

Germline Bottlenecks, Biparental Inheritance and Selection on Mitochondrial Variants: A Two-Level Selection Model

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ABSTRACT

Selection on mitochondrial mutations potentially occurs at different levels: at the mitochondria, cell, and organism levels. Several factors affect the strength of selection at these different levels; in particular, mitochondrial bottlenecks during germline development and reduced paternal transmission decrease the genetic variance within cells, while they increase the variance between cells and between organisms, thus decreasing the strength of selection within cells and increasing the strength of selection between cells and organisms. However, bottlenecks and paternal transmission also affect the effective mitochondrial population size, thus affecting genetic drift. In this article, we use a simple model of a unicellular life cycle to investigate the effects of bottlenecks and paternal transmission on the probability of fixation of mitochondrial mutants and their frequency at mutation-selection equilibrium. We find that bottlenecks and reduced paternal transmission decrease the mean frequency of alleles with $s_m > s_c$ (approximately), where s_m and s_c are the strengths of selection for an allele within and between cells, respectively, and increase the frequency of alleles with $s_m < s_c$. Effects on fixation probabilities are different; for example, bottlenecks reduce the fixation probability of mutants with $s_m > 0$ (unless s_m is very small relative to s_c) and increase the fixation probability of mutants with $s_m < 0$.

THE population of mtDNA molecules of a species presents an inherent spatial structure, as mtDNAs are distributed among mitochondria, cells, organisms, and populations. This structure gives the opportunity for selection on mitochondrial variants to act at multiple levels: at the molecule, organelle, cell, and organism levels. Several traits of organisms may influence the relative effects of selection at these different levels. One such trait is the rate of paternal transmission of mitochondria: indeed, by decreasing the genetic variance within organisms and increasing the variance between organisms, uniparental transmission (*e.g.*, the total absence of paternal transmission) decreases the effects of intra-organismal selection, while it increases the effects of selection between organisms. Building on this idea, it has been proposed that uniparental inheritance may correspond to an adaptation of the organism to reduce the spread of “selfish” mutants, *i.e.*, mutants that increase in frequency within the organism, at the expense of the organism’s survival or reproduction (HASTINGS 1992; HURST and HAMILTON 1992).

Patterns of organelle and cell division during the development of the organism also affect the partitioning of mitochondrial genetic variance and therefore may af-

fect the relative strength of selection at different levels. In many animal species, the number of mitochondria per cell does not remain constant during the development of the female germline, but decreases to a minimal value during the first cell divisions of the development and then increases back. It has been hypothesized that this bottleneck may help to purge deleterious mutations in mtDNA, by increasing the variance between cells within the germline and therefore increasing the effect of selection against deleterious mutations at the cell level (KRAKAUER and MIRA 1999). Simulation models have shown that germline bottlenecks can indeed reduce the rate of accumulation of deleterious mutations in cytoplasmic genomes (BERGSTROM and PRITCHARD 1998; RISPE and MORAN 2000).

In this article, we present a study of the joint effects of paternal leakage and bottlenecks on selection on mitochondrial variants, assuming that selection can occur both within and between cells. In particular, we derive expressions for the probability of fixation of a mutation in mtDNA and for the frequency of allelic variants at mutation-selection equilibrium. These expressions allow us to quantify the effects of bottleneck size and biparental transmission on these quantities. Previous models on the effects of germline bottlenecks used simulations to study the rate of accumulation of mutations at several loci (BERGSTROM and PRITCHARD 1998; RISPE and MORAN 2000). Here we represent a

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single selected locus, which allows us to derive analytical results; we then discuss the relation between our results and results on Muller's ratchet. To keep the analysis simple, we consider a unicellular life cycle, but the model could be extended to deal with more complicated developmental patterns. Before introducing the model, we briefly present some data about the biology of animal mitochondria. More exhaustive presentations can be found in two review articles by BIRKY (2001) and RAND (2001).

Once a mutation in mtDNA occurs, the cell becomes heteroplasmic, containing different mitochondrial allelic types. During past years, many studies have shown that heteroplasmy may be more common than previously thought, due to the constant input of new mutations (CORRAL-DEBRINSKI *et al.* 1992; CORTOPASSI *et al.* 1992; JAZIN *et al.* 1996; LIGHTOWLERS *et al.* 1997; MICHIKAWA *et al.* 1999; CHINNERY *et al.* 2000, 2001). The dynamics of heteroplasmy under neutrality have been studied using mathematical models (TAKAHATA and MARUYAMA 1981; BIRKY *et al.* 1983); critical parameters of these models are the amount of paternal transmission of mitochondria and the effective number of mitochondria per cell, which may be very different from the real number of mitochondria per cell. Organelle genome replication is stochastic, in the sense that the same genome can be replicated more than once by chance, while others are not replicated (BIRKY 1994). However, all genomes in a cell do not seem to have the same probability of being replicated: in cultured mammalian cells, it has been observed that genomes located near the nucleus are the only ones that replicate (DAVIS and CLAYTON 1996), which reduces the effective number of organelles in the cell. At cell division, organelles are thought to be partitioned randomly between the two daughter cells (BIRKY 1994); however, it has been shown in yeast that sister mitochondria tend to remain close to each other in the cytoplasm and thus have a greater chance of being in the same daughter cell than if segregation was strictly random (BIRKY 2001). This also increases the speed of intracellular drift.

The effective organelle number may also be reduced by temporal variations of the number of organelles per cell. In mammals, rapid changes in heteroplasmy levels between generations have led to the hypothesis of a mitochondrial bottleneck during the development of the female germline (HAUSWIRTH and LAIPIS 1982, 1985; LAIPIS *et al.* 1988; KOEHLER *et al.* 1991). Cytoplasm transfer experiments in mice have reinforced this hypothesis and showed that the bottleneck occurs during early oogenesis (JENUTH *et al.* 1996). Finally, direct estimates from electron micrographs have shown that, in humans, the number of mitochondria per germ cell is as low as 10 at the very beginning of germline differentiation (at the blastocyst stage) and increases steadily to reach 10^5 in mature oocytes (JANSEN and DE BOER 1998), confirming the existence of a bottleneck during early oogenesis.

Due to the bottleneck and the stochastic nature of replication and segregation of mitochondria at cell division, heteroplasmy is expected to be transient, one genotype reaching fixation after several cell divisions, and the number of germ cell generations by organismal generation is estimated to be 32–37 in *Drosophila* females, 30–35 in *Drosophila* males, and ~ 25 and 62 in female and male mice, respectively (DROST and LEE 1998). However, heteroplasmy can be created again at the next generation if mitochondria present in the sperm are transmitted to the egg at fertilization. At present, the occurrence of paternal leakage remains controversial, except for some peculiar inheritance systems as in mussels, where paternal transmission has been clearly demonstrated (ZOUROS *et al.* 1992; SKIBINSKI *et al.* 1994). In *Drosophila*, paternal transmission has been detected in both interspecific and intraspecific crosses (KONDO *et al.* 1990, 1992). In mice, paternal leakage has been observed in interspecific crosses (GYLLENSTEN *et al.* 1991), but not in experiments involving intraspecific crosses (KANEDA *et al.* 1995; SHITARA *et al.* 2000). Still, in mice, it has been shown that mitochondria in spermatids are tagged with ubiquitin and that ubiquitinated mitochondria are selectively destroyed in the egg and young embryo (SUTOVSKY *et al.* 1999). However, one cannot rule out the possibility that, sometimes, a few paternal mitochondria might escape destruction. Whether such systematic elimination of paternal transmission occurs in other species is not known. In humans, it seems that sperm mitochondria can be identified in the embryo up to the morula stage (ANKEL-SIMONS and CUMMINS 1996), and a case of paternal inheritance has been reported recently in an individual suffering from a mitochondrial myopathy (SCHWARTZ and VISSING 2002; BROMHAM *et al.* 2003). Finally, mtDNA sequence data from many animal species show evidence for recombination, which would imply the occurrence of paternal transmission (PIGANEAU *et al.* 2003). It remains unclear, however, if this result is a consequence of experimental errors or if it reflects a real biological process (see discussion in PIGANEAU *et al.* 2003).

