# Changes to the mtDNA copy number during the course of yeast culture growth

Ben Galeota-Sprung, Amy Fernandez, Paul Sniegowski

## Abstract

The yeast mitochondrial network is dynamic, with frequent fusion and fission events among mitochondria. Notable changes in mitochondrial morphology and function accompany changes in physiological state. While differences in mtDNA copy number among strains and growth conditions have been observed, it has not been previously shown that the mtDNA copy number changes substantially over time in a clonal growing culture. Here we show that there are fairly dramatic (3x-5x) changes to the mtDNA copy number per cell in growing cultures of *Saccharomyces cerevisiae*, on a timescale of hours to days. While these changes appear to be connected to the shift from fermentation to respiration, it is possible that they are more simply associated with changes in growth rate. Our work suggests that the mtDNA copy number is a dynamic phenotype, a finding that has important implications for studies of the regulation of mtDNA copy number.

## Introduction

Mitochondria are membrane-enclosed organelles, present in nearly all eukaryotic organisms, that originate from an ancient symbiosis (Gray, 2017; Sagan, 1967). Mitochondria enable a variety of capabilities, the most important of which is the production of ATP via aerobic respiration. To a varying degree across the eukaryotic tree of life, most originally mitochondrial genes have been assimilated into the nuclear genome (Malina et al., 2018), but in nearly all cases an independent mitochondrial genome (mtDNA) is maintained within the mitochondria. Generally there are multiple copies of mtDNA per individual cell. For example, in humans, there are hundreds to thousands to tens of thousands of mtDNA copies per cell with considerable variation between tissue type (D'Erchia et al., 2015; Dimmock et al., 2010; Miller et al., 2003; Wai et al., 2010) as well as substantial inter-individual variation (Kaaman et al., 2007) in copy number. Deficiency in mtDNA copy number leads to or is associated with a variety of human disease phenotypes (Elpeleg et al., 2002; Kornblum et al., 2013; Pyle et al., 2016; Zeviani & Di Donato, 2004).

The mtDNA copy number in the yeast *Saccharomyces cerevisiae* is thought to vary somewhat by strain (Miyakawa, 2017). An early study (Hall et al., 1976) found that 16-25% of all cellular DNA is mitochondrial; given an mtDNA genome of 75-85 kbp and a nuclear genome of ~12 Mbp, this suggests a copy number of ~25 to ~50 per haploid genome, a range generally borne out by subsequent studies, though copy numbers as high as 200 have been observed in diploids (Miyakawa et al., 2004). In yeast (as in other organisms) mtDNA is packed into protein-DNA complexes call nucleoids (Miyakawa, 2017; Westermann, 2014; Williamson, 2002; Williamson & Slonimski, 1976). These contain mostly linear mtDNA in polydisperse (i.e. not discrete) linear tandem arrays containing on average one to two copies of the mitochondrial genome (Freel et al., 2015; Maleszka et al., 1991), though under anaerobic conditions there may be many fewer nucleoids with many more mtDNA per nucleoid (Chen & Butow, 2005). Two recent studies have found, by whole-genome sequencing (WGS) or qPCR approaches respectively, about 20 mtDNA copies per haploid cell, and up to roughly threefold variability introduced by various gene deletions (Göke et al., 2019a; Puddu et al., 2019).

The number and arrangement of mitochondria within the yeast cell is highly dynamic. Mitochondrial fusion and fission events occur very frequently (Simon et al., 1997; Jakobs et al., 2003; Fehrenbacher et al., 2004), and rather than a collection of distinct mitochondria, a single, branched mitochondrial network is the dominant conceptual paradigm. There is generally a “giant mitochondrion” that is much bigger than the others, and the number of spatially distinct mitochondria varies with physiological state, being lowest in rapidly growing cells and highest in stationary phase cells (Stevens 1981). *S. cerevisiae* that are grown with glucose as a carbon source characteristically ferment it into ethanol and then transition into a fully respiring physiology (the “diauxic shift”) upon the exhaustion of glucose supplies. The mitochondrial network undergoes substantial changes during this transition: the total mitochondrial volume as a proportion of cell volume increases greatly (Stevens, 1981; Bartolomeo et al., 2020) and the mitochondrial role transitions from biosynthetic hub to energy generator, reflected in a dramatic shift in the mitochondrial proteome, as well as a general increase in mitochondrial protein synthesis (Bartolomeo et al., 2020).

In this study, we investigate changes in mtDNA copy number over time by sampling repeatedly from growing cultures of *S. cerevisiae*. We interrogate mtDNA copy number change on the scale of hours and days in both a common laboratory strain and a natural isolate, using both qPCR and WGS approaches. We find large (3x-5x) copy number increases as cultures shift from a rapidly growing physiological state to a stationary number of cells, with substantial differences between strains as well as important effects of ploidy. We also confirm the reverse phenomenon—that when slowly growing cells are transferred to fresh media, the mtDNA copy number drops. These results re-contextualize both longstanding observations of changes in mitochondrial volume and morphology as well as more recent findings of changes to mitochondrial protein production as the growth mode changes, and suggest that the mtDNA copy number is a dynamic and plastic phenotype.

## Results

### The mtDNA copy number increases ~4-fold over time in growing cultures

We inoculated replicate cultures by dilution into rich liquid media containing a fermentable carbon source (YPD) and tracked the relative ratio of mtDNA to genomic DNA (mt/gDNA ratio) over time by qPCR (see Methods). We used both a standard laboratory background (W303) as well as a natural isolate. In haploid and diploid laboratory strains, the mt/gDNA ratio increased approximately 4- to 5-fold over the course of ~4 days (Fig. 1, gray). The relative increase in a haploid derived from a wild isolate was somewhat less (~3.5-fold) while a wild diploid strain displayed an initial increase in mt/gDNA ratio followed by a subsequent fall. This wild diploid strain was observed to be a ready sporulator, likely explaining this particular phenomenon (see Discussion).

