Migration Restores Hybrid Incompatibility Driven By Nuclear-Mitochondrial Sexual Conflict

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Abstract

In the mitochondrial genome, sexual asymmetry in transmission favors mutations that are advantageous in females even if they are deleterious in males. Called the "Mother's Curse", this phenomenon induces a selective pressure for nuclear variants that compensate for this reduction in male fitness. Previous work has demonstrated not only the existence of these interactions but also their potential for acting as Dobzhansky–Muller loci. However, it is not clear how readily they would give rise to and sustain hybrid incompatibilities. Here, we use computer simulations in SLiM 3 to expand analytical theory to investigate the consequences of sexually antagonistic mitochondrial-nuclear interactions in a subdivided population. We consider distinct migration schemes and vary the chromosomal location, and consequently the transmission pattern, of nuclear restorers. Disrupting these co-evolved interactions results in less-fit males 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 show that these interactions may act as Dobzhansky–Muller incompatibilities, but their strength is not enough to drive population isolation. Combined, this model shows the varied ways in which populations respond to migration's disruption of co-evolved mitochondrial-nuclear interactions.

Keywords: Mitochondrial-Nuclear Interactions; Sexual Antagonism; Uniparental Inheritance; Mother's Curse; Reproductive Isolation

Introduction

A fundamental question in evolutionary genetics, and biology more broadly, is how new species form and remain distinct (1,2). Dobzhansky (3) and Mayr (4) argued that this process hinges on the evolution of reproductive isolation, which acts to limit gene flow between populations thereby advancing the process of speciation. 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 (2,4). Reproductive isolation develops as isolating barriers accumulate. These barriers may prevent members of different populations from mating or forming zygotes (prezygotic) or may act after fertilization if hybrids are incompatible (postzygotic) (5,6). Understanding the genetic basis of hybrid incompatibility, which encompasses hybrid inviability, hybrid sterility, or reduced fitness of hybrids compared to the parental populations, consequently allows us to better understand the mechanics of speciation (2).

Bateson (7), Dobzhansky (8), and Muller (9–11) first detailed how hybrid incompatibility could emerge between two allopatric populations. Populations acquire unique mutations while geographically separated. These may be mutations that confer an adaptive advantage in their local environment, or may simply be neutral. When populations reunite and hybridize, untested interactions between these newly acquired mutations are exposed and may result in reduced hybrid fitness. We now have several examples of such negative epistatic interactions, dubbed Dobzhansky–Muller incompatibilities, that generate hybrid incompatibility and, consequently, contribute to reproductive isolation (12–14). In spite of this, it remains unclear which specific genetic interactions may become Dobzhansky–Muller incompatibilities.

Interactions between the mitochondrial and nuclear genomes have been proposed as promising candidates for generating these epistatic interactions (15–17). 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, as well as 13 protein subunits that play a small but essential role in the electron transport chain and ATP synthase (18). Approximately 1,500 nuclear genes produce proteins that are actively imported, sorted, and assembled, in interaction with the mitochondrial genome, to ensure proper energy production (18–21). Coordination between these genomes is essential, as improper mitochondrial function is associated with a wide variety of pathogenic phenotypes (22–26).

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 only selection in females is effective. Consequently, sexually antagonistic mutations that are neutral or advantageous in females but deleterious in males can easily spread through a population (27–32). Coined the “Mother’s Curse” by Gemmell et al. (31), these sexually antagonistic mitochondrial mutations are best-studied in plants where they prevent pollen production in otherwise hermaphroditic species (27,33–36). The accumulation of male-harming mutations that cannot be removed places selective pressure on the nuclear genome to evolve variants that restore male fitness, at least partially counteracting the cost of these Mother’s Curse variants, and the rapid generation and fixation of these nuclear “restorer” mutations has been thoroughly documented in plants.

The ultimate dynamics of these interactions depend on the chromosomal location of the a nuclear restorer. In an XY sex-determining system with an equal 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 of a genetic element is generally higher the more time it spends in males (37–39), and the effective population sizes of the X and Y chromosomes are reduced compared to autosomes (by ¾ and ¼, respectively) which magnifies the effect of drift (40,41). Hemizygosity of sex chromosomes in males dramatics impacts the effect of selection on allele frequency dynamics. Finally, sexual antagonism can select for specific chromosomal locations to minimize the deleterious cost of specific variants in one sex (42,43).

Exploration into the chromosomal placement of nuclear genes that interact with the mitochondrial genome shows a complex landscape. Adaptive interactions should result in movement of the associated nuclear gene onto the X chromosome, while those that work to mitigate sexual conflict, such as those involved with mitochondrial Mother's Curse variants, will likely move off the X (44). The Y chromosome has been put forward as a potential harbor for nuclear restorers of Mother's Curse variants. While the Y chromosome is gene-poor, which suggests that nuclear restorers may appear on the Y with less frequency than on autosomes, the strict paternal transmission of the Y chromosome should favor the accumulation of male-beneficial mutations (45). Furthermore, the Y chromosome has a demonstrated regulatory role that may act to offset the cost of Mother's Curse variants, since genes exhibiting sex-specific sensitivity to mtDNA are overrepresented among genes known to be sensitive to Y-chromosomal variation (46,47). The 'heterochromatin sink model' suggests that the length of heterochromatin blocks on the Y chromosome may act as a sink for transcription factors or chromatin regulators, consequently affecting the distribution of these elements in the rest of the genome (48,49). Direct theoretical comparisons between autosomes, the X chromosome, and the Y chromosome suggest

that nuclear restorers on the Y most rapidly spread and fix within a single population as well (50).