Intraorganismal selection on mitochondrial variants has been documented in several species including *Drosophila*, mice, and humans (SHOUBRIDGE *et al.* 1990; YONEDA *et al.* 1992; DUNBAR *et al.* 1995; DE STORDEUR 1997; JENUTH *et al.* 1997; DOI *et al.* 1999; MORAES *et al.* 1999; TAKEDA *et al.* 2000). Both point mutations and deletions in mtDNA can be selected within the organism; moreover, selection can be tissue specific, some genotypes systematically increasing in frequency in some tissues while decreasing in others. The mechanisms of intraorganismal selection often remain obscure. While it is easy to imagine that a mutation disrupting oxidative phosphorylation compromises cell survival and therefore is counterselected at the cell level, positive selection is more difficult to explain. The popular hypothesis that deleted molecules may replicate faster due to their shorter size (WALLACE 1989) has been criticized on the

grounds that the time taken by a mtDNA molecule to replicate is a lot shorter than the time between two replication events (SHADEL and CLAYTON 1997). A well-studied example of selfish mutation is the *petite* mutation in yeast, which increases in frequency in cells at the expense of cell fitness (TAYLOR *et al.* 2002). In some *petite* mutations only a small part of the mitochondrial genome is retained and repeated many times to produce a molecule of approximately normal size, which benefits from a replication advantage because it contains several copies of the replication origin (BIRKY 2001; MACALPINE *et al.* 2001).

It is well known that mutations in mtDNA can affect the fitness of the organism, leading to selection at the organism level. In humans, pathogenic mtDNA defects affect at least 1 in 15,000 of the adult population (CHINERY and TURNBULL 2000). These defects can be caused either by point mutations or by deletions in mtDNA accumulating in postmitotic tissues. The most energy-demanding organs are the most severely impaired: the central nervous system (the visual system in particular), heart, motor muscles, kidneys, and liver (LARSSON and CLAYTON 1995; WALLACE 1999; TRIFUNOVIC *et al.* 2004). Interestingly, selection can be sex specific, some mitochondrial diseases being more severe in males than in females (FRANK and HURST 1996). Selection sometimes occurs very early in life: BARRITT *et al.* (2000) have found that the frequency of the “common” deletion is 33% among human oocytes and 8% among embryos, suggesting that oocytes with a high frequency of deleted mtDNA are less likely to be implanted as embryos. In yeast, experiments have shown that some mutants favored by intraorganismal selection do not increase in frequency when selection between organisms is allowed (TAYLOR *et al.* 2002), suggesting that selection may act in opposite directions at the two levels.

Several mathematical models have studied the dynamics of heteroplasmy and neutral variability in organelle genomes (TAKAHATA and MARUYAMA 1981; CHAPMAN *et al.* 1982; BIRKY *et al.* 1983, 1989; TAKAHATA 1983; TAKAHATA and PALUMBI 1985; CHESSEY 1998). These models derive recursions for probabilities of identity between different pairs of genes (genes from the same cell, from the same organism, from the same local population); these recursions are then used to describe the population at mutation-drift equilibrium and to obtain results about the approach to the equilibrium. Other models have considered the effects of selection. TAKAHATA and SLATKIN (1983) used a simulation model to study the effects of the number of copies of the organelle genome per individual, the number of loci in the genome, and the rate of paternal transmission on the fixation rates of new mutations; in this model, mutations can be advantageous or deleterious for the organism, while intraorganismal selection is absent. TAKAHATA (1984) derived approximations for the probability of fixation of mutations in organelle genomes, assuming that selection can occur within the organism, but not between

organisms. This model assumes that all cells are homoplasmic at the end of the development. WALSH (1992, 1993) used a birth-death model to calculate probabilities of fixation in organelle genes, including selection between molecules within organelles, genetic exchange between organelles and gene conversion, selection between organelles within cells, and selection between organisms. This model focuses on the relative effects of intracellular selection and gene conversion on fixation rates. As in TAKAHATA (1984), it makes the hypothesis that all cells are homoplasmic at the end of the development.

More recently, BERGSTROM and PRITCHARD (1998) studied the effect of germline bottlenecks on the accumulation of deleterious mutations (Muller’s ratchet) in mitochondrial genomes. They modeled a unicellular life cycle and considered the case of deleterious mutations at the cell level (while selection within cells is absent). They derived recurrence equations for the average fitness in the population and for the genetic variance within and between cells; however, as these recursions depend on higher-order moments, the system cannot be closed. Using simulations, they showed that bottlenecks reduce the speed of Muller’s ratchet, by increasing the variance in fitness between cells.

In this article, we represent a life cycle similar to the one modeled by BERGSTROM and PRITCHARD (1998). We consider a single selected mitochondrial locus and calculate the probability of fixation of a mutant allele (without recurrent mutation) and its frequency at mutation-selection equilibrium (under recurrent mutation), assuming that selection can occur between cells and between mitochondria within cells. We show that the effects of bottlenecks and of biparental transmission vary depending on the type of mutation considered (*e.g.*, selfish, uniformly deleterious, altruistic . . .); furthermore, we show that for a given type of mutation, bottlenecks can have different effects on the probability of fixation and on the mean frequency at mutation-selection equilibrium. There are several differences between our model and Bergstrom and Pritchard’s model: in particular, we include selection between mitochondria within cells, which is absent in Bergstrom and Pritchard’s model, and we derive analytical results concerning probabilities of fixation and frequency at mutation-selection equilibrium at a single locus, while Bergstrom and Pritchard obtained simulation results concerning the rate of accumulation of deleterious mutations (Muller’s ratchet) at several loci.

MODEL

Life cycle: Definitions of the different parameters and variables of the model are given in Table 1. We consider the following life cycle (represented in Figure 1). At the beginning of each generation, the population consists of n unicellular organisms, each containing N mitochondria. The number of mitochondria per cell then

measures the rate of biparental inheritance: when $\alpha = 0$, cells do not exchange mitochondria during sex (uniparental inheritance), while when $\alpha = 1/2$, mitochondria from both cells are mixed randomly. This life cycle therefore includes two events of drift within cells (when the number of mitochondria per cell drops to B , and when it goes back to N), and one event of drift between cells (when cells are sampled to form the next generation).

We assume that mitochondria are of two types: mutant (A) and nonmutant (a). p measures the frequency of A in the whole population, p_i the frequency of A in the i th cell, and p_{ij} the frequency of A in the j th mitochondrion of the i th cell; we assume that mitochondria are haploid (which seems to be true in the human germline; JANSEN 2000), so that $p_{ij} = 0$ or 1 . After the bottleneck stage (when the number of mitochondria per cell goes from B to N), selection occurs between mitochondria within cells. To represent this selection, we assume that the probability that any of the N mitochondria present in the i th cell after the bottleneck comes from the j th of the B mitochondria at the bottleneck stage is

$$\frac{1}{B} \frac{1 + s_m p_{ij}}{1 + s_m p_{i(b)}}, \quad (1)$$

where $p_{i(b)}$ is the frequency of A mitochondria in cell i at the bottleneck stage (this equation corresponds to the classical Wright-Fisher model with selection); s_m therefore measures the replication advantage (if $s_m > 0$) or disadvantage (if $s_m < 0$) of A mitochondria within cells.

