Regular bottlenecks and restrictions to somatic fusion prevent the accumulation of mitochondrial defects in *Neurospora*

E. Bastiaans, D. K. Aanen, A. J. M. Debets, R. F. Hoekstra, B. Lestrade† and M. F. P. M. Maas

Laboratory of Genetics, Wageningen University, Druivenlandsesteeg 1, Wageningen 6708 PB, The Netherlands

The replication and segregation of multi-copy mitochondrial DNA (mtDNA) are not under strict control of the nuclear DNA. Within-cell selection may thus favour variants with an intracellular selective advantage but a detrimental effect on cell fitness. High relatedness among the mtDNA variants of an individual is predicted to disfavour such deleterious selfish genetic elements, but experimental evidence for this hypothesis is scarce. We studied the effect of mtDNA relatedness on the opportunities for suppressive mtDNA variants in the fungus *Neurospora* carrying the mitochondrial mutator plasmid pKALILO. During growth, this plasmid integrates into the mitochondrial genome, generating suppressive mtDNA variants. These mtDNA variants gradually replace the wild-type mtDNA, ultimately culminating in growth arrest and death. We show that regular sequestration of mtDNA variation is required for effective selection against suppressive mtDNA variants. First, bottlenecks in the number of mtDNA copies from which a ‘Kalilo’ culture started significantly increased the maximum lifespan and variation in lifespan among cultures. Second, restrictions to somatic fusion among fungal individuals, either by using anastomosis-deficient mutants or by generating allotype diversity, prevented the accumulation of suppressive mtDNA variants. We discuss the implications of these results for the somatic accumulation of mitochondrial defects during ageing.

1. Introduction

Mitochondria are membrane-enclosed organelles with an ancient endosymbiotic origin, of an α-proteobacterium in an archaeabacterial host [1,2]. Although most mitochondrial genes have been transferred to the nuclear genome, the mitochondria have retained a small genome separate from the nuclear DNA, the mtDNA. Cells typically contain many mitochondria, and each mitochondrion has multiple copies of mtDNA, resulting in hundreds of mtDNA copies per cell. As the replication of mtDNA and its segregation during mitotic and meiotic division are not under strict control of the nucleus, mtDNA variants with an intracellular selective advantage (e.g. by a replication benefit) can increase in frequency even if these decrease individual-level fitness [3]. Such selfish mitochondrial mutants are well known from yeast [4]. With a frequency of about 1%, yeast cells mutate to form mini colonies containing far fewer cells than the wild-type. The petite phenotype is caused by mtDNA mutations giving a replication benefit to the mutated mtDNA at the cost of reduced respiration. Therefore, mutated mtDNA molecules impose a cost to cell fitness, and petite strains are selected against in competition with wild-type cells.

Selection within cells and among individuals (or cells) can thus act in opposite directions, creating a genetic conflict [4,5]. Selection between individuals favours functional mtDNA genomes, whereas selection within cells may favour selfish, less functional, variants. Various mechanisms that maintain functional mtDNA by selecting against dysfunctional mtDNA variants have been identified. For example, uniparental transmission of mtDNA binds the fate of mtDNA with a single host lineage and restricts the opportunities for selfish mtDNA variants by...
preventing ‘infection’ of other lineages [6–8]. Furthermore, an mtDNA bottleneck during egg cell formation has been shown in mice [9–11], redistributing mtDNA variation from within cells to among cells, and facilitating selection among individuals [12], as well as purifying selection in the germline [13]. A unifying characteristic of these different mechanisms is that they increase the genetic relatedness among the mtDNAs within individuals. With relatedness among mtDNAs, we refer to the genetic similarity of mtDNA copies relative to that expected from random combinations of mtDNA copies in the population where competitive interactions occur [14,15]. High within-individual relatedness exposes the effect of mtDNA mutations to selection at the level of the individual, facilitating efficient selection against selfish mtDNA variants [16,17].

Although there is substantial circumstantial evidence for the significance of high relatedness among the mtDNA within an individual, direct evidence supporting this hypothesis is scarce [4,18]. In this paper, we experimentally study the significance of high relatedness among the mtDNAs within an individual for selection among individuals against dysfunctional selfish mtDNA variants. As a model system, we use mtDNA-related ageing in the fungal genus Neurospora. In these fungi, fitness can easily be measured [19], and the syncytial nature of these fungi, the possibility of fusion between individuals and restrictions to such fusion by fusion-deficient mutants and allorecognition, allow us to manipulate the levels of selection acting on mtDNA.

