

Supplementary Materials for

Mutation dynamics and fitness effects followed in single cells

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This PDF file includes:

Materials and Methods Supplementary Text Figs. S1 to S18 Tables S1 to S12 Captions for Movies S1 and S2 References

Other Supplementary Material for this manuscript includes the following: (available at www.sciencemag.org/content/359/681/1283/suppl/DC1)

Movies S1 and S2

1 Materials and Methods

1.1 Microfabrication and microfluidics

In the chip that we used for this study, the microchannels are $\sim 25\mu$ m long and $\sim 1 \mu$ m wide and high (height between 0.95 and 1.1 μ m and width between 1 and 1.5 μ m) and they are regularly spaced with a 4 μ m period. The main trench is 15mm long, 50 μ m wide and ~ 30 μ m high.

We fabricated several master molds by laser lithography. Two steps of lithography are necessary to get different heights for the microchannels and the main trench. For aligning the mold between the two steps, Aluminium (AL3X35N Neyco) marks were first deposited on a silicon wafer (wafers4p Neyco) by metal evaporation. To improve the adhesion of photoresist to the wafer, a thin layer of Microchem's Omnicoat was deposited by spin-coating 35s at 3000rpm with acceleration 300 rpm/s, and baked 1 minute on a hotplate at 200°C. Then SU-8 2002 was spin coated at 1000 rpm for 30s and baked 1 minute on a

hotplate at 95°C. The microchannels were then patterned by laser lithography (UV laser writer DWL 66FS Heidelberg) and the photoresist was baked 2min on a hotplate at 95°C, developed 10s in Microchem's SU-8 developer and baked at 150°C for 5 min. Microchannels height was checked with a mechanical profilometer (Dektak 150 Veeco). For patterning the main trench, SU-8 2015 was spin-coated for 30S at 2000rpm, baked 4 min on a hotplate at 95°C, patterned with the laser and baked 6 min on a hotplate at 95°C, developed 2 min in Microchem's SU-8 developer and baked 5 min on a hotplate at 150°C. The height of the main trench was measured with a profilometer.

To fabricate the chips from the molds we used standard soft lithography techniques. We used RTV-615 PDMS from Momentive, with A/B ratio of 10:1. PDMS was poured on the mold, degassed under vacuum and cured overnight at 65°C. Holes were drilled in the chip using a WPI Biopsy punch of 0.75mm diameter. The PDMS was sealed to a glass cover slip using a 30s oxygen plasma treatment (Harrick Plasma). To improve the bonding, the chip was incubated 10 minutes at 100°C after sealing. To prevent bacteria from sticking to the glass slide, we then incubated the chip with 10 mg/ml Bovine Serum Albumin for 2 hrs at room temperature. To load the bacteria into the microchannels, an overnight culture was concentrated 10-20 times by centrifugation and injected into the chip. Cells can swim inside the microchannels spontaneously but with varying efficiency. Cells were usually incubated 1hr in the chip at room temperature and if channel loading was insufficient the chip was sometimes centrifuged several minutes at 300rpm (the chip was orientated such that the centrifugal force pushes the cells inside the microchannels on one side of the chip). To deliver the growth medium into the chip during the experiment, we used a Harvard Apparatus PHD Ultra syringe pump set at 2ml/hr, Monoject 140 ml syringes with LS23 needles (Phymep), Tygon S54-HL flexible tubing (Phymep) and steel couplers SC23/8 (Phymep).

1.2 Microscopy

For fluorescence imaging we used an inverted Delta Vision Elite microscope equipped with the Ultimate Focus system for automatic focalization, a 100x oil immersion objective (N.A. 1.4), a temperature-controlled chamber, and the DV Elite sCMOS Camera. Fluorescence illumination was provided by the DV Light Solid State Illuminator 7 Colors at 575 nm (mCherry excitation) and 513 nm (YFP excitation). During time-lapse experiments images were taken every 2 minutes (~10 images per cell cycle) for 24 hrs, using 0.1s exposure with illumination intensity set at 3.5% of the maximum LED intensity for mCherry, and 2s exposure at 7.5% of the maximum LED intensity for YFP. During the cell cycle,YFP-MutL foci can move in the z direction inside the cell, potentially creating variations of intensity between frames and perturbing image analysis. To limit such variations we used a special imaging mode for YFP images, called Optical axis integration, which allows collecting and integrating one continuous image through an extended Z movement. Each YFP image is therefore a 2D Z projection of the 1 μ m interval around the cell focal plane.

For μ MA experiments we used an inverted Nikon Eclipse Ti-E microscope equipped with the Perfect Focus system for automatic focalization and an external phase contrast unit, a Plan APO 100x oil immersion objective (N.A. 1.4), a temperature-controlled chamber, and a Uniblitz VMM-D1 shutter. Illumination was provided by a 100W halogen lamp and images were taken with 50ms exposure. Images were acquired using the Hamamatsu CCD C8484-05G Camera. During time-lapse experiments, images were taken every 4 minutes (\sim 5 images per cell cycle) during \sim 70h (\sim 200 cell cycles). We performed all experiments at 37°C.

1.3 Image analysis

We developed a high-throughput image analysis software for the analysis of bacteria growing in the "mother machine", based on several java-based libraries ([25, 26, 27]).

1.3.1 Fluorescence Experiments:

Bacteria are visible in the Red fluorescence channel (RFP) and foci in the Yellow fluorescence channel (YFP)

Pre-processing of RFP and YFP images

First, for each YFP or RFP image, background fluorescence is removed. Background pixels are first automatically determined in the whole image using the Kappa Sigma Clipping algorithm [28]. Then the background is removed line by line in each image by subtracting for each line the mean background pixel value. The RFP fluorescence of single-cells exhibits some heterogeneity, with rare cells reaching a fluorescence that is 5- to 20-fold higher than the average value, potentially perturbing further image processing. To solve this problem we developed an algorithm that automatically saturates the histogram of image containing such cells, based on the difference between the Otsu threshold [29] and the Kappa Sigma Clipping [28] threshold. In order to set the micro-channels along the y-axis, with the deadend at the top, both YFP and RFP images are automatically rotated by an algorithm based on the radon transform. Next, we correct the XY drift over time using the Lucas-Kanade algorithm, with a reference frame that changes every 20 frames to avoid error accumulation.

Detection of micro-channels

In fluorescence, the PDMS structures are not visible. Therefore the channels are detected using the RFP fluorescence expressed by the bacteria growing inside the channels, by a simple thresholding using Kappa Sigma Clipping algorithm [28]. The segmentation of channels is done at each frame, then the detected objects are tracked over time, then the dimensions of each micro-channel is normalized over frames.

Detection of bacteria within the channels

First, high frequency noise is removed using a median filter and low frequency signal is removed by subtracting a large-scale Gaussian transform. The image is then thresholded using a constant threshold, creating a mask containing the bacteria. At this stage, several bacteria can be fused and considered as a single object (under-segmentation). To separate the bacteria, a watershed algorithm is applied to the maximal eigenvalue of the Hessian transform within the mask. This step generates over-segmentation, that is reduced by merging adjacent regions according to a criterion based on the Hessian transform value normalized by the Gaussian transform value, at the boundaries of the regions.

Tracking of bacteria

We developed a tracking algorithm that allows local correction of segmentation errors using information at previous and next frames. In the dead-end micro-channels, the rank of the cells within one channel is directly linked to their position in the lineage, the old pole mother cell being at the closed end of the channel. Tracking is done according to this rank. In order to detect division events as well as segmentation errors, we introduced minimum and maximum thresholds for the ratio of sizes between two frames (0.85 to 1.5). When this ratio is below the minimum threshold, we consider that a division occurred. When segmented objects with very different sizes are present, several assignment solutions are possible within the same window of size ratio. To compare them, we defined an assignment score based on the median size ratio at 10 previous frames, allowing to take into account variations in growth rate. When the size ratio exceeds the maximum threshold, it can either be due to over-segmentation at previous frame(s), or under-segmentation at the current (and next) frame(s). The two correction scenarios are compared using two different criteria, a cost function based on the Hessian transform, as introduced in the segmentation step and the number of corrected tracking errors. The best scenario is applied, if it allows to correct tracking has a cost under a pre-defined threshold.

Detection of foci

In order to attenuate the effect of cell-to-cell variability in YFP fluorescence background, the distribution of pixel values inside each cell is normalized by subtracting its mean and dividing by its standard deviation. In addition, we do not work directly with fluorescent levels but on a Laplacian-of-Gaussian (LoG) transform of the image, which is more robust to fluctuations in foci and background fluorescence intensity. More precisely, foci are detected using a seeded watershed algorithm on the LoG transform. Seeds are determined as regional maxima in the LoG image, and filtered using a threshold on a quality parameter defined as the square root of the product of the LoG value and Gaussian value.

