SUPPORTING INFORMATION

Dynamics of mitochondrial inheritance in the evolution of binary mating types and two sexes.

Zena Hadjivasiliou, Nick Lane, Robert M. Seymour and Andrew Pomiankowski

A. MATHEMATICAL DERIVATIONS

We present a derivation of the equations modelling the life cycle outlined in the main text and shown in Figure S1. We begin by deriving the equations for the simplest of our models, in which wild type mitochondria are subject to deleterious mutation pressure. We define the two dimensional random variable $\mathbf{X}^t = (X_1^t, X_2^t)$ to represent the mitochondrial state and nuclear genotype of the diploid unicellular organisms (referred to from now on as 'cells') in the population at generation t where X_1^t is the number of mutant mitochondria carried by a cell and X_2^t is the mitochondrial inheritance locus of that cell. Hence, X_1^t takes values in $\{0, 1, ..., M\}$ and X_2^t takes values in $\{aa, Aa, AA\}$. It follows that there are $(M + 1)$ possible mitochondrial states. By definition their frequencies over all mitochondrial inheritance genotypes sum up to one,

$$\textstyle\sum_{i,j} P(\mathbf{X}^t = (i, j)) = 1, \forall\ t.$$

We derive the change in the relative frequency of each genotype following each step of the life cycle (Fig. S1). We assume an infinite population and so ignore drift in the nuclear locus, but include sampling (i.e. drift) of the mitochondrial population at reproduction (see below). If $P(\mathbf{X}^t = (i, j))$ denotes the population distribution at the onset of the life cycle (generation t) then $P(\mathbf{X}^{t+1} = (i, j))$ denotes the probability distributions at the onset of the next life cycle (generation $t + 1$).

During each generation the population will have gone through five steps as described in the main text (mutation, selection, meiotic step 1, meiotic step 2 and syngamy). So to go from generation t to generation $t + 1$, the population undergoes five intermediate steps. We denote the probability distribution after each step by $P(\mathbf{X}^{t,\tau_s})$ where s takes values in $\{1, 2, 3, 4, 5\}$ and $P(\mathbf{X}^{t,\tau_0})$ is the distribution at the onset of generation t. It also follows that $P(\mathbf{X}^{t,\tau_5} = (i, j)) = P(\mathbf{X}^{t+1,\tau_0} = (i, j))$.

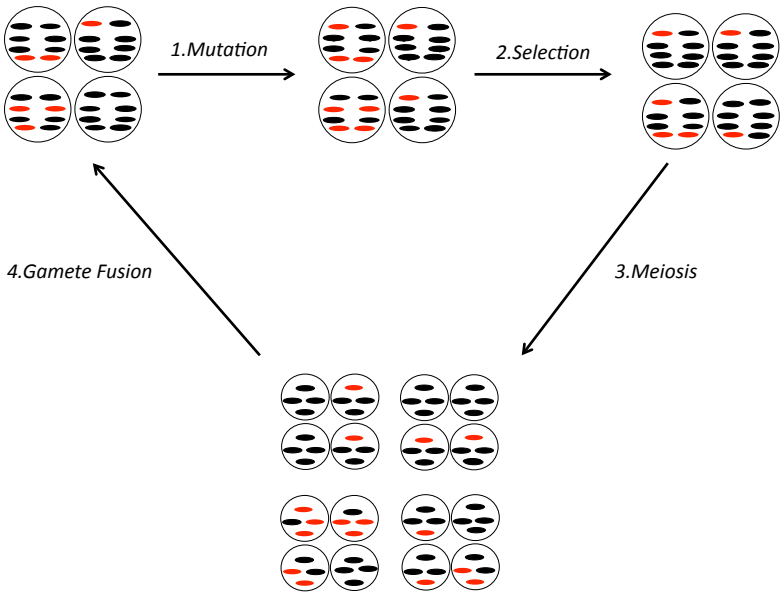

Fig. S1: Model life cycle. Big circles are cells while ovals represent mitochondria (black for wild-type and red for mutant).