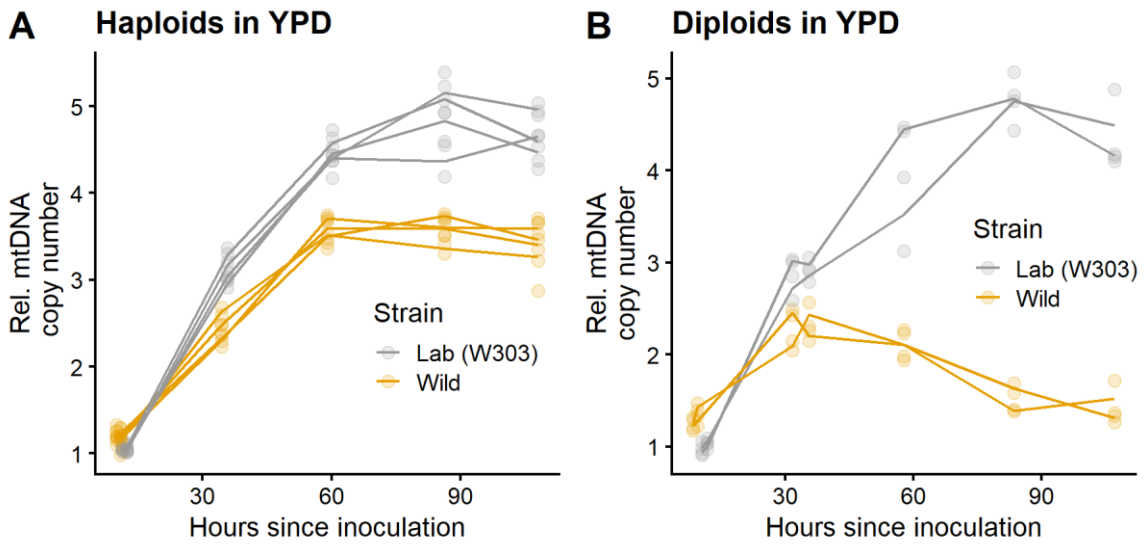


**Fig 1.** mtDNA copy number over time of replicate cultures inoculated into 50 mL YPD of (A) haploid and (B) diploid lab and wild strains. Inoculations were 1/2000 dilutions from saturated cultures. Two replicate qPCR measurements (circles) were performed for each culture at each time point; the lines track the mean of the two measurements. The first extraction was always performed at $A_{600}$ = 0.5 which corresponds to ~3e07 cells/mL in haploid strains and ~2.4e07 cells/mL in diploid strains. Four subsequent extractions were carried out for each culture at approximately 24-hour intervals. qPCR measurements of relative mtDNA copy number were performed via the relative standard curve method (see Methods).

### The timing of the mtDNA copy number increase is associated with a decrease in growth rate

In order to more carefully study the phenomenon of rising mtDNA copy number in a growing culture, we performed a denser set of DNA extractions (17 in total) over a similar period of time, along with corresponding density measurements, from a single growing culture. Characteristically, *S. cerevisiae* grown in rich media grows rapidly while fermenting glucose to ethanol (the Crabtree effect). When glucose is depleted, the culture enters the diauxic shift as the yeast transition from fermentation of glucose to oxidative respiration of ethanol. The rate of growth slows considerably at this point. In this experiment, we observed (Fig 2a) a roughly 4-fold increase in mtDNA copy number, similar to the

results from the experiment displayed in Fig 1a. Inspection of the first ~20 hours of growth (Fig 2b; note the change in slopes) reveals that the slowing of growth rate characteristic of the diauxic shift occurs at about ~14h after inoculation in our growth conditions; this is also when the mtDNA copy number begins to increase. Thus the onset of the increase in mtDNA copy number is temporally associated with the diauxic shift during growth in glucose-containing media.

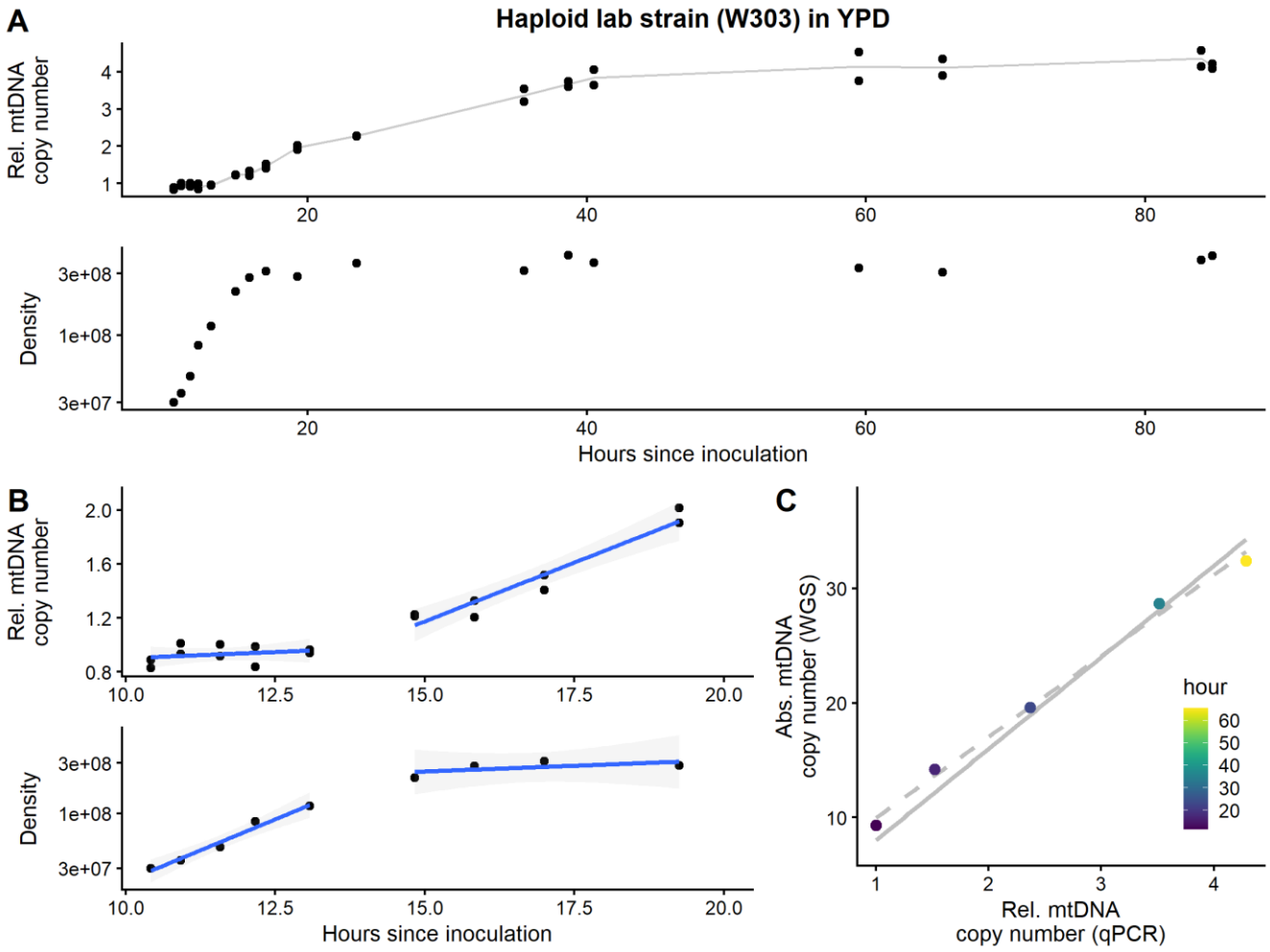


**Fig 2.** (A) As in the experiment shown in Fig. 1, the mt/gDNA ratio rises over time in a culture of the haploid lab strain. (B) The onset of the rise is associated with the slowing of growth as fermentation ends; blue lines are linear regressions of copy number, top, and cell density, bottom. (C) qPCR estimates of the mt/gDNA ratio are well-reproduced by whole genome sequencing (see Methods) although the fold change obtained is a bit less, ~3.5x in sequencing vs ~4.3x qPCR. (The difference is shown by the dotted regression line compared to the solid line which represents a perfectly matched relative increase between the two assays.)

