond over time to the disruption of co-evolved mitochondrial-nuclear interactions via migration for a specific set of migration parameters, s_f, and s_m.

All scripts were run in SLiM3 v.3.3, and scripts are on GitHub (https://github.com/

mam737/mito_nuclear_SLiMulations). All migration parameters (m under continuous symmetric migration and 1 generation of symmetric migration, m_1 and m_2 under continuous asymmetric migration, and m_f and m_m under continuous sex-specific migration) range from 0.01 to 0.1 in increments of 0.018. s_f ranges from 0.0 to 0.1 in increments of 0.02, and s_m similarly ranges from -0.1 to 0.0 in increments of 0.02. For a specific parameter set, we replicate the simulation 10 times.

Results

Continuous Symmetric Migration

Immediately upon allowing migration, we see a reduction in male-fitness in both populations. The magnitude of this reduction is driven by the magnitude of the difference between the advantageous benefit of Mother's Curse variants in females and the deleterious cost in males (s_f - | s_m|). Disrupting these co-evolved interactions leads to less fit males, a consequence of which is that many more males are killed causing the sex ratio to skew towards females.

For autosomal and X-linked restorers, fitness recovery relies on the spread of both sets of nuclear restorers (NC1 and NC2). Recombination allows individuals to obtain restorers for each population which serves to protect males from the deleterious effects of the Mother's Curse variants. As autosomal restorers spread, the fitness and sex ratio slowly recover and return back to initial levels (**Fig 3.2a,c**). X-linked restorers behave nearly identically to autosomal restorers (**Fig S3.1)**, but Y-linked restorers, however, are unable to recover male fitness. No recombination on the Y chromosome makes it impossible for males to carry both sets of nuclear restorers, and, consequently, hybrids males always suffer reduced fitness and are

killed. However, it is worth noting that, while Y-linked restorers seem to suffer from a sustained reduction in male fitness, the size of this reduction is smaller in comparison with autosomal and X-linked nuclear restorers (**Fig 3.2b,d**).

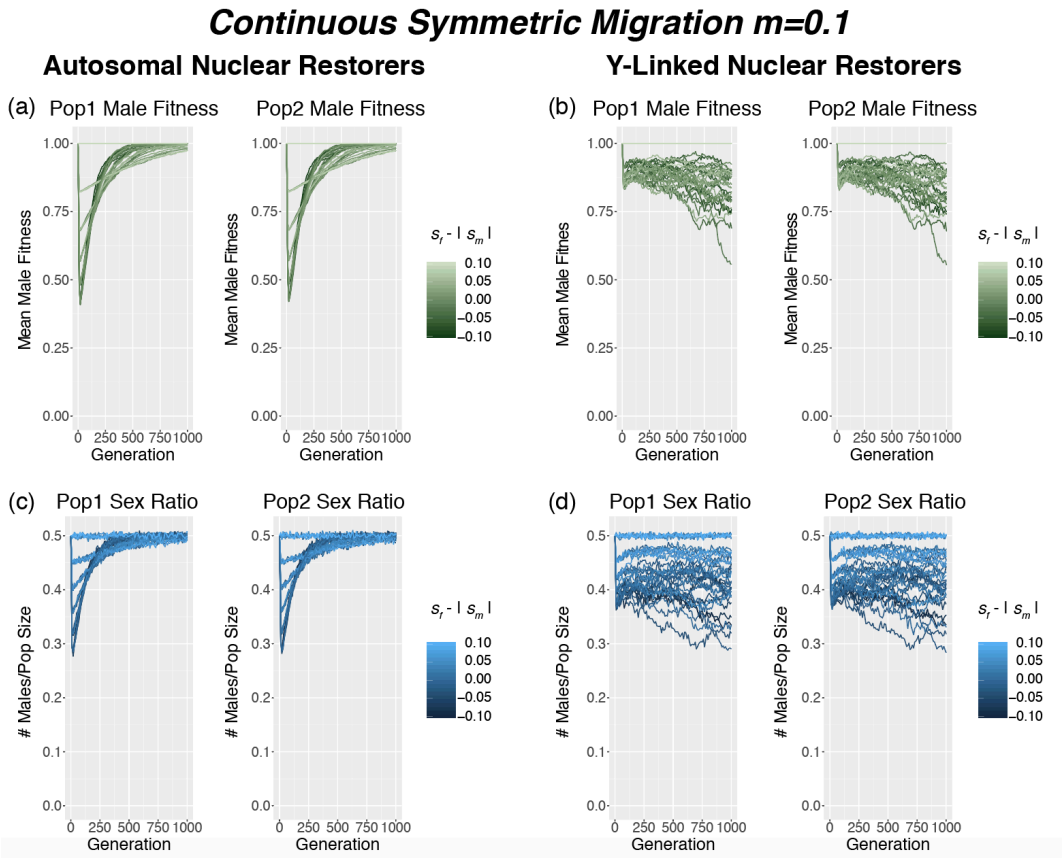

Figure 3.2 Mean Male Fitness and Sex Ratio Trajectories for population 1 (left) and population 2 (right) under Continuous Symmetric Migration rate m = 0.1. (a) Mean male fitness trajectories for autosomal nuclear restorers, (b) Mean male fitness trajectories for Y-linked nuclear restorers, (c) Sex ratio trajectories for autosomal nuclear restorers, (d) Sex ratio fitness trajectories for Y-linked restorers.

This is likely because all Y restorers from one population segregate together (another consequence of no recombination in the Y chromosome), and a male that carries the Y haplotype matching their mitochondrial haplogroup will be fully restored.

We can confirm that autosomal and X-linked restorers recover male fitness by obtaining both sets of nuclear restorers by examining the allele frequency trajectories of nuclear restorers for a specific s_f and s_m. We find that the frequency of all autosomal restorers tends towards

fixation in both populations (**Figure 3.3a**, see **Figure S3.2** for X-linked restorers**)**. This aligns with both the restoration of male fitness and the return towards an equivalent sex ratio. The more deleterious the Mother's Curse variants, the stronger the selective pressure is to obtain both sets of nuclear restorers. Once both sets of nuclear restorers are fixed, there is no fitness difference between the two mitochondrial haplogroups, and their frequency trajectory is determined by genetic drift.

With Y-linked restorers under the same conditions, we observe movement towards fixation of one Y haplotype and the loss of another, which haplotype is fixed versus lost seems to be stochastic (**Figure 3.3b**). Selection cannot remove the mitochondrial haplogroup associated with the lost Y haplotype due to the Mother's Curse variants advantage in females.

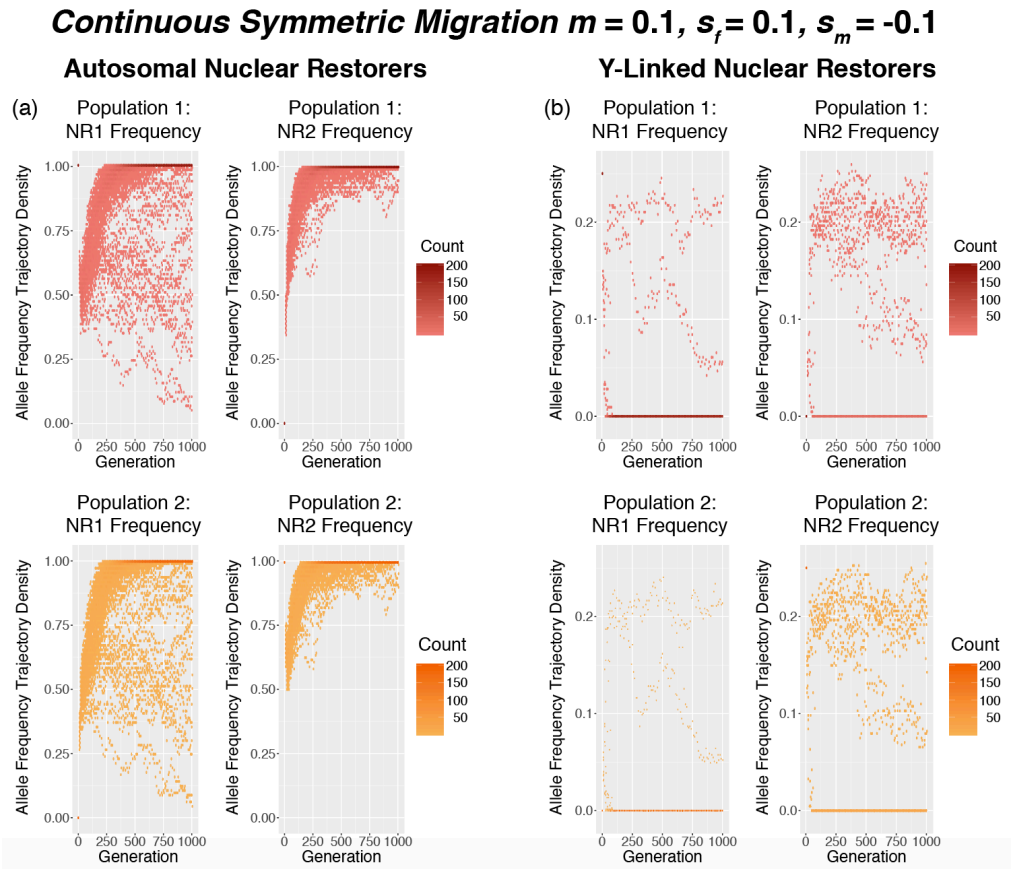

Figure 3.3 Density of Allele Frequency Trajectories for Both Sets of Nuclear Restorers, NR1 and NR2 (left and right), in population 1 and population 2 (top and bottom) under Continuous Symmetric Migration rate m = 0.1, s_f = 0.1, and s_m = -0.1. We consider density as how often the allele frequency trajectory for a specific nuclear restorer passes through that frequency at that generation. (a) 4 panel plot showing the allele frequency trajectory density for each set of autosomal nuclear restorers in each population, and (b) 4 panel plot showing the allele frequency trajectory density for each set of Y-linked nuclear restorers in each population

Consequently, some males suffer reduced fitness dependent on the frequency of the mitochondrial haplogroups, which is driven by drift as both haplogroups are equally fit in females. This explains why populations with autosomal or X-linked restorers show fitness recovery, while those with Y-linked restorers show significant variation in the final fitness.