Direct identification of Mother's Curse nuclear restorers, which would provide insight into their chromosomal placement, 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 purposely disrupt co-evolved mitochondrial and nuclear interactions and evaluate the difference in male and female fitness of hybrids. Furthermore, these interactions are often highly sensitive to environmental conditions, and so they may often be overlooked (36,51–53). This experimental design, while effective, can be laborious and may only capture a relatively small portion of the diversity of these interactions. Despite this, these studies highlight the potential strength of these interactions as Dobzhansky–Muller incompatibilities. Theoretical work on the evolution of mitochondrial-nuclear interactions, however, have mostly focused on the evolution of these interactions within a single population (54–59).

Here, we construct a theoretical framework for exploring the consequences of disrupting co-evolved mitochondrial-nuclear interactions in a multi-population setting and track their dynamics over time using computer simulations. We limit ourselves to two allopatric populations of equal size, each fixed for a unique set of mitochondrial Mother's Curse variants and corresponding nuclear restorers. A given simulation considers one of three distinct chromosomal locations for these nuclear restorers: autosomal, X-linked, or Y-linked. At the beginning of the simulation, we select one of four distinct migration schemes: continuous symmetric migration, a single generation of continuous migration, continuous asymmetric migration, or continuous sex-specific migration. This design mimics two allopatric populations that have each fixed unique sexually antagonistic mitochondrial-nuclear interactions and allows us to measure hybrid fitness

over time as migration creates gene flow between them. Ultimately, this allows us to get a sense of whether mitochondrial-nuclear interactions can act to keep populations isolated, a key step in the speciation process.

Material and Methods

Model Design

We consider two diploid, dioecious sexual populations that are initially completely geographically isolated. We start by defining two classes of 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 elements may represent either an autosome, X chromosome, or Y chromosome with the associated 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 that mutant Mother's Curse variant without impacting female fitness (see **Table 1** for a 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 fixed set of 20 distinct mitochondrial Mother's Curse variants and 20 corresponding fixed mutant nuclear restorers. These sets are disjoint, such that we are tracking 40 loci in each of the mitochondrial and nuclear genomic elements (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 (**Fig. 1.a**). As there are no good

estimates for the number of expected mitochondrial Mother's Curse variants or nuclear restorers, we choose 20 because it allows us to explore a substantial number of interactions without excessively long simulation runtimes. For simplicity, we do not allow new mutations to emerge at any point. We allow recombination to occur for autosomal and X-linked nuclear restorers, and assume these restorers are fully unlinked. Since 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 interactions between mitochondrial Mother's Curse and nuclear restorer variants.

Table 1 Genotypes and Fitnesses of Males and Females for a single interaction (1 mtDNA Mother's Curse Locus : 1 Nuclear Restorer Locus) depends on Nuclear Restorer Chromosomal Location. *M/A/X/Y* and *m/a/x/y* represent the wild type and mutant Mother's Curse and restorer alleles respectively. s_f represents the advantage given by the Mother's Curse variant, while s_m represents the cost of this mitochondrial variant in males. We assume incomplete dominance (d=0.5) for autosomal nuclear restorers.

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 $1-s_m$ $1-s_m$ 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