As a control to rule out any effect of total time in culture, we checked that the relative mtDNA copy number would remain at ~1 if a culture was repeatedly transferred while still in fermentation. This indeed was the case: a culture that had been transferred four times consecutively while in fermentation (total growth time of ~60 hours) maintained a relative mtDNA copy number of ~1, as shown in Fig S5.

## The minimum number of mtDNA copies in haploids is about 9 and increases to over 30

Our primary assay for investigating copy number change was a qPCR approach that quantified the mt/gDNA ratio. We also used read depth from WGS in order to both verify our qPCR results with an independent assay and to estimate absolute mtDNA copy number. For 5 of the 17 timepoints for which qPCR assays were performed, a WGS-based assay to determine mtDNA copy number was also carried out (see Methods). Fig 1c displays the results. The two assays correspond quite well although the fold increase is slightly attenuated in the WGS assay compared to the qPCR assay. We found that mtDNA copy number ranges from ~9 during fermentation to >30 in respiration and/or stationary phase, in haploids. Because our qPCR results indicate (Fig 1b) very similar relative mt/gDNA results in the haploid and diploid lab strains, these estimates of mtDNA copy number per cell should be doubled for those diploids. As a control for the WGS assay, we assayed the copy number of rRNA repeats, the CUP1 locus, and Ty transposons, which did not change over time (Fig. S1).

## An mtDNA copy number increase also occurs under non-fermentative conditions

Perhaps surprisingly, we also found that a substantial increase in mtDNA copy number occurs when the lab strain is grown on nonfermentable carbon sources—glycerol or ethanol+glycerol. The final copy number obtained at 100+ hours after inoculation is very similar to that obtained during growth on glucose (compare Fig 3 to Fig 1a), approximately 4- to 5-fold the copy number during rapid fermentative growth. However the first measurement of mtDNA copy number—measured in all conditions at $A_{600}$ = 0.5—is about twice as high as during fermentative growth.

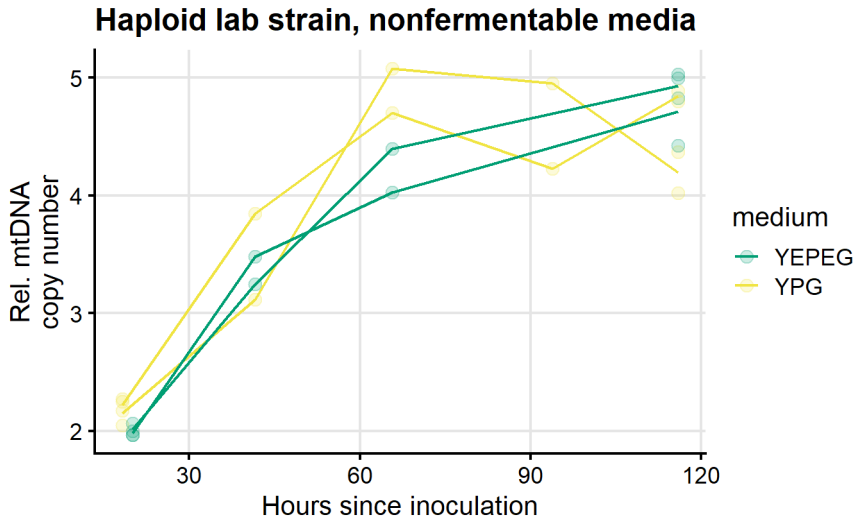


**Fig 3.** The mt/gDNA ratio, assayed by qPCR, for the haploid lab strain when grown with glycerol (green points and line) or ethanol and glycerol (yellow points and line) as the carbon source. Aside from the difference in media, conditions are identical to the experiments shown in Figs 1 and 2. As in the experiment shown in Fig 1, the first measurement was performed when $A_{600}$=0.5.

## The mtDNA copy number drops rapidly upon transfer from stationary phase

The experiment depicted in Fig. 2 closely tracks the rise in mtDNA copy number. To form a fuller picture of mtDNA copy number dynamics, we investigated the time scale of the decline in mtDNA

copy number that must by implication occur after dilutive transfer from a saturated culture to fresh media. To do so, we transferred a relatively large number of stationary cells (enough that subsequent extractions could be easily performed) to fresh YPD and began regular extractions immediately. The results of this experiment are displayed in Fig 4. The expected drop occurred fairly rapidly, mostly taking place between 1 and 4 hours after transfer, or about 2-3 generations. These observations are consistent with those obtained by (Williamson & Moustacchi, 1971)). Notably, the fall in mtDNA copy number halts before the growth rate slows, suggesting that the rate of mtDNA replication, after an initial lag, catches up to gDNA replication while the cells are still in rapid fermentative growth.

