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Supplemental Data

Evolution of Cell-to-Cell Variability in Stochastic, Controlled, Heteroplasmic mtDNA Populations

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Supplementary Information

S1. Expansion of the control law.

If we write $\epsilon_w = w - w_{ss}, \epsilon_m = m - m_{ss}$, the expansion of replication and degradation rates $\lambda(w,m)$ and $\nu(w,m)$ about the steady state $\{w_{ss}, m_{ss}\}$ gives

$$\lambda(w,m) \simeq \lambda(w_{ss},m_{ss}) + \left.\frac{\partial\lambda(w,m)}{\partial w}\right|_{(w_{ss},m_{ss})}\epsilon_w + \left.\frac{\partial\lambda(w,m)}{\partial m}\right|_{(w_{ss},m_{ss})}\epsilon_m + O(\epsilon^2) \quad (1)$$

$$\nu(w,m) \simeq \nu(w_{ss},m_{ss}) + \left.\frac{\partial\nu(w,m)}{\partial w}\right|_{(w_{ss},m_{ss})}\epsilon_w + \left.\frac{\partial\nu(w,m)}{\partial m}\right|_{(w_{ss},m_{ss})}\epsilon_m + O(\epsilon^2). \quad (2)$$

This expansion represents a model of a given control strategy $\lambda(w,m), \nu(w,m)$, which, if the original function is well behaved, we expect to reasonably reflect behaviour of the system close to $\{w_{ss}, m_{ss}\}$. Simulation results show that this expectation is fulfilled for a wide variety of cases (see figures in the Main Text).

To find w_{ss} and m_{ss} we solve the equations describing the deterministic behaviour of the system:

$$\left.\frac{dw}{dt}\right|_{(w_{ss},m_{ss})} = w_{ss}\lambda(w_{ss},m_{ss}) - w_{ss}\nu(w_{ss},m_{ss}) = 0 \quad (3)$$

$$\left.\frac{dm}{dt}\right|_{(w_{ss},m_{ss})} = m_{ss}\lambda(w_{ss},m_{ss}) - m_{ss}\nu(w_{ss},m_{ss}) = 0 \quad (4)$$

It will be observed that for this steady state to exist, the terms $\lambda(w_{ss},m_{ss})$ and $\nu(w_{ss},m_{ss})$ in Eqns. 1-2 must be equal. We can write the general expansion form of $\lambda(w,m)$ and $\nu(w,m)$, truncated to first order, as

$$\lambda(w,m) \simeq \beta_0 + \beta_w(w - w_{ss}) + \beta_m(m - m_{ss}), \quad (5)$$

$$\nu(w,m) \simeq \delta_0 + \delta_w(w - w_{ss}) + \delta_m(m - m_{ss}), \quad (6)$$

with $\beta_w = \partial\lambda/\partial w|_{w_{ss},m_{ss}}, \beta_m = \partial\lambda/\partial m|_{w_{ss},m_{ss}}, \delta_w = \partial\nu/\partial w|_{w_{ss},m_{ss}}, \delta_m = \partial\nu/\partial m|_{w_{ss},m_{ss}}$. Clearly, to support convergence to a steady state, β_w and β_w must be negative and δ_w and δ_m must be positive. Given this model for control dynamics, we next characterise the variance of the system. We can thus describe the system with a set of $R = 4$ processes with rates

$$f_1 = w\left(\beta_0 + \beta_w(w - w_{ss}) + \beta_m(m - m_{ss})\right) \quad (7)$$

$$f_2 = m\left(\beta_0 + \beta_w(w - w_{ss}) + \beta_m(m - m_{ss})\right) \quad (8)$$

$$f_3 = w\left(\delta_0 + \delta_w(w - w_{ss}) + \delta_m(m - m_{ss})\right) \quad (9)$$

$$f_4 = m\left(\delta_0 + \delta_w(w - w_{ss}) + \delta_m(m - m_{ss})\right) \quad (10)$$

and stoichiometry matrix describing the effects of these reactions on the $N = 2$ species we consider as

$$S = ((1,0),(0,1),(-1,0),(0,-1))^T. \quad (11)$$

Using index $i = 1$ to correspond to species w and $i = 2$ to correspond to species m, the master equation for the system, describing the time evolution of $P_{w,m}$ (the probability of observing w wildtype and m mutant mtDNAs) can then be written

$$\frac{\partial P_{w,m}}{\partial t} = \sum_{j=1}^{R}\left(\prod_{i=1}^{N}\mathbb{E}^{-S_{ij}} - 1\right) f_j(w,m)P_{w,m} \quad (12)$$

where $\mathbb{E}^{-S_{ij}}$ takes its normal meaning as a raising and lowering operator [1], adding $-S_{ij}$ to each occurrence of index i that follows it on the right (so e.g. as $S_{11} = 1$ and w corresponds to index 1, $\mathbb{E}^{-S_{11}} f_j(w,m)P_{w,m} \to f_j(w-1,m)P_{w-1,m}$).

The potential nonlinearities and coupling between species in this equation prevents a full general solution. To make progress, we employ Van Kampen's system size expansion [1, 2] and write $w = \phi_w\Omega + \xi_w\Omega^{1/2}, m =$

$\phi_m\Omega + \xi_m\Omega^{1/2}$, representing copy numbers as the sum of deterministic components ϕ_i and fluctuation components ξ_i scaled by powers of system size Ω. Following the standard expansion procedure, by writing $\mathbb{E}$, $P_{w,m}$ and f_i in terms of Ω and collecting powers of Ω in Eqn. 12, first gives equations for the deterministic components of the system (corresponding straightforwardly to the macroscopic rate equations):

$$\frac{\partial \phi_i}{\partial t} = \sum_{j=1}^{R} S_{ij} f_j, \tag{13}$$

then gives a Fokker-Planck equation for the time behaviour of the fluctuation components in terms of the bivariate probability distribution $\Pi(\xi, t)$ of $\xi = (\xi_w, \xi_m)$ at time t:

$$\frac{\partial \Pi(\xi,t)}{\partial t} = \sum_{i,j=1}^{N} A_{ij} \frac{\partial(\xi_j \Pi)}{\partial \xi_i} + \frac{1}{2} \sum_{i,j=1}^{N} B_{ij} \frac{\partial^2 \Pi}{\partial \xi_i \, \partial \xi_j}, \tag{14}$$