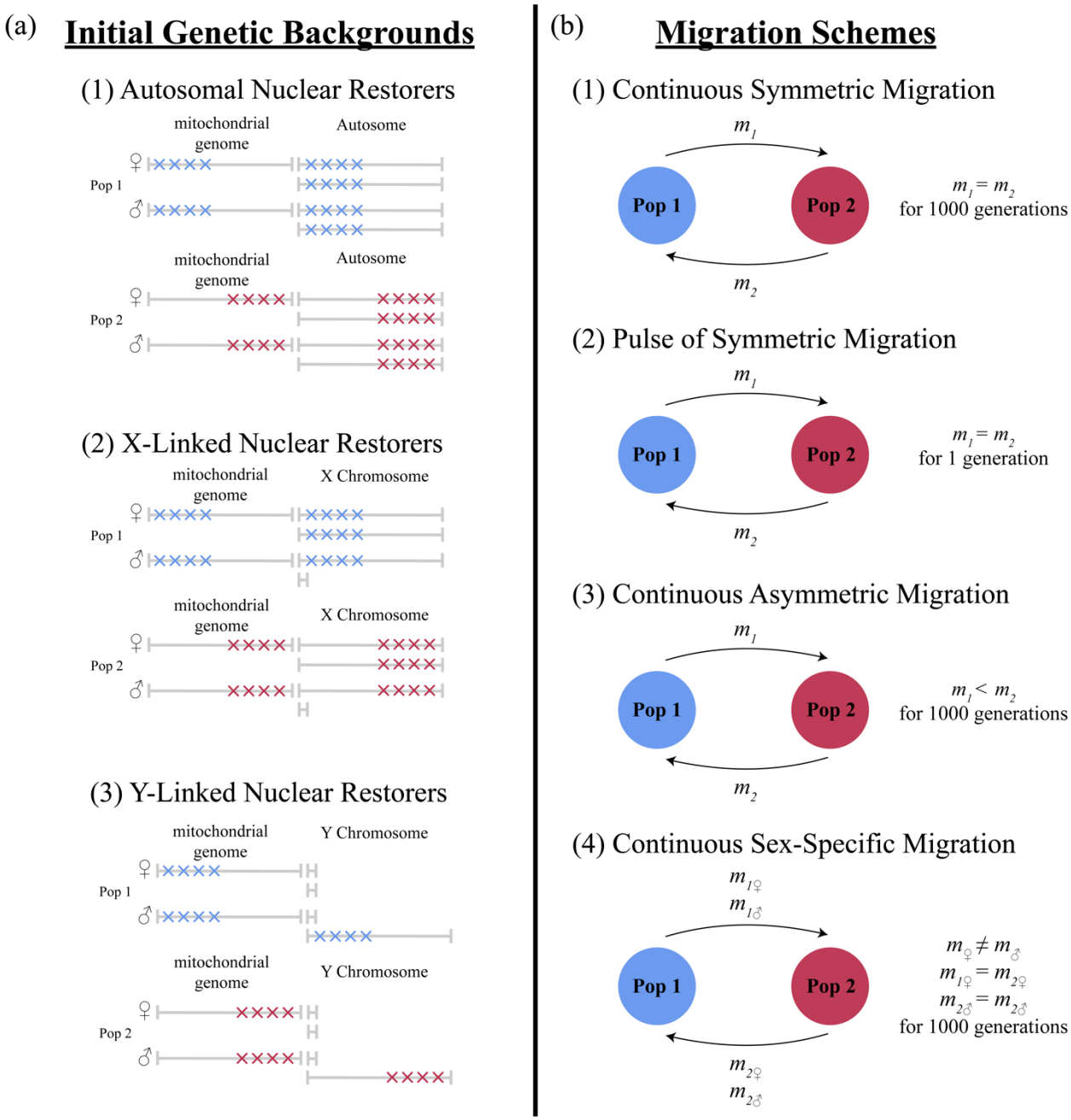

Figure 1 Visual Schematic of Model Design. Blue represents population 1, while red represents population 2. (a) Representation of genetic backgrounds for females and males in each population, depicting the chromosomal location of nuclear restorers. (b) Representation of the four distinct migration schemes explored.

We explore four different migration schemes: continuous symmetric migration, a single generation of symmetric migration, continuous asymmetric migration, and continuous sex-symmetric migration **(Fig. 1.b)**. Consequently, our model represents two allopatric populations that have evolved unique mitochondrial-nuclear interactions that are disrupted as migration

creates gene flow between them. In order to simulate this model, we use SLiM (version 3.3), a powerful and flexible genetic simulation framework, which is capable of incorporating all of these design elements into its "nonWF" model type (60).

SLiM Model Implementation

We employ the nonWF model type in SLiM since it allows us to directly control important aspects of our model, including the generation of offspring, migration events, and epistatic fitness calculations. There are two key aspects of nonWF models in SLiM we must stress: how fitness is evaluated, and how populations are regulated. Fitness, in nonWF SLiM models, influences survival, and, consequently, fitness represents absolute fitness. **Table 1** details the fitness of an individual for a specific mitonuclear interaction (1 Mother's Curse locus : 1 Nuclear Restorer locus). The final fitness of an individual is calculated multiplicatively across all mutations possessed by an individual and the density-dependence effects that regulate the population size. 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 SLiM models) by setting the fitness of non-newborns to 0 to ensure that these individuals do not survive. Additionally, population regulation is not managed automatically (i.e., there is no fixed population size) in these models. Instead, population size is emergent, determined by the number of offspring that are born minus the number that die due to selection in each generation. An important consequence of this is that the sex ratio may fluctuate if fitness differs between the sexes, which is often the case in our model. The populations can grow up to a specified carrying capacity, but we expect there to be fluctuations in both the

population size and sex ratio. Ultimately, our simulations are designed to more robustly represent the demography of natural populations.

We initialize our model by defining two genomic elements: one mitochondrial and one nuclear (an autosome, X chromosome, or Y chromosome). We set the mutation rate to 0 and recombination rates accordingly (0 for the mitochondrial genome/Y chromosome and 0.5 for the autosomes/X chromosome). We establish four mutation classes: mitochondrial Mother's Curse variants originating in population 1 (MC1), nuclear restorer variants originating in population 1 (NR1), mitochondrial Mother's Curse variants originating in population 2 (MC2), and nuclear restorer variants originating in population 2 (NR2). We evaluate their fitness as detailed in **Table 1.** Note, every Mother's Curse variant provides the same benefit to females and cost to males (i.e., s_f and s_m are constant for all Mother's Curse variants). 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 population 1 have 20 unique pairs of MC1 and NR1 variants and all individuals in population 2 have 20 unique pairs of MC2 and NR2 variants. Each MC and NR variant carries a specific tag such that each Mother's Curse variant has one corresponding nuclear restorer, originally in the same population, that compensates for that MC variant's effect on male fitness. Once our populations are established, we start the simulation and allow migration.

Within each 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, to serve as parents. Male/female pairs are chosen randomly from this pool to generate a single offspring, until 2000 offspring have been generated. By generating more offspring than

the number of parents needed, we ensure that there will always be at least 1000 individuals to serve as parents each generation.

SLiM itself has no understanding of mitochondrial DNA and always models diploid genetics, so to achieve a functionally haploid genomic element we must take additional steps. The mitochondrial genome contains a marker mutation (i.e., neutral and used only as a placeholder) 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 as a safeguard to ensure that there are no mutations on that element. This results in a functionally haploid mitochondrial genomic element that is inherited maternally. We employ a similar method for any model that uses a Y-linked nuclear restorer, but in reverse, to ensure that the Y chromosome is exclusively transmitted to males, clearing both nuclear genomic elements in females. This allows us to have two genomic elements with different ploidies and transmission patterns in the same model, which is essential to modeling mitochondrial-nuclear interactions in SLiM.

After reproduction as described above, SLiM calculates the fitness of all individuals. 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 the 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 so that each restorer is fully unlinked from the others. Because there is no recombination in the mitochondrial genome, an individual will either have the 20 MC1s from population 1 or the 20 MC2s from population 2; we can consider these as two

unchanging haplotypes that derive from their respective populations. Note that under this design, female fitness, apart from the effects of density-dependence for population regulation, is equal and constant in both populations as it depends only on the mitochondrial haplotype present, and each haplotype has the same fitness of $(1 + s_f)^{20}$ since each haplotype contains 20 Mother's Curse variants. Male fitness varies since it depends on the number of nuclear restorers present. Mean population fitness, prior to any density-dependent effects on fitness, is determined by the mean fitness of males in each population and the sex ratio.