A1. Relative frequency calculation

1. Following mutation

We let Z_k be the number of new mutants that a cell carrying k mutations may accumulate. Z_k is a random variable following a binomial distribution $\mathrm{B}(M-k,\,\mu)$ and we have,

$$P(Z_k = l) = \binom{M-k}{l}\mu^l(1-\mu)^{M-l}.$$

Hence, we obtain the cell's distribution following mutation,

$$P(\mathbf{X}^{t,\tau_1} = (i,j)) = \textstyle\sum_{k=0}^{k=i} P(\mathbf{X}^{t,\tau_0} = (k,j))P(Z_k = i-k)\ ,\ \forall\ j.$$

2. Following selection

We use a standard population genetic model of generational frequency change in a large population,

$$P(\mathbf{X}^{t,\tau_2} = (i,j)) = \frac{P(\mathbf{X}^{t,\tau_1} = (i,j))w(i)}{\bar{w}},\ \forall\ j,$$

where $\bar{w} = \sum_{i,j} P(\mathbf{X}^{t,\tau_1} = (i,j))w(i)$ and $w(i)$ is the fitness of a cell with i mutant mitochondria as defined by Equation (1) in the main text.

3. Following meiosis

Meiosis takes place in two stages and so this derivation is performed in two steps.

STEP 1: first meiotic subdivision

In the first step, the nuclear mitochondrial inheritance alleles and each mitochondrial gene are first duplicated, and then two diploid daughter cells are formed by random segregation, each with M mitochondria. The population is defined by $\mathbf{X}^{t,\tau_2}$ at the onset of meiosis. We define the random variable $\mathbf{X}^{t,\tau_3}$, which takes values (i,j) where $i \in \{0,1,...,M\}$ and $j \in \{aa, Aa, AA\}$ as before, to define the population following the first meiotic step.

We now define the random variable Y_k to be the number of mutant mitochondria sampled from a parent cell with k mutant mitochondria. Sampling takes place without replacement at this stage and so we have,

$$P(Y_k = l) = \frac{\binom{2k}{l}\binom{2(M-k)}{M-l}}{\binom{2M}{M}}.$$

Given that nuclear and mitochondrial genes are independently inherited following cell division we obtain,

$$\begin{aligned}
P(\mathbf{X}^{t,\tau_3} = (i,aa)) &= \sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k,aa)) + \frac{1}{6}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k,Aa)),\\
P(\mathbf{X}^{t,\tau_3} = (i,Aa)) &= \frac{2}{3}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k,Aa)),\\
P(\mathbf{X}^{t,\tau_3} = (i,AA)) &= \sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k,AA)) + \frac{1}{6}\sum_{k=i/2}^{M} P(Y_k = i)P(\mathbf{X}^{t,\tau_2} = (k,Aa)).
\end{aligned}$$

STEP 2: second meiotic subdivision

In this step, each diploid cell resulting from the first meiotic division randomly segregates to produce two haploid gametes, each containing $M/2$ mitochondria. We define the random variable $\mathbf{X}^{t,\tau_4}$ to represent the population of gametes following this second division step. This can take values in (p,q) where p is the number of mutant mitochondria and q is the mitochondrial inheritance gene. Then, p takes values in $\{0,1,...,M/2\}$ and q takes values in $\{a,A\}$ where we assume that M is an

even number. This is equivalent to $P(\mathbf{X}^{t,\tau_4} = (i,j)) = 0$ for $i > M/2$.

We also define the random variable Z_k to be the number of mutant mitochondria sampled from a parent cell with k mutant mitochondria following the second meiotic step. As before, sampling takes place without replacement and we have,

$$P(Z_k = l) = \frac{\binom{k}{l}\binom{M-k}{M/2-l}}{\binom{M}{M/2}}.$$

Using the fact that nuclear and mitochondrial genes are independently inherited following cell division we obtain the gamete distributions following meiosis,

$$\begin{aligned} P(\mathbf{X}^{t,\tau_4} = (p,a)) &= \sum_{k=p}^{M} P(Z_k = p) P(\mathbf{X}^{t,\tau_3} = (k,aa)) + \frac{1}{2}\sum_{k=p}^{M} P(Z_k = p) P(\mathbf{X}^{t,\tau_3} = (k,Aa)), \\ P(\mathbf{X}^{t,\tau_4} = (p,A)) &= \sum_{k=p}^{M} P(Z_k = p) P(\mathbf{X}^{t,\tau_3} = (k,AA)) + \frac{1}{2}\sum_{k=p}^{M} P(Z_k = p) P(\mathbf{X}^{t,\tau_3} = (k,Aa)). \end{aligned}$$