Tracking of foci

We use the TrackMate [30] implementation of the Jaqaman [27] algorithm, allowing efficient tracking of several foci and including a procedure for gap-closing. In order to take into account cell growth, the coordinates of detected foci used for distance calculation is expressed relatively to the nearest cell pole or cell center. In order to reduce false positives, detected foci are divided into two categories, low quality and high quality, according to the quality

parameter defined previously. Low quality spots are included in the tracking step only if they can be linked to a high quality spot. To achieve this, low and high quality are first linked all together, then tracks containing only low quality spots are removed, then tracking is performed once again, this time only with remaining objects.

1.3.2 Phase Contrast Experiments

Pre-processing

The image is first rotated as described in the section "Pre-processing of RFP and YFP images", then background is subtracted using ImageJs' subtract background.

Detection of microchannels

Phase contrast fringe is automatically detected based on an intensity criterion and image is cropped. Micro-channel end is detected as the extrema in the Y-derivative image averaged in the X-direction. To detect horizontal bounds: the image X-derivative is averaged in the Y-direction and bounds are detected as extrema separated by a gap corresponding to the micro-channel width. The segmentation is done at each frame, then the detected objects are tracked over frames, then the dimensions of each micro-channel is normalized for all frames.

Detection of Bacteria

First images are thresholded using a threshold based Otsu's method [29]. For robustness purpose, the threshold is computed using a sliding widow over frames. This step can lead to under-segmentation. Regions are then split using a watershed algorithm on the euclidean distance map, which produces over-segmented regions. Regions are then re-merged using a criterion on the 1D-curvature of the contour of the segmented objects.

Tracking of Bacteria

The tracking method is identical in the analysis of phase contrast and fluorescence images, except that the cost function for correction scenario is based on the 1D-curvature of the contour of segmented object, as defined in the segmentation step.

1.4 Mutant frequency measurement in batch cultures

We estimated the genome-wide mutation rate of WT and *mutH* with the MutL foci method $(\mu_{WT,foci} \text{ and } \mu_{mutH,foci})$. In addition, for all our strains we performed classical genetic assays that allow determining the frequency of mutants resistant to rifampicin or streptomycin arising during growth of bacterial population in batch cultures (table S1).

For these assays we grew strains overnight in LB, except the strain with the inducible dominant negative mutant of the polymerase proofreading subunit dnaQ926, which was grown in Glucose Minimal Medium. The overnight cultures were diluted 10⁶-fold in LB or Minimal Medium supplemented by 1% arabinose for inducible dnaQ926 expression, grown to saturation and plated on selective medium (LB agar containing 100 μ g/ml rifampicin or LB containing 100 μ g/ml streptomycin) to select rifampicin resistant (RifR) colonies or streptomycin resistant colonies (StrepR) and on LB agar to determine the total number of colony forming units. Colonies were scored after 24h of incubation at 37°C. The average mutant frequency was determined for each strain from three to six independent experiments.

We estimated the ratio of the mutation rate of each strain to the mutation rate of $mutH(r_{MF1}, r_{mutT} \dots)$, simply by dividing the frequency of Rif (or Strep) mutants obtained with a given strain by the frequency of Rif (or Strep) mutants obtained for mutH (e.g. $r_{mutT} = freq_{mutT}/freq_{MutH}$). Then for each strain S we estimated the genome-wide mutation rate as being this ratio r_S multiplied by $\mu_{mutH,foci}$. We therefore obtain an estimation of the mutation rate for mutT (0.37/hr) and MF1 (5.6/hr). The estimated mutation rate of mutT is therefore similar to mutH mutation rate. Consistently, measurements using whole-genome sequencing were previously performed by Lee et al and Foster et al. ([11] [12]) and showed that the mutation rate in mutT strain is the same as the mutation rate in a MMR deficient strain (0.149 mutations per generation for mutT and 0.151 for mutL).

1.5 Analysis of fitness data from μ MA to estimate the moments of the DFE

Using our image analysis software (1.3), we extracted the data on cell sizes from the phase contrast images. Single cells elongate exponentially between birth and division, while their width stays constant. Single-cell growth rate (i.e. fitness) is therefore defined as the rate of cell elongation. For each microchannel we used only the data from the cell abutting the dead-end, the so-called mother cell.

The segmentation errors generated by image analysis can create aberrant, very low or high growth rates. Therefore, we filtered our data in the following way. For each channel and each generation, we calculated the median of the growth rates for earlier generations (M_{before}) and subsequent generations (M_{after}) . Growth rate estimates that are clearly aberrant (as confirmed by visual inspection of images) are either much larger than both M_{before} and M_{after} or much smaller than both M_{before} and M_{after} (Note that M_{before} and M_{after} can be significantly different because of the occurrence of deleterious mutations). Therefore for each generation, we remove the value of the growth rate μ from the data if it differs from both M_{after} and M_{before} by more than a threshold value T ([$\mu - M_{before} > T$ and $\mu - M_{after} > T$] OR [$\mu - M_{before} < -T$ and $\mu - M_{after} < -T$]). The value T was fixed at 30% of the average growth rate in the experiment, corresponding to the mean $+/-2^*$ standard deviation of the variable $\mu - M_{after}$ (or equivalently $\mu - M_{before}$; both variables follow a distribution that is well approximated by a gaussian). The datasets for all strains (WT mutH, mutT, and MF1 such as plotted in Figure 3A and Figure S8) were all filtered using the same procedure. In order to eliminate any image analysis errors that could bias our results and would not have been removed by this filtering procedure, we selected all the channels where a growth rate smaller than 0.015 was detected and systematically checked by visual inspection cell segmentation and tracking on all the images corresponding to this channel. Therefore, small growth rate values are all valid measurements.

Death of slowly growing cells induces noise in DFE estimation (figure S14). Therefore, to estimate the DFE's first moments (figure 3C and table S3) we used a subset of the data, restricted in time and containing only cells that do not die on this restricted time window (time points before 40 hours, only channels where the mother cell is still alive at 44 hours). For selecting such data we use an automatic procedure. If the growth rate of a cell is below 0.003 (10% of its initial value) during more than 80 minutes, the cell and its potential descendants are removed from the data. We checked that the results obtained through this automatic procedure are identical to those obtained when removing dead cells detected by visual inspection of the phase contrast images, as shown in table S8 for a representative mutH experiment.

We estimated the slopes of $E_1(t)$, $E_2(t)$, ... $E_{10}(t)$, for all the μ MA experiments with WT, *mutH*, *mutT* and MF1 strains (see table S3). The slope of $E_n(t)$ is the product of the mutation rate λ with the n^{th} moment of the DFE (see section 2.3.2). For *mutH*, the mutation rate λ is determined from our MV experiments ($\lambda = 0.32 \ hr^{-1}$; see section 2.2.3). The mutation rate for *mutT* and MF1 is estimated using classical genetic assays (section 1.4), giving 5.6/hr for MF1 and 0.37/hr for *mutT*.

In order to compare the DFE to a Beta distribution we estimated the two shape parameters α and β of the Beta distribution from the estimated mean m and variance v of the DFE. $\alpha = m^2(1-m)/v - m \sim 0.0074$ and $\beta = (1-m)/m(m^2(1-m)/v - m) \sim 2.4$.

1.6 Detection of strongly deleterious mutations

For detecting strongly deleterious mutations, we detected abrupt decreases of growth rate in mother cells by computing at each generation the median of the growth rates for earlier generations (M_{before}) and subsequent generations (M_{after}) . This was done after data was filtered to remove aberrant growth rate values (see section "Estimation of the moments of the DFE from experimental data", subsection "Data analysis"). When the growth rate is stable, the difference $\Delta = M_{before} - M_{after}$ is small. In contrast, when there is an abrupt decrease of growth rate, as depicted in figure 4A, Δ has a higher, positive value. We defined abrupt decreases of growth rate by using a threshold value for Δ equal to 30% of the average growth rate, which is high enough to allow reliable detection by this procedure. In addition we systematically checked the segmentation and tracking of all the images for the channel where a decrease of growth rate was detected.