After calculating the fitness of all individuals SLiM applies viability selection, with each individual's probability of survival equal to its fitness. From the remaining individuals, we select individuals to migrate to the other population depending on the migration scheme. For continuous symmetric migration, we have one migration parameter m which defines the probability that an individual will migrate from one population to the other. We use the same migration parameter to implement a single-generation pulse of symmetric migration, but after that first generation, we set m to 0 to eliminate migration. For continuous asymmetric migration, we have two migration parameters, m_1 and m_2, where m_1 determines the probability that an individual in population 1 will migrate into population 2, and m_2 determines the reverse. We set $m_1 \leq m_2$ such that population 1 receives more migrants from population 2 than the reverse. Finally, for continuous sex-specific migration, we assign two migration parameters m_f and m_m. m_f is the probability that a female individual will move from one population to the other, while m_m is the probability that a male individual will 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. 1.b** for visual representation of all migration schemes).

After migration, the generation 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 migrational disruption of co-evolved mitochondrial-nuclear interactions for a specific set of parameters (migration parameters, s_f, and s_m).

All scripts were run in SLiM v.3.3, and scripts are on GitHub (https://github.com/mam737/mito_nuclear_SLiMulations). All migration parameters (m under continuous symmetric migration and single-generation symmetric migration, m_1 and m_2 such that $m_1 \leq m_2$ 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 each specific parameter set, we replicate the simulation 10 times. Therefore, a total of 6480 (3*6*6*6*10), 6480, 22680 (3*6*6*21*10), and 38880 (3*6*6*36*10) replicates were run in total for continuous symmetric migration, a single-generation pulse of symmetric migration, continuous asymmetric migration, and continuous sex-specific migration respectively.

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 benefit of Mother's Curse variants in females and their cost in males (s_f - | s_m |). Disrupting these co-evolved interactions leads to less fit males; as a result, many more males than females die, skewing the sex ratio 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 from both populations, protecting males from the deleterious effects of most or all Mother's Curse variants. As autosomal restorers spread, the fitness and sex ratio slowly recover to initial levels (**Fig 2.a,c**). X-linked restorers behave nearly identically to autosomal restorers (**Fig S1)**.

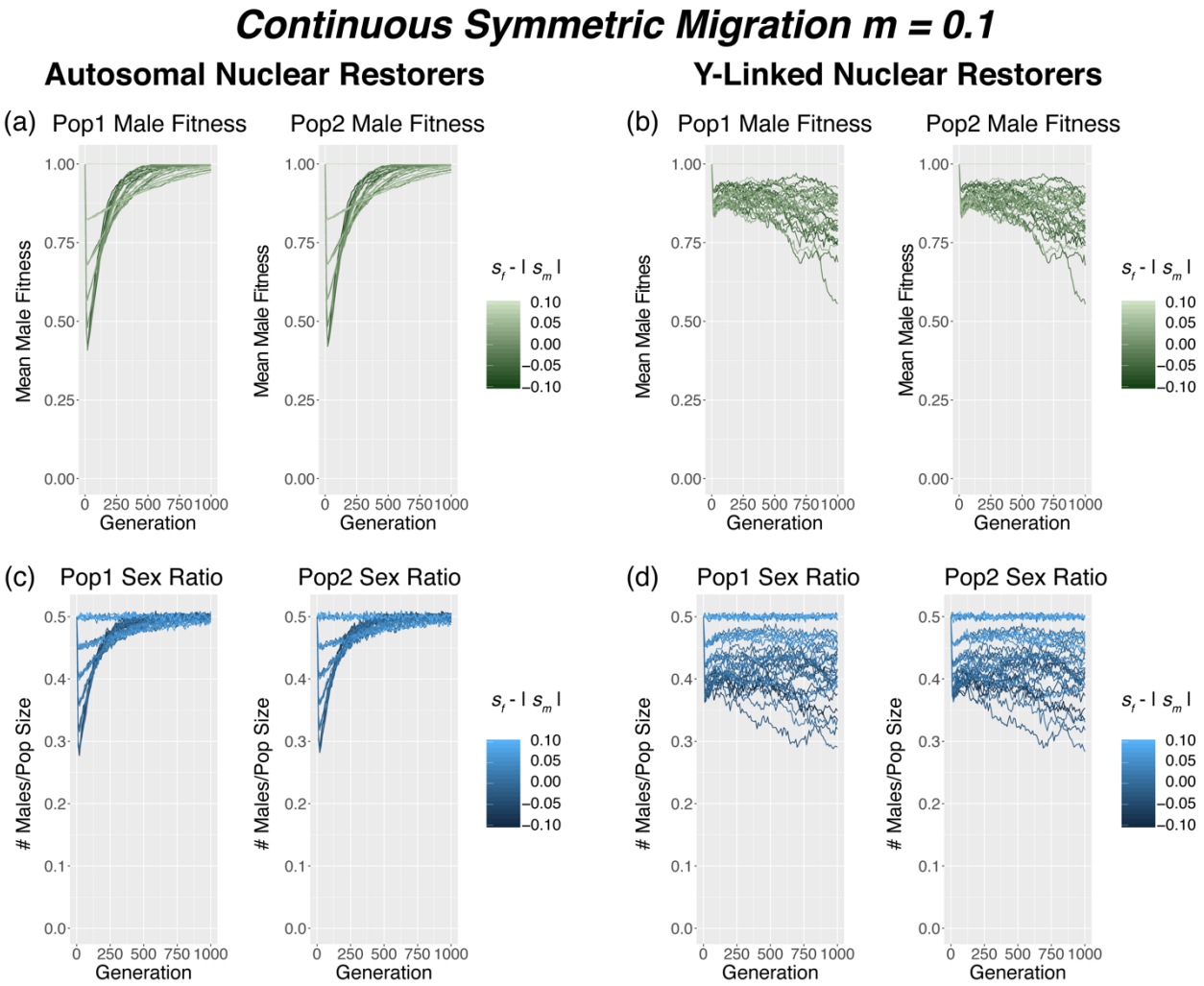

Figure 2 Mean male fitness and sex ratio trajectories for population 1 (left) and population 2 (right) under continuous symmetric migration at rate *m* = 0.1. (a) Mean male fitness trajectories for autosomal nuclear restorers, (b) Mean male fitness trajectories for Y-linked nuclear restorers, (c) Sex ratio trajectories for autosomal nuclear restorers, (d) Sex ratio fitness trajectories for Y-linked restorers. See Fig. S1 for X-linked restorerers, which behaved almost identically to autosomal restorers

In contrast, Y-linked restorers are unable to recover male fitness. The absence of recombination on the Y chromosome makes it impossible for males to acquire both sets of nuclear restorers, and, consequently, hybrid males always suffer reduced fitness.