where

$$A_{ij} = \sum_{k=1}^{R} S_{ik} \frac{\partial f_k}{\partial \phi_j}, \tag{15}$$

$$B_{ij} = \sum_{k=1}^{R} S_{ik} S_{jk} f_k. \tag{16}$$

The form of Eqns. 7-10 and Eqn. 11 gives, for steady state copy numbers and $\delta_0 = \beta_0$, $A_{11} = \kappa_w w_{ss}, A_{12} = \kappa_m w_{ss}, A_{21} = \kappa_w m_{ss}, A_{22} = \kappa_m m_{ss}, B_{11} = 2\beta_0 w_{ss}, B_{22} = 2\beta_0 m_{ss}, B_{12} = B_{21} = 0$, where $\kappa_w = (\beta_w - \delta_w), \kappa_m = (\beta_m - \delta_m)$. From this Fokker-Planck equation expressions for the moments of ξ_i can be extracted [1], leading to the expressions:

$$\frac{d\langle \xi_w^2 \rangle}{dt} = 2A_{11}\langle \xi_w^2 \rangle + 2A_{12}\langle \xi_w \xi_m \rangle + B_{11} \tag{17}$$

$$\frac{d\langle \xi_w \xi_m \rangle}{dt} = (A_{11} + A_{22})\langle \xi_w \xi_m \rangle + A_{12}\langle \xi_m^2 \rangle + A_{21}\langle \xi_w^2 \rangle + B_{12} \tag{18}$$

$$\frac{d\langle \xi_m^2 \rangle}{dt} = 2A_{22}\langle \xi_m^2 \rangle + 2A_{21}\langle \xi_w \xi_m \rangle + B_{22}, \tag{19}$$

A linear stability analysis of the deterministic ODEs describing mean behaviour is straightforward to perform. Linearising Eqns. 3-4 about (w_{ss}, m_{ss}) gives

$$\frac{dw}{dt} \simeq (\beta_w - \delta_w) w_{ss}(w - w_{ss}) + O(w^2) + O(wm) \tag{20}$$

$$\frac{dm}{dt} \simeq (\beta_m - \delta_m) m_{ss}(m - m_{ss}) + O(m^2) + O(wm), \tag{21}$$

from which it is straightforward to see that if $\kappa_w < 0$ and $\kappa_m < 0$, the mean dynamics of w and m respectively are linearly stable. This condition is met for w and m in control laws A and E, for w in B, C, and F, and for neither in D. These specific examples illustrate the principle that if a species is explicitly 'sensed' – in the sense that it modulates replication or degradation rate – its mean dynamics can be controlled to be linearly stable. If a species is not explicitly sensed (replication and degradation are not functions of its copy number) then its mean dynamics are not explicitly linearly stable, but may be 'balanced', as the corresponding κ term is zero. Perturbations in these unsensed 'balanced' variables are neither damped away by control nor guaranteed to explode with time; hence the variables are unconstrained but not explicitly unstable.

S2. Full solutions for steady state ODEs.

Eqns. 17-19 can be solved exactly for the A_{ij}, B_{ij} corresponding to the steady state condition above. The complete solutions for arbitrary $\langle \xi_w^2 \rangle, \langle \xi_w \xi_m \rangle, \langle \xi_m^2 \rangle$ at $t = 0$ are lengthy and do not allow much intuitive interpretation. For $\langle \xi_w^2 \rangle = \langle \xi_w \xi_m \rangle = \langle \xi_m^2 \rangle = 0$ at $t = 0$ (noiseless initial conditions), we can solve and separate the long-term

behaviour from the transient behaviour. The transients, given by Eqns. 25-27 below, involve terms in $t' \equiv \exp((\kappa_m m_{ss} + \kappa_w w_{ss})t)$. As the κ_i are nonpositive (for stability, β_i are nonpositive and δ_i are nonnegative), t' is either a constant or an exponentially decaying function of time t; in the cases we consider, it either decays with time (all models except D) or the associated term is always zero (model D).

$$\begin{aligned}
\langle \xi_w^2 \rangle &= F_1^{decay}(t') + \left(\frac{2\beta_0 m_{ss} w_{ss}(m_{ss} + w_{ss})\kappa_m^2}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^2} \right) t \\
&\quad + \left(\frac{-\beta_0 w_{ss}^2(w_{ss}\kappa_w^2 + m_{ss}\kappa_m(4\kappa_w - 3\kappa_m))}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^3} \right) \qquad (22) \\
\langle \xi_w \xi_m \rangle &= F_2^{decay}(t') + \left(\frac{-2\beta_0 m_{ss} w_{ss}(m_{ss} + w_{ss})\kappa_m \kappa_w}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^2} \right) t \\
&\quad + \left(\frac{\beta_0 m_{ss} w_{ss}(m_{ss}\kappa_m(\kappa_m - 2\kappa_w) + w_{ss}\kappa_w(\kappa_w - 2\kappa_m))}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^3} \right) \qquad (23) \\
\langle \xi_m^2 \rangle &= F_3^{decay}(t') + \left(\frac{2\beta_0 m_{ss} w_{ss}(m_{ss} + w_{ss})\kappa_w^2}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^2} \right) t \\
&\quad + \left(\frac{-\beta_0 m_{ss}^2(m_{ss}\kappa_m^2 + w_{ss}\kappa_w(4\kappa_m - 3\kappa_w))}{(m_{ss}\kappa_m + w_{ss}\kappa_w)^3} \right), \qquad (24)
\end{aligned}$$

where the F_i^{decay} functions characterising the transient behaviour are

$$\begin{aligned}
F_1^{decay}(t') &= \frac{\beta_0 w_{ss}^2 t'(\kappa_w^2 w_{ss} t' + \kappa_m m_{ss}(\kappa_m(t' - 4) + 4\kappa_w))}{(\kappa_m m_{ss} + \kappa_w w_{ss})^3} \qquad (25) \\
F_2^{decay}(t') &= \frac{\beta_0 w_{ss} m_{ss} t'(\kappa_m^2 m_{ss}(t' - 2) + 2(m_{ss} + w_{ss})\kappa_w \kappa_m + \kappa_w^2 w_{ss}(t' - 2))}{(\kappa_m m_{ss} + \kappa_w w_{ss}^2)^3}, \qquad (26) \\
F_3^{decay}(t') &= \frac{\beta_0 m_{ss}^2 t'(\kappa_m^2 m_{ss} t' + \kappa_w w_{ss}(\kappa_w(t' - 4) + 4\kappa_m))}{(\kappa_m m_{ss} + \kappa_w w_{ss})^3} \qquad (27) \\
&\qquad (28)
\end{aligned}$$

Eqns. 22-24 with Eqns. 25-27 then give the full transient behaviour displayed in Fig. 1 in the Main Text , decaying to the aforementioned linear behaviour with characteristic timescale $(\kappa_m m_{ss} + \kappa_w w_{ss})$.