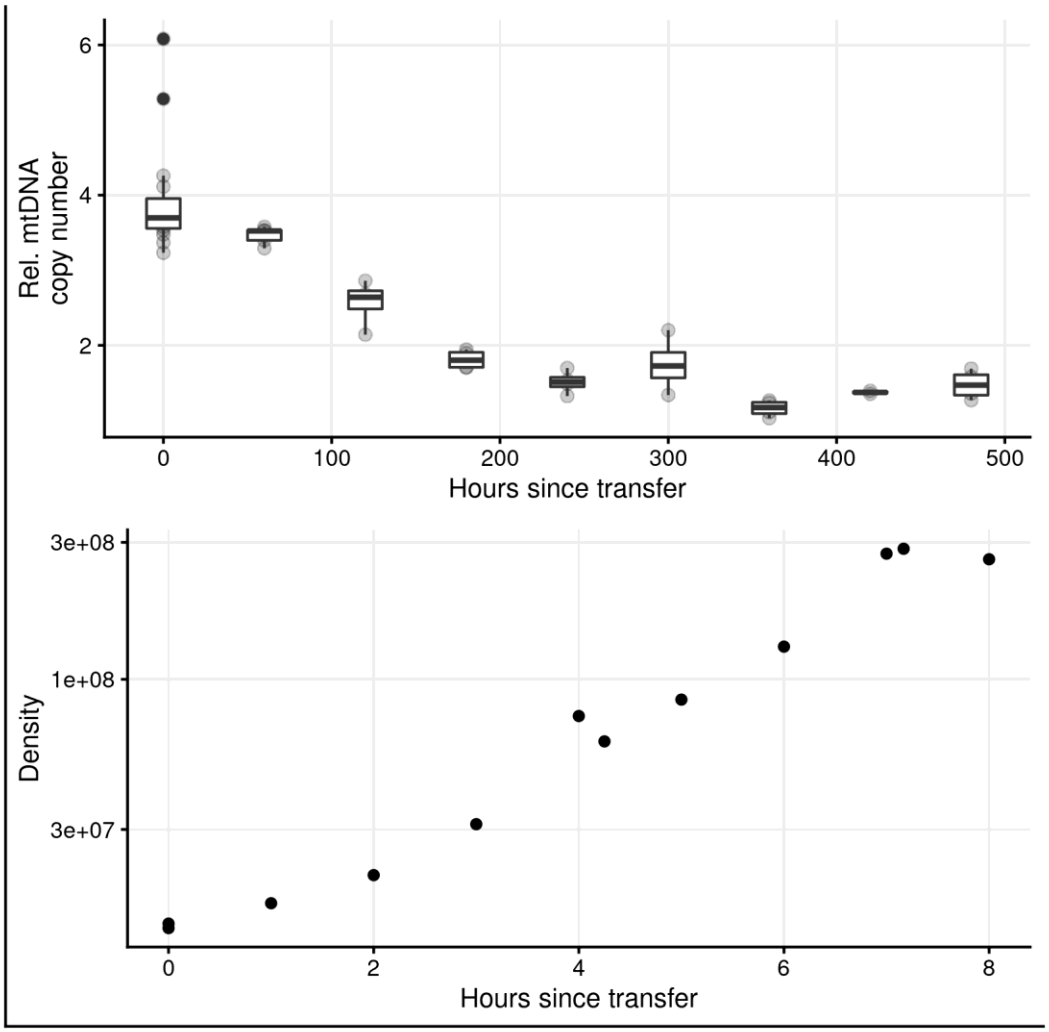


**Fig 4.** A saturated culture of the haploid lab strain was transferred, diluting 1/30, to fresh YPD. The relatively mild dilution (1/2000 dilutions were performed in all other experiments) permitted DNA extractions of small portions of the culture to begin immediately after transfer. Extractions (for subsequent qPCR) and density measurements were performed at 1-hour intervals for 8 hours.

## Discussion

It has long been recognized that the mitochondrial architecture in yeast is highly dynamic, with both fusion and fission events among mitochondrial structures occurring regularly. Yeast cultures undergo substantial physiological changes during growth, most notably, for Crabtree-positive yeasts, shifting from fermentation to respiration when grown on a fermentable carbon source such as glucose. During this physiological shift, the appearance of the mitochondrial network changes, as does the total mitochondrial volume (both proportional and absolute), the total rate of production of mitochondrial proteins, and the composition of the mitochondrial proteome. Here we show that, during these changes, the number of copies of the mitochondrial genome per nuclear genome does not remain fairly constant, but instead varies by about fourfold (in a lab strain, slightly less in a wild strain), with the copy number lowest during rapid fermentative growth and increasing thereafter. The magnitude of this change is surprising. For example, a previous study found a ~30% difference in mtDNA copy number between aerobic and anaerobic conditions (Miyakawa et al., 2004).

It is tempting to posit that because the capacities of mitochondria are most crucial during respiration, production of mitochondria is deprioritized during fermentation, accounting for the phenomenon described here, and that production of more mitochondrial genomes is deferred until they are most needed. Indeed the data bear this out in the sense that when grown under nonfermentative conditions (i.e. with glycerol or glycerol and ethanol as the carbon source) the mtDNA copy number is not as low as during growth in glucose (compare Fig 4 to Figs 1 and 2a). However, the mtDNA copy number also increases fairly sharply over time in nonfermentative conditions (Fig 4). Thus the diauxic shift from one physiological state to another is at best an incomplete explanation for the phenomena observed here.

In *S. cerevisiae*, mtDNA replication is not linked to the cell cycle (Miyakawa et al., 2004; Williamson & Moustacchi, 1971). Therefore, the rate of cellular or genomic growth (i.e. the rate of budding) is not necessarily coupled to the rate of mtDNA production in the short term. A toy model that can recapitulate both the rise and fall in mtDNA copy number observed here is that changes in mitochondrial growth rate always lag behind changes in cellular growth rate. Under this model, when cells are transferred from conditions of slow growth or stationary phase to fresh media, the genomic growth rate initially increases more rapidly than the mitochondrial growth rate, which causes the mtDNA copy number to fall. The mitochondrial growth subsequently catches up, which stabilizes the mtDNA copy number. When the cellular growth rate begins to slow as resources become exhausted, the mitochondrial growth rate does not immediately slow, causing the mtDNA copy number to increase. An example of such a model is shown in Fig. 5: the genomic and mitochondrial growth rates alternate between two arbitrary values (reflecting, for example, fermentation and respiration), with changes to the mitochondrial growth rate change always lagging changes to the genomic growth rate. This causes a cyclical fluctuation in mtDNA copy number. Under this model, the increases (Figs 1, 2) and drops (Fig 4) in mtDNA copy number that we observed are the rising and falling aspects of the full cycle that would occur with repeated serial transfers. Why such a lag should occur—whether, for instance, the lag is itself adaptive or instead an example of natural selection producing a result that is “good enough”—is open to further investigation. One potential adaptive explanation is connected to the two modes of reproduction in yeast. Asexual reproduction (budding) is associated with rapid growth, while sexual

reproduction (sporulation) tends to occur when environmental conditions become challenging and growth ceases. During sporulation, mitochondria are partitioned into four offspring, but only a little more than half of the total mtDNA makes it into one of the four ascospores (Brewer & Fangman, 1980). Thus a sporulating diploid requires a high mtDNA copy number in order for each of its four haploid offspring to possess an adequate number of mtDNA copies.