Selection between cells occurs when cells are sampled to form the next generation. To represent this selection, we assume that each time a cell is sampled to be part of the next generation, the probability of sampling the i th cell is

$$\omega_i = \frac{1}{n} \frac{1 + s_c p_{i(g)}}{1 + s_c p_{(g)}}, \quad (2)$$

where $p_{i(g)}$ and $p_{(g)}$ are the frequencies of A mitochondria in cell i and in the whole population, respectively, once the number of mitochondria per cell has grown back to N . Therefore, s_c measures the effect of A mitochondria on the cell's performances: if $s_c > 0$, A mitochondria have a beneficial effect for the cell, while if $s_c < 0$ they have a deleterious effect.

This model life cycle is similar to the one modeled by BERGSTROM and PRITCHARD (1998), to the difference that Bergstrom and Pritchard do not represent selection within cells, while paternal transmission is not modeled exactly the same way.

Method: In the following, we use a diffusion model to calculate the probability of fixation of allele A and its expected frequency at mutation-selection equilibrium. It has been shown that diffusion approximations can be applied to subdivided populations, provided that coalescence occurs at different timescales (ETHIER and

NAGYLAKI 1980; ROZE and ROUSSET 2003; WAKELEY 2003); as is discussed later, this required property is present in our model. The diffusion method considers limit processes where population size tends to infinity, while selection coefficients tend to zero, assuming that the products of population size and selection coefficients tend to finite values in this limit (which are their values under the finite population model considered). To do this, we write selection coefficients under the form $s_m = \delta \tilde{s}_m$, $s_c = \delta \tilde{s}_c$, where δ is a small term and is of order $1/n$ (thus when δ tends to zero, n tends to infinity). In the following, we express the mean and variance of the change in frequency of A over a generation to the first order in δ (and in $1/n$). Approximations for probabilities of fixation are readily obtained from such expressions (EWENS 1979; KARLIN and TAYLOR 1981).

We call Δp the change in frequency of allele A over one generation and define $M_{\delta p}$ and $V_{\delta p}$ as

$$M_{\delta p} = E[\Delta p | \mathbf{p}] \quad (3)$$

$$V_{\delta p} = E[(\Delta p)^2 | \mathbf{p}], \quad (4)$$

where $E[x]$ is the expectation of x , and \mathbf{p} is the n -dimensional vector giving the frequencies of A in the n cells of the population, at the beginning of a generation. We see in the next sections that $M_{\delta p}$ and $V_{\delta p}$ take the forms (with $q = 1 - p$)

$$M_{\delta p} = Spq + o(\delta) \quad (5)$$

$$V_{\delta p} = \frac{pq}{N_c} + o(1/n), \quad (6)$$

where S and N_c depend on the model parameters, but not on allele frequencies. From these expressions of $M_{\delta p}$ and $V_{\delta p}$, the diffusion approximation for the probability of fixation of allele A , when present in frequency p in the population, is given by (e.g., EWENS 1979)

$$u(p) = \frac{1 - \exp[-2N_c S p]}{1 - \exp[-2N_c S]}. \quad (7)$$

From S and N_c , we can also obtain the mean frequency of a mutant allele at mutation-selection equilibrium. Assuming a constant mutation rate μ from a to A (per generation) and a back mutation rate ν (from A to a), where μ and ν are small (of order δ), the mean change in frequency of A becomes

$$M_{\delta p} = Spq + \mu q - \nu p + o(\delta), \quad (8)$$

while the variance in allele frequency change, to the first order, remains unchanged. From these expressions, the probability that allele A is present in frequency p in the population at equilibrium is given by (e.g., EWENS 1979)

$$\phi(p) = K \exp[2N_c S p] p^{2N_c \mu - 1} q^{2N_c \nu - 1}, \quad (9)$$

where K is a constant such that probabilities sum to

one. The expected frequency of A at equilibrium can then be obtained by averaging over this distribution.

Expected change in allele frequency: To calculate $M_{\delta p}$, we first define the fitness W_{ij} of mitochondrion j present in cell i (at the beginning of the life cycle) as the expected number of copies that it will transmit to the next generation. With such a definition, the average fitness equals 1, and the change in frequency of allele A over a generation is given by

$$M_{\delta p} = \frac{1}{nN} \sum_{i=1}^n \sum_{j=1}^N W_{ij} p_{ij} - p. \tag{10}$$

When $\delta = 0$, $M_{\delta p} = 0$. To the first order in δ , one has

$$M_{\delta p} = \frac{\delta}{nN} \sum_{i=1}^n \sum_{j=1}^N \frac{dW_{ij}}{d\delta} p_{ij} + o(\delta). \tag{11}$$

In APPENDIX A we calculate W_{ij} under our life cycle, to the first order in δ , and obtain

$$W_{ij} = 1 + \delta \tilde{s}_c (p_{ij} - p) + \left(1 - \frac{1}{B}\right) \left[\delta \tilde{s}_m - \delta \tilde{s}_c \left(1 - \frac{1}{N}\right) \right] (p_{ij} - p_i) + o(\delta). \tag{12}$$

From this and from Equation 11 (and using the fact that $p_{ij}^2 = p_{ij}$), one arrives at

$$M_{\delta p} = s_c p q + \left(1 - \frac{1}{B}\right) \left[s_m - s_c \left(1 - \frac{1}{N}\right) \right] (p - \bar{p}_i^2) + o(\delta), \tag{13}$$

where \bar{p}_i^2 denotes the average of p_i^2 over all cells i . \bar{p}_i^2 is the probability that two mitochondria sampled with replacement from the same cell at the beginning of the life cycle are both A . As we want to express $M_{\delta p}$ to the first order in δ only, it is sufficient to express this probability when δ tends to zero, that is, in the neutral case, and when the number of individuals is infinite (remember that δ is of order $1/n$). In this limit, \bar{p}_i^2 can be expressed as a function of p using the following argument: the ancestral lineages of two mitochondria sampled in the same cell (at the beginning of the life cycle) can stay in the same cell lineage and coalesce (with a probability that we call r) or move to different cells (with probability $1 - r$), in which case they take an infinite time to coalesce as the number of cells n tends to infinity. Therefore, at a finite time t sufficiently far in the past, those two lineages have coalesced with probability r and have not coalesced with probability $1 - r$. If they have coalesced, the probability that their common ancestor was A is p (since when δ tends to zero and n tends to infinity, the frequency of A does not change over the finite number t of generations), giving

$$\begin{aligned} \bar{p}_i^2 &= r p + (1 - r) p^2 + O(\delta) \\ &= p^2 + r p q + O(\delta). \end{aligned} \tag{14}$$

Equations 13 and 14 finally give

$$M_{\delta p} = S p q + o(\delta) \tag{15}$$

with

$$S = s_c + \left(1 - \frac{1}{B}\right) (1 - r) \left[s_m - s_c \left(1 - \frac{1}{N}\right) \right]. \tag{16}$$

In the next section we express r as a function of α , N , and B . However, we can already note that when $\alpha = 0$ (uniparental inheritance), $r = 1$ (since the ancestral lineages of two mitochondria sampled in the same cell cannot move to different cells). In this case, $S = s_c$: indeed, under strict uniparental inheritance (and without recurrent mutation) cells become rapidly homoplasmic, and within-cell selection has no effect.