S3. Interpretation and behaviour of specific control strategies.

The mechanisms described in the Main Text have simple interpretations in the language of stochastic processes. Mechanism A, as discussed, corresponds to the 'relaxed replication' picture studied previously (Appendix A1). Mechanism D corresponds to independent birth-death processes acting on both species (analysed in [3]). Mechanism F corresponds to an immigration-death process acting on the wildtype (the dependence of replication rate on $1/w$ means that overall production is constant with time), and model C can be regarded as a birth-immigration-death process on the wildtype (analysed in [4]). Both these mechanisms are thus expected to tightly control wildtype behaviour (including controlling variance: immigration-death processes yield a constant steady-state variance), but do not sense (and, therefore, do not apply feedback to) mutant load. We also note that mechanisms F and G are in a sense 'dual', in that they apply similar control manifest through replication with rate $\sim 1/w$ and degradation with rate w respectively. The previous result that control applied to (a) biogenesis rates and (b) degradation rates yields similar population behaviour is visible in the behaviour of F and G in Fig. 1 in the Main Text .

Table S1 gives, for each example control strategy in the Main Text, the corresponding steady state $\{w_{ss}, m_{ss}\}$ and the expansion terms for the strategy β, δ. The corresponding behaviours of variances, from Eqns. 22-24, are given in Table S2 .

In the Main Text we discuss the implications of an increasing extinction probability of one mtDNA type. A non-negligible extinction probability challenges the validity of the linear noise approximation and leads to departure from results derived using the system size expansion. To illustrate this behaviour, we reduce the characteristic timescale of the parameterisations used to explore models A-G in the Main Text, setting $\tau = 1$ rather than $\tau = 5$, and simulate for a longer time window (see Fig. S1). It will be observed that as extinction probability increases (as $\sqrt{\langle m^2 \rangle} \to \langle m \rangle$), the numerical behaviour departs from that predicted analytically; in particular, the increase of $\langle h^2 \rangle$ slows from a linear to sublinear regime as discussed in the Main Text.

We note that, in some physiological circumstances, the representation of one mtDNA type in a cellular population may be low – for example, the appearance of one mutant mtDNA through *de novo* mutation or replication error, or the presence of a small percentage of a foreign mtDNA haplotype due to carryover in gene therapies [5]. In these cases, a non-negligible extinction probability may occur quickly and the transition of $\langle h^2 \rangle$ to a sublinear, or flat, regime will be an important aspect of the long-term dynamics. In the case of mtDNA disease inheritance, however, situations with a macroscopic fraction of mutant mtDNA are often the most important, due to the presence of a 'heteroplasmy threshold' [6] beyond which disease symptoms manifest. With two mtDNA haplotypes represented in comparable proportions in the cell, our linear analysis holds and can be used to describe heteroplasmy variance in somatic and germline cells.

S4. Steady state solution.

Eqns. 17-19 give, for steady state,

$$\begin{aligned} 2w_{ss}(\beta_0 + \kappa_m \langle \xi_w \xi_m \rangle + \kappa_w \langle \xi_w^2 \rangle) &= 0 \quad (29) \\ w_{ss}\kappa_m \langle \xi_m^2 \rangle + m_{ss}\kappa_w \langle \xi_w^2 \rangle + (m_{ss}\kappa_m + w_{ss}\kappa_w)\langle \xi_w \xi_m \rangle &= 0 \quad (30) \\ 2m_{ss}(\beta_0 + \kappa_m \langle \xi_m^2 \rangle + \kappa_w \langle \xi_w \xi_m \rangle) &= 0 \quad (31) \end{aligned}$$

Attempting to solve these equations for $\langle \xi_w^2 \rangle$, then $\langle \xi_w \xi_m \rangle$, then $\langle \xi_m^2 \rangle$ first gives $\langle \xi_w^2 \rangle = (-\beta_0 - \kappa_m \langle \xi_w \xi_m \rangle)/\kappa_w$, then $\langle \xi_w \xi_m \rangle = (\beta_0 m_{ss} - \kappa_m w_{ss} \langle \xi_m^2 \rangle)/(w_{ss}\kappa_w)$, leaving Eqn. 30 reduced to

$$\frac{2\beta_0 m_{ss}(m_{ss} + w_{ss})}{w_{ss}} = 0, \quad (32)$$

a condition only fulfilled (due to the non-negativity of m_{ss} and w_{ss}) if $m_{ss} = 0$. If one proceeds through the analysis by first solving for $\langle \xi_m^2 \rangle$, then $\langle \xi_w \xi_m \rangle$, then $\langle \xi_w^2 \rangle$, a symmetric expression is obtained

$$\frac{2\beta_0 w_{ss}(m_{ss} + w_{ss})}{m_{ss}} = 0. \quad (33)$$

Eqns. 32-33 illustrate the symmetry in the system: if the copy number of either species is zero then a situation where variance does not increase is supported (but not inevitable: compare the behaviour of relaxed replication model (A, fixed variance) and the birth-death model (D, increasing variance) in the case of zero mutant population).