1 Generation of Symmetric Migration

If symmetric migration is allowed only for 1 generation, we observe a much smaller reduction in fitness which quickly returns to the initial level. 1 generation of migration does create less fit hybrid males, but this reduction is not sustained. These F1 hybrid males are killed quickly, and any subsequent 'hybrid' males are the result of F1 hybrid females mating with males derived from the population they are in. These hybrid males are almost always killed due to their reduced fitness, which further reduces the number of hybrids. We see only minor fluctuations in male mean fitness and sex ratio as the number of hybrid males is substantially smaller than the ancestral males (**Figure S3.3-8)**. This is consistent across all nuclear restorer locations. Under our parameter range, 1 generation of symmetric migration is not enough to consistently generate less fit hybrid males. We did not directly simulate longer bursts of migration, but we expect that if enough hybrid males are generated the scenario would be similar to continuous migration where recombination would spread nuclear restorers to offset reduced male fitness.

Continuous Asymmetric Migration

We notice a distinct reduction in male fitness coupled with a skewed sex ratio as males are killed off for populations undergoing continuous asymmetric migration, but the size and duration of this reduction are dependent on the rate of migration from population 2 into population 1. When the difference in migration rates is minor, we find that the reduction in fitness is larger and sustained longer. Under asymmetric migration, populations return to their initial fitness in less than 100 generations unlike populations under continuous symmetric migration which take approximately 500 generations to return to a normal male fitness and sex ratio.

This is driven by a difference in how male fitness is restored. We see the spread of both sets of nuclear restorers under continuous symmetric migration. Under asymmetric migration, we instead see the domination of the MC2 and NR2 sets

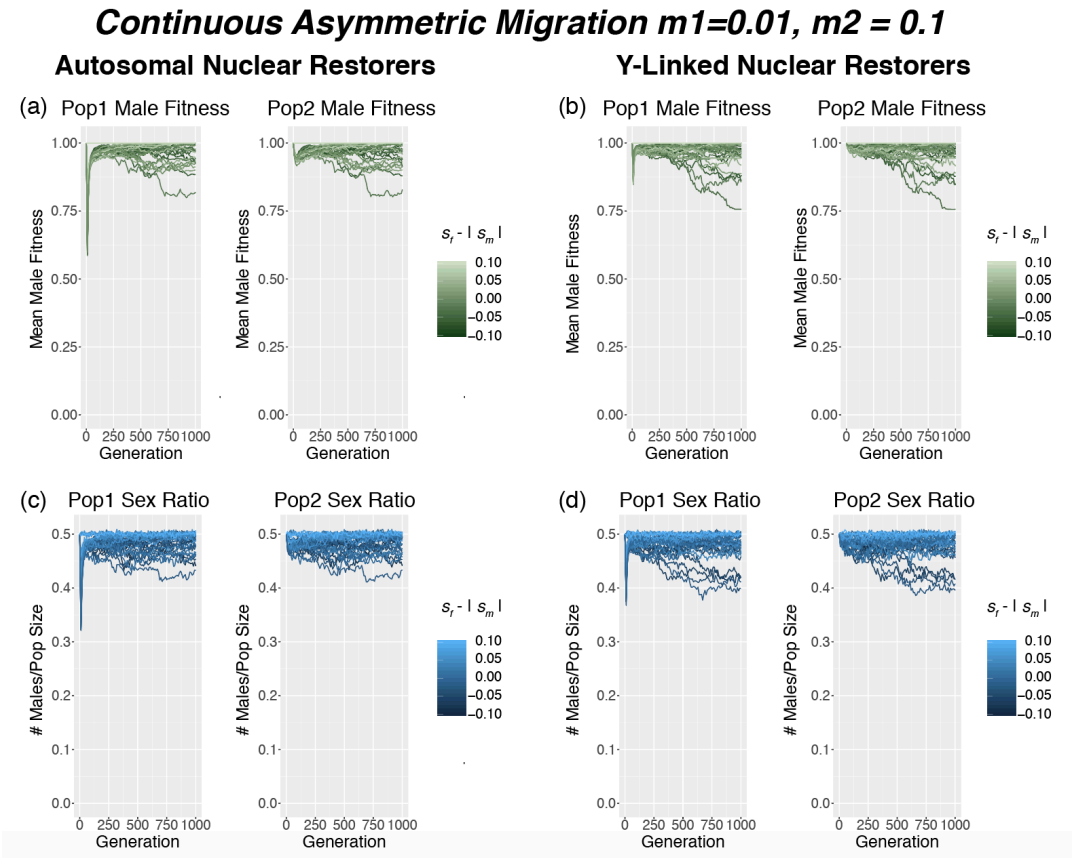

Figure 3.4 Mean Male Fitness and Sex ratio Trajectories for population 1 (left) and population 2 (right) under Continuous Asymmetric Migration rate m_1 = 0.01, m_2 = 0.1. (a) Mean male fitness trajectories for autosomal nuclear restorers, (b) Mean male fitness trajectories for Y-linked nuclear restorers, (c) Sex ratio trajectories for autosomal nuclear restorers, (d) Sex ratio fitness trajectories for Y-linked restorers.

(the sets of variants associated with population 2 that is migrating into population 1). Both populations tend to rapidly fix for MC2 and NR2; however, it is worth nothing that occasionally the MC1 haplogroup increases in frequency which drives an increase in frequency of the NR1 set of restorers.

Continuous symmetric migration with a migration rate m = 0.1 when compared with continuous asymmetric migration with migration rates m_1 = 0.01, m_2 = 0.1 (which results in population 1 receiving 10x more migrants than population 2) shows a larger reduction in fitness and a shorter time to recovery (**Fig 3.4,** see **Fig S3.9** for X-linked restorers**)**. This holds across all chromosomal locations for nuclear restorers. When looking at the allele frequency trajectories, for both autosomal and Y-linked restorers, it is clear that population 1 becomes fixed for the

mitochondrial haplogroup (MC2) and nuclear restorers (NR2) present in population 2 (**Fig 3.5**, see **Fig S3.10** for X-linked restorers**)**.

Restoration relies on replacement, not on the spread of both sets of nuclear restorers, meaning there is little difference in the ability of autosomal, X-linked, or Y-linked restores to rescue male fitness. The speed with which this occurs depends on m_2 with larger m_2 rates showing a smaller reduction in male fitness and faster time to restoration. This occurs as a higher m_2 expedites the replacement process. Population replacement tends to occur more rapidly than the spreading of both nuclear restorer sets (seen under continuous symmetric migration) except for when the difference in migration rates between the two populations is minimal. As noted earlier, the NR1 set of nuclear restorers is not always lost. The fate of the NR1 set of restorers is dependent on whether the MC1 haplogroup is able to increase in frequency. This seems to be a somewhat rare occurrence, however, and we more often see full population replacement.

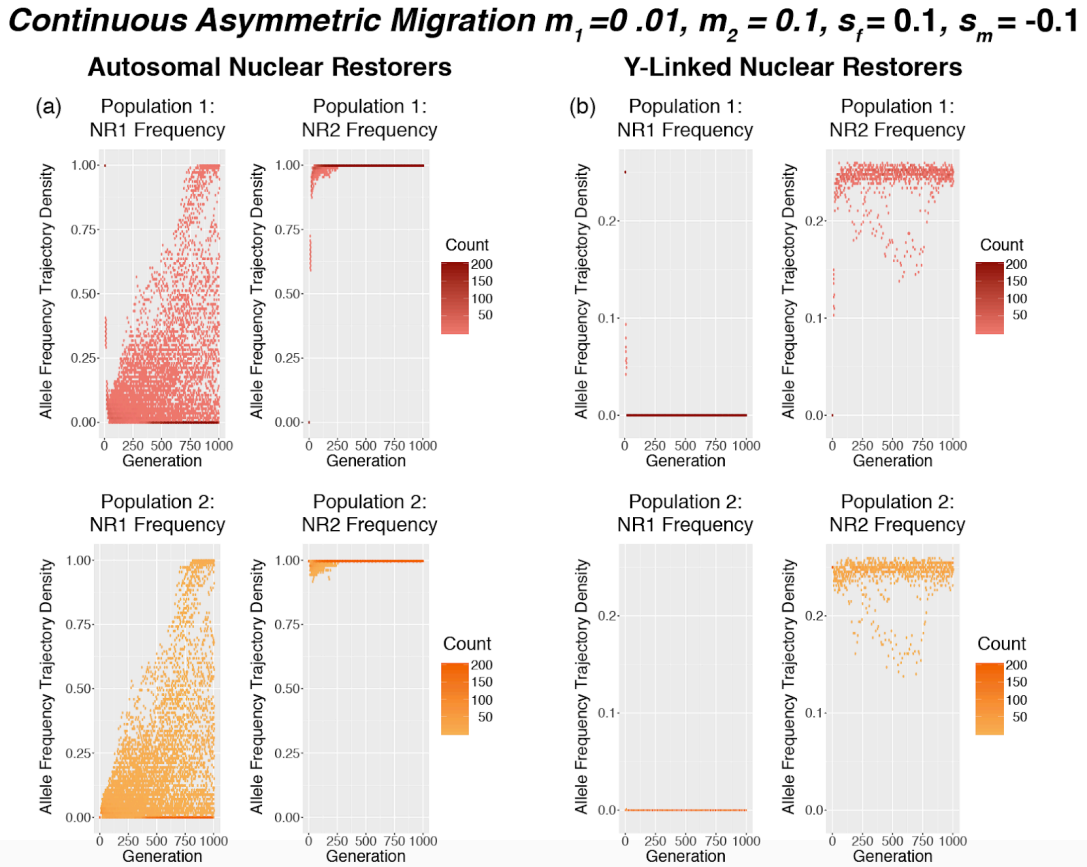

Figure 3.5 Density of Allele Frequency Trajectories for both sets of nuclear restorers, NR1 and NR2 (left and right), in population 1 and population 2 (top and bottom) under continuous asymmetric migration rate m_1 = 0.01, m_2 = 0.1, s_f = 0.1, and s_m = -0.1. We consider density as how often the allele frequency trajectory for a specific nuclear restorer passes through that frequency at that generation. (a) 4 panel plot showing the allele frequency trajectory density for each set of autosomal nuclear restorers in each population, and (b) 4 panel plot showing the allele frequency trajectory density for each set of Y-linked nuclear restorers in each population

Continuous Sex-Specific Migration

Here, we assign distinct migration rates for each sex but set them such that they are equivalent between both populations. We find that this model behaves identically to continuous symmetric migration, which suggests the symmetry between the populations is more influential in determining the fitness and sex ratio dynamics than differences in migration rate between the sexes (**Fig S3.11-16**). Once again, we see reduced male fitness and an associated skew in the sex ratio for autosomal and X-linked nuclear restorers that is slowly recovered as both sets of restorers spread to both populations. When the female migration rate exceeds the male migration

rate, there is a less gradual decline in fitness in comparison with the reverse. The final state of the populations at the end is essentially the same - recovery in the case of autosomal and X-linked restorers and a sustained reduction in fitness for Y-linked restorers.