Variance in allele frequency change: To obtain the probability of fixation of allele A , we also need to express $V_{\delta p}$. To the first order in $1/n$, this is the variance in the change in frequency of A over a generation, in the neutral case. We show in APPENDIX B that $V_{\delta p}$ is given by

$$V_{\delta p} = \frac{p q}{N_e} + o\left(\frac{1}{n}\right), \tag{17}$$

where

$$N_e = \frac{n}{1 + (1 - r) \left[1 - 2 \left(1 - \frac{1}{N}\right) \left(1 - \frac{1}{B}\right) \right]}. \tag{18}$$

As discussed previously, $r = 1$ under strict uniparental inheritance ($\alpha = 0$), and thus N_e simply equals n (the number of cells in the population) in that case.

Calculation of r : Finally, we need to express r , which measures the probability that the ancestral lineages of two mitochondria sampled with replacement from the same cell (at the beginning of the life cycle) stay in the same cell and coalesce, in the limit as population size tends to infinity, and in the neutral case (as shown above). Denoting r^D the same probability for two mitochondria sampled *without* replacement from the same cell, we have

$$r = \frac{1}{N} + \left(1 - \frac{1}{N}\right) r^D. \tag{19}$$

A recursion for r^D is given by

$$\begin{aligned} (r^D)' &= [1 - 2\alpha(1 - \alpha)] \left[\left(1 - \frac{1}{B}\right) \left(1 - \frac{1}{N}\right) r^D \right. \\ &\quad \left. + 1 - \left(1 - \frac{1}{B}\right) \left(1 - \frac{1}{N}\right) \right], \end{aligned} \tag{20}$$

Indeed, two mitochondria present in the same cell after sex were in the same cell before sex with probability $1 - 2\alpha(1 - \alpha)$; in this case, they come from two different mitochondria at the previous generation with probability $(1 - 1/B)(1 - 1/N)$, while they coalesce during the previous generation with probability $1 - (1 - 1/B)(1 - 1/N)$. Solving this recursion at equilibrium gives for r :

TABLE 2

Effects of a reduction in B (bottleneck size) and of an increase in α (rate of biparental transmission) on $u(p)$, the fixation probability of allele A , and on its average frequency at mutation-selection equilibrium (\bar{p})

	0-	-0	--	0+	+0	++	-+	+-
Effect of bottlenecks on								
$u(p)$	Increase	Increase	Increase	Decrease	Decrease	Decrease	Increase or decrease (Equation 23)	Increase or decrease (Equation 23)
\bar{p}	Decrease	Increase	Increase or decrease (Equation 26)	Increase	Decrease	Increase or decrease (Equation 26)	Increase	Decrease
Effect of α on								
$u(p)$	Increase	Decrease	Increase or decrease (Equation 25)	Decrease	Increase	Increase or decrease (Equation 25)	Decrease	Increase
\bar{p}	Increase	Decrease	Increase or decrease (Equation 26)	Decrease	Increase	Increase or decrease (Equation 26)	Decrease	Increase

The different columns correspond to different types of A alleles: in each case, the first symbol denotes the effect of A at the mitochondrial level ($-$ if deleterious, 0 if neutral, $+$ if advantageous), and the second symbol is its effect at the cell level (for example, $+-$ corresponds to the case of selfish mutants and $0-$ to mutants that are deleterious for the cell and neutral at the mitochondrial level).

$$r = 1 - \frac{2\alpha(1 - \alpha)(1 - 1/N)}{1 - (1 - 1/B)(1 - 1/N)[1 - 2\alpha(1 - \alpha)]} \tag{21}$$

$$s_m > -\frac{1}{N} \frac{2\alpha(1 - \alpha)}{1 + 2\alpha(1 - \alpha)} s_c \tag{23}$$

This expression can be plugged into Equations 16 and 18 to express S and N_c as functions of the different parameters of the model.

RESULTS

The effects of bottlenecks and of paternal transmission on fixation probabilities and average frequencies at mutation-selection equilibrium are summarized in Table 2 and Figure 5.

Fixation probabilities: In the absence of recurrent mutations ($\mu = \nu = 0$), the probability of fixation of allele A is given by Equation 7. From this expression, it is possible to show that the probability of fixation of A increases as the product $N_c S$ increases. The effect of bottlenecks on fixation probability is thus given by the sign of the derivative of $N_c S$ with respect to B : when this derivative is positive, bottlenecks (reductions in B) reduce the probability of fixation of A . From Equations 16, 18, and 21, one obtains that this derivative has the sign of

$$2\alpha(1 - \alpha)(N - 1)(s_c + Ns_m) + N^2s_m \tag{22}$$

When the number of mitochondria per cell is not small, so that $N - 1 \approx N$, one thus obtains that bottlenecks reduce the probability of fixation of A when

Condition (23) indicates that, when the number of mitochondria per cell N is large, bottlenecks reduce the fixation probability of A roughly when $s_m > 0$ (selfish and uniformly advantageous mutants) and increase the fixation probability of A when $s_m < 0$ (altruistic and uniformly deleterious mutants). It also shows that, when selection within cells is absent ($s_m = 0$), bottlenecks reduce the fixation probability of mutations that are advantageous for the cell ($s_c > 0$) and increase the fixation probability of mutations that are deleterious for the cell ($s_c < 0$). This result may seem surprising at first, because bottlenecks increase the variance between cells, thus increasing the effect of selection at the cell level. However, bottlenecks have another effect, which is to decrease the effective population size of mitochondria, N_c (as is shown from Equations 18 and 21). Therefore, bottlenecks increase the effect of genetic drift, which reduces the fixation probability of advantageous mutants and increases the fixation probability of deleterious mutants. From the overall effect of B on the $N_c S$ product, one finds that, in our model, the effect on N_c predominates: bottlenecks increase the fixation probabilities of mutants that are deleterious for the cell and decrease the fixation probability of advantageous mutants for the cell.

The effect of the rate of biparental transmission on fixation probabilities is given by the sign of the derivative