For determining more precisely the timing of occurence of the growth rate decrease, we used a different quantity δ_t , defined as the difference between the average growth rate of the six generations before and the average growth rate of the six generations after. δ_t allows a precise timing of the event because it shows a sharp peak when the growth rate decreases (see figure S9). Because of mortality, the number of cells decreases substantially between the beginning and the end of the experiment (in the *mutH* strain, typically 2/3 of the mother cells are dead at the end of the experiments). Therefore, the time series of growth rates (W_t in section 2.3.1) can have very different lengths for different channels, introducing an important data censoring effect. To take this into account in our study of deleterious mutations occurence (as shown in figure 4A), we performed an analysis as described in section 1.9 for survival. The instantaneous rate of strongly deleterious mutations is calculated as the number of cells acquiring such a mutation during a time interval divided by the number of cells "at risk", i.e. still alive at the beginning of the time interval. The bottom panel of figure 4A is a reconstruction using these rates (therefore correcting for the decreasing number of cells during the experiment). Confidence intervals are calculated with the formula mentioned in section 1.9 for survival analysis (see [31]).

1.7 Strains

For the experiment on the mutation accumulation dynamics, we used the MG1655 strain 63ME121R, constructed for this study. It expresses yfp-mutL from the Plac promoter and tdCherry from the PRNA1 promoter on the chromosome. It is inactivated for the native mutL and *mutH* genes. *ufp-mutL* expression allows real-time mutation detection, where mutations are detected as bright spots of YFP-MutL [15]. tdCherry expression allows cell segmentation during image analysis. *mutL* deletion prevents the obstruction of mutation visualization by the non fluorescent version of MutL while *mutH* inactivation results in all replication errors being converted to mutations. We constructed the strain 63ME121R in four steps. First, we inactivated the mutL gene of the wild-type sequenced MG1655 strain obtained from E. coli Genetic Stock Center (CGSC 6300) by replacing the wild-type mutL allele with the *mutL::kan* allele using P1 transduction. This resulted in the strain MG1655mutL. Second, we replaced the *lacZ* gene of MG1655mutL by the *yfp-mutL-cat* construct from the strain ME120 using P1 transduction. We described the ME120 strain previously in [32]. This resulted in the strain 63ME120. Third, we integrated into the strain 63ME120, between two Tn7 insertion sites, the PRNA1 promoter-*tdCherry* sequence. This was done by transforming into 63ME120, the pNDL-32 temperature-sensitive, ampicillin-resistant plasmid obtained from Johan Paulsson's lab (http:/openwetware.org/wiki/Paulsson:Strains). Upon transformation, we selected on LB agar plates supplemented with $100\mu g/mL$ ampicillin, then streaked twice at 30°C and once at 42°C on LB agar to get rid of the plasmid. We confirmed the plasmid loss by checking that selected clones were red and ampicillin-sensitive. This resulted in the strain 63ME120R1. The CatR and KanR cassettes were then removed from 63ME120R1 by site-specific recombination between FRT sites, using the pCP20 plasmid, as described by Wanner et al. [33], producing the strain 63ME120R. Finally, we inactivated the *mutH* gene of the 63ME120R strain by replacing the wild-type *mutH* allele with the *mutH::cat* allele using P1 transduction. This resulted in the strain 63ME121R.

For inducible expression of dnaQ926 mutant from PBad promoter on the chromosome we integrated the araC-PBad-dnaQ926 DNA into prophage P21 attachement site using the Clonetegration method [34]. This was done in five steps. First, we PCR-amplificied the araC-PBad-dnaQ926 fragment from the plasmid pMQ [21]. Second, we cloned araC-PBad-dnaQ926 fragment into kanamycin resistant pOSIP-KT plasmide containing attP21 and P21 integrase[34]. This resulted in pOSIP-KTdnaQ926. Third, we transformed pOSIP-KTdnaQ926 into MG1655 by selecting on kanamycin. Fourth, we checked by PCR that pOSIP-KTdnaQ926 integrated into attP21 site on the MG1655 chromosome. This resulted in strain MF0. Fifth, to remove from the chromosomal attP21 site the pOSIP-KTdnaQ926 integration module including the antibiotic marker (KanR) we transformed MF0 with temperature sensitive pE-FLT plasmid expressing the FLR recombinase [34] and selected on ampicilin at 30°C. We checked for the excision of the antibiotic marker and neighbouring sequences by PCR and we got rid of the plasmid pE-FLT by growing MF0 ON at 37°C. This resulted in the strain MF1.

For all other experiments we used the wild-type $E.\ coli\ MG1655\ (CGSG\ 6300)\ strain$ and its derivatives listed in table S9, generated by P1 transduction and transformation.We verified the strain genotypes by testing for different phenotypes such as the capacityto generate mutations conferring resistance to rifampicin or expression of the fluorescentprotein.

1.8 Media

We grew cells in LB medium at 37°C. During growth in microchannels, LB medium was supplemented with 100 μ g/ml Bovine Serum Albumin (BSA) to prevent cell adhesion in the main channel. For mutation visualization we added 1mM IPTG to induce *yfp-mutL* expression. For μ MA experiments with the inducible dominant negative mutant of the polymerase proofreading subunit dnaQ926 (strains pMQ and MF1), cells were grown overnight in Glucose minimal medium (M9, 2mM MgSO4, glucose 0.4%) to repress dnaQ926 expression (supplemented for pMQ with Streptomycin 10 μ g/ml and Spectinomycin 100 μ g/ml to prevent plasmid loss). After cells were loaded into the chip, a first flow of LB+BSA+glucose 0.4% (+Streptomycin 10 μ g/ml +Spectinomycin 100 μ g/ml for pMQ) was applied during 1 hr, to re-initiate cell growth without induction of dnaQ926. Then the medium was switched to LB+BSA+arabinose 1% (+Streptomycin 10 μ g/ml for pMQ) to induce dnaQ926 expression and time-lapse experiment started 1 hr after to ensure full induction.

1.9 Survival analysis

After death, the cell's aspect can change, in particular its morphology and intensity in phase contrast. This can result in segmentation and tracking errors in automatic image analysis that limit the precision with wich cell death can be automatically detected. To estimate the mortality rates (figure 4B), we therefore detected the death of cells abutting the channels dead end by visual inspection of phase contrast images. We define cell death as an abrupt cessation of growth and division (fig S7). Also, during the experiment, some cells can escape the channels, by swimming away. Such incomplete observation events, so-called censored data, can bias the survival curve and the estimation of the death rate if not properly taken into account. Altought they are rare in our experiments (typically less than 10% of the channels) we took them into account by performing an appropriate, classical survival analysis, as described in [35]. The principle of this analysis is to calculate an instantaneous death rate (q_t) as the number of cells that die in a given time interval (d_t) divided by the number of cells that were "at risk" at the beginning of the interval (i.e. that were alive and had not escaped, $n_{atrisk,t}$): $q_t = d_t/n_{atrisk,t}$. Survival curves such as presented in figure 4B are then reconstructed using these instantaneous death rates : denoting S_t the percent of

survivors (as plotted in figure 4B), $S_t = 100 \prod_{i=0}^{t-1} (1 - q_i)$. These survival curves therefore correspond to a fictive cohort. Approximate 95% confidence intervals are calculated as in [35], using the formula in Peto et al. [31] : The 95% confidence interval is $[S_t - 1.96 \times SE_t]$, $S_t + 1.96 \times SE_t$], with $SE_t = S_t \sqrt{\frac{(1 - S_t/100)}{n_{atrisk,t}}}$

2 Supplementary Text

2.1 Validation of fluorescent MutL foci method for mutation visualization

2.1.1 Foci quantification with our software is robust to variations in detection parameters

As explained in section 1.3, regional maxima in the LoG image are considered as true foci only if their quality parameter is above a certain threshold. In addition, the detected foci are then divided into two categories corresponding to different levels of confidence ("high quality" and "low quality" foci), based on another threshold on the quality parameter. The values for these two thresholds have been tested in order to yield the best results (as assessed by visual inspection). Importantly, we checked that our foci detection procedure is robust to changes in these parameters. We chose a reasonable range for each threshold (such that outside this range the segmentation was clearly aberrant), and we measured the mutation rate for several combinations of parameter values within these ranges. We found only 8% variation in the estimated mutation rate, i.e. the coefficient of variation of the distribution of estimated mutation rate is 8%. This low variation demonstrates that our method is robust to parameter changes and allows a precise quantification of MutL foci.