However, it is worth noting that while Y-linked restorers 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 2.b,d**). This is likely because all Y restorers from one population segregate together (another consequence of no recombination in the Y chromosome); as a result, a male that carries the Y haplotype matching their mitochondrial haplotype will be fully restored, while one that carries the other will experience the full cumulative cost of the Mother's Curse variants.

If we examine the allele frequency trajectories of nuclear restorers for a specific s_f and s_m, we can assess the degree to which autosomal and X-linked restorers fully recover male fitness by obtaining both sets of nuclear restorers. We find that the frequency of all autosomal restorers tends towards fixation in both populations (**Figure 3.a**, see **Figure S2** for X-linked restorers**)**. This aligns with both the restoration of male fitness and the return towards an equal 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 haplotypes, and their frequency trajectory is determined by genetic drift thenceforth.

With Y-linked restorers under the same conditions, we observe movement towards fixation of one Y haplotype and loss of the other; which haplotype is fixed versus lost seems to be initially stochastic, with positive feedback toward fixation driving whichever haplotype initially increases in frequency (**Figure 3.b**).

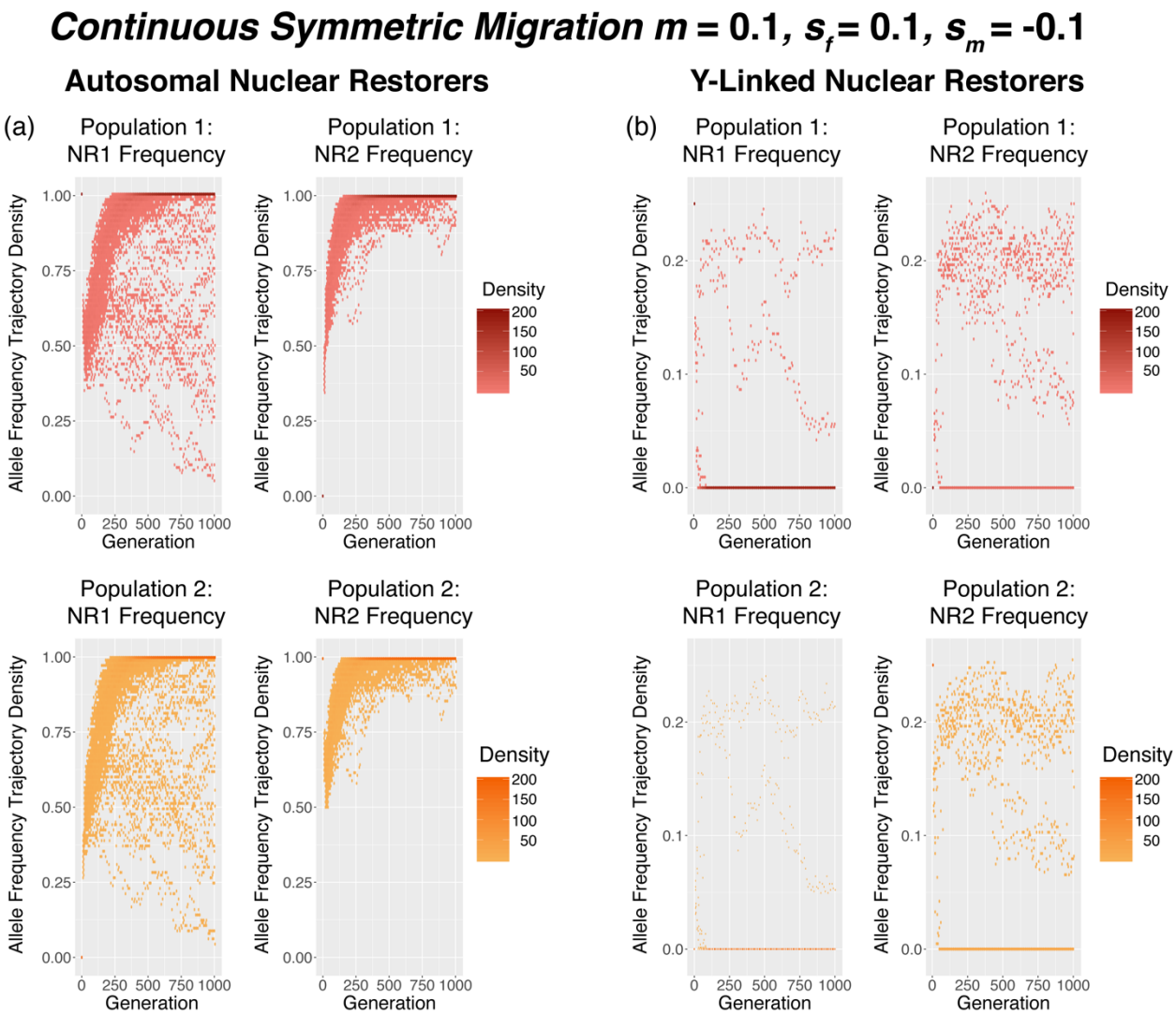

Figure 3 Density of Allele Frequency Trajectories for both sets of nuclear restorers, NR1 and NR2 (left and right), in population 1 and population 2 (top and bottom) under continuous symmetric migration rate *m* = 0.1, s_f = 0.1, and s_m = -0.1. Density represents how often the allele frequency trajectory 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

Selection cannot remove the mitochondrial haplotype associated with the lost Y haplotype due to the advantage of the Mother's Curse variants in females. If this mitochondrial haplotypeincreases in frequency, there is no longer any way to offset the reduction in male fitness, as the associated Y haplotype has been lost. Consequently, some males suffer reduced fitness depending on the frequency of the mitochondrial haplotypes, which is driven by drift as both haplotypes 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.