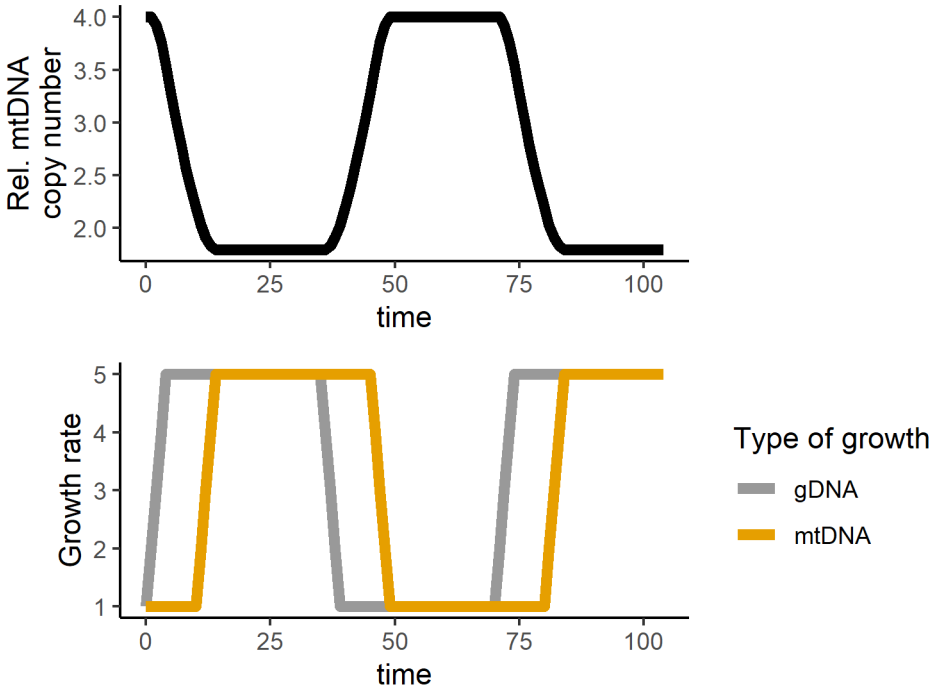


**Fig 5.** A simple model that could explain the patterns observed. Both cellular (i.e. gDNA) and mitochondrial growth rates alternate between the same two values, but changes to the mtDNA growth rate lag behind changes to the gDNA growth rate. This causes an increase in the relative mtDNA copy number when the cellular growth rate slows, and a decrease in mtDNA copy number when the cellular growth rate increases. Simulation code for this model is provided in the online data supplement.

It appears that sporulation has a significant effect on mtDNA copy number relative to genomic copy number: our wild diploid strain, which is a ready sporulator (microscopic inspection revealed that the cultures were dominated by ascii by the end of our experiments), displayed very different mtDNA copy number dynamics (Fig 1b) than the lab diploid strain, which does not sporulate readily, and which behaved essentially identically to the lab haploid strain. We speculate that this is because the single genome doubling that occurs in meiosis is not accompanied by a corresponding increase in mtDNA copy number (Brewer & Fangman, 1980); thus the mt/gDNA ratio falls by a factor of ~2 as a result of meiosis.

A recent paper (Göke et al., 2019) systematically investigated the effect of all viable gene deletions on mtDNA copy number, and found that mutants that increased cell size tended to increase mtDNA copy number, probably because mitochondrial network length correlates with the number of nucleoids (Osman et al., 2015). Yet, from fermentation to respiration, cell volume decreases (Bartolomeo et al.,

2020), suggesting that cell size changes are unlikely to be causative in the mtDNA copy number rise we have observed.

Our findings show that mtDNA copy number is a dynamic phenotype in which the average copy number per cell depends on physiological state and culture time. This suggests that differences in mtDNA copy number between different lab strains or wild isolates, or between ancestral and evolved strains in evolution experiments, should be investigated carefully, ideally with time-series data, to ensure that apples-to-apples comparisons are made, especially between strains with difference growth rates. One limitation of our work is that we have measured the mean copy number per cell as a bulk property of the culture. Future work might examine the distribution of mtDNA copy number per individual cell over time in order to more fully elucidate the manner in which these changes occur.

## Methods

### Strains

yJHK112, a haploid, prototrophic, heterothallic, MATa, BUD4-corrected, and ymCherry-labeled W303 strain (Koschwanez et al., 2013) was used as the haploid lab strain in this paper. We created the diploid lab strain from this strain by transforming (Gietz and Schiestl 2007) the haploid lab strain with plasmid pRY003, temporarily providing a functional HO locus allowing mating type switching and subsequent mating. pRY003 was a gift from John McCusker (Addgene plasmid #81043; http://n2t.net/addgene:81043; RRID:Addgene_81043). The ploidy of the resulting strain was confirmed by (1) ability to produce tetrads after plating to sporulation media; (2) by flow cytometry for total genomic content; and (3) by the presence of a PCR product for both the *MATa* and *MATα* loci. YPS623, the wild diploid strain used in this paper, was isolated from Mettler's Woods, NJ as described in (Sniegowski et al., 2002). YPS 2066, the haploid wild strain used in this paper, originated from an ascospore of YPS623, isolated by microdissection, and subsequently made heterothallic by knocking out the *HO* locus.

### Growth conditions

All growth was carried out in liquid media in Erlenmeyer flasks kept at 30C and shaken at 200 rpm. An initial growth cycle was carried out in 6 mL media in a 50 mL flask. Unless otherwise noted, all experiments described in this paper were carried out in 50 mL media in a 250 mL flask. Except for the experiment described in Fig. 4, all transfers to start experiments were 1:2000 (25 ul into 50 mL) dilutions from pregrown cultures. Three kinds of media were used: YPD (2% peptone, 2% glucose, 1% yeast extract); YPG (2% peptone, 3% glycerol, 1% yeast extract); and YPEG (2% peptone, 2.6% glycerol, 2.6% ethanol, 1% yeast extract).

### qPCR

qPCR assays for mtDNA content were performed using the relative standard curve method. A single large-scale extraction from the haploid lab strain was performed when the culture was in fermentation, at absorbance $A_{600}$=0.5 (about 3e07 cells/mL). For every qPCR run, a fivefold serial dilution of this extract served as the standard curve. Each sample was run with two sets of primers, identical to the primers employed in (Puddu et al., 2019). We employed one set of primers to target a region of genomic DNA and another to amplify a region of mitochondrial DNA. The gDNA primer set (fwd: `TGCTTTGTCAAATGGATCATATGG` , rev: `CCTGGAACCAAGTGAACAGTACAA`) targeted a short region of GAL1 (chr II: 280382–280459) and the mtDNA primer set (fwd: `CACCACTAATTGAAAACCTGTCTG`, rev: `GATTTATCGTATGCTCATTTCCAA`) targeted a short region of COX1 in the mtDNA (25574–25686). For each sample, the quantity of mtDNA and gDNA relative to the standard curve was computed, and the ratio of the two recorded as the relative mtDNA copy number. Threshold calculations, curve fitting, and quantity calculations were performed by the QuantStudio 3 software. All wells were run in triplicate per run and outliers from each triplet were removed manually whenever needed. Each qPCR run was performed on a 96-well plate allowing a maximum of 11 unknown samples to be assayed for relative mtDNA copy number (11 samples x 2 primer sets x in triplicate = 66 wells, plus 5 standards x 2 primer sets x in triplicate = 30 wells, for a total of 96 wells).

## DNA extractions

DNA extractions were performed using Zymo Quick-DNA Fungal/Bacterial kits (D6005), which employ glass beads to physically lyse cells followed by a spin column-based purification. Because extractions were performed from growing cultures at different time points, within a single experiment the extraction volume was adjusted so that the absolute number of cells extracted was similar for each timepoint, in order to limit any effects of different extraction conditions. As an exception to this practice, extractions destined for whole-genome sequencing were larger in cell number than extractions destined for qPCR only.

Similar results as to those described here were obtained with both purely enzymatic extraction protocols (Zymo D2002) and a non-kit-based glass beads + phenol:chloroform protocol.

## Density measurements

Density measurements were performed by manually counting the number of cells, via microscope, in an improved Neubauer-style hemocytometer. Depending on culture density, dilutions of up to 1/200 (in water) were performed before counting. Still-attached buds were counted as a separate cell if having a diameter greater than half that of the mother. Density was also tracked by measuring absorbance at 600 nm ($A_{600}$), and referring to a standard curve created from many simultaneous measurements of absorbance and cell count.