Discussion

Our results provide novel insights into the consequences of disrupting co-evolved mitochondrial-nuclear interactions. Continuous migration leads to a marked reduction in male fitness which skews the sex ratio as males are killed off. Populations respond to this in one of two ways depending on whether or not that migration is symmetric or asymmetric. Under symmetric migration, populations acquire both sets of nuclear restorers to shield males from the deleterious effects of either mitochondrial haplogroup. Populations with Y-linked restorers are incapable of doing this, and so they continue to suffer from reduced male fitness. However, the magnitude of this effect is mitigated as all nuclear restorers for a specific Y haplotype segregate together meaning that any male with the corresponding mitochondrial haplogroup is fully restored. Under asymmetric migration, the population receiving an influx of migrants is usually replaced by the other, which eliminates the potential for less fit hybrid males. This occurs more rapidly than the spreading of both sets of nuclear restorers shortening the duration of reduced male fitness and a female-biased sex-ratio.

Under the parameters explored, we found little evidence that mito-nuclear interactions led to population isolation. Disrupting these interactions clearly generated less-fit male hybrids, implying that they do in fact act as Dobzhansky-Muller incompatibilities, but this was not enough to keep populations isolated. As long as migration continued, populations responded to reduced male fitness by either absorbing all nuclear restorers or by the replacement of one population by the other. It is possible under stronger deleterious effects that all hybrid males

would be killed which could generate population isolation. However, we are unaware of any Mother's Curse variants that induced lethality in hybrid males. Of documented Mother's Cure variants, male fertility seems to be a targeted trait (Lewis 1941, Smith, Turbill et al. 2010, Case, Finseth et al. 2016, Patel, Miriyala et al. 2016). It is possible that with complete or near complete male sterility populations may remain isolated, but it is our expectation that with continued migration the scenarios detailed here would occur to try and offset the reduction in male fitness.

Along with reduced male fitness, we see a distinctive skew in the sex ratio as less fit males are killed. A 1:1 sex ratio is not a universal trait, even among dioecious species (Hamilton 1967, Barrett, Yakimowski et al. 2010), but there are consequences for a biased sex ratio. The effective population size is reduced in populations with divergent sex ratios away from the theoretically predicted 1:1, which will decrease allelic diversity through increased genetic drift (Hedrick 2011). This may be so impactful that the consequences of a drastically reduced effective population size may outweigh the benefits of an obligate outcrossing mating system (Dubreuil, Riba et al. 2010). A significantly reduced number of males (the sex ratio shifts as far as 1:3 males to females in our models) for a sustained period of time is likely to impact the genetic diversity of the Y chromosome. Even if nuclear restorers are autosomal or X-linked, the skewed sex ratio that occurs while they slowly spread through the population under continuous symmetric migration should reduce the genetic diversity of the Y chromosome. This may have distinct consequences as the Y chromosome as it is known to influence a wide variety of traits (Lemos, Araripe et al. 2008, Lemos, Branco et al. 2010).

Previous work has put forward the Y chromosome as a promising candidate for housing nuclear restorers that counteract the male-harming effects of mitochondrial mutations (Dean, Lemos et al. 2015, Yee, Rogell et al. 2015, Ågren, Munasinghe et al. 2019). Early empirical

evidence by Innocenti et al. (2011) and Rogell et al. (2014) suggests an overlap in genes showing differential expression dependent on both mitochondrial haplogroup and Y haplotype. However, our results show that under continuous symmetric migration Y-linked restorers perform worse than their autosomal or X-linked counterparts. The lack of recombination on the Y chromosome hinders a population's ability to respond in the face of the continual disruption of co-evolved mitochondrial-nuclear interactions. Parapatric populations with limited gene flow may benefit more autosomal and X-linked restorers than Y-linked in order to restore fitness of hybrid offspring.

Our theoretical framework and simulations serve as a robust exploration of the impact both migration and chromosomal location of nuclear restorers have on mitochondrial-nuclear interactions. However, there are many unexplored aspects that merit additional follow-up. We did not model the emergence of novel mitochondrial Mother's Curse variants and nuclear restorers or the consequences of some linkage between our autosomal and X-linked restorers - mostly due to our inability to find robust estimates of those values. We also assumed that both populations were initially fixed for these interactions, as previous theory suggests these interactions do in fact move towards either fixation or loss (Clark 1984, Arnold, Asmussen et al. 1988, Connallon, Camus et al. 2018, Ågren, Munasinghe et al. 2019), but it may be of interest to explore these dynamics under the assumption that these interactions are segregating. It is also worth noting that we did not explore the genetic disequilibria between the mitochondrial and nuclear genomic elements. It is well-established that several evolutionary forces, including genetic drift, epistatic selection, and nonrandom mating, may lead to cytonuclear disequilibria (i.e. departures from random association between nuclear and cytoplasmic genotypes) (Clark 1984, Asmussen, Arnold et al. 1987, Arnold, Asmussen et al. 1988). Hybrid zones with

directional and strong assortative mating will exacerbate cytonuclear disequilibria and epistatic interactions, like those we explored, and may only further this association.

There are also facets of both Mother's Curse variants and nuclear restorers that may influence our results. We reduce the dynamics of the mitochondrial genome down for simplicity, as is traditional. Mitochondrial DNA copy number ranges from hundreds to thousands of copies per cell depending on the cell's energetic needs (Cavelier, Jazin et al. 2000). If these copies are identical, they are considered homoplasmic and reduce down to a haploid element (which is what we assume in our models). However, if there is variation between the mtDNA copies, which there often is, fitness is not as simple as the presence or absence of a specific variant. It can instead be considered as a sort of 'threshold effect' where a certain proportion of mutant DNA must be present in order to impact the phenotype (Rossignol, Faustin et al. 2003). We also assume exclusive maternal inheritance of the mitochondrial genome as only a handful of examples exist of partial to full paternal inheritance or paternal leakage (Havey 1997, Schwartz and Vissing 2002, Wolff, Nafisinia et al. 2013, Worth, Yokogawa et al. 2014). Paternal transmission would introduce purifying selection on the male-deleterious Mother's Curse mutations, but this is likely a rare occurrence.

We also assume that nuclear restorers are able to fully rescue male fitness for the mitochondrial Mother's Curse variant they interact with and that restorer mismatch (i.e. a nuclear restorer negatively interacts with the wild-type mitochondrial variant) does not occur. It is likely that types of restorers do exist in natural populations (see Montgomery, Bailey et al. 2014) for details on the differential strength of two nuclear restorers in *Brassica napus*), and it is our belief that these scenarios would influence the dynamics of our models.

Finally, our models are also limited by the assumption of random mating, but there is increasing evidence suggesting that specific demographic processes may influence these processes. Both inbreeding and kin selection have been shown to limit the spread of mitochondrial Mother's Curse variants - as it couples a mother's fitness with those of her sons or a sister's fitness with those of her brothers (Unckless and Herren 2009, Wade and Brandvain 2009). There are also additional modes of migration and admixture that may be of interest. We limit ourselves to exploring only two populations, but our framework may be easily extended to incorporate additional populations. Complex patterns of migration between any number of populations could be simulated, which may be useful when trying to test hypotheses about mitochondrial-nuclear interactions for specific populations.

Our models lay the groundwork for simulating complex, multi-locus mitochondrial -nuclear interactions using SLiM3. The highly flexible nature of SLiM3 means that our models can be extended in several ways to continue exploring the dynamics of these interactions. In fact, many of the scenarios detailed above can be considered in SLiM3. Our models provide novel insight into how populations respond to the disruption of co-evolved mitochondrial-nuclear interactions due to migration, and they highlight the distinct ways this response occurs depending on both the migration scheme and the chromosomal placement of nuclear restorers for Mother's Curse. Extensions of our models that continue to leverage SLiM3's capabilities will provide additional insight into how asymmetrically inherited genomic elements both cause and resolve genetic and sexual conflict.

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Author Contributions

M.M and A.G.C. conceived the study and designed the theoretical framework. M.M. and B.H incorporated the framework into SLiM3 and wrote all associated scripts. M.M analyzed and visualized the results. M.M wrote the first draft and all authors contributed to the writing of the manuscript. A.G.C supervised the project.

Data Accessibility

The scripts for all simulations can be found on GitHub:

https://github.com/mam737/mito_nuclear_SLiMulations

Supporting Information

Figure S3.1. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under Continuous Symmetric Migration Rate $m = 0.1$ for X-linked Nuclear Restorers

Figure S3.2. Density of Allele Frequency Trajectories for Both Sets of X-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under Continuous Symmetric Migration Rate $m = 0.1$, $s_f = 0.1$, $s_m = -0.1$

Figure S3.3. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$ for autosomal Nuclear Restorers

Figure S3.4. Density of Allele Frequency Trajectories for Both Sets of autosomal Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$, $s_f = 0.1$, $s_m = -0.1$

Figure S3.5. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$ for X-linked Nuclear Restorers

Figure S3.6. Density of Allele Frequency Trajectories for Both Sets of X-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$, $s_f = 0.1$, $s_m = -0.1$

Figure S3.7. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$ for Y-linked Nuclear Restorers

Figure S3.8. Density of Allele Frequency Trajectories for Both Sets of Y-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under 1 Generation of Symmetric Migration Rate $m = 0.1$, $s_f = 0.1$, $s_m = -0.1$

Figure S3.9. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under Continuous Asymmetric Migration Rate $m = 0.1$ for X-linked Nuclear Restorers

Figure S3.10. Density of Allele Frequency Trajectories for Both Sets of X-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under Continuous Asymmetric Migration Rate $m = 0.1$, $s_f = 0.1$, $s_m = -0.1$

Figure S3.11. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ for autosomal Nuclear Restorers

Figure S3.12. Density of Allele Frequency Trajectories for Both Sets of autosomal Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ $s_f = 0.1$, $s_m = -0.1$

Figure S3.13. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ for X-linked Nuclear Restorers

Figure S3.14. Density of Allele Frequency Trajectories for Both Sets of X-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ $s_f = 0.1$, $s_m = -0.1$

Figure S3.15. Mean Male Fitness and Sex Ratio Trajectories for population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ for Y-linked Nuclear Restorers

Figure S3.16. Density of Allele Frequency Trajectories for Both Sets of Y-linked Nuclear Restorers, NR1 and NR2, in population 1 and population 2 under Continuous Sex-Specific Migration Rate $m_f = 0.01$, $m_m = 0.1$ $s_f = 0.1$, $s_m = -0.1$

CHAPTER 4

Mitochondrial-Y Chromosome Epistasis in *Drosophila melanogaster*[3]

Abstract

The coordination between mitochondrial and nuclear genes is crucial to eukaryotic organisms. Predicting the nature of these epistatic interactions can be difficult because of the transmission asymmetry of the genes involved. While autosomes and X-linked genes are transmitted through both sexes, genes on the Y chromosome and in the mitochondrial genome are uniparentally transmitted through males and females respectively. Here, we generate 36 otherwise isogenic *Drosophila melanogaster* strains differing only in the geographical origin of their mitochondrial genome and Y chromosome to experimentally examine the effects of the uniparentally inherited parts of the genome, as well as their interaction, in males. We assay longevity and gene expression through RNA-sequencing. We detect an important role for both mitochondrial and Y-linked genes, as well as extensive mitochondrial-Y chromosome epistasis. In particular, genes involved in male reproduction appear to be especially sensitive to such interactions, and variation on the Y chromosome is associated with differences in longevity. Despite these interactions, we find no evidence that the mitochondrial genome and Y chromosome are co-adapted within a geographic region. Overall, our study demonstrates a key

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role for the uniparentally inherited parts of the genome for male biology, but also that mito-nuclear interactions are complex and not easily predicted from simple transmission asymmetries.