2.1.2 Mutation rate estimation using MutL foci in *mutH* are in excellent agreement with previous estimates from the literature

From MV experiments with mutH strain, we estimated the MutL foci rate from the number of new foci occurrence per frame or from the mean foci inter-arrival time (these two estimates are coherent, showing less than 10% difference). From the rate of MutL foci, we estimated the mutation rate as explained in section 5.3. Our mutation rate estimate is 0.32 mutations/hr. In order to compare with previous literature we can convert this rate per time unit in a rate per generation, simply by multiplying it by the population doubling time (here 26 minutes). We find 0.14 mutations/generations, which is in excellent agreement with the recent estimate of mutation rate in a MMR-deficient mutL strain, determined by Mutation Accumulation and Whole Genome Sequencing ([11]; 0.15 mutations/generation). This strongly supports the fact that MutL foci in mutH correspond to unrepaired errors that are converted to mutations.

2.1.3 The lifetime of MutL foci in *mutH* confirms that all tagged mismatches are converted into mutations

We checked that each MutL focus is converted to a mutation in mutH by testing the prediction that the foci lifetime should equal the arrival time of the next replication fork. Note that the inter-arrival time of replication forks is equal to the doubling time [36]. We found that the foci lifetime distribution resembles a gaussian, with a mean very close to the doubling time (see figure S1; for this experiment mean focus lifetime is ~ 23min and mean doubling time is ~ 25 min). This indicates that the focus generated by a replication fork stays at the replication error until the next fork comes and converts the error into a mutation This strongly indicates that as expected in a mutH strain, replication errors are not repaired and MutL foci tags nascent mutation.

2.1.4 No false-positive, mismatch-independent MutL foci

We quantified the rate of false positive, i.e. replication error-independent MutL foci, by performing MV experiment using a double mutant mutH mutS. We found that the rate of false positives is very low, potentially accounting for only 0.08% of MutL foci in mutH strain.

2.1.5 mutH inactivation results in all replication errors being converted to mutations

Very similar mutation rate in MMR- mutH strain (our data) and MMR- mutL strain ([11]) suggests that MutH is as responsible as MutL for repair of replication errors, in agreement with previous genetic studies [37]. In order to confirm this in our conditions we inactivated uvrD, the helicase acting downstream of MutH, and performed MV experiment using the uvrD strain. The rate of MutL foci in uvrD is similar to the rate in mutH (0.31 mutations/hr vs 0.32 mutations/hr) and importantly, foci lifetimes are also similar in uvrD and mutH (mean 24 min vs 23 min). This result indicates that MutH is essential to mismatch repair. Likewise, we found very similar MutL foci rate and foci lifetime distributions in mutH rnhB and mutH (0.25 vs 0.32 mutations/hr, and mean foci lifetime of 22min vs 23 min), suggesting that unlike in *B. subtilis* or in eukaryotes [38], Ribonucleotide Excision Repair (RER) RnhB ribonuclease can not substitute for MutH during MMR.

2.1.6 MutL foci can be used to follow mutations in MMR proficient cells

Contrarily to *mutH*, where the repair of errors by MMR is inactivated, in wild type (WT) cells with proficient MMR repair, two kinds of foci are distinguishable: very short-lived foci with lifetime < 4 min, accounting for 98% of all foci (total number of foci analyzed :1572), and 2% of long-lived foci, with lifetime similar to *mutH* foci. Short-lived foci may correspond to repaired errors. In agreement with this hypothesis, MMR is known to repair $\sim 99\%$ of mismatches [39]. Nevertheless, demonstrating that short-lived foci are indeed

repaired replication errors requires further investigation. In contrast, the lifetime of longlived foci (similar to the lifetime of *mutH* foci) strongly suggests they are unrepaired errors that will be converted into mutations. In agreement with this interpretation, the number of long-lived foci in WT is ~ 130 x lower than the number of foci in a *mutH* background, which has a 100-200x higher mutation rate (in particular MMR deficient strain has a rate ~ 150 x higher than WT according to whole genome sequencing of MA lines [11]). From the rate of occurrence of long-lived foci we can estimate the rate of mutation in WT, as explained in section 2.2.3, and we find 0.0022 mutations/hr. Simply by multiplying this value by the population doubling time (here ~ 0.43 hr), we find a rate per generation of $\sim 10^{-3}$ mutations/generations, in excellent agreement with previously reported estimations of mutation rate in WT E. coli (in particular Lee et al. reported 0.87 10^{-3} and 1.14 10^{-3} for whole genome sequencing of two MA experiments [11]). In addition, this number of longlived foci is increased in a mutant expressing a mutant allele of the polymerase proof-reading subunit, of a factor consistent with its elevated mutation rate. All this constitutes strong evidence that long-lived foci correspond to unrepaired replication errors that are converted to mutations. Our method therefore also allows reliable estimation of the mutation rate of MMR proficient strains.

2.1.7 Biological contexts limiting mutation visualization using MutL foci

We found that the number of MutL foci in mutT is ~ 60 times lower than in mutH, whereas the mutation rate of the two strains are similar. This discrepancy is expected since the incorporation of oxidatively-damaged nucleotides is poorly recognized by MMR [40]. This shows that MutL foci cannot be used to detect mutations arising from incorporation of oxidatively-damaged nucleotides. Mutations originating from other chemically-altered bases may be also poorly detected by MutL foci. Importantly, MMR can be subject to downregulation in some stressful conditions [41, 42] or can become limiting in some conditions [43, 44]. In such conditions our method is not appropriate to detect mutations.

2.2 Data analysis of fluorescent foci dynamics

2.2.1 Discrete observation of a poisson process at regular time intervals deforms the exponential distribution of inter-arrival times

To test if mutations follow a poisson process we determined the distribution of foci interarrival times, i.e. time intervals between two successive foci. In a Poisson process, the interarrival times are exponentially distributed. Nevertheless, the observation of a Poisson process at discrete, regular times (time interval δ) introduces a bias in the estimated distribution. If the observed inter-arrival time is 0, the real inter-arrival time is in $[0,\delta]$. In contrast, observed inter-arrival times of $n\delta$ (n > 0) correspond to events occuring with a real time delay in $](n-1)\delta, (n+1)\delta]$, an interval twice larger than for n = 0. This leads to a deformation of the exponential distribution, with an under-representation of very small inter-arrival times. We can derive an analytical formula for such distribution as follows. Let us consider the inter-arrival time X between fluorescent foci following an exponential law of parameter λ . The first observation of the first focus will occur a time delay U after its appearance. The times of observation being independent of the mutations process, U is a random variable following a uniform distribution between 0 and δ (δ : time between consecutive images). Therefore the observed inter-arrival time T is given by : $T = \delta \left(\lfloor \frac{X-U}{\delta} \rfloor + 1 \right) \mathbb{1}_{X>U}$. From this, we find that the law of observed inter-arrival times is :

$$P(T=0) = 1 - \frac{(1 - e^{-\lambda\delta})}{\lambda\delta}$$
(2.1)

$$P(T = n\delta) = \frac{e^{-(n-1)\lambda\delta}}{\lambda\delta} (1 - e^{-\lambda\delta})^2 \quad \text{, for } n > 0 \tag{2.2}$$

To validate this formula we performed simulations of a Poisson process observed at regular time intervals. We show in figure S2 that the distribution of simulated inter-arrival times indeed follows the law defined by equations 2.1 and 2.2.

2.2.2 Estimation of the number of replication forks in single cells

We estimate the number of replication forks in a single cell depending on its rate of growth and cell cycle stage. This estimation is based on common knowledge of the bacterial cell cycle ([36], [45]), such as explained in [36], where a nice program of Cell Cycle Simulation (CCSim) is presented [36]. Our model is similar to the one in [36], the required parameters are the C and D periods, which are respectively the constant time required to replicate the chromosome (C period) and the constant time between replication termination and division (D period), and a cell size parameter (i.e. the average size of bacterial cells growing at a given rate). We use classical values for C and D periods, i.e. respectively 40min and 20 min and the cell size parameter is fitted such that the average cell size we measure corresponds to the average rate of growth we measure. Using only these parameters we can estimate the number of forks of any single cell from its size and its rate of growth. We can then estimate the mean error rate per fork in a cell population, such as in Figure 2F, by normalizing the number of MutL foci occurrences in a single cell at a given time step by its predicted number of forks.