## Sequencing and copy number analysis

The mtDNA copy number analysis was conducted essentially following (Puddu et al., 2019) was determined from short-read (Illumina) WGS as follows. Reads were first processed by `trim_galore` with options `--nextera --stringency 3 --paired --quality 20 --length 50`. Reads were mapped to the S288C R64 reference genome by `bwa`. Duplicate reads were removed by `biobambam2`'s `bammarkduplicates` and then read depth computed by `samtools depth`. The mtDNA genome includes many regions of high AT content that are not covered well in WGS. Hence the mtDNA copy number was computed as the median read depth in a region of the mtDNA genome with regular coverage (COX1 from 14000-20000; see Fig. S3) divided by the median read depth in the nuclear genome. mtDNA copy number calculated using COX3 instead (79213–80022) was very similar (Fig S1). We validated our copy-number estimation procedure by calculating copy numbers for other repetitive elements (Fig S1).

For long-read (Nanopore) data, reads were mapped via `minimap2`, and then `samtools depth` used to obtain read depths as for the short-read data. mtDNA coverage for long-read data in shown in Fig. S3; it is somewhat more regular than for short-read sequencing. mtDNA copy number estimates from the two sequencing methods, performed on the same extract, were extremely similar (Table S2).

## Data and code

[[Specify where data and code are hosted online.]]

## Supplementary Figures and Tables

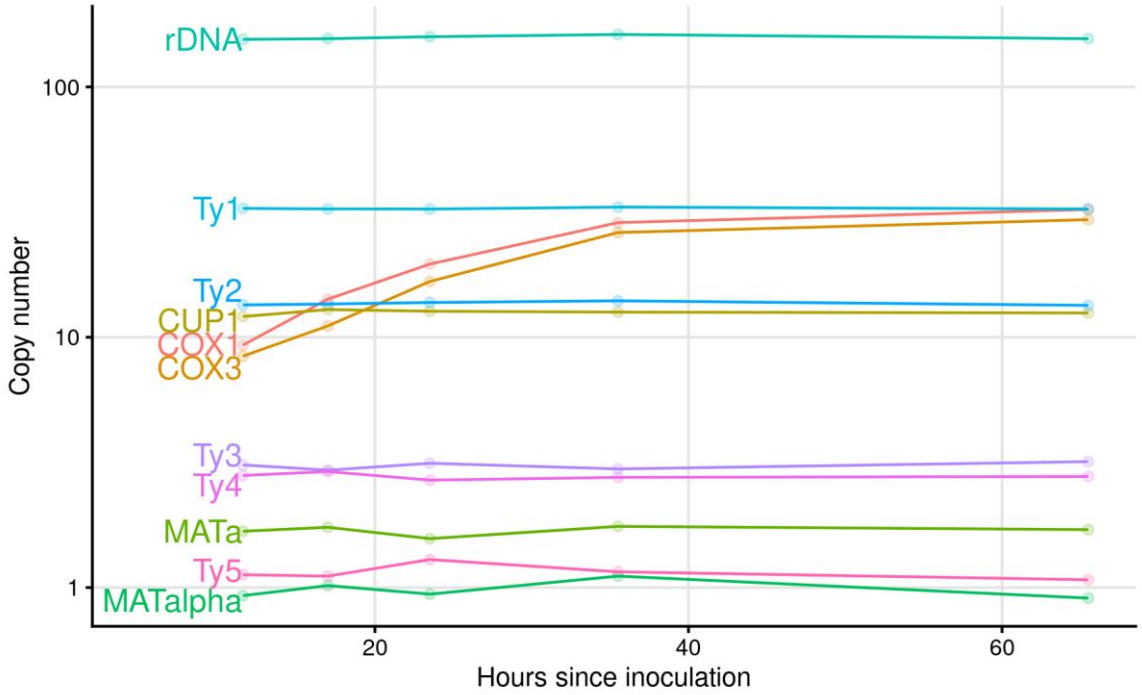


Fig. S1. The mitochondrial *COX1* and *COX3* genes increase in copy number over time. *COX1* provides the data for Fig. 1C. As controls, we assayed the copy number of 9 gDNA loci: ribosomal DNA, *CUP1*, *MATa*, *MATα*, and the 5 Ty transposons.

Table S2. Long-read sequencing gives near identical copy number results to short-read sequencing for identical extracts.

| | Illumina | Nanopore |
|---|---|---|
| Fermentation | 8.6 | 8.2 |
| Respiration | 27.8 | 28.5 |