Introduction

The co-evolution between mitochondrial and nuclear genes is one of the oldest and best-studied examples of symbiosis (Gillham 1994, Rand, Haney et al. 2004, Lane 2017). Orchestrated interaction between genes in the two genomes is essential for a number of eukaryotic traits, especially metabolism and energy production (Blier, Dufresne et al. 2001, Burton, Pereira et al. 2013, Hill 2015, Ghiselli and Milani 2020), and this intimate coordination has been taken as evidence for positive selection for cooperative mito-nuclear combinations (Wade and Goodnight 2006, Wade and Drown 2016). Moreover, there has also been a well-documented transfer of genes from the mitochondrial to the nuclear genome (Berg and Kurland 2000, Adams and Palmer 2003, Lotz, Lin et al. 2014), and bilaterian animal mitochondrial genomes, with a limited number of exceptions, contains only 37 genes. Finally, the case for the importance of adaptive mito-nuclear epistasis is further strengthened by the observation that placing mitochondrial genomes on novel nuclear backgrounds is often, though not always, associated with adverse fitness effect (see for example (Reinhardt, Dowling et al. 2013) and (Eyre-Walker 2017) for alternate perspectives).

A key factor governing mito-nuclear co-evolution is the difference in transmission between the two genomes. For example, because the mitochondrial genome is almost exclusively maternally inherited (Birky 1995), mutations that are deleterious in males can spread in a population, given that they are beneficial or neutral in females (Lewis 1941, Charlesworth and

Charlesworth 1978, Frank 1989, Frank and Hurst 1996, Gemmell, Metcalf et al. 2004, Connallon, Camus et al. 2018). The occurrence of male-deleterious mitochondrial mutations is particularly well studied in plants (Kaul 1988, Budar and Pelletier 2001, Budar, Touzet et al. 2003, Havird, Forsythe et al. 2019), where such mutations usually prevent pollen production in hermaphroditic plants, essentially rendering individuals female, guaranteeing the mutation's transmission through ovules. This phenomenon is therefore called cytoplasmic male sterility. In the zoological literature, following Gemmell et al. (2004), the presence of mitochondrial mutations with deleterious effects in males is known as the Mother's Curse.

Other than the mitochondrial genome, other uniparentally inherited genes are those located on the sex-determining chromosome, i.e. on the Y in an XY system, where males are the heterogametic sex, and on the W in a ZW female heterogametic system. The strict paternal inheritance makes the Y an especially interesting candidate for mito-nuclear epistasis. In particular, it has been suggested to be an ideal location for modifiers that counteract the male deleterious effects of mitochondrial mutations (Dean, Lemos et al. 2015, Yee, Rogell et al. 2015), a scenario formally modeled by Ågren, Munasinghe, and Clark (Ågren, Munasinghe et al. 2019). Despite its heterochromatic structure and paucity of protein coding genes, the Y chromosome is now recognized as being able to affect a wide variety of traits (Lemos, Araripe et al. 2008, Jiang, Hartl et al. 2010, Lemos, Branco et al. 2010). For example, in *Drosophila* it underlies variation in traits ranging from susceptibility to bacterial infection (Kutch and Fedorka 2017), male reproductive success, and sex-specific aging (Brown, Nguyen et al. 2020).

The extent of mito-Y interactions and their importance to male fitness remain poorly understood. Some suggestive evidence comes from empirical work by Innocenti et al. (2011), who found that loci in the mitochondrial genome can affect the expression of a large number of

autosomal loci in male, but not in female *Drosophila melanogaster*. Such sexual dimorphism in expression is consistent with a sex-specific selective sieve being central to mitochondrial genome evolution. Furthermore, several of the loci identified by Innocenti et al. (2011) to be sensitive to mitochondrial variation overlap with loci shown by Lemos et al. (2008) to be sensitive to variation on the Y chromosome (Rogell, Dean et al. 2014). The extent to which male autosomal gene expression is subject to mito-Y interactions, however, is unclear.

Recent attempts to empirically address these questions in the fruitfly *D. melanogaster* have revealed some suggestive patterns. Dean et al. (2015) found that both mitochondrial and Y-linked genes independently affected male locomotor activity, but only under certain diets and social environments. Similarly, Yee et al. (2015) used combinations from three populations (a total of 9 mito-Y combinations) to provide proof-of-concept evidence of how mito-Y combinations may affect aspects of male fitness. The latter was also interested in testing for evidence of co-evolution between mitochondrial and Y-linked genes. If co-evolution is important, the prediction is that males that carry a Y-chromosome and a mitochondrial genome that have co-evolved in the same sympatric population may differ from males where the mitochondrial genome and Y chromosome are from diverged populations and therefore represent a novel mito-Y combination. However, in this relatively low-powered study, Yee et al. (2015) did not find evidence that males with sympatric mito-Y combinations had higher fitness than males with novel combinations.

We extend these studies in three ways. First, we increase the sample size considerably, by including 36 mito-Y combinations of *D. melanogaster* males with mitochondrial and Y chromosomes sampled from five worldwide locations (**Table 4.1**). Second, we assay longevity, another major fitness trait previously shown to be sensitive to mito-nuclear epistasis (Rand, Fry

et al. 2006, Clancy 2008, Zhu, Ingelmo et al. 2014, Tower 2017). Finally, we performed RNA-sequencing on all lines to identify the importance of mitochondrial and Y chromosome variation, as well as mito-Y epistasis, for differential gene expression. In line with Dean et al. (2015) and Yee et al. (2015), we found an important role for both mitochondrial and Y-linked genes and an abundance of mito-Y chromosome epistasis.

Materials and Methods

Drosophila melanogaster strains

Both the longevity and the gene expression assays were performed on isogenic *D. melanogaster* males differing only in the geographical origin of their mitochondrial genome and their Y chromosome (referred to as mito-Y combinations throughout). These lines were constructed by crosses to an isogenic strain of Bloomington stock 4361, which has recessive markers on all chromosomes (y^1; bw^1; e^4; ci^1 ey^R, also see **Supplement** for details). Each Y chromosome was crossed into the isogenic 4361 genetic background in a two-generation cross, avoiding recombination between the original GDL strain and the 4361 strain by only having them in a heterozygous state in male parents. These lines were Illumina-sequenced to 1x depth, and across all the lines we only detected residual heterozygosity at 196 sites. The confidence in the background replacement of these lines is therefore high (details in Kelsey et al., *in prep.*) The mitochondrial replacement lines we constructed by recurrent backcrossing to the 4361 stock for 10 generations. In this case, there could be recombination between the GDL and 4361 backgrounds, but the expected removal of the GDL background by dilution exceeded one-half of each generation because of the selectable markers. We applied a simple computer simulation to determine that more than 99% of the 1000 independent trials had no residual heterozygosity, and

in the cases where the replacement was not complete, the expected portion of the genome with residual heterozygosity was 0.0088 (see **Supplement** for details). This residual heterozygosity could be confounded with an inferred mitochondrial effect, but its contribution is expected to be

Table 4.1 Geographical origin of strains used to generate mito-Y combinations. Sympatric combinations are shown on white background and novel on grey.

		Y chromosome genotype					
		B04 Beijing	**B11 Beijing**	**N03 Netherlands**	**N07 Netherlands**	**ZWH23 Zimbabwe**	**ZW139 Zimbabwe**
Mitochondrial genotype	**B38 Beijing**	Beijing × Beijing	Beijing × Beijing	Beijing × Netherlands	Beijing × Netherlands	Beijing × Zimbabwe	Beijing × Zimbabwe
	B39 Beijing	Beijing × Beijing	Beijing × Beijing	Beijing × Netherlands	Beijing × Netherlands	Beijing × Zimbabwe	Beijing × Zimbabwe
	I02 Ithaca	Ithaca × Beijing	Ithaca × Beijing	Ithaca × Netherlands	Ithaca × Netherlands	Ithaca × Zimbabwe	Ithaca × Zimbabwe
	N01 Netherlands	Netherlands × Beijing	Netherlands × Beijing	Netherlands × Netherlands	Netherlands × Netherlands	Netherlands × Zimbabwe	Netherlands × Zimbabwe
	N02 Netherlands	Netherlands × Beijing	Netherlands × Beijing	Netherlands × Netherlands	Netherlands × Netherlands	Netherlands × Zimbabwe	Netherlands × Zimbabwe
	N23 Netherlands	Netherlands × Beijing	Netherlands × Beijing	Netherlands × Netherlands	Netherlands × Netherlands	Netherlands × Zimbabwe	Netherlands × Zimbabwe

very small, and the coherence of gene ontologies impacted by mitochondrial replacements would not be expected if driven by incomplete background replacement.

We used a 6 x 6 design (**Table 4.1**), crossing females from six mitochondrial replacement lines with males from six Y chromosome replacement lines, resulting in male offspring with 36 mito-Y combinations. Mitochondrial genomes came from Beijing, China (2 lines; B38 and B39), Ithaca, NY, USA (1 line: I02), and the Netherlands (3 lines; N01, N02, and N23), and the Y chromosome from Beijing, China (2 lines; B04 and B11), the Netherlands (2 lines; N03 and N07), and Zimbabwe (2 lines; ZWH123 and YZW139). These populations represent deeply divergent mitochondrial and Y chromosome clades chosen from the Global Diversity Lines (Grenier, Arguello et al. 2015). The central aim of this study is to assess the extent of mito-Y epistasis. Following Yee et al. (2015), a secondary aim is to investigate if there is any evidence of mito-Y co-adaptation. If the mtDNA and Y chromosome were from the same geographic region, they are therefore labeled "sympatric" and otherwise they are labeled "novel".