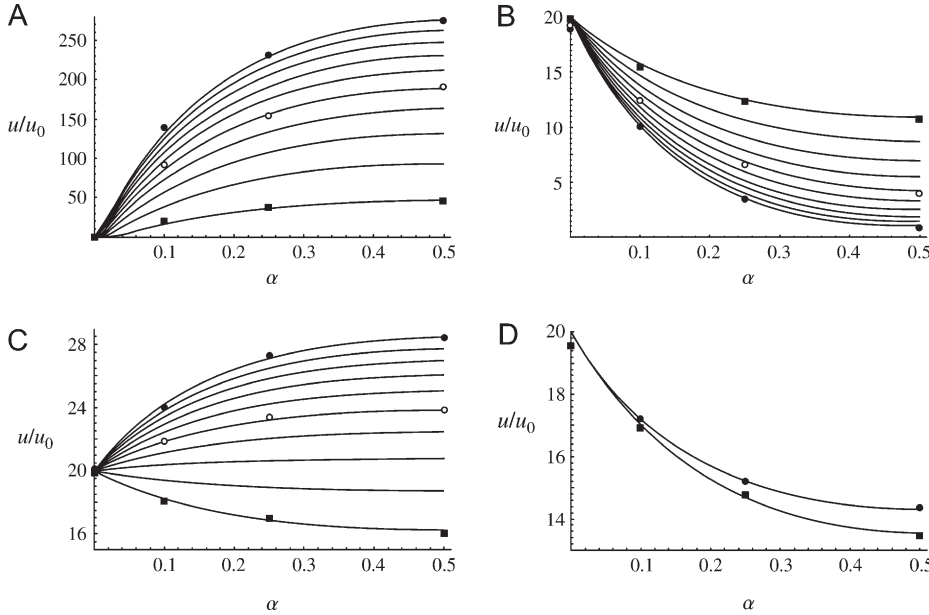


FIGURE 2.—Probability of fixation of allele *A* when present as a single copy at the beginning of a generation, relative to the probability of fixation of a neutral allele, as a function of the rate of biparental inheritance α , and for different values of the bottleneck size *B*. (A) Selfish mutant ($s_m = 0.02$, $s_c = -0.01$); (B) altruistic mutant ($s_m = -0.001$, $s_c = 0.02$); (C) uniformly advantageous mutant ($s_m = 0.001$, $s_c = 0.02$); (D) advantageous mutant at the cell level ($s_m = 0$, $s_c = 0.02$). The values of the other parameters are $n = 500$; $N = 100$; and $B = 10, 20, 30, 40, 50, 60, 70, 80, 90$, and 100 (from bottom to top in A and C and from top to bottom in B); $B = 10$ (bottom) and 100 (top) in D. Simulation results: $B = 10$ (squares), $B = 50$ (open circles), and $B = 100$ (solid circles).

of $N_c S$ with respect to α . From Equations 16, 18, and 21, one obtains that this derivative has the sign of

$$-s_c(N + B - 1) + s_m N(B - 1) \tag{24}$$

(when $0 \leq \alpha \leq \frac{1}{2}$) and therefore the probability of fixation of *A* increases as α increases if

$$s_m > \frac{N + B - 1}{N(B - 1)} s_c. \tag{25}$$

Thus, increasing the rate of biparental transmission increases the fixation probability of selfish mutants ($s_m > 0$, $s_c < 0$) and decreases the fixation probability of altruistic mutants ($s_m < 0$, $s_c > 0$), while it can increase or decrease the fixation probability of uniformly deleterious and uniformly advantageous mutants, depending on the values of s_m , s_c , N , and B . This is due to the fact

that biparental transmission reduces selection between cells, while it increases selection within cells. Effects of paternal transmission and bottlenecks on probabilities of fixation are illustrated by Figure 2, which also compares our analytical solution with simulation results.

Mutation-selection equilibrium: In the presence of recurrent mutations from *a* to *A* and from *A* to *a*, the average frequency of *A* at equilibrium can be obtained by integrating over the frequency distribution given by Equation 9. Although we could not obtain any simple result concerning the effects of B and α from this frequency distribution, it is useful to consider the two following limit cases.

In the first case, population size is very small, so that $|N_c S| \ll 1$, $N_c \mu \ll 1$, and $N_c \nu \ll 1$. In this case, the population is fixed most of the time for one of the two alleles *a* or *A*. The expected frequency of *A* is then determined by the probability of fixation of *A* in a population fixed for *a* and by the probability of fixation of *a* in a population fixed for *A*. In this case, increasing the probability of fixation of *A* will also increase the average frequency of *A* at mutation-selection equilibrium. Therefore in a very small population, the effects of bottleneck size and of paternal leakage on the probability of fixation of *A* and on the mean frequency of *A* at mutation-selection equilibrium are the same.

When population size is very large, so that $|N_c S| \gg 1$, $N_c \mu \gg 1$, and $N_c \nu \gg 1$, the average frequency of *A* can be approximated by the deterministic equilibrium (calculated for the case of an infinite population); when $S < 0$, this deterministic equilibrium is $p = -\mu/S$ (neglecting back mutations). In this case, one thus neglects the effects of drift in the total population, and the different parameters of the model affect average allele frequencies only through their effects on the selection coefficient *S*. Figure 3 compares the results obtained

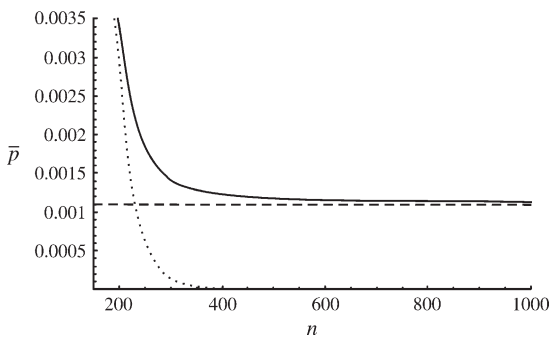


FIGURE 3.—Average frequency of a selfish mutant at mutation-selection equilibrium, as a function of population size *n*. The solid line corresponds to integration over the distribution given by Equation 9, the dotted line to the approximation obtained by assuming that the population is fixed most of the time for one of the two alleles (small population size), and the dashed line to the deterministic approximation $-\mu/S$ (infinite population size). Parameter values are $N = 100$, $B = 10$, $s_c = -0.02$, $s_m = 0.005$, $\alpha = 0.05$, $\mu = 10^{-5}$, and $\nu = 10^{-6}$.

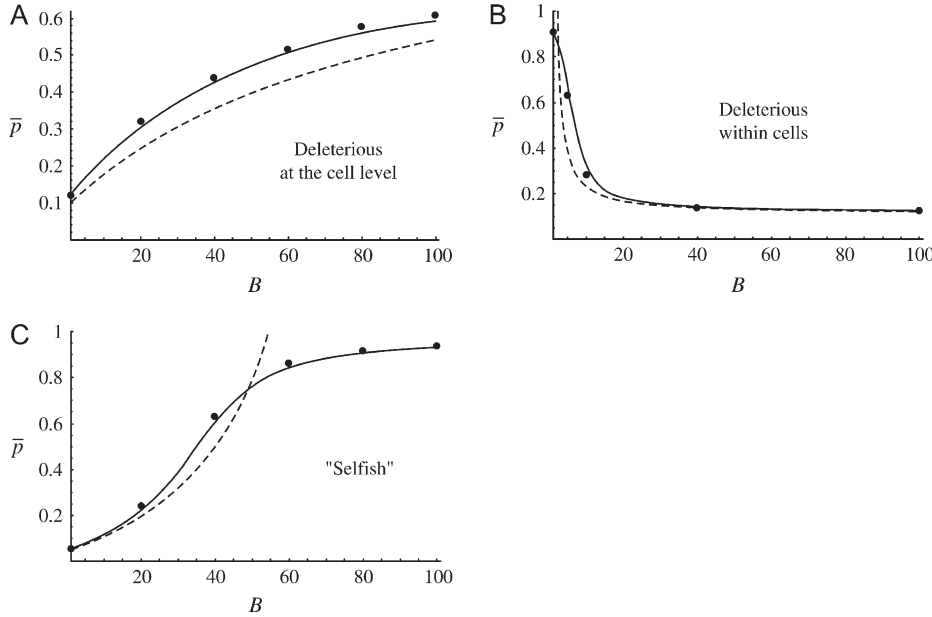


FIGURE 4.—Average frequency of allele *A* at mutation-selection equilibrium, as a function of bottleneck size *B*. Solid lines correspond to integration over the distribution given by Equation 9, dashed lines to the deterministic approximation $-\mu/S$, and dots to simulation results. Parameter values are $n = 500$, $\alpha = 0.05$, $\mu = 10^{-3}$, $\nu = 10^{-4}$, and $N = 100$. (A) $s_m = 0$, $s_c = -0.01$; (B) $s_m = -0.01$, $s_c = 0$; (C) $s_m = 0.005$, $s_c = -0.02$.