4. Following syngamy

We now have $\mathbf{X}^{t,\tau_4}$ be the gametes right before syngamy and we let $\mathbf{X}^{t,\tau_5}$ be the new cells following syngamy. When inheritance of mitochondria is biparental, the number of mutant mitochondria of the new cell is given by the sum of the mutants that the two gametes carry. Using the assumption that fusions between two A or two a gametes are biparental we get,

$$\begin{aligned} P(\mathbf{X}^{t,\tau_5} = (i,aa)) &= \sum_{k=0}^{i} P(\mathbf{X}^{t,\tau_4} = (k,a)) P(\mathbf{X}^{t,\tau_4} = (i-k,a)), \\ P(\mathbf{X}^{t,\tau_5} = (i,AA)) &= \sum_{k=0}^{i} P(\mathbf{X}^{t,\tau_4} = (k,A)) P(\mathbf{X}^{t,\tau_4} = (i-k,A)). \end{aligned}$$

When inheritance is uniparental, the mitochondria of the passive gamete (not passing on its mitochondria) are discarded and we sample with replacement from the active gamete to obtain M mitochondria for the new zygote (note, this is better than simply doubling the number of mitochondria, otherwise we always have even numbers of mutant mitochondria in UPI zygotes). We define the random variable Q_k to be the number of mutant mitochondria sampled from a gamete which carries k mutants. This follows a Binomial distribution $\mathrm{B}(M, 2k/M)$ from which we have,

$$P(Q_k = l) = \binom{M}{l} \left(\frac{2k}{M}\right)^l \left(\frac{M-2k}{M}\right)^{M-l}.$$

Using the assumption that fusions between an a and an A gamete are uniparental we then obtain,

$$P(\mathbf{X}^{t,\tau_5} = (i, Aa)) \quad = \quad \sum_{l=0}^{M/2} P(\mathbf{X}^{t,\tau_4} = (l, a)) \sum_{k=0}^{M/2} P(Q_k = i) P(\mathbf{X}^{t,\tau_4} = (k, A)).$$

A2. Mating types

The derivations above are for the simplest model presented in the main text which only addresses mitochondrial mutational pressure. Also, we only considered nuclear genes a and A. When mating types A_1, a_1, A_2, a_2 are implemented, the calculations are essentially the same but extended to more than three genotypes appropriately. In addition, if there was recombination between the two nuclear loci (inheritance of mitochondria and mating type), this was applied to generate the resulting nuclear genotype frequencies. In the final step (syngamy) the same probabilistic rules are followed and uniparental or biparental inheritance is assumed according to the gamete's mitochondrial inheritance locus.

A3. Simulations

The biological complexity encompassed by this model prevents us from solving analytically for the equilibrium states. So the asymptotic behavior and equilibria of the life cycle were explored using numerical simulation. The simulations were coded in C and the code can be accessed at https://github.com/UCL/SexesProceedings. We assumed that equilibrium had been reached when the maximum changes in mitochondrial state frequency and nuclear gene frequency across a generation are smaller than an appropriately value ϵ, taken to be 10^{-9}. Our results were qualitatively robust to changes in the parameter values and an equilibrium point was reached within approximately 2000 generations in most simulations.

B. SUPPLEMENTARY TO MAIN TEXT

In this section we provide results that are supplementary to each of the four result subsections in the main text.

B1. Mitochondrial mutation pressure

High M and μ

In Section 3.1 in the main text we discuss the impact of M and μ on the E_1 equilibrium value for p_A. As M and μ increase so does p_A and eventually this pushes the first equilibrium E_1 to merge with E_2. The reason for this relates to the capacity of A cells to allow leakage of UPI benefits to a cells.

When M and μ are low, UPI is very effective at keeping a high proportion of cells in the fittest states in the A population. A significant proportion of A cells have nearly perfect fitness (see Fig.S2). This means that A x a fusions generate highly fit a gametes whose frequency may then be amplified via selection to produce a highly fit population of aa zygotes. However, when M and μ increase, the ability of UPI to maintain such a high proportion of the population at high fitness is impaired (Fig.S2 second and third columns, for increased μ and M respectively). This in turn means that the leakage of fitness advantage from A to a cells is impaired. So p_A can increase further before an equilibrium is reached (Fig.S2).