Longevity Assay

Scoring Survival

Individual life span was quantified for all 36 mito-Y combinations. For each cross, 4 males and 4 females were put into a single vial. Flies were collected upon eclosion, sexed, and placed into vials. Because collections took place over several days, the starting number of individuals per vial varied and so did the number of vials per cross. Most vials had around 12 individuals (**Figure S4.9**) and most crosses had 7 replicate vials (**Supplementary Table S4.1**). The total number of flies per cross ranged from 74 to 138, with an average of 107. Seven crosses had less than 90 individuals. Every other day, flies were transferred without using CO_2 to vials with fresh sucrose-yeast medium, and individual deaths were recorded. Investigators scoring

deaths were blind to which mito-Y combination a given vial contained. Vials were kept in climate-controlled growth chambers at 25 °C and at a 12:12 hours light:dark cycle.

In total, approximately 4,500 individuals were scored. Because we were interested in documenting differences in intrinsic mortality, we excluded individuals that died within the first 11 days of the experiment (**Table S4 .1**). In total, 671 individuals lived less than 11 days, which represents just under 15% of all individuals. There appears to be little correlation between the starting number of individuals in a vial and the number of individuals in a vial that died before 11 days (Kendall rank correlation = 0.056). In addition, with the exception of the few vials with only a couple of individuals, there seems to be no correlation between the starting number in a vial and the average life span of individuals in a given vial (Kendall rank correlation coefficient = 0.11; **Figure S4.10**).

Differential Gene Expression

RNA Extraction and Sequencing

3-5 day old males from each of the 36 mito-Y combinations were maintained in vials on standard sucrose-yeast medium in climate-controlled growth chambers with a 12:12 hours light:dark cycle at 25 °C. For each mito-Y combination, 10 males were flash frozen. We used two biological replicates for each combination, with individuals for each replicate being collected from separate crosses between females from the mitochondrial-replacement and males from the Y-replacement parental lines performed on the same day.

RNA was extracted from the 10 pooled whole-fly males and RNA seq libraries were prepared using the Lexogen QuantSeq 3’ mRNA-Seq Library Prep Kit FWD. Samples were

sequenced in a single-end 75 bp run on a NextSeq500 at the Genomics Facility in the Cornell Biotechnology Resource Center.

Read Processing and Alignment

The quality of the RNA sequences was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimmomatic was then used to clip adapters, the leading 10 bp, and reads once the average quality of a sliding window of 4 bp dropped below 20 (Bolger, Lohse et al. 2014). Reads shorter than 20 bp were dropped from subsequent analysis. Reads were aligned to the *D. melanogaster* reference genome (Release 6; (Hoskins, Carlson et al. 2015)) using STAR (Dobin, Davis et al. 2013) and HTseq-count was used to determine the raw number of read counts per gene (Anders, Pyl et al. 2015).

Quantifying Gene Expression

Differential transcript abundance was analyzed using DESeq2 (Love, Huber et al. 2014). Read counts were normalized using DESeq2's internal normalization function estimateSizeFactors, which corrects for both library size and RNA composition bias. Lowly expressed genes were removed from subsequent analysis, such that a given gene was only kept in the dataset if it had at least 20 normalized counts in at least half of the samples. After filtering, 9,533 out of 17,324 (~55%) of the originally identified genes were kept for subsequent analysis.

To identify nuclear genes sensitive to variation in the mitochondrial genome, the Y chromosome, and mito-Y epistasis, we conducted three separate differential expression analyses using linear models. Principal component analyses first suggested the presence of a batch effect, likely the result of samples undergoing RNA extraction and library preparation on two different

days (**Figure S4.2**). These analyses also indicated a higher level of heterogeneity between replicates than expected. To mitigate this, we used the R package SVA to identify surrogate variables, or covariates constructed directly from the RNA sequencing data, that could adjust for noise and unmodeled artefacts ((Leek 2014); see **Figure S4.2** for details). SVA considers all expression levels simultaneously in order to recover the effects of potentially missed variables by identifying sets of genes affected by each unobserved variable and then estimating the variable based on the expression of those genes. We characterized a gene as being differentially expressed if they maintain significance after performing independent filtering for multiple test corrections using the Benjamini-Hochberg method set with a false discovery rate of 0.05. **Supplemental Tables S4.2-S4.4** contain the results of these tests rank-ordered by significance after multiple test correction.

We performed a gene enrichment analysis to determine whether certain gene families were differentially expressed across Y haplotypes, mitochondrial haplogroups, and mitochondrial:Y interactions. We used the R Bioconductor package 'goseq' (Young, Wakefield et al. 2010) to perform gene ontology (GO) analyses. Taking length bias into account, we identified GO categories as either significantly over- or under-represented using a 0.05 false discovery rate cutoff. REVIGO was used to semantically cluster the lists of enriched GO terms to find a representative subset that could be easily analyzed and visualized (Supek, Bošnjak et al. 2011). The results from 'goseq' and REVIGO can be found in **Supplemental Tables S4.5-S4.7**.

For each set of differentially expressed genes, we identified enriched transcription factor binding motifs using the R Bioconductor package 'RcisTarget' (Aibar, González-Blas et al. 2017). We used the motif ranking file of *Drosophila* species 'dm6-5kb-upstream-full-tx-11species.mc8nr.feather' (accessed on 04-20-20202) with a search space of 5kb upstream of

transcription start site and transcript introns. We searched each gene set independently (i.e. Y haplotype, mitochondrial haplogroup, and mitochondrial-Y interaction sensitive) with parameters aucMacRank = 5% and nesThreshold = 3. We consider high confidence transcription factor annotations, those directly inferred or inferred by orthology, to determine which transcription factors bind to enriched binding motifs. The full list of enriched transcription factor binding motifs and a list of the annotated transcription factors for these motifs can be found in **Supplementary Tables S4.8 – 4.13**.

To determine whether any differentially expressed genes showed testis- or accessory gland-biased expression, we downloaded data from FlyAtlas, which measures the expression levels of a gene in each adult male (Leader, Krause et al. 2018). The bias metric used is simply the expression of the gene in the tissue of interest divided by the sum of expression, in males, over all other tissues. We considered a gene biased for expression in a tissue of interest if the bias metric for that tissue is greater than 0.5, as that indicates that more than half the reads collected for that gene come from that tissue. **Supplemental Figures S4.6-S4.8** highlight all genes found to show biased expression in male reproductive tissue in both the Y and mitochondrial differential expression analysis. All scripts used for the RNA-sequencing analysis are on GitHub: (https://github.com/mam737/mitoY_RNASEQ).

Results

Extensive Variation in Lifespan Across Mito-Y Combinations

We detected extensive variation in the average lifespan across the 36 mito-Y combinations (**Figure 4.1; Table S4.1**). To assess the contribution of mitochondrial type and Y chromosome and their interaction we performed several types of analysis. First, we performed a two-way

ANOVA based on vial means. We performed the analysis with and without individuals that lived less than 11 days, and with and without vials with fewer than 6 individuals. The results were qualitatively the same and reveal a role for variation on the Y chromosome in governing longevity ($F = 3.5$, $P = 0.00836$; individuals with lifespan <11 days and vials with <6 individuals not included). These results also hold if we include the starting number of individuals per vial as a covariate in the linear model. In these models, the only significant effect remains the Y chromosome. Second, we also performed a mixed-model ANOVA including vial as a random factor nested within the mtDNA × Y interaction. Again, we performed the analysis with and without individuals with life-span less than 11. These results were qualitatively the same and found a significant effect of the Y chromosome ($F = 3.7$, $P = 0.0034$; individuals with life-span < 11 days not included). Finally, we pooled all individuals into two categories, sympatric (N = 1235) and novel (N = 2618), and found no evidence that individuals with a sympatric mito-Y combination live longer than individuals with a novel combination (Wilcoxon rank-sum test, $W = 1536800$, $P = 0.5114$).

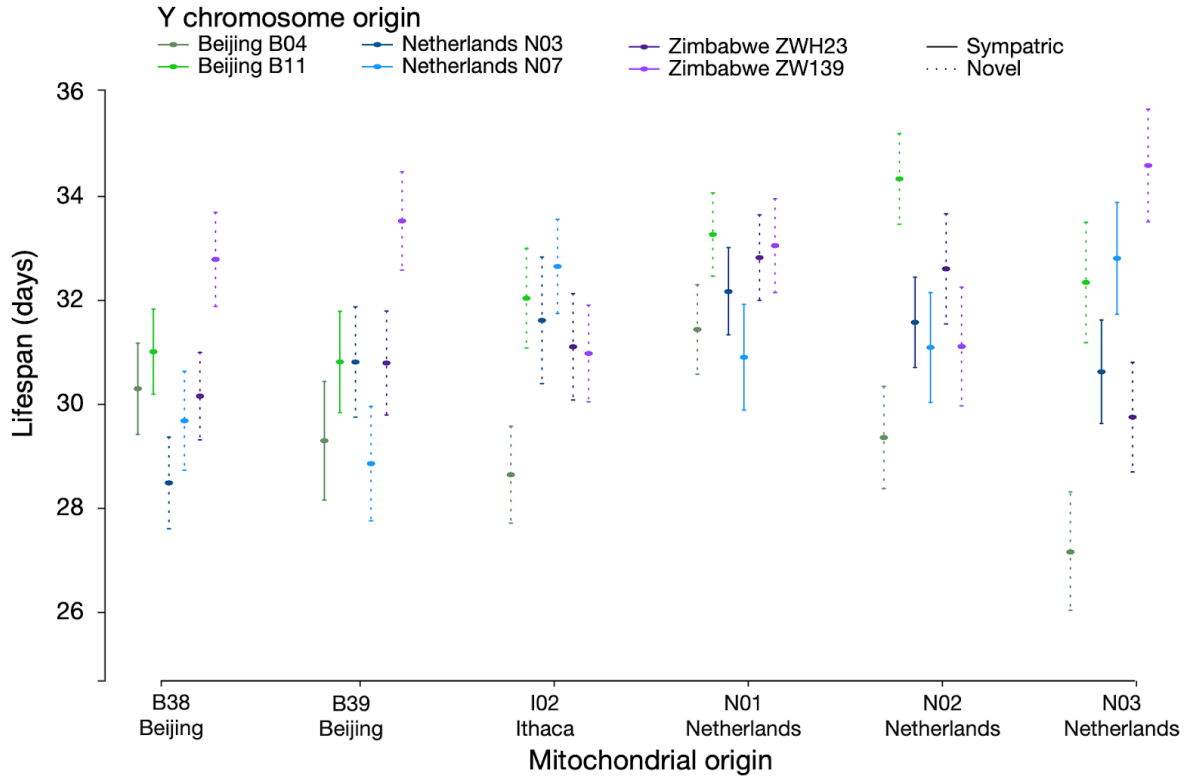

Figure 4.1. Mean lifespan (days) ± standard error across 36 mito-Y combinations. Geographical origin of the mitochondrial genome is stated at the bottom and colour coded for the Y chromosome. Solid and dashed lines represent sympatric and novel mito-Y combinations respectively.