A Single Generation of Symmetric Migration

If symmetric migration is allowed only for one generation, we observe a much smaller reduction in fitness which quickly returns to the initial level. One generation of migration does create less fit hybrid males, but this reduction is not sustained. These F1 hybrid males have greatly reduced fitness and a large number of them die before producing any offspring. Later generation hybrids are consequently mostly the result of F1 hybrid females mating with males native to the population they are in. Without new migrants to continue generating hybrids, the number of hybrids in both populations declines until there are few to no hybrids left. Consequently, we see only minor fluctuations in male mean fitness and sex ratio since there are substantially fewer hybrid males than ancestral males (**Figure S3-8)**. This is consistent across all nuclear restorer locations. Under our parameter values, one generation of symmetric migration is not enough to allow less-fit hybrid males to persist. 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 die off for populations undergoing continuous asymmetric migration, but the size and duration of

this reduction depends on the migration rates between the two populations. The impact on male fitness depends on the number of migrants the population receives. When the migration rates are asymmetric, we find that one population experiences a much more severe reduction in male fitness and a larger sex ratio skew. We saw before that with continuous symmetric migration it takes both populations approximately 500 generations to return to the initial male fitness and sex ratio. With continuous asymmetric migration, in contrast, even with the smallest difference in migration rates explored in our simulations (m_1=0.01, m_2=0.028), populations recover male fitness in approximately 150 generations, and this occurs even faster as the difference in migration rates increases.

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 (the sets of variants initially associated with population 2). Both populations tend to rapidly fix for MC2 and NR2; however, it is worth noting that occasionally the MC1 haplotype increases in frequency despite the asymmetric gene flow, which drives an increase in frequency of the NR1 set of restorers.

Continuous asymmetric migration with rates m_1 = 0.01, m_2 = 0.1 (which results in population 1 receiving ten times more migrants than population 2) shows a smaller reduction in fitness and a shorter time to recovery than continuous symmetric migration with a rate of m = 0.1 (**Fig 4,** see **Fig S9** 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 haplotype (MC2) and nuclear restorers (NR2) present in population 2 (**Fig 5,** see **Fig S10** for X-linked restorers**)**.