from this approximation and from the previous approximation for small population size, with the more exact results obtained by integrating over the distribution given by Equation 9. The derivative of $-\mu/S$ with respect to *B* has the sign of $Ns_m - (N - 1)s_c$. Therefore, when *N* is not small (*i.e.*, $N \approx N - 1$), bottlenecks reduce the mean frequency of allele *A* approximately when

$$s_m > s_c. \tag{26}$$

The derivative of $-\mu/S$ with respect to α has also the sign of $Ns_m - (N - 1)s_c$ (when $0 \leq \alpha \leq 1/2$). Thus, increasing the rate of biparental inheritance increases the frequency of *A* when $s_m > s_c$. Therefore, in a large population, bottlenecks (and reduced paternal transmission) decrease the frequency of selfish mutants, increase the frequency of altruistic mutants, and either increase or decrease the frequency of uniformly deleterious and uniformly advantageous mutants, depending on the relative strength of selection within and between cells. Figure 4 illustrates the effect of bottlenecks on the mean frequency of mutations that are deleterious for the cell (Figure 4A), deleterious for the mitochondrion (Figure 4B), and selfish (Figure 4C). It also compares predictions from the model with simulation results.

Figure 5 summarizes results about fixation probabilities and mean frequency at mutation-selection equilibrium. Mutants located in the top left corner of Figure 5 are selfish (+ -), those in the bottom left are uniformly deleterious (- -), those in the top right are uniformly advantageous (+ +), and those in the bottom right are altruistic (- +). Bottlenecks (reductions in *B*) decrease the fixation probability of mutations with values of s_c and s_m located in the area above the dashed line; although this line appears horizontal, it has a slightly negative slope (given by Equation 23). Reductions in α decrease the fixation probability of mutants located above the

dotted line (obtained from Equation 25) and increase the fixation probability of mutants located below. In a large population, reductions in both *B* and α decrease the mean frequency of mutants located above the solid line and increase the frequency of mutants located below. In a small population, effects on fixation probabilities and on mean frequencies are the same: reductions

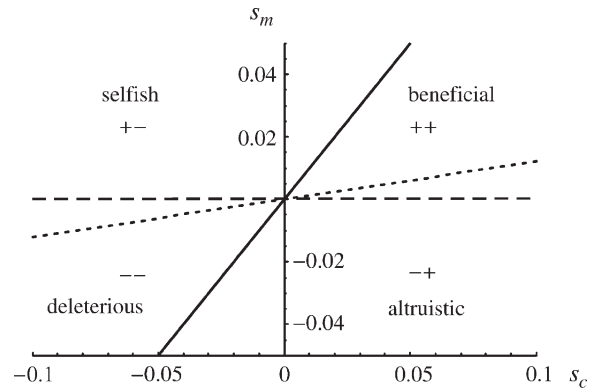


FIGURE 5.—Effects of bottlenecks and reduction in α on the fixation probability and mean frequency of mitochondrial mutants. The axes are selection coefficients at the cell and mitochondrial levels; mutants in the top left are selfish (+ -), those in the bottom left are uniformly deleterious (- -), those in the top right are uniformly advantageous (+ +), and those in the bottom right are altruistic (- +). In a large population, bottlenecks and reductions in α decrease the mean frequency of mutants (at mutation-selection equilibrium) above the solid line (from Equation 26) and increase the frequency of mutants below it. Bottlenecks decrease the fixation probability of mutants above the dashed line (from Equation 23) and increase the fixation probability of mutants below it. Reductions in α decrease the fixation probability of mutants above the dotted line (from Equation 25) and increase the fixation probability of mutants below it. Parameter values are $N = 100$, $B = 10$, and $\alpha = 0.05$.

in B decrease the mean frequency of mutations located in the area above the dashed line, while reductions in α decrease the mean frequency of mutants located above the dotted line.

DISCUSSION

Our simplest results concern the effects of bottlenecks and paternal transmission on the mean mutant frequency in a large population: in this case, qualitative effects can be predicted from effects on $M_{\delta p}$. Both bottlenecks and reduced paternal transmission increase the strength of selection between cells and decrease the strength of selection within cells; therefore, reducing B and/or α decreases the frequency of selfish mutants and increases the frequency of altruistic mutants. When selection acts in the same direction at both levels (uniformly deleterious and uniformly advantageous mutants), bottlenecks and reduced paternal transmission decrease the mutant frequency approximately when $s_m > s_c$ (Figure 5, solid line).

The effects of paternal transmission on fixation probabilities are qualitatively similar, but in the case of uniformly deleterious and uniformly advantageous mutants, the sign of the effect is now given by condition (25). Therefore, decreasing α will have different effects on the mean mutation-selection equilibrium frequency and on the probability of fixation of some of these mutants (unless population size is very small, in which case effects on fixation probability and on mean frequency are the same, as we argued earlier): reductions in α decrease the probability of fixation, but increase the mean frequency of uniformly advantageous (++) mutants located between the dotted and solid lines in Figure 5, while reductions in α increase the fixation probability, but decrease the mean frequency of uniformly deleterious (--) mutants located between those lines.

Bottlenecks decrease the effective population size N_e and thus increase the effects of drift. Regarding probabilities of fixation, this effect compensates the increased strength of selection at the cell level: when selection within cells is absent ($s_m = 0$), we find that bottlenecks increase the probability of fixation of mutations that are deleterious for the cell and decrease the probability of fixation of advantageous mutations for the cell. Therefore in the case of these mutants, bottlenecks have different qualitative effects on probabilities of fixation and on frequencies at mutation-selection equilibrium; this is due to the fact that drift affects fixation probabilities to a greater extent than mutation-selection equilibrium frequencies (in a very large population, one can neglect drift when calculating mutation-selection equilibrium frequencies, but not when calculating fixation probabilities). When selection occurs only within cells ($s_c = 0$), the effects of bottlenecks through drift and through selection go in the same direction: bottlenecks increase drift and decrease the strength of selection; therefore, in this case, bottlenecks have the same effect on fixation

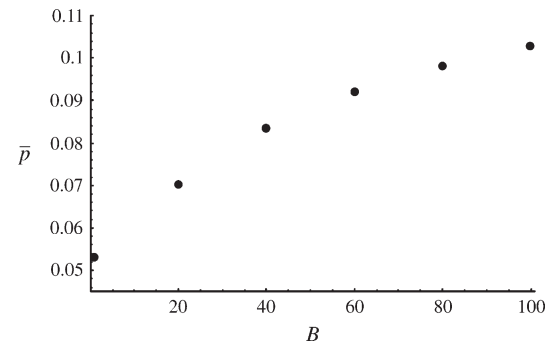


FIGURE 6.—Average frequency of a selfish allele at mutation-selection equilibrium, as a function of bottleneck size B , in the absence of paternal transmission ($\alpha = 0$): simulation results. Parameter values are $n = 500$, $N = 100$, $\mu = 10^{-3}$, $\nu = 10^{-4}$, $s_m = 0.005$, and $s_c = -0.02$.

probabilities and on mutation-selection equilibrium frequencies. Finally, bottlenecks decrease the probability of fixation of selfish mutants and increase the probability of fixation of altruistic mutants, except when selection within cells is very weak [condition (23)].