(a) Neurospora as a model system for studying levels of selection acting on mtDNA

Like many other modular organisms, the filamentous ascomycetes Neurospora crassa and Neurospora intermedia reproduce both sexually and asexually. Individuals normally develop from a single cell. In the sexual cycle, they develop from a uninucleate ascospore and in the asexual cycle from a multinucleate macroconidium. New individuals may, however, also develop from multiple cells, for example from a number of macroconidia that fuse upon germination. Upon germination, spores form specialized hyphae called germ tubes, but also form anastomosis or fusion tubes [20]. The former differentiate into the vegetative hyphae of a fungal colony, whereas the latter fuse with other tubes or hyphae (figure 1a) [21]. The hyphae of a growing colony constantly branch and fuse, ultimately generating an intricate network of hyphae called the mycelium, which makes up the fungal body. The mycelium is compartmentalized by septa, but cytoplasm and even nuclei can pass the septa through pores, so that the mycelium effectively is a syncytium. Hyphal fusion is especially important for communication and homeostasis within the mycelium [22]. However, between genetically different individuals, fusion usually is heavily restricted. A single genetic difference at any of several recognition loci results in compartmentalization and death of the fused hyphae. This form of allorecognition is generally known in fungi as somatic or heterokaryon incompatibility [23,24].

PKALILO (pKAL) is a linear mitochondrial plasmid found in the natural population of Neurospora species. It acts as an insertional mutagen and is associated with a senescence syndrome [25,26]. Like almost all other fungi, Neurospora strains without the plasmid do not show senescence, meaning that their vegetative growth potentially is unlimited, but strains carrying the plasmid have a limited lifespan. In senescing cultures, the plasmid inserts into the mitochondrial genome, and it is often found at sites located within or near the mitochondrial rRNA loci. For yet unknown reasons, mtDNA molecules carrying an integrated copy of pKAL are ‘suppressive’, i.e. they accumulate during growth, gradually replacing the wild-type molecules [27]. Isolates carrying the mutator plasmid thus become deficient in functional mitochondrial ribosomes and ultimately die. Essentially, this means that the mutated mtDNA has become a selfish element that is selected, even though it comes at a cost for the multicellular mycelium at a higher level of selection.

Senescence in Neurospora can be demonstrated using ‘race tubes’ containing long slants of agar, where survival is measured in millimetres of growth or days of growth. Alternatively, it can also be demonstrated by serially transferring large numbers of conidia. After a certain number of subcultures, usually within a matter of weeks, strains that carry the pKAL plasmid can no longer be propagated, in contrast to strains not carrying the pKAL plasmid, which do not show senescence at all, even after many sub-cultures. The pKAL plasmid can be seen as a truly parasitic element and presumably survives by virtue of its ability to spread horizontally. Though the system of vegetative incompatibility is effective most of the time, sometimes it does not completely prevent cytoplasmic exchange. Transmission of pKAL can thus sometimes be observed even between somatically incompatible isolates [28]. Transmission of the senescent state has, however, never been observed between somatically incompatible isolates, while this easily occurs between compatible isolates. This shows that allorecognition effectively prevents the exchange of mtDNAs. Furthermore, during the formation of sexual spores, ‘rejuvenation’ occurs, meaning that suppressive mtDNAs are not transmitted to the sexual spores [27,29].

(b) This study

Here, we systematically assess the significance of relatedness among mtDNA within individuals for the opportunities of dysfunctional selfish mtDNAs causing senescence. The mutagenic action of the linear plasmid pKAL causes variation among mtDNAs resulting in lowered genetic similarity among the mtDNAs of a single mycelium. We manipulated mtDNA relatedness within mycelia by manipulating the bottleneck size of transferred conidia and the degree of fusion, using mixtures of incompatible strains and fusion mutants. We show that high among-mtDNA relatedness within individuals facilitates selection among individuals against dysfunctional selfish mtDNAs.