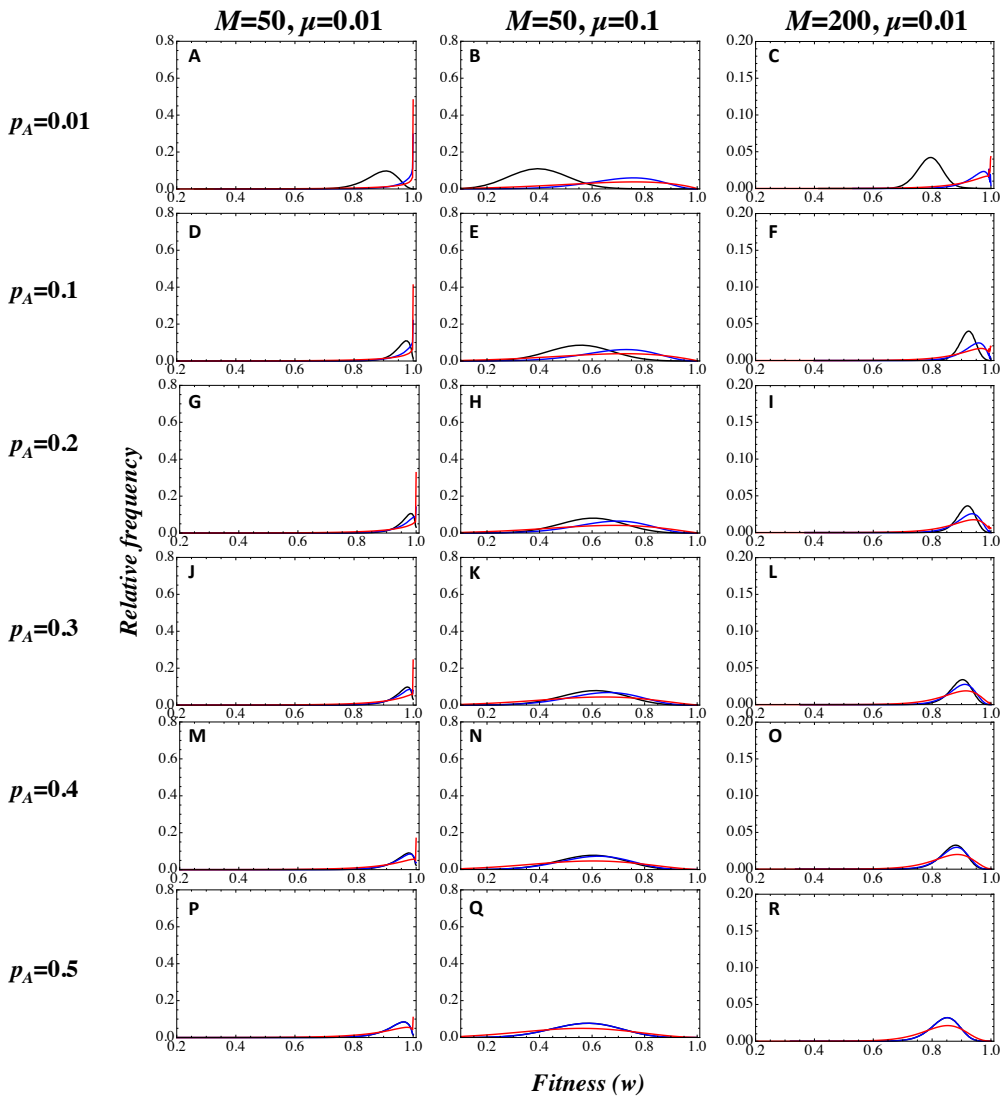

Fig. S2: Fitness distributions for each genotype (aa:black; Aa:red; AA:blue) for different values of M and μ indicated at the top off each column. The population was held at a fixed value of p_A (indicated at the beginning of each row) and the mitochondria were allowed to evolve. The figures illustrate that depending on the values of M and μ, different values of p_A affect the distribution of mitochondrial fitness resulting from selection and 'leakage'.

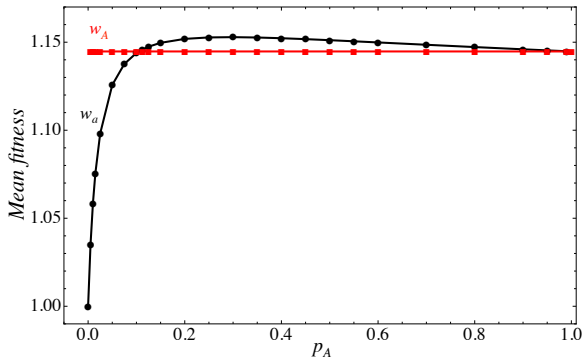

Fig. S3: Mean fitness of a ($\bar{w}_a$, black line) and A ($\bar{w}_A$, red line) for fixed values of p_A using a concave fitness function and assuming that A x A matings are uniparental. Parameter values: M=50, μ=0.01.