Our model stresses the fact that, without paternal transmission ($\alpha = 0$), bottlenecks and within-cell selection have no effect on probabilities of fixation: in this case, $S = s_c$ and $N_e = n$. Indeed under strict uniparental inheritance, cells become rapidly homoplasmic, and selection occurs only at the cell level. The mean frequency at mutation-selection equilibrium also does not depend on B or on s_m in our model when $\alpha = 0$; this comes from the fact that we assumed that both selection and mutation were weak (intuitively, when the mutation rate is small and without paternal transmission, cells are homoplasmic most of the time, and within-cell selection has little effect). Under sufficiently strong mutation rates, however, bottlenecks affect mutant frequencies even under strict uniparental transmission, as shown by Figure 6; however, one cannot use the diffusion method in that case. RISPE and MORAN (2000) analyzed a simulation model of Muller's ratchet in endosymbiotic bacteria; their model includes a bottleneck stage during transmission to the next generation. Although transmission is strictly uniparental, they found that bottlenecks decrease the rate of accumulation of selfish mutations and can increase or decrease the rate of accumulation of uniformly deleterious mutations, depending on the relative strength of selection at the within- and between-organism levels.

Because bottlenecks and paternal transmission affect differently the different types of mitochondrial mutations, the evolution of modifiers affecting these traits should depend on the relative frequencies of the different types of mutational events. For example, reduced paternal transmission decreases the frequency of selfish mutants and uniformly deleterious mutants with $s_m > s_c$, but increases the frequency of mutants with $s_m < s_c$; therefore, uniparental inheritance decreases the frequency of some mutations that are deleterious for the

organism (selfish mutations in particular), but increases the frequency of others, which are also deleterious for the organism (those with $s_m < s_c < 0$). Similarly, uniparental inheritance can either increase or decrease the frequency of advantageous mutations for the organism, depending on the relative values of s_m and s_c . Theoretical models investigating the conditions for the evolution of uniparental inheritance (HASTINGS 1992; HURST and HAMILTON 1992; LAW and HUTSON 1992) have always considered the case of selfish mutations occurring in organelle genomes; although these mutations can be present at higher frequencies within populations, uniformly deleterious mutations may occur more frequently. Therefore, new insights could be gained from models that consider distributions of mutational effects at the mitochondria and organism levels.

How do these results relate with previous results on the effects of mitochondrial bottlenecks? As we said in the Introduction, KRAKAUER and MIRA (1999) proposed that germline bottlenecks may slow the rate of accumulation of deleterious mutations in mitochondrial genomes, by increasing the genetic variance between cells and thus increasing the effect of selection at the cell level against deleterious mitochondrial mutations. Indeed, in the simulations of BERGSTROM and PRITCHARD (1998), bottlenecks reduce the rate of accumulation of mutations that are deleterious for the organism (which is a single cell in their model, as in ours) and neutral within cells. In our model, we find that bottlenecks decrease the mean frequency of these mutations, but increase their fixation probability (through a reduction in N_e and increased drift). For very low mutation rates, at most one deleterious mutation segregates in the population at any time; in this case, deleterious mutations can accumulate by drift, if selection against them is sufficiently weak (KONDRASHOV 1995). In this case, what determines the rate of accumulation of deleterious mutations is the fixation probability of individual mutations; in our model, bottlenecks would accelerate this accumulation, since they increase the fixation probability of mutations that are deleterious for the cell, but neutral within cells. When selection also occurs within cells, however, bottlenecks decrease the fixation probability of selfish mutants (unless s_m is very small), while they increase the fixation probability of uniformly deleterious mutants, depending on the relative values of s_m and s_c . Under higher mutation rates, several deleterious mutations will coexist at several loci. In this case, what determines the rate of accumulation of mutations is the expected frequency of organisms without mutation in the population (HAIGH 1978; GORDO and CHARLESWORTH 2000). Although our model does not cover that case (as it represents a single locus), the fact that bottlenecks reduce the mean frequency of mutants that are deleterious for the cell goes in the same direction as Bergstrom and Pritchard's results.

Although other life cycles should be investigated using specific models, bottlenecks and paternal transmission

should have similar qualitative effects on within- and between-cell selection and on genetic drift. However, the relative strength of these effects may depend on the details of the life cycle. The method presented in this article can be applied to more complex life cycles, and we have worked on various extensions of our model to represent multicellular life cycles: for example, models that represent organisms in which mitochondria do not replicate during the first cell divisions of the development (as in humans) so that the number of mitochondria per cell decreases to reach a minimum and then increases back. These multicellular models are more complicated, but lead to essentially similar results concerning the effects of bottlenecks and paternal transmission.

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APPENDIX A

We calculate here W_{ij} , the expected number of copies of mitochondrion j in cell i , at the next generation, to the first order in δ . Since we assume that δ is of order $1/n$, it is sufficient to calculate W_{ij} in the limit as population size (n) tends to infinity.

In the following, $p_{(b)}$ and $p_{i(b)}$ denote the frequency of A in the whole population and in cell i , respectively, at the bottleneck stage, while $f_{ij(b)}$ denotes the frequency of copies of mitochondrion ij in cell i , also at the bottleneck stage. We then define $p_{(g)}$ as the frequency of A in the whole population when the number of mitochondria per cell has grown back to N (just before cell selection), while $p_{i(g)}$ is the frequency of A in cell i , and $f_{ij(g)}$ is the frequency of copies of mitochondrion ij in cell i .

The expected number of copies of mitochondrion ij at the next generation is given by

$$W_{ij} = nN \times E[\omega_i f_{ij(g)}], \quad (\text{A1})$$

where ω_i is given by Equation 2. To the first order in δ , this is

$$\begin{aligned} W_{ij} &= N \times E[f_{ij(g)}(1 + s_c(p_{i(g)} - p_{(g)}))] + o(\delta) \\ &= N(E[f_{ij(g)}] + s_c E[f_{ij(g)} p_{i(g)}] - s_c E[f_{ij(g)} p_{(g)}]) + o(\delta). \end{aligned} \quad (\text{A2})$$

Since we want to express W_{ij} to the first order only, it is sufficient to express the last two expectations of Equation A2 when $\delta = 0$ (neutral case). We have

$$E[f_{ij(g)} p_{(g)}] = \frac{1}{N} \times p + O(\delta). \quad (\text{A3})$$

$E[f_{ij(g)} p_{i(g)}]$ is the probability of sampling one copy of mitochondrion ij and one A mitochondrion, after sampling two mitochondria with replacement from cell i , just before selection between cells. With a probability that we call C_1 , these two mitochondria come from the same mitochondrion in cell i (at the beginning of the life cycle); this mitochondrion is mitochondrion j with probability $1/N$, and mitochondrion j is of type A with probability p_{ij} . With probability $1 - C_1$, the two mitochondria come from two different mitochondria of cell i ; in this case, the first is mitochondrion j with probability $1/N$, while the second is A with probability $(Np_i - p_{ij})/(N - 1)$. We therefore have

$$E[f_{ij(g)} p_{i(g)}] = C_1 \frac{p_{ij}}{N} + (1 - C_1) \frac{1}{N} \frac{Np_i - p_{ij}}{N - 1} + O(\delta), \quad (\text{A4})$$

where C_1 is given by

$$C_1 = 1 - \left(1 - \frac{1}{B}\right) \left(1 - \frac{1}{N}\right)^2. \quad (\text{A5})$$