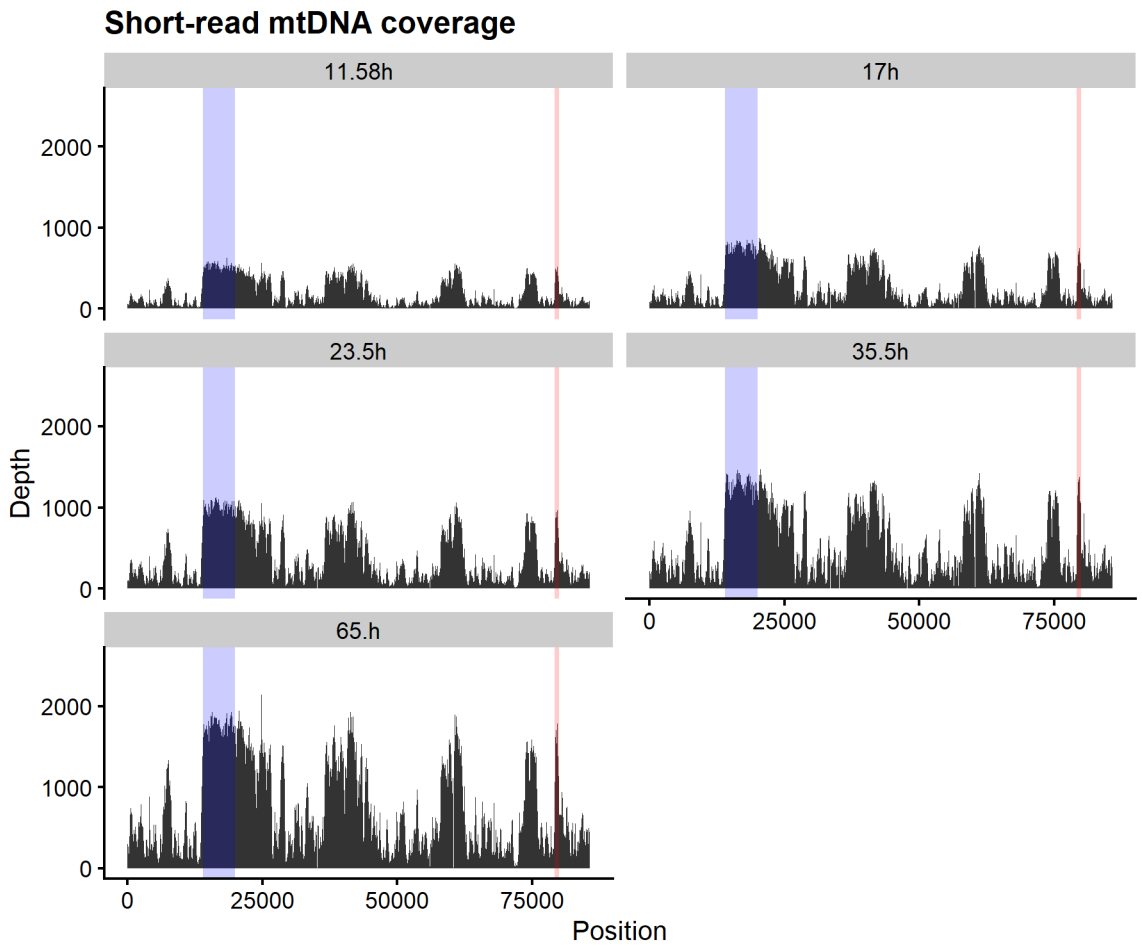


Fig S3. From short-read (Illumina) sequencing, mtDNA read depth from the 5 timepoints sequenced, with regions of mtDNA used for determining copy number highlighted (*COX1* in blue; *COX3* in red).

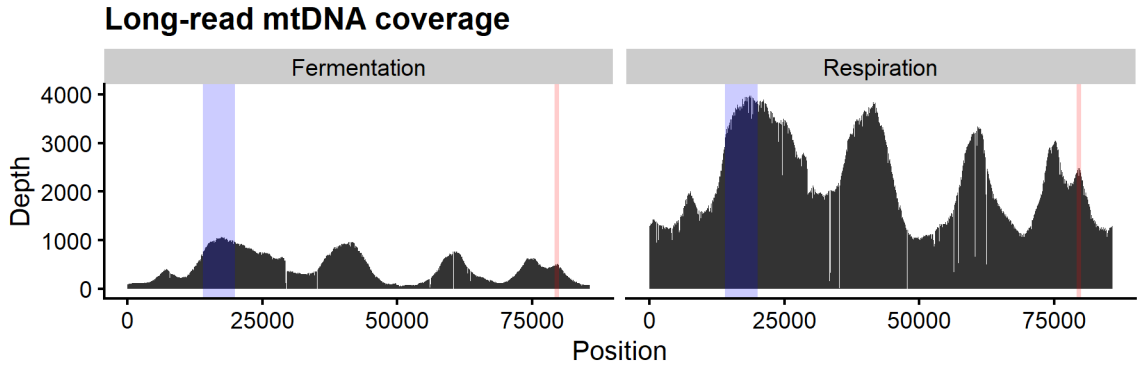


Fig S4. From long-read (Nanopore) sequencing, mtDNA read depth, with regions of mtDNA used for determining copy number highlighted (*COX1* in blue; *COX3* in red).

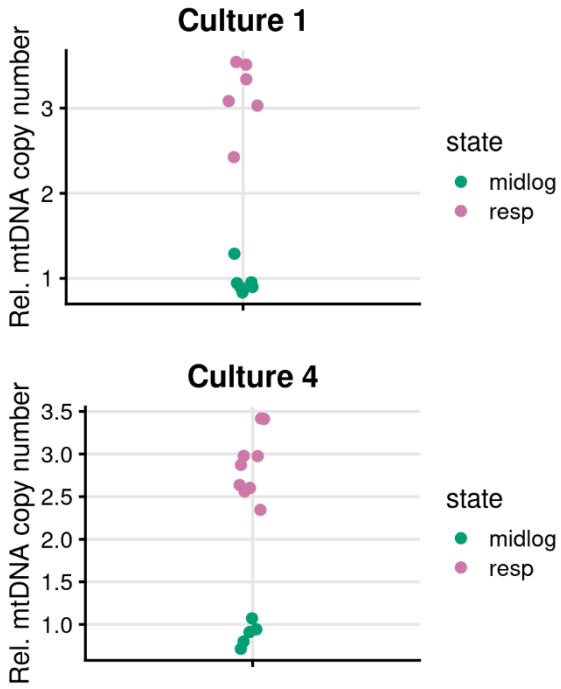


Fig S5. A culture of the haploid lab strain was inoculated as described in Methods, and then three consecutive serial transfers were performed during the fermentation phase, to create Cultures 1-4. Extracts were taken from both fermentation and respiration phase of Cultures 1 and 4. This experiment shows that even after ~60 hours in fermentation, the mtDNA copy number remains low, ruling out the possibility that copy number increases solely as a function of time.

## References


Bartolomeo, F. D., Malina, C., Campbell, K., Mormino, M., Fuchs, J., Vorontsov, E., Gustafsson, C. M., & Nielsen, J. (2020). Absolute yeast mitochondrial proteome quantification reveals trade-off between biosynthesis and energy generation during diauxic shift. *Proceedings of the National Academy of Sciences*, *117*(13), 7524–7535. https://doi.org/10.1073/pnas.1918216117

Berger, K. H., & Yaffe, M. P. (2000). Mitochondrial DNA inheritance in Saccharomyces cerevisiae. *Trends in Microbiology*, *8*(11), 508–513. https://doi.org/10.1016/S0966-842X(00)01862-X

Brewer, B. J., & Fangman, W. L. (1980). Preferential inclusion of extrachromosomal genetic elements in yeast meiotic spores. *Proceedings of the National Academy of Sciences*, *77*(9), 5380–5384. https://doi.org/10.1073/pnas.77.9.5380

D'Erchia, A. M., Atlante, A., Gadaleta, G., Pavesi, G., Chiara, M., De Virgilio, C., Manzari, C., Mastropasqua, F., Prazzoli, G. M., Picardi, E., Gissi, C., Horner, D., Reyes, A., Sbisà, E., Tullo, A., & Pesole, G. (2015). Tissue-specific mtDNA abundance from exome data and its correlation with mitochondrial transcription, mass and respiratory activity. *Mitochondrion*, *20*, 13–21. https://doi.org/10.1016/j.mito.2014.10.005

Dimmock, D., Tang, L.-Y., Schmitt, E. S., & Wong, L.-J. C. (2010). Quantitative Evaluation of the Mitochondrial DNA Depletion Syndrome. *Clinical Chemistry*, *56*(7), 1119–1127. https://doi.org/10.1373/clinchem.2009.141549

Elpeleg, O., Mandel, H., & Saada, A. (2002). Depletion of the other genome-mitochondrial DNA depletion syndromes in humans. *Journal of Molecular Medicine*, *80*(7), 389–396. https://doi.org/10.1007/s00109-002-0343-5

Freel, K. C., Friedrich, A., & Schacherer, J. (2015). Mitochondrial genome evolution in yeasts: An all-encompassing view. *FEMS Yeast Research*, *15*(fov023). https://doi.org/10.1093/femsyr/fov023