2.2.3 Estimation of the mutation rate from the rate of MutL foci occurrence, taking into account multifork replication and DNA segregation

The distribution of observed inter-arrival times between two fluorescent foci follows an exponential distribution of parameter Λ , corrected for discrete observation as explained in section 2.2.1. The mean inter-arrival time is therefore $\frac{1}{\Lambda}$. This allows estimating Λ , the rate of fluorescence focus occurrence. For the *mutH* strain in LB medium we find $\Lambda = 2.7$ hr⁻¹ and $\Lambda = 2.6$ hr⁻¹ in two independent experiments. Because a focus tags a replication error that will give rise to a mutation and not the mutation itself, the rate of foci occurrence cannot be directly interpreted as the rate of mutation. We estimated the mutation rate from the replication error rate based on the following considerations. The passage of the

replication fork generates two double-stranded DNA molecules, therefore four DNA strands. If a replication error occurs, one strand carries a mutated base, whereas the three other strands carry the parental base. Therefore 1/4 of the strands at this fork are mutated (figure S_{6A}). Consequently, in conditions where DNA replication cycles do not overlap, 1/4 of the descendants of the cell where the error occurs will be mutants (figure S6B). An additional complexity comes from the fact that at fast growth, the cell undergoes multifork replication, i.e. several cycles of DNA replication overlap, and all replication forks are not equivalent. When a given fork incorporates an erroneous base at a given locus, the proportion of mutants in the cell's descendants depends on the number of copies of that locus inside the cell. As an example, if 6 forks are present in the cell (see figure $S_{6}C$ and D), corresponding to two overlapping replication cycles, two of them (red dots in figure S6) replicate loci that have not already been replicated and are present as a single copy, leading to a proportion of mutants in the progeny of 1/4 (figure S₆C). In contrast, the 4 other forks (yellow dots in figure S_6) replicate loci that are already present in two copies inside the cell, on the two partly replicated chromosomes, leading to a mutated base in one of the 8 strands of this locus in the cell, i.e. 1/8 of mutants in the cell's progeny (figure S6D). Therefore, in this example a replication error can lead to a proportion of mutants in the descendants of 1/4or 1/8, depending on which fork generates the error. Taking all this into account, we can calculate the proportion p of mutant cells in the descendants of a cell where a fluorescent focus appears. The mutation rate when only one cell is kept at each generation, as in our μ MA experiment will therefore be given by the product Λp . In our Mutation Visualization experiments, the doubling time is around 26 minutes. Therefore the cell has in average 7 replication forks : 6 forks between age 0 min and 6min, 4 forks between age 6 min and age 18 min, and 12 forks between age 18 min and 26 min (see section 2.2.2 and [36]). The case with 6 replication forks is shown in figure $S_{6}C$ and D. The case with 4 replication forks is as shown in fig S6B but with two chromosomes. Likewise, the case with 12 forks corresponds to two chromosomes with 6 forks such as shown in figure $S_{6}C$ and D. We can therefore calculate that in our MV experiments, p = 0.12.

In conclusion, in our conditions the rate of mutation λ in a *mutH* strain is $\lambda = 0.12\Lambda = 0.32 \text{ hr}^{-1}$. Note that this also corresponds to the rate of replication errors in a MMR-proficient strain.

As explained in the main text (p.6), in our MV experiments where fluorescent foci are followed, a small subpopulation of cells exhibits a lower rate of growth and therefore has a lower number of replication forks and a lower mutation rate (see figure S15). In order to investigate quantitatively whether this subpopulation of cells has a substantial impact on our mutation rate estimation we performed the following numerical simulations. We measured the growth rate of each single cell in the population of cells of a typical mutH MV experiment. We estimated the average number of replication forks corresponding to each growth rate, using common knowledge of the bacterial cell cycle (see section 2.2.2). The number of replication forks is a good estimation of replication error rate, as shown in the main text (p.6). We then simulated inter-arrival times for the heterogeneous cell population using exponential laws with parameters corresponding to the estimated single-cell replication error rates. From the distribution of inter-arrival times that we obtained we estimated the replication error rate of the population, as done for the experimental data. We found that the estimated mutation rate is only 2% below the rate of the "normally" growing cells. Therefore the small subpopulation of slowly growing cells has a very low impact (< 2%) on the estimation of the global mutation rate.

2.3 Non-parametric characterization of the DFE in μ MA

In this section we first describe a probabilistic framework that is classical in MA studies and allows modeling fitness evolution during mutation accumulation (2.3.1). Then we derive analytical formula for all the moments of the DFE in this framework (2.3.2) and extend the classical model to the single-cell level (2.3.4). Finally, we investigate quantitatively the effect of mutation rate decrease through time during μ MA (due to slowing down of growth during mutation accumulation) on our estimation of DFE moments (2.3.5).

2.3.1 Probabilistic model of fitness evolution during mutation accumulation

We used a simple mathematical description of the mutation accumulation process. We consider a series of mutations occuring according to a Poisson process of intensity λ ($\mathcal{P}(\lambda t)$), with relative effects s_i on fitness (W_t):

$$s_i = \frac{W_{t_{i-1}} - W_{t_i}}{W_{t_{i-1}}}, \quad i > 0$$

 W_t : fitness at time t; s_i : relative effect of mutation number i

Hence, assuming that mutations have independent effects, s_i are independent, identically distributed variables and :

$$\frac{W_t}{W_0} = \prod_{i=1}^{N_t} (1 - s_i), \qquad N_t \sim \mathcal{P}(\lambda t)$$
(2.3)

Therefore, the model is based solely on two assumptions, the poissonian occurence of mutations, which is validated by the results of our Mutation Visualization (MV) experiments as shown in Figure 2, and the independent effects of mutations, which is validated by our results on fitness evolution in μ MA experiments. Our results indeed show that accumulation of mild-effects mutations does not lead to any significant acceleration or deceleration of fitness reduction (see figure S16) and that strongly deleterious and lethal mutations occur at a constant rate (Figure 4), independently of the accumulated mild-effects mutations.

2.3.2 Calculating the moments of the DFE from the moments of the fitness distribution

Using the mathematical model introduced in section 2.3.1 (Equation 2.3), we can calculate the moments of all orders of W_t from the moments of the DFE. We will assume $W_0 = 1$ without loss of generality. $\mathbb{E}()$ stands for mathematical expectation. The k^{th} moment of W_t is :

$$\mathbb{E}(W_t^k) = \mathbb{E}(\mathbb{E}(\prod_{i=1}^{N_t} (1-s_i)^k | N_t)), \quad k > 0$$
$$= \mathbb{E}(\mathbb{E}((1-s)^k)^{N_t})$$
$$= \sum_{i=1}^{\infty} e^{-\lambda t} \frac{\lambda^i t^i \mathbb{E}[(1-s)^k]^i}{i!}$$
$$= e^{-\lambda t} e^{\lambda t \mathbb{E}[(1-s)^k]}$$

We define $E_n(t) = \sum_{k=1}^n (-1)^k \binom{n}{k} \ln(\mathbb{E}(W_t^k))$. We can calculate $E_n(t)$ as follows:

$$E_n(t) = \sum_{k=1}^n (-1)^k \binom{n}{k} [-\lambda t + \lambda t \mathbb{E}[(1-s)^k]]$$
$$= \lambda t \sum_{k=1}^n (-1)^k \binom{n}{k} \sum_{l=1}^k (-1)^l \binom{k}{l} \mathbb{E}(s^l)$$
$$= \lambda t \sum_{l=1}^n \mathbb{E}(s^l) \sum_{k=l}^n (-1)^{k-l} \binom{k}{l} \binom{n}{k}$$

We have

$$\binom{k}{l}\binom{n}{k} = \frac{n!}{l!(n-k)!(k-l)!} = \binom{n-l}{k-l}\binom{n}{l}$$

Thus,

$$E_n(t) = \lambda t \sum_{l=1}^n \mathbb{E}(s^l) \binom{n}{l} \sum_{k=l}^n (-1)^{k-l} \binom{n-l}{k-l}$$
$$= \lambda t \sum_{l=1}^n \mathbb{E}(s^l) \binom{n}{l} (1-1)^{n-l}$$
$$= \lambda t \mathbb{E}(s^n)$$

In our μ MA experiments, W_t is measured in a thousand independent cell lines, allowing calculating $\mathbb{E}(W_t^k)$ for any k > 0. Therefore, in order to estimate the moment of order n of the DFE ($\mathbb{E}(s^n)$), we plot $E_n(t) = \sum_{k=1}^n (-1)^k \binom{n}{k} \ln(\mathbb{E}(W_t^k))$ as a function of time t and estimate the slope, which is $\lambda \mathbb{E}(s^n)$, as illustrated in Figure S16. It is worth noting that we make no assumptions on the deleterious or beneficial nature of mutations and our procedure estimates the moments of the DFE of all mutations.