The effect of selection, mutations, and replicative errors on mtDNA variances can straightforwardly be included in this analysis. In this general case, we replace Eqns. 1-4 in the Main Text with:

$$\begin{aligned} \{w, m\} &\xrightarrow{\epsilon_1 + (1+\epsilon_2) w \lambda(w,m)} \{w+1, m\} \quad (34) \\ \{w, m\} &\xrightarrow{\epsilon_3 + (1+\epsilon_4) m \lambda(w,m)} \{w, m+1\} \quad (35) \\ \{w, m\} &\xrightarrow{\epsilon_5 + (1+\epsilon_6) w \nu(w,m)} \{w-1, m\} \quad (36) \\ \{w, m\} &\xrightarrow{\epsilon_7 + (1+\epsilon_8) m \nu(w,m)} \{w, m-1\} \quad (37) \\ \{w, m\} &\xrightarrow{w\mu_1} \{w-1, m+1\} \quad (38) \\ \{w, m\} &\xrightarrow{w\mu_2} \{w, m+1\} \quad (39) \\ \{w, m\} &\xrightarrow{w\mu_3} \{w-1, m+2\}. \quad (40) \end{aligned}$$

Here, we have added processes corresponding to spontaneous mutation of a given wildtype mtDNA (μ_1), and two types of replicative error affecting wildtype mtDNA, giving rise to one (μ_2; original molecule remains intact, new molecule is mutated) and two (μ_3; both original and new molecules are mutated) mutant mtDNAs respectively. Differences manifest either through replicative or degradation advantages (or both) are incorporated with even-indexed ϵ_i (providing multiplicative changes to the bare rates) and odd-indexed ϵ_i (providing additive changes). We thus have two ways of provoking selective advantages in each case: increasing wildtype biogenesis, increasing mutant biogenesis, increasing wildtype degradation, and increasing mutant degradation.

Fig. S2 shows example trajectories arising from each of our control mechanisms in the presence of the mutation processes above, and the selective pressures $(\epsilon_3, \epsilon_4, \epsilon_5, \epsilon_6)$ that favour mutant mtDNA. An excellent agreement between ODE theory and stochastic simulation is again illustrated, and there is substantial similarity between

the behaviours caused by selection (favouring mutant mtDNA) and mutation (producing mutant mtDNA). In several cases (mechanisms B, C, F, G), $\langle m \rangle$ and $\langle m^2 \rangle$ simply increase exponentially with time under favourable selective or mutational pressures; this situation straightforwardly gives rise to a sigmoidal change in heteroplasmy $\langle h \rangle \sim 1/(1 + e^{-\Delta f t}(1 - h_0)/h_0)$, with Δf an effective selective difference, as used in previous work [7, 8]. In mechanisms coupling wildtype and mutant content (A and E), mutant increase is slower and accompanied by a decrease in wildtype, attempting to keep total copy number constant. In these circumstances, variance behaviour can be more complex: for example, under relaxed replication with pressure favouring mutant mtDNA, $\langle w^2 \rangle$ initially increases then subsequently decreases as $\langle w \rangle$ decreases in magnitude. Mechanism D, where control does not couple mutant and wildtype, has correspondingly perpendicular trajectories in $(\langle w \rangle, \langle m \rangle)$ space under different selective pressures, but the coupling action of the mutation operations lead to curved trajectories under mutational influence.

S5. Heteroplasmy.

For a general function $h = h(x, y)$,

$$\langle h^2 \rangle = \langle (h - \langle h \rangle)^2 \rangle. \tag{41}$$

We will consider an expansion about (x_0, y_0), a state such that $h(x_0, y_0) = \langle h \rangle$. Using the first-order Taylor expansion of $h(x, y)$ around (x_0, y_0):

$$\begin{aligned}
\langle h^2 \rangle &= \langle (h - \langle h \rangle)^2 \rangle & (42)\\
&\simeq \left\langle \left(h(x_0, y_0) + (x - x_0) \left.\frac{\partial h}{\partial x}\right|_{(x_0,y_0)} + (y - y_0) \left.\frac{\partial h}{\partial y}\right|_{(x_0,y_0)} - h(x_0, y_0) \right)^2 \right\rangle & (43)\\
&= \left\langle (x - x_0)^2 \left(\frac{\partial h}{\partial x}\right)^2_{(x_0,y_0)} + (y - y_0)^2 \left(\frac{\partial h}{\partial y}\right)^2_{(x_0,y_0)} + 2(x - x_0)(y - y_0) \left(\frac{\partial h}{\partial x}\frac{\partial h}{\partial y}\right)_{(x_0,y_0)} \right\rangle & (44)\\
&= \langle x^2 \rangle \left(\frac{\partial h}{\partial x}\right)^2_{(x_0,y_0)} + \langle y^2 \rangle \left(\frac{\partial h}{\partial y}\right)^2_{(x_0,y_0)} + 2\langle xy \rangle \left(\frac{\partial h}{\partial x}\frac{\partial h}{\partial y}\right)_{(x_0,y_0)}. & (45)
\end{aligned}$$

We now consider $h(x, y) = x/y$, so that

$$\begin{aligned}
\langle h^2 \rangle &\simeq \left(\langle x^2 \rangle \frac{1}{y^2} + \langle y^2 \rangle \frac{x^2}{y^4} - 2\langle xy \rangle \frac{x}{y^3} \right)_{(x_0,y_0)} & (46)\\
&= \left(\frac{x^2}{y^2} \left(\frac{\langle x^2 \rangle}{x^2} + \frac{\langle y^2 \rangle}{y^2} - \frac{2\langle xy \rangle}{xy} \right) \right)_{(x_0,y_0)}. & (47)
\end{aligned}$$

Finally, given that $x_0 = \langle x \rangle$ and $y_0 = \langle y \rangle$, and setting $x \equiv m$ and $y \equiv w + m$, we obtain

$$\langle h^2 \rangle \simeq \frac{\langle m \rangle^2}{\langle w + m \rangle^2} \left(\frac{\langle m^2 \rangle}{\langle m \rangle^2} + \frac{\langle (w + m)^2 \rangle}{\langle w + m \rangle^2} - \frac{2\langle m(w + m) \rangle}{\langle m \rangle \langle w + m \rangle} \right) \tag{48}$$

To see that exponential growth or decay in one mtDNA type while the other remains constant gives rise to sigmoidal heteroplasmy dynamics, consider (without loss of generality) $m = h_0 n_0 e^{\beta t}, w = (1 - h_0)n_0$, where n_0 is an initial population size which will cancel. Then, as m (and hence $n = m + w$) increases with time,

$$h = \frac{m}{m + w} = \frac{h_0 n_0 e^{\beta t}}{n_0(h_0 e^{\beta t} + (1 - h_0))} = \frac{1}{1 + \frac{1-h_0}{h_0} e^{-\beta t}}, \tag{49}$$

as used in Refs. [5] and [3], with β corresponding to a selective pressure (in this derivation, positive β favours mutant mtDNA).