2. Material and methods

(a) Strains and culturing conditions in test for the effect of somatic bottleneck size and defective fusion

For testing the effect of bottleneck size, a N. crassa strain with standard Oak-Ridge genetic background was used, in which the prototypic pKAL plasmid was introduced via transient anastomosis and several rounds of introgression. To test the effect of defective fusion, ham-2KIP cultures deficient in hyphal fusion [30] were obtained by crossing the ham-2KIP null allele (provided by C. Rasmussen) into a ‘Kahilo’ background, using the

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pKAL-carrying partner as the female and the ham-2RIP partner as a male. Crosses were done at 25°C, on Westergaard’s medium (WM), according to the protocol given by Davis & deSerres [31]. The progeny was screened for the presence of pKAL using polymerase chain reaction (PCR) as described further on.

Senescence was tested by serially sub-culturing the isolates in small tubes containing 1.3 ml of solid minimal medium (VMM), as previously described by Griffiths & Bertrand [29], or by growing them in 50 cm long glass race tubes containing 18 ml VMM, at 25°C. To control for inoculum size, spores were harvested by adding water to the cultures and suspending the spores by vortex mixing. Then the spore densities of the obtained spore suspensions were estimated by counting, using a haemocytometer. With these estimates, the spore suspensions were diluted to obtain appropriate inoculum sizes (of 10^7–10^8 spores/10 μl). From these suspensions, 10 μl was transferred to a tube with fresh solid medium.

(a) A large inoculation size leads to a relatively low relatedness among the mitochondria within a single mycelium, increasing the probability that suppressive mitochondria are present in the mycelium, which will cause senescence of the culture. By contrast, a smaller inoculation size creates a bottleneck and increases relatedness among the mtDNAs of a single mycelium, reducing the probability that suppressive mitochondria will be in the culture. (b) Successful fusion between conidia allows the exchange of mitochondria and will transmit the suppressive mitochondria from any of the conidia with suppressive mitochondria to the entire mycelium. Restriction to fusion prevents exchange of mitochondria, creating an effective bottleneck of one conidium. Selection between mycelia growing from one spore will now disfavour mycelia grown from conidia with suppressive mitochondria. Fusion between mycelia and germinating spores can be restricted in two ways: using a fusion-defective mutant or by allorecognition.

(b) Strains and culturing conditions in the test of the effects of allorecognition

To test the effects of allorecognition, wild N. intermedia isolates collected from Hawaiian soil samples were used, described earlier by Maas et al. [32]. Samples that contained pKAL and had proved to senesce were selected. The conidia used were taken from serially transferred cultures in an advanced stage of ageing (two transfers before the cultures died). For this experiment, the conidia were harvested in water. The suspensions were used to make the mixed cultures. Then suspensions from mixed strains and from single strains were inoculated by spreading the suspension evenly over the agar surface. All mixed and monocultures were grown as three replicate serial transferred cultures. Cultures were grown in 16 × 100 mm glass tubes containing 3.5 ml VMM solidified under an angle of 60°. Further serial transferring of these cultures was done in a similar way,
as we hypothesized that spreading the conidia ensures that the fusion rates are lowered in the mixed cultures.

(c) Detection of pKALILO
For DNA extraction, cultures were grown in liquid VMM for approximately 24 h at 25°C. Mycelium was harvested, dried between filter paper and ground using liquid nitrogen, followed by a standard phenol/chloroform-based DNA extraction [33]. Prior to the addition of phenol and chloroform, samples were incubated for 1 h at 37°C with proteinase-K (100 μg ml⁻¹ final concentration). Diagnostic PCRs were done using primers located within the open reading frame of the plasmid: 5′-GGT GGA ATC TGT GAG CTA TA-3′ and 5′-TGC ATC TCT CTC TTC AC-3′ [32]. Nested semi-random two-step (ST-)PCRs were done as described earlier [34].

(d) Calculations of the Weibull parameter estimates
Weibull parameter estimates for k and λ were obtained using the Solver tool in Microsoft Excel which uses quasi-Newton methods to minimize the sum of squares but with derivatives replaced by finite differences.

3. Results

(a) The survival rate of senescing ‘Kalilo’ cultures depends on vegetative ‘bottleneck’ size
Using the traditional way of demonstrating senescence in Neurospora (i.e. via the serial transfer of large numbers of macroconidia), pKAL-carrying cultures generally survive for several weeks. The survival of these cultures fits well with a two-parameter Weibull function \( S(t) = \exp(-x/\lambda)^k \) (\( x \) is time to failure, \( \lambda > 0 \) is the scale parameter and \( k > 0 \) is the shape parameter) using an increasing failure rate (i.e. with a ‘Weibull slope’ or ‘shape’ parameter \( k \) progressively decreased (table 2). At the narrowest bottleneck size used, \( k \) no longer differed from one. This means that by narrowing the bottlenecks, the survival function was essentially reduced to an exponential one (i.e. with a hazard rate that is constant over time rather than increasing; figure 2). With these results, we predicted that further reducing the bottleneck size and its frequency would reduce the chance of accumulating suppressive mtDNA variants even more, and thus increase the maximum lifespan. We tested this by reducing the frequency of somatic fusion.