A x A Uniparental

We also considered the case where matings between two A cells are uniparental (like in (1)). In this case there is no cost to $\bar{w}_A$ from increasing p_A, as A x A matings are still uniparental, so the average fitness of A is independent of p_A. However, $\bar{w}_a$ increases in a frequency-dependent manner with p_A due to increasing leakage from matings between a and A (Fig. S3). This still results in an E_1 equilibrium when $\bar{w}_a = \bar{w}_A$.

Values of p_A above E_1 result in an increase in $\bar{w}_a$. This is because A uniparental matings generate high variance at each generation with more lower fitness individuals. This short term disadvantage is offset by the longer term cleansing of the mitochondrial mutation load, hence $\bar{w}_A > \bar{w}_a$ when p_A is infrequent and leakage is weak. But for higher values of p_A, $\bar{w}_a > \bar{w}_A$ as a enjoys the benefits of leakage while being able to avoid the disadvantage of short-term increased variation and more lower fitness individuals. This explains why E_3 is unstable. At $p_A = 1$, all matings are uniparental and the mitochondrial mutation load is minimised. So the mitotypes of a mutants are equally cleared of mitochondrial mutations. But the a mutant has the additional advantage of a x a matings, that are BPI and have lower variance. Even though these are initially rare, they have higher fitness on average, so the a mutant will invade a population fixed for A.

Note that a biological mechanism that allows A x A matings to have random uniparental inheritance is unlikely to occur without any costs (2). Such costs will decrease the fitness of AA zygotes and therefore the fitness of A cells as p_A increase. This would result in a frequency-

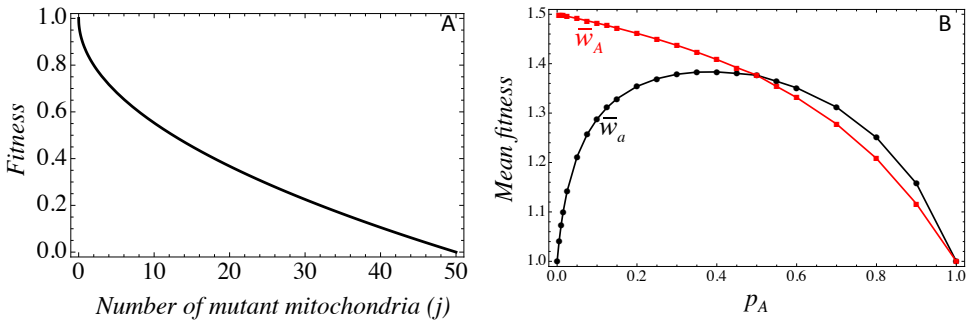

Fig. S4: (A): Convex fitness curve (B): Mean fitness of a ($\bar{w}_a$, black line) and A ($\bar{w}_A$, red line) for fixed values of p_A using a convex fitness function. Parameter values: $M = 50$, $\mu = 0.01$.

dependent decrease in the fitness of A, hindering its spread.

Convex Fitness Curve

We repeated our analysis with the assumption of a convex fitness curve given by,

$$w(j) = 1 - \sqrt{\frac{j}{M}},$$

where M is the number of mitochondria in each cell and j is the number of mutants (Fig.S4A). Here we assume that mitochondrial mutants cause a sharp and increasing fall in oxidative phosphorylation, and hence fitness. This assumption is difficult to justify biologically as it implies that the steepness in fitness decline is higher with fewer mutants and becomes less steep as the number of mutants increases. This is against both intuition and empirical evidence but is included here for completeness (and perhaps there are situations where it might apply).

The assumption of a convex curve causes a much greater benefit when moving from BPI to UPI. For example, the relative advantage at $p_A = 0.1$ ($M = 50$ and $\mu = 0.01$) with a convex curve is $\sim$0.5, (Fig.S4B), compared to only $\sim$0.15 with a concave curve (Fig.1c). This is because there is a short-term advantage to increased variance with a convex curve (Fig.S4A), and leakage is not fast enough to bring $\bar{w}_a = \bar{w}_A$ before the uniparental inheritance allele (A) suffers from a significant decrease in fitness due to frequent biparental A x A matings. It follows that E_1 merges with E_2 even for lower values of M and μ.