S6. Fokker-Planck terms for nonequilibrium regimes.

The system size expansion approach above can be applied to the general system without employing an expansion of the control strategy about a steady state, by considering the processes

$$
\begin{aligned}
f_1 &= w\lambda_w(w,m) && (50)\\
f_2 &= m\lambda_m(w,m) && (51)\\
f_3 &= w\nu_w(w,m) && (52)\\
f_4 &= m\nu_m(w,m) && (53)
\end{aligned}
$$

If the expansion about steady state is not used, the corresponding terms are

$$
\begin{aligned}
A_{11} &= \lambda_w(\phi_w,\phi_m) - \nu_w(\phi_w,\phi_m) + \phi_w(\partial_w\lambda_w(\phi_w,\phi_m) - \partial_w\nu_w(\phi_w,\phi_m)) && (54)\\
A_{12} &= \phi_w(\partial_m\lambda_w(\phi_w,\phi_m) - \partial_m\nu_w(\phi_w,\phi_m)) && (55)\\
A_{21} &= \phi_m(\partial_w\lambda_m(\phi_w,\phi_m) - \partial_w\nu_m(\phi_w,\phi_m)) && (56)\\
A_{22} &= \lambda_m(\phi_w,\phi_m) - \nu_m(\phi_w,\phi_m) + \phi_m(\partial_m\lambda_m(\phi_w,\phi_m) - \partial_m\nu_m(\phi_w,\phi_m)) && (57)\\
B_{11} &= \phi_w(\lambda_w(\phi_w,\phi_m) + \nu_w(\phi_w,\phi_m)) && (58)\\
B_{22} &= \phi_m(\lambda_w(\phi_w,\phi_m) + \nu_w(\phi_w,\phi_m)) && (59)\\
B_{12} &= B_{21} = 0, && (60)
\end{aligned}
$$

where $\partial_x f(\phi_i,\phi_j)$ means $\left.\frac{\partial f}{\partial x}\right|_{\phi_i,\phi_j}$. We include the mutational processes in the text by adding $f_5 = \mu_1 w, f_6 = \mu_2 w, f_7 = \mu_3 w$ and setting the corresponding stoichiometry matrix to

$$
S = ((1,0),(0,1),(-1,0),(0,-1),(-1,1),(0,1),(-1,2))^T. \quad (61)
$$

If $\lambda_w = \lambda_m = \lambda$ and $\nu_w = \nu_m = \nu$ (no selective differences between mtDNA types), the Fokker-Planck terms become

$$
\begin{aligned}
A_{11} &= -\mu_1 - \mu_3 + \lambda(\phi_w,\phi_m) - \nu(\phi_w,\phi_m) + \phi_w(\partial_w\lambda(\phi_w,\phi_m) - \partial_w\nu(\phi_w,\phi_m)) && (62)\\
A_{12} &= \phi_w(\partial_m\lambda(\phi_w,\phi_m) - \partial_m\nu(\phi_w,\phi_m)) && (63)\\
A_{21} &= \mu_1 + \mu_2 + 2\mu_3 + \phi_m(\partial_w\lambda(\phi_w,\phi_m) - \partial_w\nu(\phi_w,\phi_m)) && (64)\\
A_{22} &= \lambda(\phi_w,\phi_m) - \nu(\phi_w,\phi_m) + \phi_m(\partial_m\lambda(\phi_w,\phi_m) - \partial_m\nu(\phi_w,\phi_m)) && (65)\\
B_{11} &= \phi_w(\mu_1 + \mu_3 + \lambda(\phi_w,\phi_m) + \nu(\phi_w,\phi_m)) && (66)\\
B_{22} &= (\mu_1 + \mu_2 + 4\mu_3) + \phi_m(\lambda(\phi_w,\phi_m) + \nu(\phi_w,\phi_m)) && (67)\\
B_{12} &= B_{21} = -(\mu_1 + 2\mu_3)\phi_w. && (68)
\end{aligned}
$$

Including selection terms (without mutation) requires no change to the original structure of reactions and stoichiometries and immediately gives

$$
\begin{aligned}
A_{11} &= (1+\epsilon_2)\lambda(\phi_w,\phi_m) - (1+\epsilon_6)\nu(\phi_w,\phi_m) + \phi_w((1+\epsilon_2)\partial_w\lambda(\phi_w,\phi_m) - (1+\epsilon_6)\partial_w\nu(\phi_w,\phi_m)) && (69)\\
A_{12} &= \phi_w((1+\epsilon_2)\partial_m\lambda(\phi_w,\phi_m) - (1+\epsilon_6)\partial_m\nu(\phi_w,\phi_m)) && (70)\\
A_{21} &= \phi_m((1+\epsilon_4)\partial_w\lambda(\phi_w,\phi_m) - (1+\epsilon_8)\partial_w\nu(\phi_w,\phi_m)) && (71)\\
A_{22} &= (1+\epsilon_4)\lambda(\phi_w,\phi_m) - (1+\epsilon_8)\nu(\phi_w,\phi_m) + \phi_m((1+\epsilon_4)\partial_m\lambda(\phi_w,\phi_m) - (1+\epsilon_8)\partial_m\nu(\phi_w,\phi_m)) && (72)\\
B_{11} &= \epsilon_1 + \epsilon_5 + \phi_w((1+\epsilon_2)\lambda(\phi_w,\phi_m) + (1+\epsilon_6)\nu(\phi_w,\phi_m)) && (73)\\
B_{22} &= \epsilon_3 + \epsilon_7 + \phi_m((1+\epsilon_4)\lambda(\phi_w,\phi_m) + (1+\epsilon_8)\nu(\phi_w,\phi_m)) && (74)\\
B_{12} &= B_{21} = 0, && (75)
\end{aligned}
$$

The same approach as above can be used to obtain Eqns. 17-19 for the time evolution of fluctuation moments, this time valid for a full temporal trajectory of the system.