Table 4.2. Two-way ANOVA for mito-Y Interactions in Vial Mean Longevity

Source	Degrees of freedom	F	*P*
Mitochondrial genome	5	1.1	0.3473
Y chromosome	5	3.5	0.0044
Mito-Y interaction	25	0.7	0.8415
Residuals	206		

Individuals with lifespan <11 days not included

To assess how mortality changed over time we fitted a number of survival functions based on different assumptions (Gompertz, Gompertz-Makeham, Logistic, and Logistic-Makeham models; see **Supplement** for details). This comparison revealed significant variation across lines, but no difference between novel and sympatric mito-Y combinations (**Figure S4.1**).

Expression of Many Nuclear Genes is Sensitive to Variation on the Y chromosome, in the mitochondrial genome, or both

We detected 71, 760, and 29 genes whose expression was sensitive to variation on the Y chromosome, in the mitochondrial genome, and mito-Y epistasis respectively. To gain insight into the biological function of these genes, we searched for gene ontology (GO) terms (Ashburner, Ball et al. 2000, The Gene Ontology Consortium 2019) that were either over- or under-represented among our significant hits. We found that genes associated with visual perception (GO:0007601; $P_{adj} = 0.006$), response to stimulus (GO:0050896; $P_{adj} = 0.0144$), and rhabdomere, a central compartment in compound eyes (GO:0016028; $P_{adj} = 0.0435$) were over-represented among genes sensitive to Y haplotype. For those sensitive to mitochondrial haplotype, we find 44 over- and 3 under-represented GO categories with an enrichment of terms belonging to categories such as purine biosynthesis, metabolism, and immune responses (**Figure 4.2**). Whereas genes sensitive to mito-Y interactions show substantial variation in expression across samples (**Figure 4.4**), we detect no enrichment or depletion of GO categories among the genes sensitive to mito-Y epistasis.

Next, we identified any substantial enrichment of transcription factor binding motifs among differentially expressed genes. We found 156, 207, and 379 motifs overrepresented

among genes sensitive to either Y haplotype, mitochondrial haplogroup, or mitochondrial-Y interactions respectively.

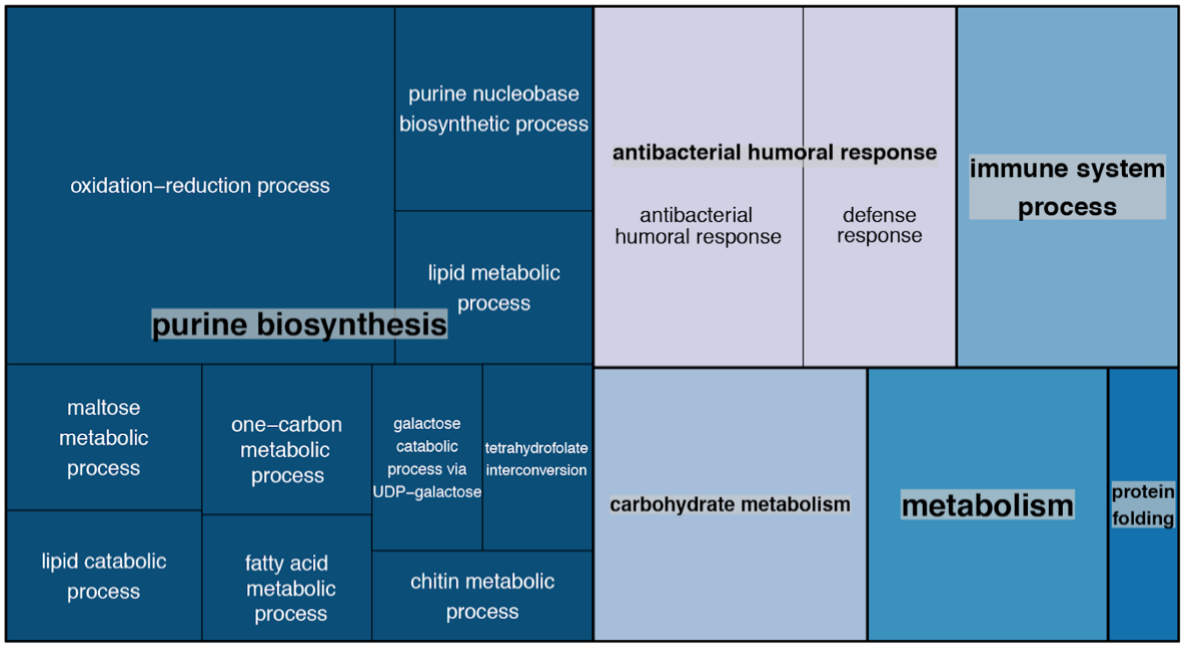

Figure 4.2. Semantic Clustering of Over-Represented GO terms Found Among Genes Sensitive to Mitochondrial Haplogroup. REVIGO semantically clustered GO terms involved in biological processes enriched among genes sensitive to mitochondrial haplotype. Each rectangle represents a single cluster, which in are joined into larger 'superclusters' as visualized by color. Size of the rectangles indicates *p*-value significance (absolute value of the log 10 transformed *p*-value).

Several of these binding motifs contained high confidence transcription factor annotations (**Supplementary Tables S4.9, S4.11, and S4.13**). Notably, all gene sets showed an enrichment of motifs binding all five GATA transcription factors–*GATAd, GATAe, grn, pnr,* and *srp* (Okumura, Matsumoto et al. 2005)–which play a well-documented role in cell differentiation and proliferation (Patient and McGhee 2002). GATA transcription factors have also been demonstrated to influence the effects of dietary amino acids on lifespan, and it is suspected that they operate in a sex-specific manner (Camus, Piper et al. 2019). Below, we discuss certain biological trends that emerge among our most significant hits.

Discussion

As in most species, male *Drosophila* live shorter than females (Yoon, Gagen et al. 1990, Tower and Arbeitman 2009), and recent studies have demonstrated a central role for both the mitochondria (Camus, Clancy et al. 2012) and the Y chromosome (Brown, Nguyen et al. 2020) in explaining this difference. Here, we find that the Y chromosome contributes to variation in longevity. Consistent with previous work on mito-Y interactions (Dean, Lemos et al. 2015, Yee, Rogell et al. 2015), our results also reveal no evidence that males with sympatric mito-Y combinations differed in these traits compared to males with their mitochondrial genome and Y chromosome sampled from different populations.

Mitochondrial haplotype influences the expression of metabolic genes

Figure 4.2 highlights the abundance of genes related to metabolism that show differential expression across mitochondrial haplogroups. Many metabolic reactions involve mitochondria, so while the emergence of genes involved in several metabolic processes showing differential expression may not be surprising, the sheer number of nuclear genes whose expression is affected by mitochondrial variation is. Some of these genes exhibit consistent differences between the haplogroups. Several maltases involved in maltose/carbohydrate metabolism show reduced expression among the Netherlands haplogroups compared to the others, whereas 3 of the 4 enzyme-encoding genes involved in the reduction of NADP to NADPH (*Men, Idh,* and *Zw)* show reduced expression in the Beijing B38 haplogroup (**Figure S4.3**).

Transcription factors with motifs enriched among mitochondrial haplogroup sensitive genes also show a demonstrated role in regulating metabolism. *Hnf4* regulates both lipid mobilization and fatty acid catabolism (Palanker, Tennessen et al. 2009, Storelli, Nam et al. 2019), and it is thought to be required for the transcription of nuclear and mitochondrial genes

involved in oxidative phosphorylation (Barry and Thummel 2016). *SREBP* (Lim, Wang et al. 2011, Bertolio, Napoletano et al. 2019) is involved in lipid metabolism and regulates the transcription of lipogenesis when lipid of cholesterol levels drop.

Genes related to male fertility show sensitivity to both Y and mitochondrial haplotype

Both the Y chromosome, which contains six essential male fertility factors, and the mitochondrial genome have previously been demonstrated to affect male fertility in *D. melanogaster* (Morgan 1910, Brosseau 1960, Kennison 1981, Clark 1990, Carvalho, Lazzaro et al. 2000, Chippindale and Rice 2001, Yee, Rogell et al. 2015, Patel, Miriyala et al. 2016). Whereas there is no statistical enrichment of differentially expressed genes belonging to male fertility processes, there are several notable hits that not only show sensitivity across both Y and mitochondrial haplotypes but also show elevated expression in both the testis and the accessory glands (**Figures S4.5-S4.8**). Among our Y sensitive hits, genes such as *Testis EndoG-Like 1 (Tengl1)*, Adenosine deaminase-related growth factor A2 (*Adgf-A2), Male-specific RNA 98CA (Mst98Ca), gonadal (gdl),* and *Ductus ejaculatorius peptide 99B (Dup99B)* show markedly higher, if not exclusive, expression in either the testis or the accessory glands. Furthermore, *Adgf-A2*, *Mst98Ca,* and *gdl* are all thought to be involved with spermiogenesis, spermatogenesis, or sperm function, while *Dup99B* is a sex-peptide that influences the female post-mating response (Schäfer, Börsch et al. 1993, Matsushita, Fujii-Taira et al. 2000, Saudan, Hauck et al. 2002, Ayyar, Jiang et al. 2003, Jiang and White-Cooper 2003).

Several genes sensitive to mitochondrial haplogroup also show higher expression in the testis and accessory glands. Whereas many of these genes are not functionally characterized, there are a few worth highlighting. *Protamine B* (*ProtB)* packages the paternal genome in sperm

during spermiogenesis (Jayaramaiah Raja and Renkawitz-Pohl 2005, Rathke, Barckmann et al. 2010), *Otefin (Ote)* encodes a nuclear membrane-associated protein involved in transcriptional silencing of *bag-of-marbles (bam)*, which is a key protein involved in gametogenesis. *Tim17a1*, a subunit of the TIM23 complex, is involved in transporting proteins across the inner mitochondrial membrane and shows markedly higher expression in the testis compared to all other tissues (Thurmond, Goodman et al. 2019). *Seminase (Sems),* which is expressed predominantly in the accessory glands, is not only transferred during mating but is also thought to be involved in sperm release from storage in females (LaFlamme, Ram et al. 2012). Finally, we also found several accessory gland proteins, *lectin-46Ca, Acp53C14a*, *Acp33A, CG14034,* and *CG9029. lectin-46-Ca, Acp53C14a,* and *CG13309* (which is significant, but not enriched in the accessory gland) all show reduced expression among the Netherlands mitochondrial haplogroups compared to the others.