Göke, A., Schrott, S., Mizrak, A., Belyy, V., Osman, C., & Walter, P. (2019a). Mrx6 regulates mitochondrial DNA copy number in Saccharomyces cerevisiae by engaging the evolutionarily conserved Lon protease Pim1. *Molecular Biology of the Cell*, *31*(7), 527–545. https://doi.org/10.1091/mbc.E19-08-0470

Göke, A., Schrott, S., Mizrak, A., Belyy, V., Osman, C., & Walter, P. (2019b). Mrx6 regulates mitochondrial DNA copy number in Saccharomyces cerevisiae by engaging the evolutionarily conserved Lon protease Pim1. *Molecular Biology of the Cell*, *31*(7), 527–545. https://doi.org/10.1091/mbc.E19-08-0470

Gray, M. W. (2017). Lynn Margulis and the endosymbiont hypothesis: 50 years later. *Molecular Biology of the Cell*, *28*(10), 1285–1287. https://doi.org/10.1091/mbc.e16-07-0509

Kaaman, M., Sparks, L. M., van Harmelen, V., Smith, S. R., Sjölin, E., Dahlman, I., & Arner, P. (2007). Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. *Diabetologia*, *50*(12), 2526–2533. https://doi.org/10.1007/s00125-007-0818-6

Kornblum, C., Nicholls, T. J., Haack, T. B., Schöler, S., Peeva, V., Danhauser, K., Hallmann, K., Zsurka, G., Rorbach, J., Iuso, A., Wieland, T., Sciacco, M., Ronchi, D., Comi, G. P., Moggio, M., Quinzii, C. M., DiMauro, S., Calvo, S. E., Mootha, V. K., … Prokisch, H. (2013). Loss-of-function mutations in MGME1 impair mtDNA replication and cause multisystemic mitochondrial disease. *Nature Genetics*, *45*(2), 214–219. https://doi.org/10.1038/ng.2501

Koschwanez, J. H., Foster, K. R., & Murray, A. W. (2013). Improved use of a public good selects for the evolution of undifferentiated multicellularity. *ELife*, *2*. https://doi.org/10.7554/eLife.00367

Maleszka, R., Skelly, P. J., & Clark-Walker, G. D. (1991). Rolling circle replication of DNA in yeast mitochondria. *The EMBO Journal*, *10*(12), 3923–3929.

Malina, C., Larsson, C., & Nielsen, J. (2018). Yeast mitochondria: An overview of mitochondrial biology and the potential of mitochondrial systems biology. *FEMS Yeast Research*, *18*(foy040). https://doi.org/10.1093/femsyr/foy040

Miller, F. J., Rosenfeldt, F. L., Zhang, C., Linnane, A. W., & Nagley, P. (2003). Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: Lack of change of copy number with age. *Nucleic Acids Research*, *31*(11), e61–e61. https://doi.org/10.1093/nar/gng060

Miyakawa, I. (2017). Organization and dynamics of yeast mitochondrial nucleoids. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*, *93*(5), 339–359. https://doi.org/10.2183/pjab.93.021

Miyakawa, I., Miyamoto, M., Kuroiwa, T., & Sando, N. (2004). DNA Content of Individual Mitochondrial Nucleoids Varies Depending on the Culture Conditions of the Yeast *Saccharomyces cerevisiae*. *Cytologia*, *69*(1), 101–107. https://doi.org/10.1508/cytologia.69.101

Osman, C., Noriega, T. R., Okreglak, V., Fung, J. C., & Walter, P. (2015). Integrity of the yeast mitochondrial genome, but not its distribution and inheritance, relies on mitochondrial fission and fusion. *Proceedings of the National Academy of Sciences*, *112*(9), E947–E956. https://doi.org/10.1073/pnas.1501737112

Puddu, F., Herzog, M., Selivanova, A., Wang, S., Zhu, J., Klein-Lavi, S., Gordon, M., Meirman, R., Millan-Zambrano, G., Ayestaran, I., Salguero, I., Sharan, R., Li, R., Kupiec, M., & Jackson, S. P. (2019). Genome architecture and stability in the Saccharomyces cerevisiae knockout collection. *Nature*, *573*(7774), 416–420. https://doi.org/10.1038/s41586-019-1549-9

Pyle, A., Anugrha, H., Kurzawa-Akanbi, M., Yarnall, A., Burn, D., & Hudson, G. (2016). Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. *Neurobiology of Aging*, *38*, 216.e7-216.e10. https://doi.org/10.1016/j.neurobiolaging.2015.10.033

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology*, *14*(3), 225-IN6. https://doi.org/10.1016/0022-5193(67)90079-3

Shiiba, D., Fumoto, S.-I., Miyakawa, I., & Sando, N. (1997). Isolation of giant mitochondrial nucleoids from the yeastSaccharomyces cerevisiae. *Protoplasma*, *198*(3), 177–185. https://doi.org/10.1007/BF01287567

Sniegowski, P. D., Dombrowski, P. G., & Fingerman, E. (2002). Saccharomyces cerevisiae and Saccharomyces paradoxus coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. *FEMS Yeast Research*, *1*(4), 299–306. https://doi.org/10.1111/j.1567-1364.2002.tb00048.x

Unal, E., Kinde, B., & Amon, A. (2011). Gametogenesis Eliminates Age-Induced Cellular Damage and Resets Life Span in Yeast. *Science*, *332*(6037), 1554–1557. https://doi.org/10.1126/science.1204349

Wai, T., Ao, A., Zhang, X., Cyr, D., Dufort, D., & Shoubridge, E. A. (2010). The Role of Mitochondrial DNA Copy Number in Mammalian Fertility1. *Biology of Reproduction*, *83*(1), 52–62. https://doi.org/10.1095/biolreprod.109.080887

Westermann, B. (2014). Mitochondrial inheritance in yeast. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(7), 1039–1046. https://doi.org/10.1016/j.bbabio.2013.10.005

Williamson, D. H. (2002). The curious history of yeast mitochondrial DNA. *Nature Reviews Genetics*, *3*(6), 475–481. https://doi.org/10.1038/nrg814

Williamson, D. H., & Moustacchi, E. (1971). The synthesis of mitochondrial DNA during the cell cycle in the yeast Saccharomyces cerevisiae. *Biochemical and Biophysical Research Communications*, *42*(2), 195–201. https://doi.org/10.1016/0006-291X(71)90087-8

Williamson, D. H., & Slonimski, P. P. (1976). Packaging and Recombination of Mitochondrial DNA in Vegetatively Growing Yeast Cells. In *Genetics, Biogenesis, and Bioenergetics of Mitochondria* (pp. 117–124). De Gruyter. https://www.degruyter.com/document/doi/10.1515/9783111522241-012/html

Zeviani, M., & Di Donato, S. (2004). Mitochondrial disorders. *Brain*, *127*(10), 2153–2172. https://doi.org/10.1093/brain/awh259