(b) The survival rate of senescing pKALILO cultures depends on somatic fusion
Fusion between the conidia and mycelia in a starting culture brings the mitochondria of different individuals together in a fused individual and thus reduces the relatedness among mtDNA. We argued that, without the possibility to fuse, a culture consists of many separate mycelia each of which goes through a bottleneck of a single conidium during each transfer. Such a small bottleneck maximizes the relatedness among the mtDNAs within individual mycelia, each transfer redistributing new mtDNA variation, arisen via mutation, across spores. This then enables the sequestering of suppressive mtDNA variants in the culture, and selection against them at the level of between-mycelia competition (figure 1b). We therefore tested whether a ham-2RIP strain, which is defective in fusion, would show suppression of pKAL-based senescence.

First, we tested this using race tubes containing long slants of agar; in these cultures, the single-cell bottleneck will occur only once when the culture is started. Lifespan of the ham-2RIP strain was increased about twofold compared with the wild-type ham-2 strain (Wald’s Z = 11.980, d.f. = 1, \( p < 0.001 \); table 3). We hypothesized that the effect of ham-2 inactivation would be larger when using serial sub-culturing tests than when using race tubes: using race tubes, suppressive mtDNA variants would be able to invade the culture from
behind the growth front through cytoplasmic contact, and due to the narrow growth front of a race tube, genetic drift and between-mycelium selection would quickly eliminate the variation between spores of the starting inoculum, even in the absence of fusion (figure 3). Using serial sub-culturing, this would not be the case either. It thus appeared that pKAL was merely tolerated as an autonomously replicating plasmid and that suppressive mtDNA variants either did not arise, or, more probably, that they arose but were highly effectively selected against.

Using ST-PCR, we tested whether pKAL was lost in the surviving ham-2RIP cultures after 25 transfers. This was not the case. Using PCR, we tested whether pKAL was lost in the surviving ham-2RIP cultures after 25 transfers. This was not the case.

(c) Allorecognition effectively protects against pKALILO-driven senescence

Allorecognition potentially has a similar effect as reduced fusion rates. If conidia from cultures of different allotypes are mixed, they will have a reduced chance of successfully fusing to neighbouring conidia. Only if neighbouring conidia are from the same allotype successful can fusion occur (figure 1b). The more allotypes present in a culture, the lower the probability that neighbouring conidia are of the same allotype, so that the rate of fusion is inversely related to the number of allotypes present in a culture. With this in mind, we hypothesized that if multiple allotypes are present in a well-mixed culture, senescence could be postponed and even be prevented if sufficient allotypes were present, just as effective as in the ham-2RIP cultures.

We found a clear effect of allotype diversity in a culture on senescence. In an experiment using cultures that were near the end of their lifespan, we found that, with one exception, almost all monocultures aged after two or three more transfers. By sharp contrast, senescence was effectively delayed in cultures consisting of minimally three different allotypes (figure 4). Cultures consisting of a mix of three or more allotypes survived significantly longer than the monocultures of the strains used in these mixes (Mann–Whitney U-test \( p < 0.05 \)). Four out of nine of these mixed cultures still lived after 18 transfers when we stopped the experiment.
sub-culturing: within the time frame of the experiment (approx. three months), we did not observe any stoppers among the serially sub-cultured
ham approximately twofold longer than wild-type strains (Wald’s
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Neurospora as a model system.
selection against selfish mtDNAs using the ascomycete genus
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gate, and leads to a conflict between two levels of selection.
The replication and segregation of mtDNA is not under strict
control of the nuclear DNA. This provides opportunity to self-
ish mtDNA variants that bear a selective advantage within
the cell but a deleterious effect on organisinal fitness, to propa-
gate, and leads to a conflict between two levels of selection.
We experimentally addressed the importance of genetic relat-
edness among the mitochondria of a single mycelium for
selection against selfish mtDNAs using the ascomycete genus
Neurospora as a model system.