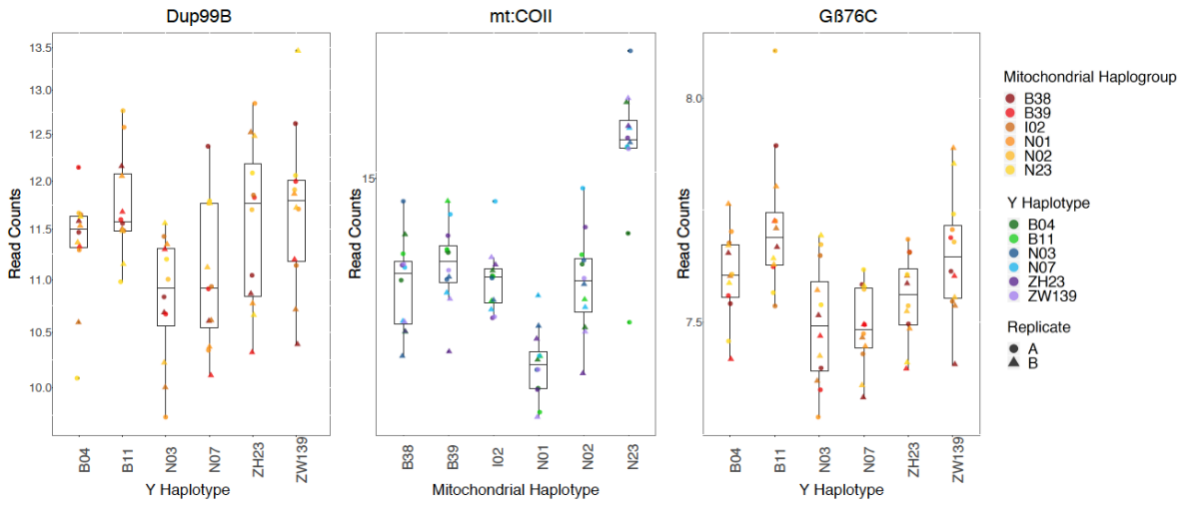

Figure 4.3. Read Count Visualization of Genes Sensitive Across Each Differential Expression Model. Box and scatter plots visualizing differential expression of genes sensitive to (a) Y haplotype, (b) mitochondrial haplogroup, and (c) mitochondrial-Y interactions. Normalized read counts for (a) *Dup99B*, a male sex peptide, which shows reduced expression in the Netherlands haplotypes, (b) *mt:COII*, a mitochondrial subunit of cytochrome oxidase II, which shows variable expression across all 6 mitochondrial haplotypes, (c) *Gβ76C*, a Y-sensitive hit, linked to visual perception that shows significant differences in expression across all 6 Y haplotypes.

In addition to genes that are enriched for expression in either the testis or the accessory gland, we find a handful of other genes related to male fertility. *mt:COII* and *mt:Cyt-B* have not only been demonstrated to influence male fertility, but also show no effect on females, leading authors to cite them as examples of Mother's Curse variants in *Drosophila* (Clancy, Hime et al. 2011, Yee, Sutton et al. 2013, Dowling, Tompkins et al. 2015, Patel, Miriyala et al. 2016). For *mt:COII*, we see variable expression across mitochondrial haplogroups with the Netherlands N23 haplogroup showing the highest levels of expression (**Figure 4.3b**). Another top mitochondrial hit, the heat shock protein *Hsp83*, has been demonstrated to affect spermatid development and differentiation in *D. melanogaster* (Yue, Karr et al. 1999, Wasbrough, Dorus et al. 2010).

We also find transcription factors involved in male reproduction with binding motifs enriched among genes differentially expressed across both Y haplotype and mitochondrial haplogroup. Enriched among Y sensitive genes are motifs for *SOX100B*, which is required for testis differentiation (Nanda, DeFalco et al. 2009),and *FoxP*, which influences levels of courtship behavior in males (Lawton, Wassmer et al. 2014). For mitochondrial sensitive genes, motifs for *ERR* (Yu, Wu et al. 2015, Misra, Pandey et al. 2017), a transcription factor directly involved in testicular development and spermatogenesis in *Drosophila* males, are overrepresented.

Despite several genes related to male fertility showing sensitivity to both Y and mitochondrial haplotype, we see little evidence for epistatic interactions between them. Simply looking at the overlap of genes that show sensitivity to Y or mitochondrial haplotype yields only one gene: *mino.* Furthermore, when specifically testing for mito-Y epistasis, the only gene related to male fertility with significant differential expression is the seminal fluid protein *Sfp24Ba*, which, surprisingly, is not found to be significant when testing for sensitivity to just Y or mitochondrial haplotype.

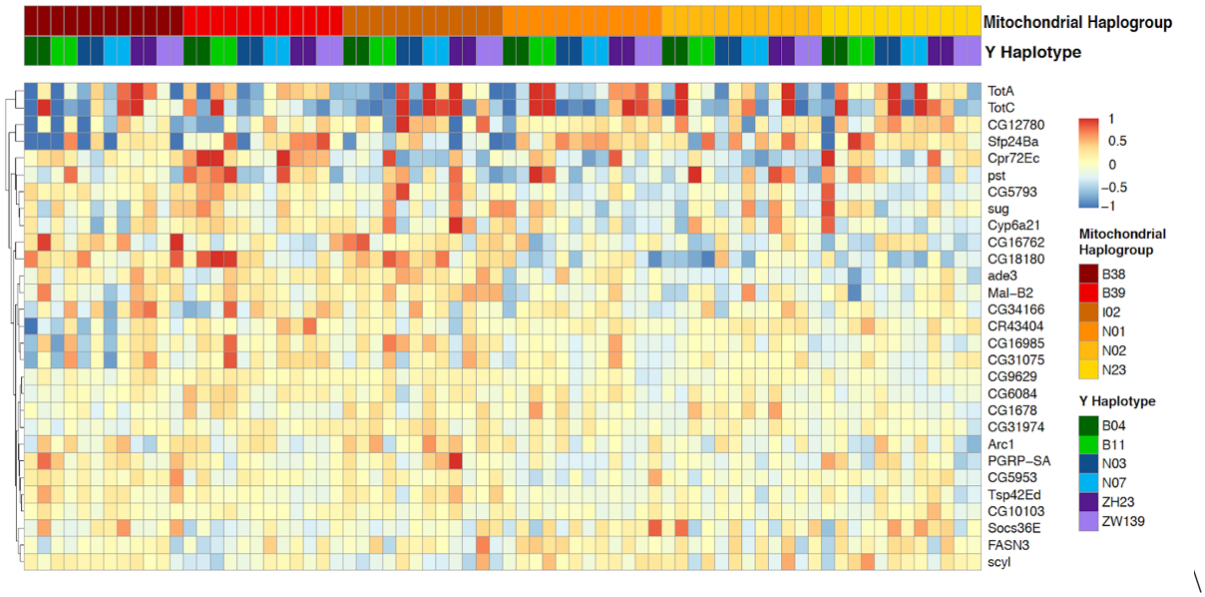

Figure 4.4. Expression Profile of Genes Sensitive to mitochondrial-Y Interactions. Samples (36 mito-Y combinations with 2 replicates, labelled A and B, for each) and genes of interest are listed along the *x*- and *y*-axis respectively. Colour indicates either overexpression (red) or underexpression (blue) compared to the mean expression level of that gene across all samples.

In summary, this analysis identifies several differentially expressed genes that present a strong potential to have downstream consequences for male fertility. The pattern of expression levels seen in **Figure 4.4** suggests no simple additive role of mitochondrial and Y variants but instead points to specific genotypic combinations having the most aberrant expression.\

Visual perception and nervous system processes show Y haplotype sensitivity

Genes belonging to both visual perception (GO:0007601) and rhabdomere (GO:0016028), a key compartment in compound eyes, are not only enriched among our Y sensitive hits but they are also some of our most significant hits. We see less expression of these

genes among the Netherlands haplotypes, yet a markedly higher expression of these genes for the Beijing B11 haplotype (**Figure S4.4**). Previous work has shown that variable expression in *Arr1*, *Arr2, Rh4, Gβ76C, trpl,* and G*γ30A*, all of which show differential expression, may lead to phenotypic differences in rhodopsin inactivation, phototransduction, and the photoresponse (Dolph, Man-Son-Hing et al. 1994, Niemeyer, Suzuki et al. 1996, Hardie and Raghu 2001, Gu, Oberwinkler et al. 2005, Satoh and Ready 2005, Shieh, Kristaponyte et al. 2014, Hanlon and Andrew 2015, Katz and Minke 2018). Further, we find several binding motifs for transcription factors regulating vision including *oc*, *lola*, *Optix*. *Oc* is required for photoreceptor development and rhabdomere morphogenesis (Vandendries, Johnson et al. 1996, McDonald, Xie et al. 2010, Mishra, Oke et al. 2010), and *Optix* is involved in eye formation and development of the optic lobe (Kenyon, Yang-Zhou et al. 2005, Gold and Brand 2014). *Lola* is not only involved in axon growth but also acts during photoreceptor neuron differentiation (Giniger, Tietje et al. 1994, Mishra, Bargmann et al. 2016). In contrast, we are not aware of any previous work linking variation on the Y chromosome to variation in visual perception in *Drosophila* or any other system. However, further dissection of the influence of the Y on differential gene expression may provide additional clues.

Concluding Remarks

In this study we used 36 mito-Y combinations to experimentally examine the effects of the uniparentally inherited parts of the genome on male *Drosophila melanogaster*. We detect an important role for Y-linked genes but little sign that individuals with their Y chromosome and mitochondrial genome sampled from the same population being any different than individuals where the two are from geographically isolated populations. We also detect many genes that are

sensitive to variation on the Y chromosome, in the mitochondrial genome or both. The biological function of these genes range from metabolic to visual and neuronal phenotypes, with the strongest effect being for genes involved in male reproduction. Although the results presented here fall short of demonstrating a clear co-evolutionary process between the Y chromosome and mitochondrial genome, the extensive gene expression and longevity interactions highlight the opportunity for these uniparentally inherited segments of the genome to influence male biology.

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Author Contributions

J.A.Å, M.M, and A.G.C conceived the study. J.A.Å performed the fly work and analyzed the longevity data. M.M. analyzed the expression data. J.A.Å wrote the first draft and all authors contributed to the writing of the manuscript. A.G.C supervised the project.