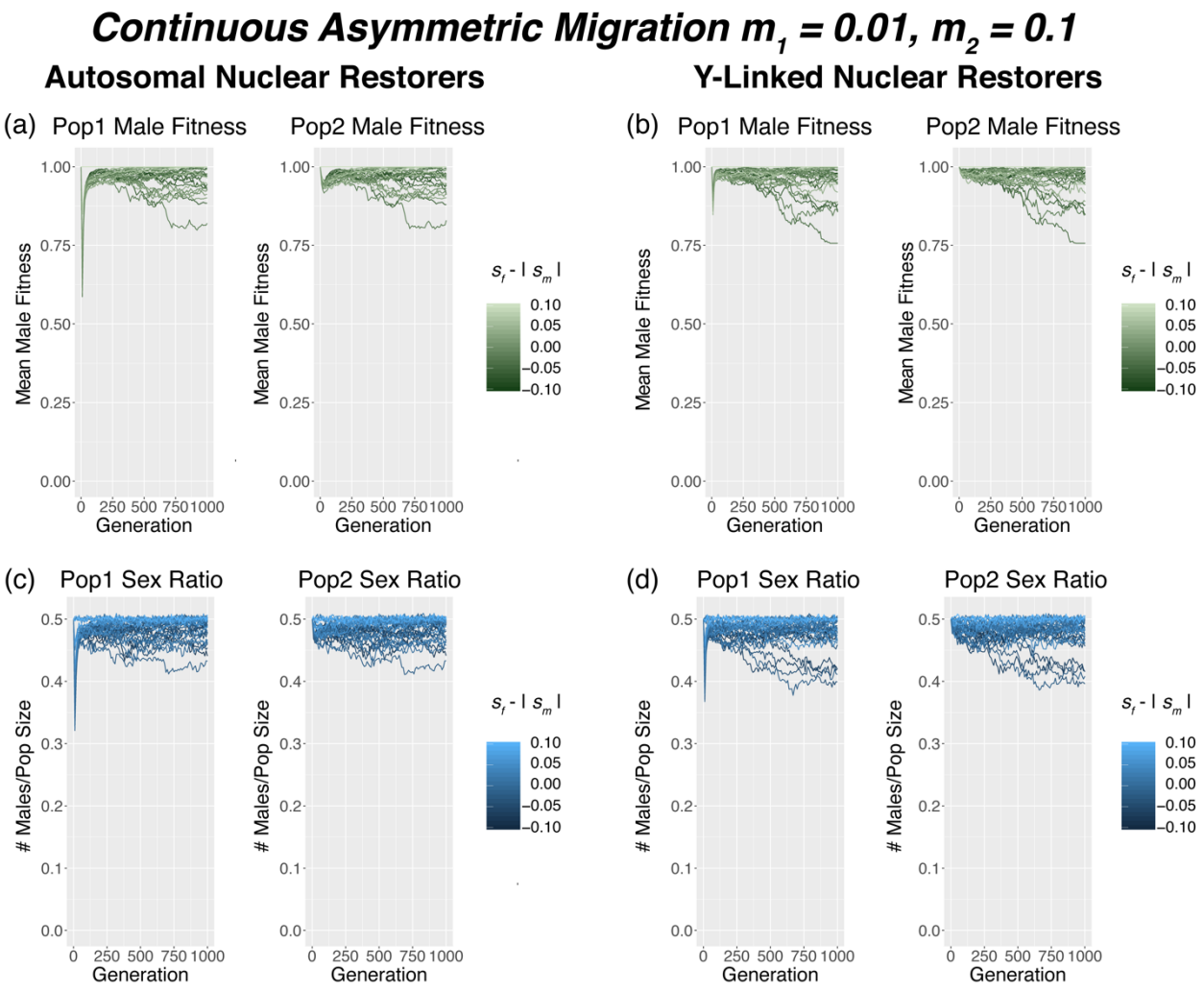

Figure 4 Mean male fitness and sex ratio trajectories for population 1 (left) and population 2 (right) under continuous asymmetric migration rate m_1 = 0.01 and m_2 = 0.1. (a) Mean male fitness trajectories for autosomal nuclear restorers, (b) Mean male fitness trajectories for Y-linked nuclear restorers, (c) Sex ratio trajectories for autosomal nuclear restorers, (d) Sex ratio trajectories for Y-linked restorers.

Restoration in this scenario relies on replacement due to the asymmetric gene flow, 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 restorers 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 because a higher m_2 expedites the replacement process.

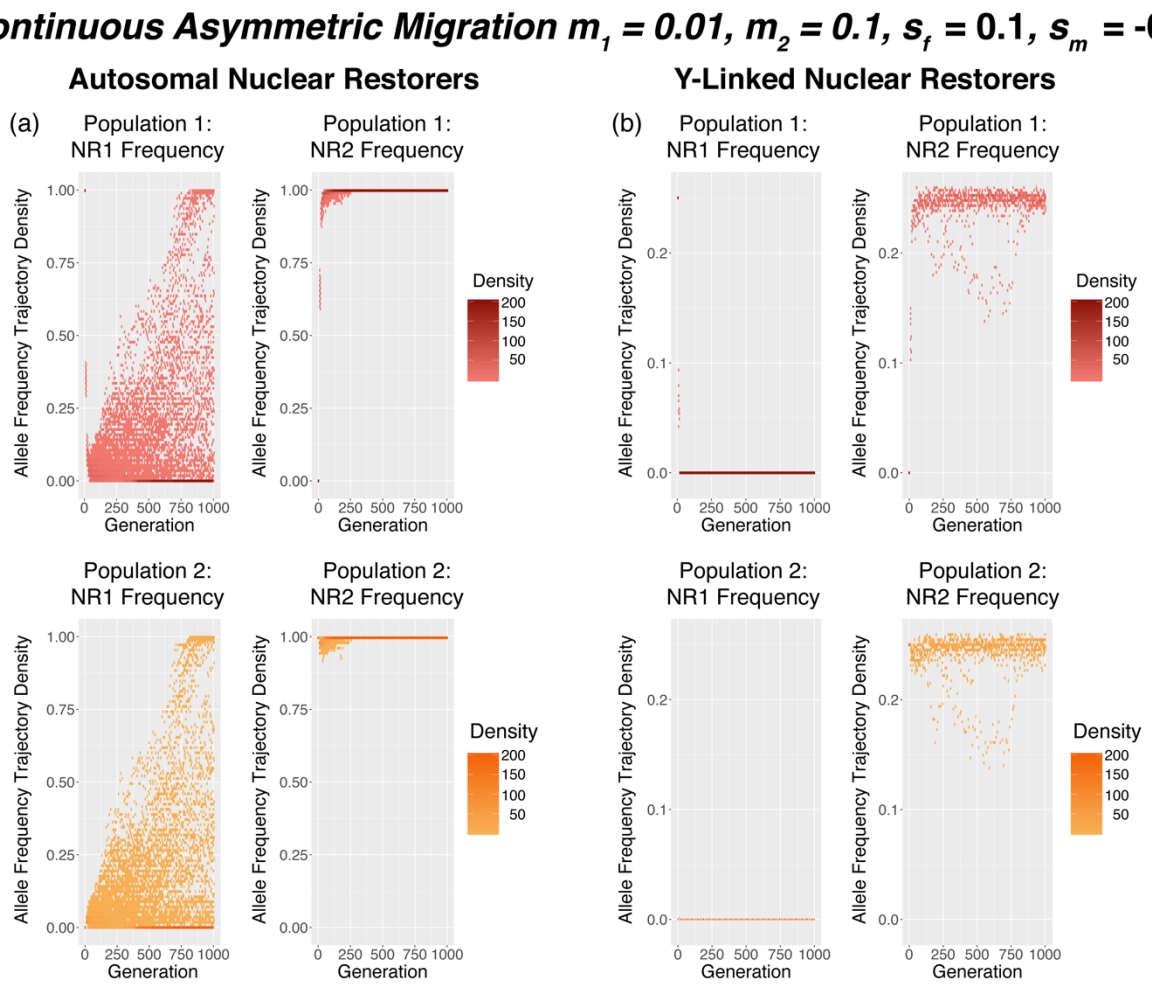

Figure 5 Density of Allele Frequency Trajectories for both sets of nuclear restorers, NR1 and NR2 (left and right), in population 1 and population 2 (top and bottom) under continuous asymmetric migration rate *m* = 0.1, s_f= 0.1, and s_m = -0.1. Density represents how often the allele frequency trajectory 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.

Population replacement tends to occur more rapidly than recombination can merge the nuclear restorer sets (as seen under continuous symmetric migration), although the speed of this process depends on the number of migrants moving into population 1 – larger influxes of migrants cause population replacement to occur more rapidly.

As noted earlier, the NR1 set of nuclear restorers is not always lost. The fate of the NR1 set of restorers depends on whether the MC1 haplotype is able to increase in frequency. This seems to be a somewhat rare occurrence since the MC1 haplotype is also being driven downward

in frequency by the influx of migrants. Consequently, we more often see full population replacement, and this scenario may resemble older, simpler models of genetic drift in the face of asymmetric gene flow.