S7. Experimental observations to distinguish mechanisms.

Our theoretical results suggest measurements to further elucidate the control mechanisms underlying mtDNA evolution within cells, without using heteroplasmy variance $\langle h^2 \rangle$ (the shortcomings of which are manifest because

seven different feedback controls all yield the same dynamics in $\langle h^2 \rangle'$ – Fig. 1 in the Main Text), and in conjunction with further molecular elucidation of processes governing mtDNA [9, 10] which providing bounds on the types and rates of molecular processes involved (for example, disallowing unphysically high rates of mtDNA replication).

If $\langle w^2 \rangle$ increases with time, mechanisms with weaker constraints on wildtype copy number are more likely (including relaxed replication (A), mechanisms sensing a combination of mutant and wildtype copy number (E), and the case with no feedback (D)). If $\langle w^2 \rangle$ is low and constant, mechanisms involving differential (B) or ratiometric (C) control are likely. If $\langle w^2 \rangle$ is high and constant (of the order of $\langle w \rangle$), mechanisms resembling immigration-death processes (with propagation scaled by inverse copy number, F and G) are more likely. The behaviour of $\langle wm \rangle$ can be used to further distinguish mechanisms which strongly couple wildtype and mutant (including relaxed replication and total copy number control) from those with less coupling.

In all these cases, the likelihood functions associated with specific biological observations will be complicated. Model selection and inference in this case could be performed through comparison to simulation, or using likelihood-free inference [11] for the mean and variance of mtDNA populations [12].

S8. Back-of-the-envelope calculations for leukocyte heteroplasmy measurements.

Average cellular mtDNA copy number measurements in Ref. [13] are made by normalising the signal from the mtDNA-encoded *ND1* gene by that from the nuclear-encoded *GADPH* genes using real-time PCR using iQ Sybr Green on the BioRad ICycler. The published protocol [14] for this technique suggests using 50ng-5pg of genomic DNA. Diploid human cells contain $\sim$ 6pg of genomic DNA; the mass of several hundred (much smaller) mtDNA genomes is negligible by comparison. The protocol thus implies the presence of 1-10000 cells' genomic DNA content; we assume 1000 as an estimate consistent with qPCR standards (Joerg Burgstaller, personal communication).

In our analysis of the data from Ref. [13] we use $\tau = 5$ days and the processes:

$$\{w, m\} \xrightarrow{w\lambda} \{w+1, m\} \tag{76}$$

$$\{w, m\} \xrightarrow{m\lambda} \{w, m+1\} \tag{77}$$

$$\{w, m\} \xrightarrow{w\nu} \{w-1, m\} \tag{78}$$

$$\{w, m\} \xrightarrow{(1+\epsilon_8)m\nu} \{w, m-1\} \tag{79}$$

with $\lambda = \nu = 1/\tau$, and ϵ_8 a selective difference acting to increase degradation of the mutant mtDNA species. We first estimate a value for ϵ_8 consistent with the heteroplasmy changes involved. Using the transformation

$$\beta t = \log\left(\frac{h(h_0 - 1)}{h_0(h - 1)}\right), \tag{80}$$

from Eqn. 49 above, where h_0 is initial heteroplasmy and h is heteroplasmy at time t, we obtain an estimate $\bar{\beta} = -1.2 \times 10^{-4}$ day^{-1}. We thus set $\epsilon_8 = 1.2 \times 10^{-4}$ day^{-1}, to produce the required selective difference manifest through mutant degradation.

Solving the ODEs arising from our theoretical approach (Eqns. 17-19) then give values for $\langle w^2 \rangle$ and $\langle m^2 \rangle$ over time for a given initial condition. Assuming that each datapoint consists of a sample of 10^3 cells, we divide these values by 10^3 to obtain an estimated distribution for each later w, m pair, given the paired initial w, m state. We combine these distrbutions to build an overall distribution over later results, and use the Kolmogorov-Smirnov test to test the alternative hypothesis that the later results were incompatible with draws from this distribution. The results were $p = 0.054$ for wildtype mtDNA copy number and $p = 0.861$ for mutant mtDNA copy number. As highlighted in the text, the absence of a $p < 0.05$ result cannot be interpreted as support for the null hypothesis, but this analysis suggests that the available data is not incompatible with the predictions of our model.