The third equilibrium E_3 with $p_A = 1$ also exists but is unstable. When $p_A \approx 1$, all a matings are uniparental, whereas almost no A matings are. So $\bar{w}_a > \bar{w}_A$ even though there is no cumulative

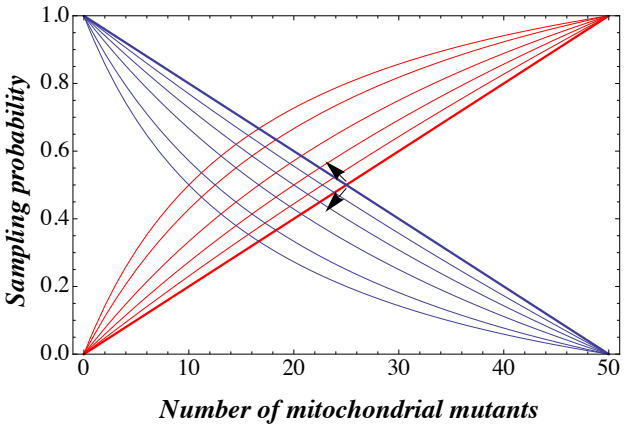

Fig. S5: Probability of sampling a mutant (red) or wild-type (blue) mitochondrion under the assumption of selfish conflict. Number of mitochondria M=50.The lines are for increasing values of replicative advantage k in the direction of the arrows.

benefit in the a population, because a is associated with higher variance in mitotypes and this confers a short-term fitness advantage. This makes E_3 unstable and drives the population to $p_A = 0.5$. This is an important finding as it shows that even modifiers of the "kill your own mitochondria" type can spread under some circumstances. Note that leakage take places both with a convex fitness curve as with a concave fitness curve. It results in an increase in $\bar{w}_a$ as p_A increases, reducing the relative fitness difference between UPI and BPI.

B2. Selfish mitochondrial mutants

For the selfish mutant case, we implement a step after mutation and before selection. At this step mutant mitochondria are given an advantage. So if a cell carries l mutant mitochondria the probability of sampling a mutant mitochondrion should be higher than $\frac{l}{M}$. We defined this probability to be $\frac{l(1+k)}{M+lk}$ where the parameter k determines the mutant advantage. This is an appropriate function, as the overall advantage of mutant over wild-type mitochondria increases with the ratio of mutant mitochondria, due to the additive effect of the advantage of mutant mitochondria. The sampling probabilities for different values of k can be seen on Fig.S5.

To derive the equations implementing this step in the life cycle we define Y_r as the number of mutant mitochondria sampled from a cell carrying r mutants. Then, Y_r follows $\mathrm{B}\left(M, \frac{r(1+k)}{M+rk}\right)$ and we have,

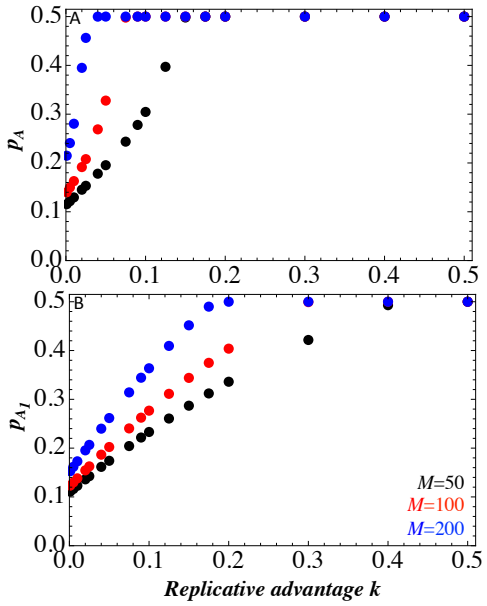

Fig. S6: Frequencies of A and A_1 at equilibrium for different values for the advantage of mutant mitochondria k. Mutation rate μ=0.01.

$$P(Y_r = l) = \binom{M}{l} \left(\frac{r(1+k)}{M+rk}\right)^l \left(1 - \frac{r(1+k)}{M+rk}\right)^{M-l}.$$

Letting $\mathbf{X}^{t,\tau_s}$ and $\mathbf{X}^{t,\tau_{s+1}}$ define the population before and after this step takes place we obtain,

$$P(\mathbf{X}^{t,\tau_{s+1}} = (i,j)) = \sum_{r=0}^{r=M} P(\mathbf{X}^{t,\tau_s} = (r,j)) P(Y_r = i).$$

The frequency of p_A at equilibrium increased when this step was implemented. This increase was higher for larger values of k (Fig.S6A).

B3. Mitonuclear coadaptation

Mitonuclear coadaptation is somewhat more complex as a nuclear gene interacting with the mitochondria has to be defined. The equations for this case are modified following their definition and derivation in (7).