Continuous Sex-Specific Migration

Here, we assign distinct migration rates for each sex but set them such that they are symmetric between the populations. We find that this model behaves identically to continuous symmetric migration, which suggests the fitness and sex-ratio dynamics are influenced more by the symmetry between the populations than by the differences in migration rate between the sexes (**Fig S11-16**). 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. The larger the female migration rate is relative to the male migration rate, the more rapid the decline in fitness is. The final state of the populations 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 die off. Populations respond to this in one of two ways, depending on whether the migration is symmetric or asymmetric. Under symmetric migration, populations acquire both sets of nuclear restorers to shield males from the deleterious effects of both mitochondrial haplotypes. 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, since all nuclear restorers for a specific Y haplotype segregate together; this means that any male with the corresponding mitochondrial haplotype is fully restored. Under asymmetric migration, one population's genetic variation is usually replaced by the other, as a result of swamping due to gene flow, which eliminates the potential for less fit hybrid males. This occurs more rapidly than recombination and selection can merge the two sets of nuclear restorers, shortening the duration of reduced male fitness and the female-biased sex ratio in this scenario.

Under the parameters explored, we found little evidence that mito-nuclear interactions can lead to reproductive 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 incorporating all nuclear restorers through recombination, or by replacement after being swamped by gene flow. It is possible all hybrid males would die out if the deleterious effects of the Dobzhansky–Muller incompatibilities were stronger, which could generate reproductive isolation. However, we are unaware of any Mother's Curse variants that induced lethality in hybrid males, likely because such a mutation could drive a population extinct before a nuclear restorer emerges to counteract it. Of documented Mother's Curse variants, male infertility seems to be the most commonly observed trait (27,36,53,61). 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 in such a way as to offset the reduction in male fitness.

Along with reduced male fitness, we see a distinct skew in the sex ratio as less-fit males are removed from the population. A 1:1 sex ratio is not a universal trait, even among dioecious

species (62,63), but there are consequences to having a biased sex ratio. A skewed sex ratio is known to reduce the effective population size, which decreases the efficacy of natural selection (64). This increases the rates of genetic drift and inbreeding, ultimately resulting in a loss of genetic variability (65). A significantly reduced number of males (in our model, the sex ratio shifts as far as 1:3 in favor of females) for a sustained period of time is likely to affect the genetic diversity of the Y chromosome even if the nuclear restorers are autosomal or X-linked. This may have large fitness consequences, since the Y chromosome is known to influence a wide variety of traits (47,66).

Previous work has proposed the Y chromosome as a promising site for nuclear restorers that counteract the male-harming effects of mitochondrial mutations (50,67,68). Early empirical evidence by Innocenti et al. (69) and Rogell et al. (46) examined genes that are male-biased in their sensitivity to mitochondrial variation and found that these same genes are over-represented on a list of genes known to be sensitive to Y chromosomal regulation, which suggests a potential interaction between these two elements to influence male fitness. 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 regain fitness more rapidly with autosomal and X-linked restorers than with Y-linked restorers when contact with other populations leads to hybrid offspring. The proposed advantages of Y-linked restorers may therefore only be realized in fully allopatric populations.

Our theoretical framework and simulations explore the effects of migration and chromosomal location of nuclear restorers on mitochondrial-nuclear interactions. Among the

many unexplored aspects of our model, there are several that merit further research. We did not model the emergence of novel mitochondrial Mother's Curse variants and nuclear restorers, nor the consequences of linkage for autosomal and X-linked restorers – mostly due to our inability to find robust empirical estimates of the relevant parameter values. We also assumed that both populations were initially fixed for these interactions, as previous theory suggests these interactions move rapidly towards either fixation or loss (50,70–72), but the evolutionary dynamics of these interactions while they are still segregating may be of interest. It is also worth noting that we did not explore the effects of genetic disequilibria between 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 linkage disequilibria (i.e., departures from random association between nuclear and cytoplasmic genotypes) (70,71,73). Hybrid zones with directional and strong assortative mating will exacerbate cytonuclear disequilibria and epistatic interactions, like those we explored, and may only further this non-random association between nuclear and cytoplasmic genotypes.

There are also facets of both Mother's Curse variants and nuclear restorers that may influence our results. Mitochondrial DNA copy number ranges from hundreds to thousands of copies per cell depending on the cell's energetic needs (74). If these copies are identical, they are "homoplasmic" and can be treated as a haploid element (which we assume in our model). However, if there is variation among the mtDNA copies in a cell, 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 change the phenotype (75). We also assume exclusive maternal inheritance of the mitochondrial genome, since only a handful of examples exist of paternal mitochondrial inheritance (whether

partial or full) (76–79). Paternal transmission would introduce purifying selection in males on the male-deleterious Mother's Curse mutations, but this is likely a rare occurrence.

We also assume that a nuclear restorer is able to fully rescue male fitness for its complementary mitochondrial Mother's Curse variant, and that restorer mismatch (i.e., a negative fitness interaction between a nuclear restorer and the wild-type mitochondrial variant) does not occur. It is likely that restorer mismatch does exist in natural populations (see (80) 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 model.

Finally, departures from random mating, especially inbreeding and assortative mating, have been shown to influence the spread of mitochondrial Mother's Curse variants. Kin selection could also hinder Mother's Curse mitochondrial variants, since it couples a mother's fitness with that of her sons or a sister's fitness with that of her brothers (58,59).

The model presented here provides novel insight into how populations respond to the disruption of co-evolved mitochondrial-nuclear interactions by migration, and they highlight the distinct forms this response takes depending on both the migration scheme and the chromosomal placement of nuclear restorers. Extensions of our model will provide additional insight into how asymmetrically inherited genomic elements can 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 SLiM 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.

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Data Accessibility

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

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