2.3.3 Data filtering during analysis of μ MA does not bias the estimation of the moments of the DFE

As explained in section 1.5, we applied a filtering procedure to clean the data from aberrant growth rate values. This filtering and the exact value of the threshold parameter T has little impact on the quantification of the moments of the DFE, as shown in table S5 and S6.

We limited the noise induced by mortality by using a subset of the data containing only cells that do not die on a restricted time window (see section 1.5). Although less precise, an analysis on the whole data leads to similar conclusions. Table S7 shows the result of this analysis, performed on the data containing all time points and all channels (for cells dying in the course of the experiment, 10 generations before death were removed to avoid a potential decrease of growth rate linked to cell death). This analysis also shows a heavy-tailed, very skewed and leptokurtic distribution, although the quantification is less precise.

2.3.4 Extending the model to the single-cell level shows that measurement noise and phenotypic variability do not affect our estimation of the DFE's moments

In our experiments, growth rate is measured at the single cell level. In addition to the fitness of the cell's genotype at time t W_t , our measure G_t is affected by the measurement noise and a potential phenotypic variability of growth. We show in this section, by including in our model a noise term accounting for phenotypic variability and mesurement noise, that these two components do not affect estimation of the DFE moments with our method. As the singlecell growth rates measured in a clonal population (corresponding to variations of G_t when W_t is constant) are normally distributed with limited inheritance from mother to daughter ([13] and Figure S17 A and B), we introduce a white noise ϵ_t in the model (i.e. the derivative of the Brownian motion). This noise could be introduced in a multiplicative $(G_t = W_t(1+\epsilon_t))$ or in an additive way $(G_t = W_t + \epsilon_t)$. If the mean single-cell growth rate changes, for instance due to a mutation or a change of environment, a multiplicative noise leads to a proportionnal change in the standard deviation of measured growth rates (a change in W_t leads to a proportional change in G_t). In contrast, in the case of an additive noise, a change of the mean of single-cell growth rate does not affect the growth rate standard deviation (standard deviation of G_t does not change when W_t changes). It has been shown experimentally that single-cell growth rate has higher standard deviation in a rich environment, compared to a poor medium [46], suggesting a multiplicative noise. To confirm that this holds as well in our experiments, we calculate the mean and the standard deviation of growth rates for cells that grow slowly due to strongly deleterious mutations. More precisely, for each channel where a strongly deleterious mutation occur, we can compute the growth rates for all the generations after the mutation and estimate the mean and standard deviation of these growth rates.

The analysis shows that the standard deviation and the mean are correlated (figure S17 C). We therefore use in our model a multiplicative noise.

We define G_t as the measured fitness (measured single-cell growth rate), while W_t is the

real underlying fitness of the cell's genotype.

$$G_t = W_t(1 + \epsilon_t)$$
 with ϵ_t a white noise independent of W_t

We can calculate the moments of G_t as a function of the moments of W_t and ϵ_t . By independence of ϵ_t and W_t :

$$ln(\mathbb{E}(G_t^k)) = ln(\mathbb{E}(W_t^k)) + ln(\mathbb{E}[(1+\epsilon_t)^k])$$

 ϵ_t is stationary so the right-hand term does not depend on time t. Therefore the slope of $ln(E(G_t^k))$ is the same as the slope of $ln(E(W_t^k))$. The noise does not affect our estimation of the moments of the DFE.

Note that our estimation method is also robust to the introduction of a phenotypic lag τ (time delay between the occurrence of a mutation and its effect on fitness). In this case Equation 1.1 is changed for

$$\frac{W_{t+\tau}}{W_0} = \prod_{i=1}^{N_t} (1 - s_i), \qquad N_t \sim \mathcal{P}(\lambda t)$$
(2.4)

Following the calculations in section 1.2, it is easy to show that in this case $E_n(t) = \lambda(t - \tau)\mathbb{E}(s^n)$. Consequently, the phenotypic lag does not change the slope of $E_n(t)$ and our estimation of the DFE moments.

2.3.5 Impact of mutation rate decrease during μ MA on DFE characterization

During μ MA experiments, cells accumulate mutations and their growth rate changes. In our conditions, mutations are mostly deleterious and the average growth rate decreases through time. At the single-cell level, the replication error rate depends on the rate of growth, through the number of replication forks present in the cell (see figure S15). Therefore, the mutation rate should decrease during our μ MA experiments, which is not taken into account by the model developped in section 2.3.1. We therefore investigated quantitatively, through numerical simulations, in which conditions this effect could significantly bias our estimation of DFE moments.

We performed numerical simulations of μ MA experiments according to the two following scenarios:

A. First we simulated a thousand independent cell lines accumulating mutations according to a simple Poisson process (with a single, constant rate). The effect of each mutation on growth rate is randomly drawn from a Beta distribution with parameters alpha=0.0074 and beta=2.4 (i.e. the parameters that give the same mean and variance as the DFE that we estimated experimentally, see figure 3). We added a gaussian noise to the single-cell growth rate, as observed in the data (see section 2.3.4).

B. Then we performed the same kind of simulations but instead of a constant mutation rate we used a mutation rate that depends on growth rate, through the number of replication forks (see section 2.2.2 and figure S15). Therefore a deleterious mutation decreases both the growth rate and the mutation rate of the cell.

We first used the mutation rate estimated for our mutH strain. Figure S18 shows a representative example of simulations of scenario A (constant mutation rate), which resemble the experimental results of mutH and mutT. We performed 30 simulations of scenario A and 30 simulations of scenario B and found that the evolution of the growth rate distribution was very similar for the two scenarios and the difference between estimated moments was very small (see table S10).

Then we used a 20 times higher mutation rate, corresponding to our MF1 strain (with a chromosomic dnaQ926 allele). We found that for this higher mutation rate a significant difference appears between the moments estimated from simulations with scenario A or B (see table S11). Nevertheless, if moments of the DFE are estimated only on the beginning of the experiment where the fraction of slowly growing cells is still limited, the difference between scenario A and B vanishes. In particular, as shown in table S12, for this mutation rate, taking only the 10 first hours of the experiment reduces the bias in DFE moments estimation to a reasonable extent, the difference between scenario A and B being of the same order of magnitude as the variability between replicate experiments.

2.4 Mortality in pMQ strain is due to mutations

In the pMQ strain, the dnaQ926 allele is located on a streptomycin-resistant plasmid. Therefore we checked that death is not due to plasmid loss in the growth medium containing streptomycin. We therefore compared the mortality with or without induction of dnaQ926. Although there is some residual dnaQ926 expression in the absence of inducer (arabinose), the death rate was more than 30 times lower (fig S11). Therefore, mortality induced by plasmid loss is negligible compared to mutation-induced mortality.

Figure S 1: Distribution of MutL foci lifetime for a representative *mutH* MV experiment In this experiment the mean cell doubling time is 25 min



Figure S 2: Inter-arrival time distribution of a Poisson process with discrete observation

Probability Density Function of inter-arrival times for simulated data (red dots; we simulated a Poisson process of intensity 0.1 and in order to simulate the effect of discrete observation with time interval $\delta = 1$ we approximated the times of arrival T_i by $\lfloor T_i \rfloor + 1$; the inter-arrival times are calculated as $T_{i+1} - T_i$, for i > 0). Our analytical derivation of the probability law (equations 2.1 and 2.2) is represented with blue triangles.



Figure S 3: The number of foci occuring between two frames is Poissondistributed. Histogram of the number of new foci occuring during the two minutes time interval between two frames (light blue, log-scale, n=11275) and a Poisson distribution (red dots) with a parameter λ estimated from panel Fig. 2B. PDF: Probability Density Function.



Figure S 4: The replication error rate is proportionnal to the cell size.

Black dots represent experimental data, binned by cell size (i.e. rates of foci occurrence are averaged for subpopulations of cells with similar size) and the red line is a linear regression with a null intercept (i.e. the slope is the only fitting parameter). The bin size here is much larger than for figure 2F. Such large bin size smoothes the step-like variations that can be observed in figure 2F and emphasizes the global proportionnality between error rate and cell size.



Figure S 5: Slow-growing and long cells do not present elevated rates of replication errors per fork Mean error rate per fork for subpopulations of cells with particular size or growth rate (see Fig.2F; error bars are Mean +/- 2 SEM). Left : mean error rate for cells with length smaller (light blue) or longer (dark blue) than 12 μ m; right: mean error rate for cells with growth rate smaller (dark green) or larger (light green) than 1.7 doublings/hrs. NS: no significant difference according to t-test (p-value =0.2) ; * significant according to t-test (p-value 0.0005).