Fluctuating external factors

In addition, we considered a regularly changing environment. To model this we assumed that the nuclear optimum fluctuates. We imposed a cost (q) at the selection step either on the 00 nuclear

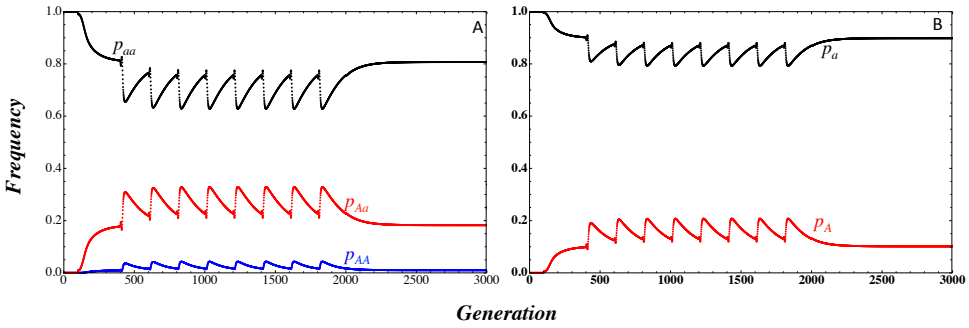

Fig. S7: Change in (A) genotype (p_{aa}, p_{Aa}, p_{AA}) and (B) gene (p_a, p_A) frequency across time (generation), from introduction of the uniparental inheritance mutation A allele (initial frequency 10^{-2} at generation 100) until stability is reached at equilibrium E_1. Here we assume fluctuating environmental conditions force the dominant mitonuclear state to switch by imposing a cost $q = 0.75$ every $p = 200$ generation for a duration of $d = 20$ generations. Once the fluctuations are removed the population returns to its initial equilibrium. Other parameters: (M, μ, ν)= (50, 0.01, 0.0001).

state (when at that optimum) or on the 11 nuclear state (when at that optimum). This was imposed on the population periodically (every p generations) for a duration of d generations.

We found that if the cost was high enough (high q) and was imposed on the population for long enough (high d), then the population switched states during the time the cost was imposed. This caused an increase in the frequency of A in the population. During the switch mitochondria inherited uniparentally are more efficient at adapting to a new nuclear background than those inherited biparentally, explaining the increase in the degree of uniparental inheritance. Once the fluctuations were removed however, the population returned to its initial equilibrium (Fig.S7).

B4. Mating types

In the main text we consider the effect of introducing mating types (A_1) to a population where $p_a = 1$. We saw that this resulted in the spread of A_1 to an equilibrium equivalent to E_1 (Fig.4a). Once at E_1, we then introduced a second mating type allele p_{a_2} into this polymorphic A_1/a population. However, the a_2 allele did not invade, but simply monotonically decreased in frequency (Fig.S8). This is a general finding. Namely that once a polymorphic equilibrium is reached (with some degree of uniparental inheritance), further alleles that potentially increase the degree of uniparental inheritance are not favoured.

This was true even when a_2 was introduced at a higher frequency (Fig.S8C) and when a_2 was