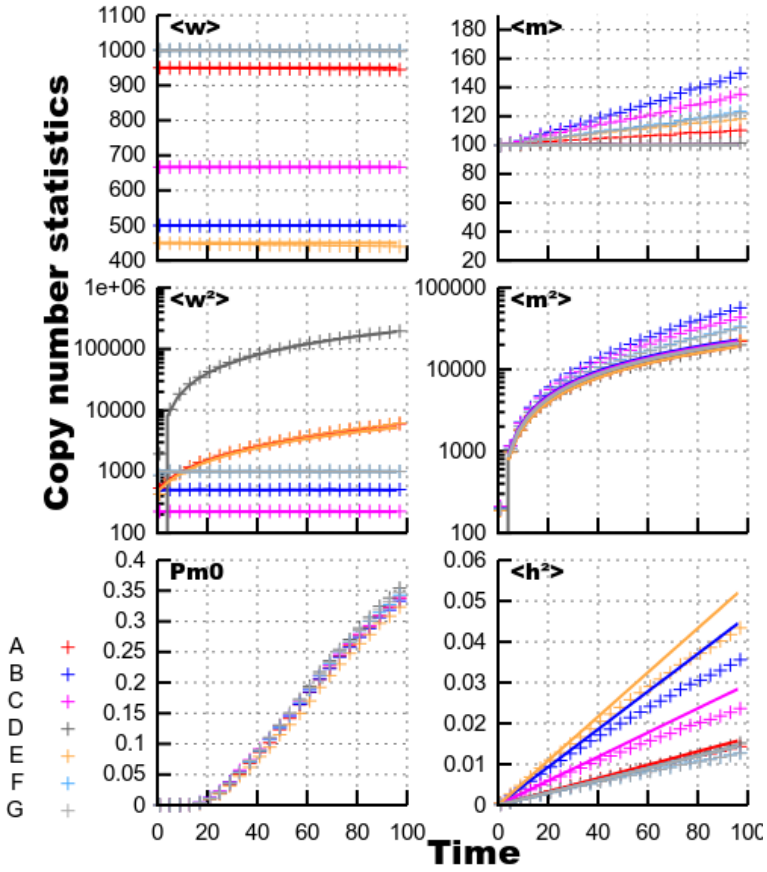

Figure S1: **Influence of fixation on expansion analysis.** The models from the Main Text, simulated for a longer time window and for a shorter characteristic timescale τ, illustrating the behaviour of the systems when extinction becomes possible. $Pm0$ gives the numerically computed probability that $m = 0$; it can be seen that an increase in this quantity corresponds to a moderate increase of $\langle m \rangle$ and $\langle m^2 \rangle$ relative to their predicted values, and a decrease of $\langle h^2 \rangle$ relative to its predicted value (shifting towards a sublinear increase as discussed in the Main Text).

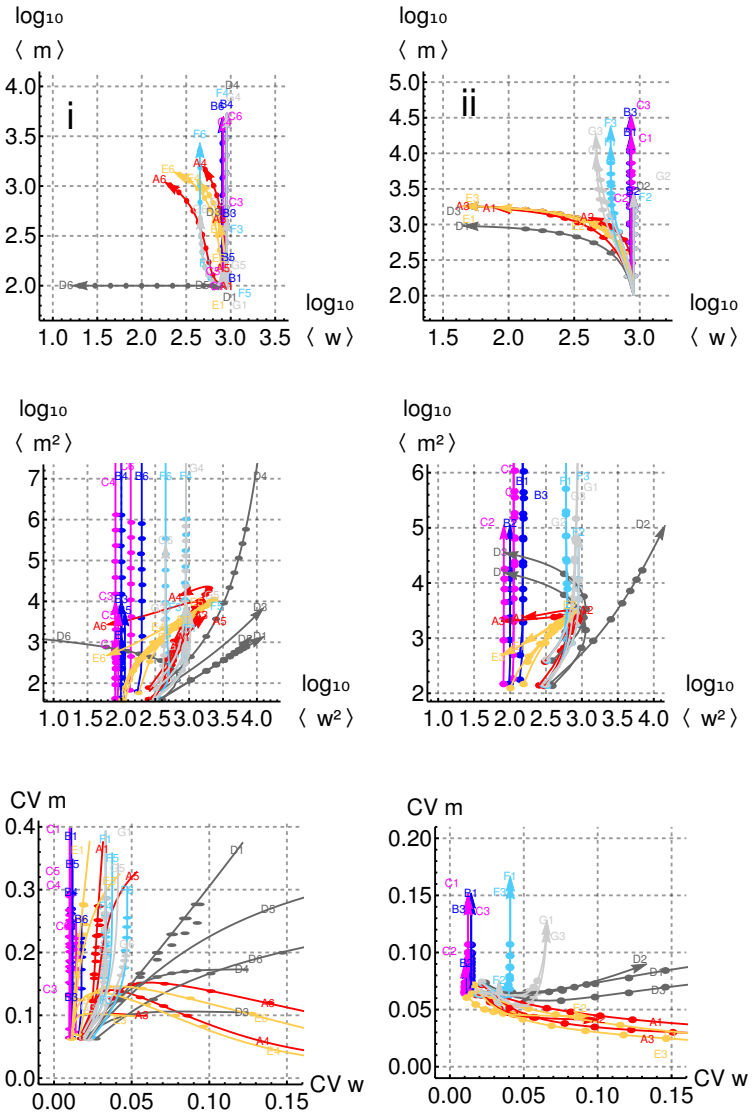

Figure S2: **MtDNA copy number and variability under mutational and selective advantages for mutant mtDNA.** Mean, variance, and CV trajectories with (i) selection pressures, and (ii) mutation rates favouring mutant mtDNA, under models A-G from the text. Some control strategies (A, E) keep mutant relatively bound but sacrifice wildtype and provoke large increases in variability; others (B, C, F, G) focus on wildtype stability, allowing mutant to grow unbound. Labels give the control model (letter) and the parameter varied ($\epsilon_3 = 20; \epsilon_4 = 1; \epsilon_5 = 20; \epsilon_6 = 1$ for (i); $\mu_1 = 0.1; \mu_2 = 0.1; \mu_3 = 0.1$ for (ii)); all other ϵ, μ parameters are set to zero. Results are shown for theory (lines) and stochastic simulation (points), progressing from an initial condition with $w_0 = 900, m_0 = 100$ with the parameterisations in Fig. 1 in the Main Text .

Control	$\lambda(w,m)$ ($\nu(w,m)$ for G)	w_{ss}	m_{ss}	β_w (δ_w for G)	β_m (δ_m for G)
A	$\frac{\alpha(w_{opt}-w-\gamma m)+w+\gamma m}{\tau(w+m)}$	$\frac{w_0 w_{opt}\alpha}{m_0+w_0\alpha+\gamma m_0(\alpha-1)}$	$\frac{m_0 w_{opt}\alpha}{m_0+w_0\alpha+\gamma m_0(\alpha-1)}$	$\frac{-m_0-w_0\alpha-m_0\gamma(\alpha-1)}{m_0 w_{opt}\tau+w_0 w_{opt}\tau}$	$\frac{-(1+(\alpha-1)\gamma)(m_0+w_0\alpha+m_0(\alpha-1)\gamma)}{(m_0+w_0)w_{opt}\alpha\tau}$
B	$\alpha(w_{opt}-w)$	$w_{opt}-1/\alpha\tau$	$\frac{m_0}{w_0}(w_{opt}-1/\alpha\tau)$	$-\alpha$	0
C	$\alpha\left(\frac{w_{opt}}{w}-1\right)$	$\frac{w_{opt}\alpha\tau}{1+\alpha\tau}$	$\frac{m_0}{w_0}\frac{w_{opt}\alpha\tau}{1+\alpha\tau}$	$\frac{-(1+\alpha\tau)^2}{w_{opt}\alpha\tau^2}$	0
D	$1/\tau$	w_0	m_0	0	0
E	$\alpha w_{opt}-\alpha w-\alpha_m m$	$\frac{w_0(w_{opt}\alpha\tau-1)}{\tau(w_0\alpha+m_0\alpha_m)}$	$\frac{m_0(w_{opt}\alpha\tau-1)}{\tau(w_0\alpha+m_0\alpha_m)}$	$-\alpha$	$-\alpha_m$
F	$1/w$	$\alpha\tau$	$\frac{m_0}{w_0}\alpha\tau$	$\frac{-1}{\alpha\tau^2}$	0
G	$1/\tau-\frac{w_{opt}-w}{w_{opt}\tau}$	w_{opt}	$\frac{m_0 w_{opt}}{w_0}$	$\frac{1}{w_{opt}\tau}$	0