It remains to express $E[f_{ij(g)}]$, to the first order in δ . We have from Equation 1:

$$\begin{aligned} E[f_{ij(g)}] &= E\left[\frac{(1 + s_m p_{ij}) f_{ij(b)}}{1 + s_m p_{i(b)}}\right] \\ &= E[f_{ij(b)}(1 + s_m(p_{ij} - p_{i(b)}))] + o(\delta) \\ &= (1 + s_m p_{ij}) E[f_{ij(b)}] - s_m E[f_{ij(b)} p_{i(b)}] + o(\delta). \end{aligned} \quad (\text{A6})$$

We have $E[f_{ij(b)}] = 1/N$, while the same reasoning as above gives

$$E[f_{ij(b)} p_{i(b)}] = C_2 \frac{p_{ij}}{N} + (1 - C_2) \frac{1}{N} \frac{Np_i - p_{ij}}{N - 1} + O(\delta), \quad (\text{A7})$$

where C_2 is the probability that two mitochondria sampled with replacement in cell i at the bottleneck stage come from the same mitochondrion in cell i before the bottleneck:

$$C_2 = 1 - \left(1 - \frac{1}{B}\right)\left(1 - \frac{1}{N}\right). \quad (\text{A8})$$

Equations A2–A8 lead to

$$W_{ij} = 1 + s_c(p_{ij} - p) + \left(1 - \frac{1}{B}\right)\left[s_m - \left(1 - \frac{1}{N}\right)\right](p_{ij} - p) + o(\delta). \quad (\text{A9})$$

APPENDIX B

To the first order in $1/n$, $V_{\delta p}$ equals the variance of p' (the frequency of A at the next generation), in the neutral case. Since the frequency of A does not change during cell fusion, we have $p' = p_{(c)}$, where $p_{(c)}$ is the frequency of A in the whole population, just before cell fusion. Denoting $p_{i(c)}$ the frequency of A in cell i at this stage, we have

$$\begin{aligned} V_{\delta p} &= \text{Var}\left[\frac{1}{n}\sum_{i=1}^n p_{i(c)}\right] + o\left(\frac{1}{n}\right) \\ &= \frac{1}{n^2}\sum_{i=1}^n \text{Var}[p_{i(c)}] + \frac{1}{n^2}\sum_{i=1}^n \sum_{\substack{j=1 \\ j \neq i}}^n \text{Cov}[p_{i(c)}, p_{j(c)}] + o\left(\frac{1}{n}\right). \end{aligned} \quad (\text{B1})$$

By symmetry, $\text{Var}[p_{i(c)}]$ and $\text{Cov}[p_{i(c)}, p_{j(c)}]$ are the same for all i and j , and we can write

$$V_{\delta p} = \frac{1}{n}\text{Var}[p_{i(c)}] + \left(1 - \frac{1}{n}\right)\text{Cov}[p_{i(c)}, p_{j(c)}] + o\left(\frac{1}{n}\right). \quad (\text{B2})$$

To obtain $V_{\delta p}$ to the first order in $1/n$, we therefore need to express $\text{Var}[p_{i(c)}]$ in the limit when n tends to infinity and $\text{Cov}[p_{i(c)}, p_{j(c)}]$ to the first order in $1/n$. We have

$$\text{Var}[p_{i(c)}] = E[(p_{i(c)})^2] - E[p_{i(c)}]^2. \quad (\text{B3})$$

By symmetry, $E[p_{i(c)}] = E[p_{(c)}] = p$ under neutrality. $E[(p_{i(c)})^2]$ is the probability that two mitochondria sampled with replacement from the same cell, just before cell fusion, are both A . With probability $(1 - 1/N)(1 - 1/B)(1 - r)$, the ancestral lineages of these two mitochondria move to different cells before coalescence has occurred, in which case the probability that both mitochondria are A is p^2 in the limit as n tends to infinity. With probability $1 - (1 - 1/N)(1 - 1/B)(1 - r)$ coalescence occurs, and the probability that both mitochondria are A is p (still as n tends to infinity). This gives

$$\text{Var}[p_{i(c)}] = \left[1 - \left(1 - \frac{1}{N}\right)\left(1 - \frac{1}{B}\right)(1 - r)\right]pq + o\left(\frac{1}{n}\right). \quad (\text{B4})$$

To express $\text{Cov}[p_{i(c)}, p_{j(c)}]$, we proceed as follows. We have

$$\text{Cov}[p_{i(c)}, p_{j(c)}] = E[p_{i(c)}p_{j(c)}] - E[p_{i(c)}]E[p_{j(c)}]. \quad (\text{B5})$$

In the neutral case, $E[p_{i(c)}]E[p_{j(c)}] = p^2$. $E[p_{i(c)}p_{j(c)}]$ is the probability of sampling two A mitochondria, by sampling two mitochondria from different cells just before cell fusion. We can distinguish two cases:

With probability $1 - 1/n$, these two mitochondria come from different cells (of the present generation, at the beginning of the life cycle). In this case, the probability that they are both A is the probability of sampling two A mitochondria from different cells of the present generation (at the beginning of the life cycle); we denote this probability $\overline{p_i p_j}$. Since we need to calculate $E[p_{i(c)}p_{j(c)}]$ to the first order in $1/n$, we need to express $\overline{p_i p_j}$ to the first order in $1/n$. This can be done as follows: the probability of sampling two A mitochondria at the present generation after sampling two mitochondria with replacement is p^2 . With probability $1/n$, both mitochondria are sampled from the same cell, and the probability that they are both A is $p^2 + rpq + O(1/n)$ (Equation 14), while with probability $1 - 1/n$, they are sampled in different cells, and the probability that they are both A is $\overline{p_i p_j}$, giving

$$p^2 = \frac{1}{n}(p^2 + rpq) + \left(1 - \frac{1}{n}\right)\overline{p_i p_j} + o\left(\frac{1}{n}\right) \quad (\text{B6})$$

and therefore

$$\overline{p_i p_j} = p^2 - \frac{1}{n} r p q + o\left(\frac{1}{n}\right). \quad (\text{B7})$$

With probability $1/n$, the two mitochondria come from the same cell of the present generation (at the beginning of the life cycle). In this case, it is sufficient to calculate the probability that they are both A in the limit as n tends to infinity. In this limit, the probability that their ancestral lineages coalesce is $1 - (1 - 1/N)(1 - 1/B)(1 - r)$, in which case they are both A with probability p , while with probability $(1 - 1/N)(1 - 1/B)(1 - r)$ they move to different cells before coalescence has occurred, in which case the two mitochondria are A with probability p^2 .

Putting everything together gives

$$\text{Cov}[p_{i(c)}, p_{j(c)}] = \frac{1}{n}(1 - r) \left[1 - \left(1 - \frac{1}{N}\right) \left(1 - \frac{1}{B}\right) \right] p q + o\left(\frac{1}{n}\right). \quad (\text{B8})$$

And finally, Equations B2, B4, and B8 lead to

$$V_{\delta p} = \frac{1}{n} \left[1 + (1 - r) \left[1 - 2 \left(1 - \frac{1}{N}\right) \left(1 - \frac{1}{B}\right) \right] \right] p q + o\left(\frac{1}{n}\right). \quad (\text{B9})$$