Data Accessibility

The RNA-seq data reported here are posted on the GEO resource with reference number GSE155395. Fly lines and other resources are available on request to A.G.C. The scripts for all RNASeq analyses can be found on GitHub: https://github.com/mam737/mitoY_RNASEQ

Supporting Information

Appendix S4.A. *Drosophila* stock construction

Appendix S4.B. Simulation of the mitochondrial replacement line construction

Appendix S4.C. Analyzing Longevity Data

Appendix 4.D. Surrogate Variable Analysis

Figure S4.1. Pairwise Z-tests of α, the initial mortality rate (a), and β, age-dependent increase in mortality (b) in the Gompertz model

Figure S4.2. PCA Analysis Showing Effect of Surrogate Variable Analysis

Figure S4.3. Expression of Metabolic Genes Showing Differential Expression Across Mitochondrial Haplogroups

Figure S4.4. Expression of Vision Genes Showing Differential Expression Across Y Haplotype

Figure S4.5 Y-Sensitive Hits that are Enriched for Expression in the Testis

Figure S4.6. Y-Sensitive Hits that are Enriched for Expression in the Accessory Glands

Figure S4.7. Mito-Sensitive Hits that are Enriched for Expression in the Testis

Figure S4.8. Mito-Sensitive Hits that are Enriched for Expression in the Accessory Glands

Figure S4.9. Distribution of the Starting Number of Flies in Each Vial for Longevity Assays

Figure S4.10. Correlation between Starting Number of Flies in a Vial and the Average Lifespan of that Vial

Table S4.1. Survival Statistics

Table S4.2. Y Differential Expression Analysis Results Rank Ordered By P-Value

Table S4.3. Mitochondrial Differential Expression Analysis Results Rank Ordered By P-Value

Table S4.4. Mitochondrial:Y Interaction Differential Expression Analysis Results Rank Ordered By P-Value

Table S4.5. GO Categories Over (+) or Under (-) Represented Among Y Haplotype Sensitive Hits

Table S4.6. Go Categories Over (+) or Under (-) Represented Among Mitochondrial Haplogroup Sensitive Hits

Table S4.7. REVIGO Clustering of GO terms on Overrepresented Mitochondrial Biological Process Terms

Table S4.8. Enriched Transcription Factor Binding Motifs Among Genes Differentially Expressed Across Y Haplotypes

Table S4.9. Transcription Factors Annotated for Binding Motifs Enriched Amongst Y Haplotype Sensitive Genes

Table S4.10. Enriched Transcription Factor Binding Motifs Among Genes Differentially Expressed Across Mitochondrial Haplogroups

Table S4.11. Transcription Factors Annotated for Binding Motifs Enriched Amongst Mitochondrial Haplogroup Sensitive Genes

Table S4.12. Enriched Transcription Factor Binding Motifs Among Genes Differentially Expressed Across Mitochondrial:Y Interactions

Table S4.13. Transcription Factors Annotated for Binding Motifs Enriched Amongst Mitochondrial:Y Interactions

Table S4.14. Percentages of Genes that are Enriched for Expression in the Testis and Accessory Gland

CHAPTER 5

Conclusions and Future Directions

Summary

In this thesis, I explored the role of asymmetric transmission as a driver of genetic and sexual conflict. The maternally inherited mitochondrial genome creates sexual conflict when female-advantageous, male-harming mutations emerge that cannot be purged by selection due to the transmission pattern of mtDNA. Mutations emerge within the nuclear genome that act to restore male fitness, but their evolutionary dynamics are greatly impacted by their chromosomal location due to the differential transmission of autosomes, X chromosomes, and Y chromosomes. In **Chapter 2**, I performed a comprehensive analysis of how transmission asymmetries between mitochondrial, autosomal, and sex-linked genes differ in both causing and resolving sexual conflict. We expand this framework in **Chapter 3** to explore how disruption of these co-evolved mitochondrial-nuclear interactions via migration impact hybrid fitness and population isolation with a specific focus on the differences between autosomal, X-linked, and Y-linked nuclear restorers. Finally, in **Chapter 4** I directly test for evidence of mitochondrial-Y interactions to explore the effects uniparentally inherited genetic elements, as well as their interactions, play in males.

This work directly shows the differential behavior of nuclear restorers dependent on chromosomal placement. In a single population, a biallelic 2-locus model clearly shows that nuclear restorers readily evolve and move towards fixation to counteract the male-harming cost of mitochondrial Mother's Curse variants. However, the rate of this trajectory depends on the exact transmission pattern of the chromosome harboring the restorer locus. The Y chromosome

shows the most rapid dynamics and restoration of male fitness. However, when we expand this framework to a two population biallelic multilocus model, we find contradictory results. If co-evolved mitochondrial-nuclear interactions are disrupted via continuous symmetric or sex-specific migration, we find that populations with Y-linked nuclear restorers struggle to restore hybrid male fitness. Consequently, the fitness of the population can readily decline depending on the frequency of the competing mitochondrial haplogroups. Scenarios with autosomal or X-linked restorers under the same migration scheme are easily able to rescue male fitness over time as nuclear restorers can spread to both populations via recombination. This suggests that autosomal and X-linked restorers may be preferable under certain circumstances to Y-linked restorers.

To directly examine the effect of uniparentally inherited genetic elements in males, we leveraged 36 otherwise isogenic *Drosophila melanogaster* strains differing only in the geographical origins of their mitochondrial haplogroup and Y chromosome. We assayed longevity and gene expression through RNA-sequencing, two phenotypes with a previously demonstrated sensitivity to these two genetic elements. We detect an important role for both mitochondrial and Y-linked genes, as well as substantial mitochondrial-Y epistasis. We found that variation on the Y chromosome influenced longevity, and both the mitochondrial haplogroup and Y chromosome had a notable impact on the expression of genes involved in reproduction. However, we did not find any evidence that combinations of mitochondrial haplogroups and Y haplotypes within the same geographic region outperformed novel combinations.

Combined our results clearly implicate transmission dynamics in causing and resolving genetic conflict driven by sexually antagonistic mitochondrial mutations. However, there is still

substantial work to do to fully understand the role of these interactions in contributing to population isolation and divergence. These interactions act as Dobzhansky-Muller incompatibilities leading to reduced hybrid male fitness when disrupted, but it remains to be seen whether these interactions can drive population isolation on their own. It is likely that they contribute substantially to this process, but they may not be powerful or frequent enough to do so on their own.

Future Directions

This thesis began as an investigation into the influence of transmission dynamics on mitochondrial-nuclear interactions, and both the theoretical and empirical work done as part of this project serve as a robust step forward in understanding the evolutionary dynamics and consequences of these interactions. There are, however, several extensions to the projects presented and experimental avenues to explore given our results.

The modelling framework detailed in both **Chapter 2** and **Chapter 3** serve as useful scaffolding for expanding theoretical analyses on mitochondrial-nuclear interactions (several of which are detailed in those chapters). One of the most notable extensions involves our understanding of nuclear restorers. We, for the most part, assume that nuclear restorers fully restore male fitness and that there is no negative interaction between a wild-type mitochondrial variant and a nuclear restorer. However, the same logic that suggests the nuclear genome would evolve in response to mitochondrial mutations that alter the functionality of any of the protein complexes involved in OxPhos also indicates that there should be a negative fitness consequence when a nuclear restorer interacts with a wild-type mitochondrial variant. At no point do we explore this possibility, and it is very likely that incorporating this would impact the evolutionary

dynamics of nuclear restorers as their spread will be limited by the negative fitness interaction with the wild-type mitochondrial variant. We do briefly explore the impact of incomplete fitness restoration of nuclear restorers in **Chapter 2**, and our results do indicate that fixation is dependent on the amount of restoration exceeding the deleterious cost in males. It is currently unclear how often nuclear restorers emerge and how capable they are of fully restoring the effects of Mother's Curse variants. As empirical work comes in that allows us to better quantify the mutation rates and distributions of fitness effects, these models should be revisited to make more robust predictions on these dynamics.

In that vein, **Chapter 4** highlights the potential for exploring gene expression data to gain insight into the consequence of variation in the mitochondrial genome. Human cybrid cell lines that place different mitochondrial haplogroups on a shared nuclear background also show distinctive nuclear gene expression patterns (Hwang, Kwak et al. 2011, Cohen, Levin et al. 2016). While these cybrids allow researchers to directly link differences in nuclear gene expression to mitochondrial haplogroup, their generation is arduous and time-consuming, limiting the size and scope of these studies. An interesting follow-up to our experiments would be to leverage the Genotype-Tissue Expression project (GTEx), arguably the most comprehensive transcriptomic dataset in humans (Consortium 2020). The most recent version of the GTEx database includes whole-genome sequencing data which now makes the exploration of the effect of mitochondrial haplogroup on gene expression possible with this resource. The statistical challenge of this analysis will involve disentangling mtDNA genotype from population structure in order to quantify the role of mtDNA variants in affecting the differential expression of nuclear genes. Vegesna et al. recently explored the influence of high variation in Y ampliconic gene copy number in gene expression levels in human testis using the GTEx dataset, and while

they do not directly account for population structure, their analysis may serve as a useful framework for considering the effect of mitochondrial haplogroup on human gene expression (Vegesna, Tomaszkiewicz et al. 2019). We have obtained access to the GTEx database and look forward to pursuing this experimental analysis.

We also comment in **Chapter 2** on a novel form of sexual conflict driven by transmission asymmetry. Dubbed Father's Curse, this phenomenon arises when a derived Y-linked allele impacts expression of an autosomal locus such that one allele favors males over females. We showed that as long as the Y mutant restorer allele provides a large enough selective advantage for *aa* males, the *a* allele will increase in frequency even if it is disfavorable in females. While our theoretical analyses predict this form of sexual conflict, it has yet to be explicitly confirmed in natural or experimental populations. A potential example of this is the high heterochromatin content of the Y chromosome in *Drosophila* which is demonstrated to influence the chromatin state of the *white*^{mottled4} allele and suppress position effect variegation (Elgin and Reuter 2013), but more experimental work is necessary to confirm this as a form of Father's Curse. An interesting approach would be to adapt an experimental evolution approach undertaken in Patel et. al (2018) designed to isolate male-harming mtDNA mutations. There, they decoupled male versus female evolution by mating virgin females each generation to naive males from an external stock every generation to eliminate indirect selection against male-harming mtDNA mutations. We propose an analogous approach but in reverse to eliminate indirect selection against female-harming autosomal variants that interact with the Y chromosome. This may be a useful strategy for validating our theoretical predictions of Father's Curse.

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