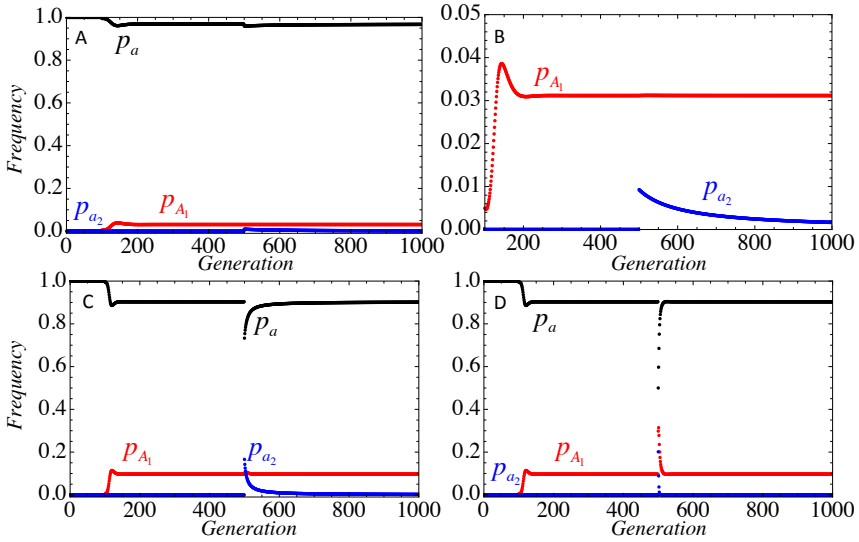

Fig. S8: Change in genotype frequency (p_{aa}, p_{A_1a}, p_{aa_2}, $p_{A_1a_2}$), from introduction of the uni-parental inheritance allele A_1 at generation 100, and the allele a_2 at generation 500 (initial frequency 10^{-2}). B: Zoomed-in to illustrate the monotonic decrease in p_{a_2}. C: Allele a_2 is introduced at a higher frequency (0.2). D: Allele a_2 is introduced at a higher frequency (0.3) and is only allowed to fuse with A_1 (and not with a). A,B:(M, μ) = (50, 0.01) and C,D:(M, μ) = (100, 0.01).

allowed to fuse only with A_1 (and so have strict UPI fitness) (Fig.S8 D) . This is because a benefits from the presence of A_1 in the population through leakage. When a_2 was subsequently added there was no significant additional fitness benefits from A_1 x a_2 matings that could give $\bar{w}_{a_2} >> \bar{w}_a$. This along with the slight disadvantage a_2 suffers by not being able to fuse with self (very low when the frequency of a_2 is low) result in a monotonic decrease of the frequency of a_2.

Alternatively, we assumed that two mating types a_1 and a_2 pre-exist and then introduced A_1. This causes A_1 to displace a_1 up to a degree equivalent to E_1. For higher M and μ, A_1 displaced a_1 altogether, leading to an equilibrium at which there is complete uniparental inheritance of mitochondria (Fig.S9).

When recombination was allowed ($R = 0.5$), the frequency of A reached similar levels to those seen without recombination. Recombination allowed both A_1 and A_2 alleles to spread, until both reached E_1-like equilibria with complementary frequencies of the a_1 and a_2 alleles. The frequency of the uniparental inheritance alleles rises with higher values of M and μ. However, in this case complete UPI is not possible because each mating type is equally associated with uniparental (A) and biparental (a) mitochondrial inheritance alleles, so at equilibrium $p_{a_1} = p_{a_2} = p_{A_1} = p_{A_2} =$

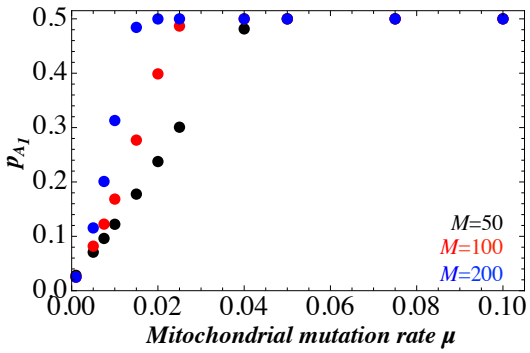

Fig. S9: Equilibrium frequency of A_1 when introduced into a population with $p_{a_1} = p_{a_2} = 0.5$ for different M and μ

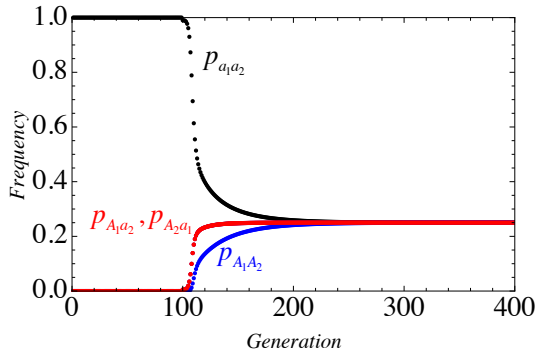

Fig. S10: Change in genotype frequency ($p_{a_1a_2}$, $p_{A_1a_2}$, $p_{a_1A_2}$, $p_{A_1A_2}$), from introduction of the uniparental inheritance mutation A_1 allele (initial frequency 10^{-2} at generation 100) until stability is reached. Full recombination between the mating type and mitochondrial inheritance loci is assumed. Parameters used: (M, μ) = (50, 0.01).

0.25 (Fig.S10). So when mating types pre-exist, strict UPI requires complete linkage between the mating type and mitochondrial inheritance loci.

References

[1] I M Hastings. Population genetic aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction. *Genetical Research*, 59(3):215–25, 1992.

[2] J P Randerson and L D Hurst. Small sperm, uniparental inheritance and selfish cytoplasmic elements : a comparison of two models. *Science*, 12:1110–1124, 1999.