Table S1: **Steady states and expansion terms for control strategies A-G.**

Control	Time-independent part of $\langle \xi_w^2 \rangle$	Time-independent part of $\langle \xi_w \xi_m \rangle$	Time-independent part of $\langle \xi_m^2 \rangle$
A	$\frac{w_0^2(m_0+w_0)w_{opt}\alpha(w_0\alpha^2-m_0(1+\gamma(\alpha-1))(3-4\alpha+3\gamma(\alpha-1)))}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^4}$	$\frac{-m_0w_0(m_0+w_0)w_{opt}\alpha(m_0(1+\alpha(\gamma-2)-\gamma)(1+\gamma(\alpha-1))+w_0\alpha(\alpha-2+2\gamma-2\alpha\gamma))}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^4}$	$\frac{m_0^2(m_0+w_0)w_{opt}\alpha(m_0(1+\gamma(\alpha-1))^2+w_0\alpha(4-3\alpha+4\gamma(\alpha-1)))}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^4}$
B	$\frac{1}{\alpha\tau}$	$\frac{-m_0}{w_0\alpha\tau}$	$\frac{-3m_0^2}{w_0^2\alpha\tau}$
C	$\frac{w_{opt}\alpha\tau}{(1+\alpha\tau)^2}$	$\frac{-m_0w_{opt}\alpha\tau}{w_0(1+\alpha\tau)^2}$	$\frac{-3m_0^2w_{opt}\alpha\tau}{(w_0+w_0\alpha\tau)^2}$
D	0	0	0
E	$\frac{w_0^2(w_0\alpha^2+\alpha_m m_0(4\alpha-3\alpha_m)}{(\alpha w_0+\alpha_m m_0)^3\tau}$	$\frac{m_0w_0(2\alpha\alpha_m(m_0+w_0)-w_0\alpha^2-m_0\alpha_m^2)}{(\alpha w_0+\alpha_m m_0)^3\tau}$	$\frac{m_0^2(m_0\alpha_m^2+\alpha w_0(4\alpha_m-3\alpha)}{(\alpha w_0+\alpha_m m_0)^3\tau}$
F	$\alpha\tau$	$\frac{-m_0\alpha\tau}{w_0}$	$\frac{-3m_0^2\alpha\tau}{w_0^2}$
G	w_{opt}	$\frac{-m_0w_{opt}}{w_0}$	$\frac{-3m_0^2w_{opt}}{w_0^2}$

Control	Time coefficient of $\langle \xi_w^2 \rangle$	Time coefficient of $\langle \xi_w \xi_m \rangle$	Time coefficient of $\langle \xi_m^2 \rangle$
A	$\frac{2m_0w_0(m_0+w_0)w_{opt}\alpha(1+(\alpha-1)\gamma)^2}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^3\tau}$	$\frac{-2m_0w_0(m_0+w_0)w_{opt}\alpha^2(1+(\alpha-1)\gamma)}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^3\tau}$	$\frac{2m_0w_0(m_0+w_0)w_{opt}\alpha^3}{(m_0+w_0\alpha+m_0(\alpha-1)\gamma)^3\tau}$
B	0	0	$\frac{2m_0(m_0+w_0)(w_{opt}\alpha\tau-1)}{w_0^2\alpha\tau^2}$
C	0	0	$\frac{2m_0(m_0+w_0)w_{opt}\alpha}{w_0^2(1+\alpha\tau)}$
D	$2w_0/\tau$	0	$2m_0/\tau$
E	$\frac{2m_0w_0(m_0+w_0)\alpha_m^2(w_{opt}\alpha\tau-1)}{(w_0\alpha+m_0\alpha_m)^3\tau^2}$	$\frac{-2m_0w_0(m_0+w_0)\alpha\alpha_m(w_{opt}\alpha\tau-1)}{(w_0\alpha+m_0\alpha_m)^3\tau^2}$	$\frac{2m_0w_0(m_0+w_0)\alpha^2(w_{opt}\alpha\tau-1)}{(w_0\alpha+m_0\alpha_m)^3\tau^2}$
F	0	0	$\frac{2m_0(m_0+w_0)\alpha}{w_0^2}$
G	0	0	$\frac{2m_0(m_0+w_0)w_{opt}}{w_0^2\tau}$

Control	Time coefficient of $\langle h^2 \rangle$ increase
A	$\frac{2m_0w_0(\alpha w_0+m_0+\gamma(\alpha-1)m_0)}{(m_0+w_0)^3w_{opt}\alpha\tau}$
B	$\frac{2m_0w_0^2\alpha}{(m_0+w_0)^3(w_{opt}\alpha\tau-1)}$
C	$\frac{2m_0w_0^2(1+\alpha\tau)}{(m_0+w_0)^3w_{opt}\alpha\tau^2}$
D	$\frac{2m_0w_0}{(m_0+w_0)^3\tau}$
E	$\frac{2m_0w_0(w_0\alpha+m_0\alpha_m)}{(m_0+w_0)^3(w_{opt}\alpha\tau-1)}$
F	$\frac{2m_0w_0^2}{(m_0+w_0)^3\alpha\tau^2}$
G	$\frac{2m_0w_0^2}{(m_0+w_0)^3w_{opt}\tau}$

Table S2: **Post-transient time behaviour of copy number and heteroplasmy variances in control strategies A-G.**

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