4. Discussion
The replication and segregation of mtDNA is not under strict
control of the nuclear DNA. This provides opportunity to self-

(a) High relatedness among mtDNA facilitates selection
against selfish mtDNA
First, we varied relatedness among mitochondria by the
number of conidia inoculated in serially transferred cultures.
An mtDNA bottleneck resulted in more variation in lifespan
and a longer maximum lifespan. We then effectively decreased
the bottleneck to a single conidium by using a fusion-defective
mutant, so that conidia and mycelia cannot fuse. This preven-
ted senescence in serially transferred cultures altogether.
Relatedness among mitochondria within starting mycelia is
maximized this way, and the suppressive mtDNA variants
are sequestered and selected against due to their deleterious
effect on mycelial fitness (figure 1). However, we showed that
this fusion mutant dsl senesce in a race tube. In a race tube,
there is only a single bottleneck during inoculation (figure 3).
During subsequent growth, no further bottlenecks occur, and
thus no further sequestration of different mtDNA variants is
possible. Owing to genetic drift and selection at the level of
the mycelium, we expect a loss of individuals, so that ultimately
within-mycelium selection in the initially most successful
individual will dominate, which then will senesce (figure 3).
Finally, we maximized relatedness among mtDNAs by
allorecognition in a similar way as defective fusion does. In a
situation where multiple allototypes occur together, neighbour-
ing mycelia will often be of different allotype and thus not
successfully fuse. We show that mixing at least three different
allootypes in a serial culturing experiment effectively delays
senescence. An interesting question for follow-up research is
whether allotype diversity is maintained during the exper-
iment, in which case the culture would be protected against
senescence. Alternatively, owing to drift and/or positive fre-
quency-dependent selection, allototypes are lost, in which case
we will expect only a delay in senescence. We detected a
large phenotypic instability during the extended life of the
mixed cultures. A culture sometimes seemed to be almost
aged while after one or more transfers this culture appeared

Figure 3. Schematic of mycelial growth of a fusion mutant in a race tube. A culture starts (on the left) with the inoculation of multiple conidia. These will form
multiple mycelia competing at the growth front. Owing to drift and selection for fast growth, the number of mycelia will decrease until only a single mycelium will
be left at the growth front. At this point, there will only be selection among the mitochondria within this mycelium. (Online version in colour.)

Table 3. Effect of hyphal fusion on the survival of ‘Kalilio’ cultures, using two different culturing methods. On race tubes, fusion-defective ham-2RIP strains live
approximately twofold longer than wild-type strains (Wald’s Z = 11.980, d.f. = 1, p < 0.001). This difference is much more pronounced when using serial
sub-culturing: within the time frame of the experiment (approx. three months), we did not observe any stoppers among the serially sub-cultured ham-2RIP
lines.

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Figure 4. Bar diagram showing the effects on lifespan of allotype diversity in
pKAL containing cultures that have been serially transferred. Cultures consisting
of a mix of two, three, four or seven allototypes are compared to cultures
that contain the same allotypes in monoculture. Survival is defined as
number of transfers made from the start of the experiment until the culture
died. Significant differences between mixed cultures and monocultures are
indicated by an asterisk (Mann–Whitney U-test, p < 0.05, N = 3); error
bars: ± 2 s.e. Treatment: dark grey denotes mixed allotypes, light grey,
mono cultures.
two levels of selection, viz. among mycelia and among mtDNA within mycelia.

A likely explanation for the difference in shape between the highest and the lowest inoculum sizes is the potential number of suppressive mtDNAs. A large inoculum increases the probability that the inoculum contains a suppressive mtDNA variant. The frequency of this suppressive mtDNA will initially be small since it finds itself in a large inoculum but will gradually increase during the serial transfers until the culture becomes unviable. With a bottleneck genetic drift becomes more important. On the one hand, the probability that the inoculum contains a suppressive mtDNA variant is smaller. This explains why the maximum lifespan is much higher with a bottleneck. On the other hand, however, if the inoculum contains a suppressive mtDNA variant, it will have a higher starting frequency. As a consequence, senescence will then take place earlier in life. This explains why the minimum lifespan is reduced for cultures transferred with a small bottleneck.