Figure S 6: Occurrence of replication error and proportion of mutants in the progeny



Figure S 7: **Representative example of cell death** Temporal montage of phase contrast images of a single channel where a mother cell dies; result of automatic image analysis are also shown in the bottom panel.



Figure S 8: Temporal evolution of the single cell growth rate distribution in μ MA experiment with a *mutT* strain.

For each time point, all growth rates are plotted. Color depicts the local growth rate density (from blue to yellow). The red line is the evolution of the mean growth rate.



Figure S 9: Automatic detection of abrupt decreases of growth rate Growth rate trajectory for a microchannel where a strongly deleterious mutation occur (top panel) and evolution of δ as a function of time (bottom panel; δ is the difference between the average growth rate of the six generations before and the average growth rate of the six generations after); δ presents a sharp peak when the growth rate suddenly decreases; the maximum value of delta gives the timing of the growth rate decrease, as shown with the vertical red line.



Figure S 10: Survival of the mutT strain in a μ MA experiment. Survival is plotted in log-scale. In blue : survival analysis of experimental data (95% confidence interval is plotted in thin blue lines); in red: exponential fit (a line here in log scale)



Figure S 11: Survival of the pMQ strain in a μ MA experiment, with or without induction of the mutator allele dnaQ926 by arabinose



Figure S 12: Our automatic filtering procedure removes image analysis errors producing aberrantly low or high growth rate values.

Raw data from the $mutH \mu$ MA experiment shown in figure 3A. Red dots : growth rate values that are detected as aberrant and removed by our procedure; black dots: growth rate values that are kept in our analysis. Importantly, the noise does not affect our estimation of the moments of the DFE, as shown in section 2.3.4. Therefore our filtering procedure improves the visualization of growth rate evolution but does not impact significantly the estimation of DFE moments (see table S5).



Figure S 13: Distributions of single-cell growth rate in μ MA experiments.

Growth rate distributions (growth rates for all times are pooled) for A) the WT experiment shown in figure 3A (WT n^o1), B) another WT experiment (WT n^o2), C) the *mutH* experiment shown in figure 3A. Red curves in A and B are gaussian fits. D) growth rate evolution in WT n^o2. The slight asymmetry shown here in panel A creates a slight asymmetry in figure 3A, left panel, where we can see a few more data points in high growth rates than in low growth rates. This is due to some slight variations between experiments (we believe it is caused by slight differences in the quality of our images and image analysis) that are not strain-specific, as shown by the other WT experiment represented in panel B and D, which has a more symmetrical distribution



Figure S 14: Mortality induces noise in the estimation of the DFE's moments.

 $E_2(t) = \ln(\langle W_t^2 \rangle) - 2\ln(\langle W_t \rangle)$, whose slope is $\lambda \langle s^2 \rangle$, is plotted for a representative mutH μ MA experiment, taking all cells (bottom panel, green line) or only the cells that are still alive at 44 hours after the beginning of the experiment (top panel, blue line). R^2 coefficient of determination for linear regressions are indicated in each case (a good fit with a linear regression gives R^2 close to one).



Figure S 15: The dependency of the replication error rate on growth rate can be explained by the variations in the number of replication forks The average number of replication forks increases with growth rate. In balanced growth, this translates into a simple relation between these two variables. Nevertheless, here cells that grow slowly are stressed and their cell size is often larger than expected for their rate of growth. This adds some complexity to the expected relation between the average fork number and the growth rate (since the number of forks increases with cell size). Consequently, in order to determine whether the observed dependency of the error rate on growth rate can be explained simply by variations in the number of forks we estimate the number of forks for each single cell in the data, from its size and growth rate. We multiply this estimation by the mean error rate per fork in the cell population (i.e. the average rate of foci occurrence divided by the average estimated number of forks) to get an estimation of the error rate in each single cell. Here black dots represent experimental data, binned by growth rate (i.e.rates of foci occurrence are averaged for subpopulations of cells with similar rate of growth) and the red line shows the theoretical estimations on all single-cells, also binned by growth rate.



Figure S 16: Non-parametric estimation of the DFE moments.

Plot of $E_n(t) = \sum_{k=1}^n (-1)^k {n \choose k} \ln(\mathbb{E}(W_t^k))$ for n=1,..4, for a representative WT (blue) and mutH (cyan) experiment. Red and magenta lines are linear regressions for WT and mutH respectively. The WT experiment is a control showing negligible variations of $E_n(t)$. The slope of $E_n(t)$ in mutH is the product of the mutation rate and the nth moment of the DFE.



Figure S 17: Noise in single-cell growth rate . A) Probability Density Function of single-cell growth rate in WT; B) Autocorrelation function of single cell growth rate in WT as a function of generations; C) Mean and standard deviation of single-cell growth rate are plotted for 50 subpopulations of cells (blue dots), corresponding each to a single microchannel in a $mutH \mu$ MA experiment; we selected 35 microchannels where a strongly deleterious mutation occurred, extracted for each microchannel the growth rates of the mother cells after the mutation occurred and computed the mean and standard deviation; we also randomly selected 15 microchannels where no strongly deleterious mutation occurred and performed the same analysis; the red line is a linear regression



Figure S 18: μ **MA** simulations are in good agreement with experimental data. Numerical simulations of μ MA experiments, with a constant mutation rate (scenario A section 2.3.5) of 0.32 mutations per hour (identical to our estimation for *mutH* strain) and a Beta distribution with parameters $\alpha = 0.0074$ and $\beta = 2.4$ for the DFE. For each time point, all growth rates are plotted (as in figure 2) and color depicts the local growth rate density (from blue to yellow) and the red line is the evolution of the mean growth rate.



Table S 1: Mutant frequency measurements in batch cultures

As explained in section 1.4, we measured the frequency of antibiotic resistant mutants arising during growth of bacterial population in batch cultures. We used either rifampicin or streptomycin and cultured the cells either in LB or Minimal Medium supplemented with arabinose (to induce expression of dnaQ926 in MF1). The Mean and Standard Error of the Mean of mutant frequencies are given

Strain	Antibiotics	Growth	Mean mutant	SEM mutant	
Stram	Antibiotics	medium	frequency	frequency	
mutH	Streptomycin	LB	$9.4 \ 10^{-8}$	$1.6 \ 10^{-8}$	
mutT	Streptomycin	LB	$1.1 \ 10^{-7}$	$0.27 \ 10^{-7}$	
mutH	Rifampicin	MM arabinose	$1.0 \ 10^{-6}$	$0.8 \ 10^{-6}$	
MF1	Rifampicin	MM arabinose	$1.8 \ 10^{-5}$	$3.7 \ 10^{-6}$	

Table S 2: Mutation rate, death rate and growth rate decrease in μ MA for *mutH*, *mutT* and MF1. WT dies from aging [13], independently of mutations. Therefore we calculate the mutation-related death rate of each strain by subtracting the death rate of the WT to the death rate of the other strains. The mutation rate is calculated with MutL foci method for WT and *mutH* and from classical genetic assays (see section 1.4) for *mutT* and MF1

	mutH	mutT	MF1	WT
Decrease of average growth rate $(\%/hr)$	0.1	0.07	2.3	< 0.01
Death rate (per hour)	0.004	0.004	0.06	0
mutation rate (per hour)	0.32	0.37	5.6	0.0022

Table S 3: Estimated slopes of $E_1(t)$, $E_2(t)$, ... $E_{10}(t)$ for all μ MA experiments with WT, mutH and mutT strains. The moments of the DFE $\langle s \rangle, \langle s^2 \rangle, \ldots \langle s^{10} \rangle$ are estimated from the slopes of $E_1(t)$, $E_2(t)$, ... $E_{10}(t)$. The coefficient λ is the mutation rate. For mutH $\lambda = 0.0054 \ min^{-1}$, for mutT $\lambda = 0.0063 \ min^{-1}$, for MF1 $\lambda = 0.1 \ min^{-1}$, and for WT $\lambda = 2.7 \ 10^{-5} \ min^{-1}$.