These results provide experimental evidence for the importance of high relatedness among mtDNAs in an individual or cell to prevent the invasion of selfish mtDNA variants. In this system, a regular single-celled bottleneck is sufficient to protect individuals against the invasion by suppressive mtDNA that are constantly generated by the mutagenic action of the KALILO plasmid. If suppressive mtDNA is present in an individual it will increase in frequency during growth because of the selective advantage of this mtDNA within the individual. Many cells with multiple mtDNA copies per cell will be formed during the formation of single-celled dispersal units. The frequency of suppressive mtDNA will vary among these cells, resulting in high genetic similarity among the mtDNA copies within individuals originating from these spores relative to genetic similarity between these individuals. The resulting high within-mycelium relatedness among mtDNAs creates the opportunity for selection against suppressive mtDNAs at the level of the individual.

**MtDNA evolution in fungi**

The cells of the mycelium of *Neurospora* are incompletely closed and allow the passage of cytoplasm and even nuclei, which results in efficient exchange of mtDNA throughout an individual. This syncytial nature, also found in other filamentous fungi, increases the opportunities for suppressive mtDNA to spread through an individual. However, in nature, *Neurospora* will go through a regular bottleneck of one cell or sometimes a small group of cells during both the vegetative and the sexual stage of its life cycle. Furthermore, during sexual reproduction rejuvenation of the sexual spores occurs [27,29]. A recent study shows that also in yeast sexual reproduction reduces the frequency of respiratory-deficient mtDNA variants [18]. A possible explanation is that meiosis requires respiration, providing a selective sieve against respiratory-deficient mtDNA variants [18].

MtDNA-induced senescence has been found in a few other fungi [35]. The evolution of senescence in fungi has been explained by their ecology: senescence usually occurs in species from ephemeral substrates, such as herbivore dung [35]. The ecology of *Neurospora* is consistent with this pattern, as strains with the KALILO plasmid have been isolated from sugar cane fields in Hawaii. On this island, fields are burnt yearly, extinguishing all mycelia and asexual spores, while triggering the germination of the sexual spores. External mortality causes thus limit the lifespan of the vegetative state in *Neurospora* in this habitat, reducing the power of natural selection with age, thus ’allowing’ the evolution of senescence [36,37].

**The benefits and risks of somatic fusion**

Our results demonstrate that fusion between mycelia increases the possibilities for selfish mtDNA variants and thus poses a high risk to a mycelium. Fusion-deficient mutants are protected against selfish mtDNA variants and one could therefore ask why natural selection has not favoured such variants in nature. The reason is probably that fusion also provides benefits. For example, fusion of conidia increases the growth speed of a starting mycelium [38] and under conditions of high density, fusion results in a higher conidia yield [39]. Furthermore, the fusion mutant has a much lower spore production, indicating the importance of hyphal anastomosis within a fungal individual [30].

However, somatic fusion carries risks because it provides opportunities to selfish genetic elements. Successful fusion in fungi is severely restricted by allorcognition. We show that allorcognition can prevent mitochondria-related ageing, suggesting that this may be an adaptation to prevent diseases caused by cytoplasmic selfish genetic elements [40]. *Vice versa*, cytoplasmic selfish genetic elements may contribute to the evolutionary stability of allorcognition [41]. However, the balance between selection favouring allorcognition and selection favouring fusion remains to be established [42,43].

**MtDNA evolution in other organisms**

In contrast to fungi, in most animals the opportunities for somatic fusion are much more restricted. Only in the sexual stage can gametes fuse, and unrelated mtDNAs be united in a single zygote. However, the vast majority of sexually reproducing organisms have uniparental transmission of mtDNA [8,44], maintaining high relatedness. Furthermore, regular single-celled bottlenecks, usually in combination with an additional bottleneck for mtDNA [9,10], facilitate efficient selection against suppressive mtDNA.

Nevertheless, some aspects of human mitochondrial diseases are consistent with the existence of conflicts between levels of selection [4]. Especially during somatic growth, mtDNA mutations may occur and accumulate within tissues and contribute to ageing. Tissues may differ in the relative importance of within-cell selection among mtDNA variants versus among-cell selection. Long-lived cells that undergo few divisions and/or cells with high energetic demands that require large amounts of mitochondria are predicted to be most sensitive to accumulating deleterious mitochondrial mutations. Indeed, mtDNA abnormalities are most frequently found in tissues, such as brain, heart and muscle [45,46]. The model system explored in this study may be a good model for approaching mtDNA-related diseases as an evolutionary problem of conflicts between the levels of selection.

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**Data accessibility.** The raw data are deposited in datadryad (www.data dryad.com) under doi:10.5061/dryad.5s67r.

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