	mutH	mutH	mutH	· · · · · / //		WT	WT	WT
	Exp1	Exp2	Exp3	mut1	IVIF I	$\mathbf{Exp1}$	$\mathbf{Exp2}$	Exp3
Slope of E_1 : $\lambda < s > (\cdot 10^5)$	1.9	1.6	1.5	1.2	39	-0.3	0.1	0.3
Slope of E_2 : $\lambda < s^2 > (\cdot 10^6)$	6.8	5.2	3.0	2.2	166	-1.5	-1.6	-0.1
Slope of $E_3: \lambda < s^3 > (\cdot 10^6)$	3.9	2.7	1.2	1.6	89	-0.1	-0.09	0.03
Slope of $E_4: \lambda < s^4 > (\cdot 10^7)$	25	16	7.3	9.4	560	0.1	-0.2	0.6
Slope of E_5 : $\lambda < s^5 > (\cdot 10^7)$	18	11	4.8	5.5	390	-0.01	0.06	0.4
Slope of $E_6: \lambda < s^6 > (\cdot 10^7)$	13	7.5	3.3	3.5	290	-0.05	0.01	0.2
Slope of $E_7: \lambda < s^7 > (\cdot 10^7)$	9.7	5.5	2.3	2.3	220	-0.04	-0.007	0.1
Slope of E_8 : $\lambda < s^8 > (\cdot 10^7)$	7.4	4.2	1.6	1.6	180	-0.03	0.002	0.07
Slope of $E_9: \lambda < s^9 > (\cdot 10^7)$	5.6	3.3	1.2	1.1	150	-0.02	0.001	0.05
Slope of $E_{10}: \lambda < s^{10} > (\cdot 10^7)$	4.3	2.7	0.8	0.8	130	-0.02	-0.002	0.03

Table S 4: Mean, Coefficient of Variation (CV), skewness and kurtosis of the DFE estimated from μ MA experiments with mutH (3 independent experiments), mutT (1 experiment) and MF1 (1 experiment)

	mutH	mutH	mutH	man + T	MTF1
	Exp1	$\mathbf{Exp2}$	Exp3	muti	
Mean $(\%)$	0.35	0.30	0.28	0.22	0.35
CV	10.0	10.3	8.2	9.2	11
Skewness	16.0	16.6	17.3	35	14
Kurtosis	302	329	446	1040	220

Table S 5: Estimated slopes of $E_1(t)$, $E_2(t)$, $E_3(t)$, $E_4(t)$ for a representative *mutH* μ MA experiment, with or without filtering of aberrant growth rate values (with different threshold parameters *T* for filtering).

	MutH T=0.15	MutH T=0.2	$\begin{array}{c} \mathbf{MutH} \\ \mathbf{T}=0.25 \end{array}$	$\begin{array}{c} \mathbf{MutH} \\ \mathbf{T}=0.3 \end{array}$	MutH T=0.35	$\begin{array}{c} \mathbf{MutH} \\ \mathbf{T}=0.4 \end{array}$	MutH T=0.45	MutH No filter
Slope of E_1 (.10 ⁵)	2.0	1.9	1.9	1.9	2.0	2.0	2.0	1.9
Slope of E_2 (.10 ⁶)	6.8	6.5	6.7	6.9	7.1	7.2	7.2	7.8
Slope of E_3 (.10 ⁶)	3.9	3.7	3.7	3.9	4.0	4.0	4.0	4.2
Slope of E_4 (.10 ⁷)	26	24	24	25	26	26	26	29

Table S 6: Estimated slopes of $E_1(t)$, $E_2(t)$, $E_3(t)$, $E_4(t)$ for a representative WT μ MA experiment, with or without filtering of aberrant growth rate values (with different threshold parameters T for filtering).

	WT T=0.15	WT T=0.2	WT T=0.25	WT T=0.3	$\begin{vmatrix} \mathbf{WT} \\ T=0.35 \end{vmatrix}$	WT T=0.4	$\left \begin{array}{c} \mathbf{WT} \\ \mathbf{T}=0.45 \end{array}\right $	WT No filter
Slope of E_1 (.10 ⁵)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2
Slope of E_2 (.10 ⁶)	-0.9	-1.3	-1.5	-1.6	-1.7	-1.9	-1.9	-1.6
Slope of E_3 (.10 ⁶)	-0.005	-0.05	-0.08	-0.09	-0.1	-0.09	-0.1	-0.1
Slope of E_4 (.10 ⁷)	-0.2	-0.1	-0.1	-0.2	-0.2	-0.2	-0.3	-1.2

Table S 7: The 4 first moments of *mutH* DFE estimated from the whole data (including cells that die before the end of the experiment). As in Figure 3C, mean +/-2 Standard Error of the Mean for the 3 *mutH* experiments are shown.

	Experimental
	DFE
Mean	0.0032 + / - 0.0004
Coefficient of Variation	7 + / - 2.3
Skewness	17 + / - 1
Kurtosis	500 + / - 200

Table S 8: Estimated slopes of $E_1(t)$, $E_2(t)$, $E_3(t)$, $E_4(t)$ for one representative $mutH \mu MA$ experiment with visual detection of cell death or with our automatic procedure to eliminate mortality effects. λ is the mutation rate of the mutH strain : $\lambda = 0.0054 \ min^{-1}$

	Visual de-	Automatic
	tection	Procedure
Slope of $E_1 : \lambda < s > (.10^5)$	1.97	1.92
Slope of $E_2 : \lambda < s^2 > (.10^6)$	6.69	6.79
Slope of $E_3 : \lambda < s^3 > (.10^6)$	3.77	3.85
Slope of $E_4 : \lambda < s^4 > (.10^7)$	25.0	25.3

Strain/plasmid	Genotype	Source
MG1655		E. coli GSC
ME63-01	MG1655, but mutH::cam	This study
ME63-02	MG1655, but mutT::kan	This study
ME63-03	MG1655, but pMQ	This study
63ME120R	MG1655, but $lacZ::yfp-mutL$ -frt, $mutL::frt$, $attTn7::pRNA1-tdCherry$	This study
63ME121R	63ME120R, but <i>mutH::cam</i>	This study
63ME122R	63ME121R, but <i>mutS::spec/strep</i>	This study
63ME123R	63ME120R, but <i>uvrD::phleo</i>	This study
63ME124R	63ME121R, but rnhB::kan	This study
63ME125R	63ME120R, but mutT::kan	This study
MF1	MG1655 attP21::araC-PBad-dnaQ926	This study
pMQ		[21]
pNDL32		Paulsson lab

Table S 9: Bacterial strains and plasmids \mathbf{S}

Table S 10: Estimated DFE moments for simulations of μ MA experiments, with *mutH* mutation rate, for scenario A (constant mutation rate) and scenario B (growth rate-dependent mutation rate). The mutation rate was the one estimated for our *mutH* strain. Mean +/- 2 Standard Error of the Mean $(\frac{\sigma}{\sqrt{(n)}})$ for 30 simulations are shown

	Scenario A	Scenario B
1st moment (mean) $.10^3$	3.1 +/- 0.1	3.1 +/- 0.1
2nd moment $.10^4$	9.4 + - 0.5	8.9 +/- 0.5
3 rd moment $.10^4$	4.4 +/- 0.4	3.9 + - 0.3
4 th moment $.10^4$	2.5 + - 0.3	2.1 +/- 0.2

Table S 11: Estimated DFE moments for simulations of μ MA experiments, with MF1 (dnaQ926) mutation rate (20x higher than *mutH*), for scenario A (constant mutation rate) and scenario B (growth rate-dependent mutation rate). Mean +/-2 Standard Error of the Mean $(\frac{\sigma}{\sqrt{(n)}})$ for 30 simulations are shown

	Scenario A	Scenario B
1st moment (mean) $.10^3$	3.1 +/- 0.03	2.0+/- 0.02
$2nd moment .10^4$	9.4 +/- 0.1	3.5 + - 0.07
3 rd moment $.10^4$	4.3+/- 0.1	1.1 + - 0.04
4 th moment $.10^4$	2.4 + - 0.08	0.5 + / - 0.03

Table S 12: Estimated DFE moments for simulations of μ MA experiments, with MF1 (dnaQ926) mutation rate (20x higher than *mutH*), for scenario A (constant mutation rate) and scenario B (growth rate-dependent mutation rate). The moments were estimated only on the beginning of the experiment (the first 10 hours). Mean +/-2 Standard Error of the Mean $(\frac{\sigma}{\sqrt{(n)}})$ for 20 simulations are shown

	Scenario A	Scenario B
1st moment (mean) $.10^3$	3.1 + - 0.05	2.8 + - 0.06
2nd moment $.10^4$	9.0 +/- 0.3	7.3 +/- 0.3
3 rd moment $.10^4$	4.0 +/- 0.2	3.0 + - 0.2
4 th moment $.10^4$	2.2 + - 0.2	1.5 + - 0.2

Movie S1. μ MA experiment with *mutH* strain (3 days of growth).

Movie S2. MV experiment with *mutH* strain. Top panel : Red fluorescence (constitutive expression of tdCherry). Bottom panel: Yellow fluorescence (YFP-MutL tags replication errors).

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