Temporal scaling of aging as an adaptive strategy of *Escherichia coli*

Yifan Yang\textsuperscript{1,2*}, Ana L. Santos\textsuperscript{1}, Luping Xu\textsuperscript{1,2†}, Chantal Lotton\textsuperscript{1}, François Taddei\textsuperscript{1,2}, Ariel B. Lindner\textsuperscript{1,2*}

Natural selection is thought to shape the evolution of aging patterns, although how life-history trajectories orchestrate the inherently stochastic processes associated with aging is unclear. Tracking clonal growth-arrested *Escherichia coli* cohorts in an homogeneous environment at single-cell resolution, we demonstrate that the Gompertz law of exponential mortality characterizes bacterial lifespan distributions. By disentangling the rate of aging from age-independent components of longevity, we find that increasing cellular maintenance through the general stress pathway reduces the aging rate and rescales the lifespan distribution at the expense of growth. This trade-off between aging and growth underpins the evolutionary tuning of the general stress response pathway in adaptation to the organism’s feast-or-famine lifestyle. It is thus necessary to involve both natural selection and stochastic physiology to explain aging patterns.

**INTRODUCTION**

The biology of aging and senescence revolves around the duality of individual frailty and population resilience. Throughout the course of normal metabolism, components of living systems such as cells, lipids, proteins, and DNA inevitably suffer from wear and tear such as free-radical damage. This constant and collective decay eventually leads to the loss of vital functions and collapse of individuals, measured as increased mortality and decreased fertility rates for nearly any living organism or even engineered systems. As an example, for humans, the Gompertz empirical law of mortality, one of the earliest quantitative laws in life sciences published in 1825 (1), demonstrates that human death probability doubles every 8 years. While this functional senescence approach is broadly applicable to nearly all living organisms and even engineered systems, identifying the causal molecular processes for senescence of any particular organism remains a major challenge for the aging field. Beyond that, understanding how system-level failures emerge out of microscopic damage could even reveal general organization principles that give rise to self-maintaining individuals in the first place (2, 3).

Yet, for some organisms in the tree of life, aging does not lead to increased mortality and declined fertility. For organisms such as hydrids, netleaf oaks, desert tortoises, and naked mole rats, mortality rates remain constant or may even decrease with age (4). More generally, the “immortal germline” theory (5) exemplified that organisms are able to repair or replace most of the damage to their components. Evolutionary biologists attribute the apparent senescence of metazoan somas to the inadequate investment in cellular maintenance as an adaptive strategy to maximize lifetime reproductive success, due to the trade-offs between the survival and reproduction of the young on one hand and maintenance for the benefit of the old on the other (6, 7). Presumably, natural selection has to operate through the molecular “levers” of damage accumulation and/or repair to achieve such life-history optimization.

*Escherichia coli,* a single-cell prokaryote, has historically served as a model organism that resolved many fundamental questions in biology. *E. coli* cells, as their metazoan counterparts, suffer from damage to their components, which lead to cellular senescence (8–10). In exponential growth, these damages are quickly diluted by de novo biosynthesis, and the effects of cellular senescence mitigated by rapid and robust reproduction (11). Yet, the natural life cycle of *E. coli* entails a much wider range of physiological conditions than exponential growth. Most bacterial cells spend much of their lives in resource-limited growth arrested conditions, where de novo biosynthesis rates are slower (12). While *E. coli* undergoes substantial changes in adaptation to these conditions, once the depletion of nutrients is complete, without growth-associated dilution, the accumulation of molecular damage such as protein misfolding and oxidation eventually leads to functional senescence (fig. S1). Despite the lack of fixed separation of germ and soma cells, the ability to survive the wear and tear of cellular components during growth arrest contributes to bacterial overall fitness as much as the ability for exponential growth. Therefore, we adopted a biodemographic approach (2, 3) to understand how modulation of molecular damage repair could shape aging dynamics in growth-arrested *E. coli.*

**RESULTS**

**Longitudinal tracking of vitality at single-cell level**

We first set out to acquire high-quality longitudinal physiological and life-history data at single-cell resolution of clonal populations of *E. coli*. For decades, limitations of traditional cell culture methods have frustrated quantitative physiologists’ attempts to understand bacterial maintenance (13, 14), due to both media-cell interactions and cellular interaction such as cross-feeding and cannibalism. Here, we designed a novel microfluidic device with cell-dimension chambers to measure individual *E. coli* cell lifespan in constant and homogeneous environmental settings (Fig. 1). Cells are trapped in an array of single-cell–wide dead-ended wells, with openings to a main flow channel. Constant flow of fresh media in this main channel provides necessary nutrients and eliminates metabolic waste, cell debris, and intercellular cross talk so that the environmental conditions are maintained constant over time. Cells with appropriately expressed fluorescent markers are imaged bottom-up and appear as fluorescent spots (Fig. 1B). At the end of the experiments, 70% ethanol was injected into the device to
To observe cell mortality, we included in the carbon source–free medium a red-fluorescent, DNA-binding, bacterial viability dye propidium iodide (PI), which penetrates the cells only when cellular membrane potentials are disrupted. PI staining has been established as an effective proxy of cellular death and correlates well with cell viability assessed by proliferating potential (15). We used PI at a concentration fourfold lower than the concentration that previously had been shown to have no effect on E. coli viability and growth (15). Automatic time-lapse fluorescence microscopy and fixed geometry of our devices allowed longitudinal quantification of the PI signal for every single cell in the population.

Despite being genetically clonal and environmentally controlled, cells did not share the same time or manner of death, as measured by the PI time series. The transition from life to death of a representative cohort (N = 4744) could be visualized by the boundary between dark and light in Fig. 1D. Because cells were sorted vertically according to estimated lifespan, the shape of this boundary represents the survival function of the population. Considering aging as a stochastic process of system reliability reduction, the observed time of death could be modeled as the first time to hit death as an absorbing barrier, in accordance with the classic first passage time problem for stochastic models (2, 3). In addition, we observed individual differences in death trajectories. Short-lived cells tended to have very sharp PI increases that are associated with abrupt losses of membrane integrity, while those that die late tended to suffer a type of “slow death” characterized by a gradual PI increase over the course of 10 to 15 hours. Dark strips after cell deaths indicate decaying DNA or empty wells after cell debris have been washed away.

**E. coli lifespan distributions follow the Gompertz law**

For both demographers and reliability engineers, the age-associated increase in death probability, also known as the hazard rate $h(t)$, is considered to be a hallmark of aging (2–4). The detailed population statistics derived from our experiments are particularly useful in estimating the shape of the hazard functions over the whole lifespan. We find that E. coli lifespan distributions have, as their main feature, regimes with exponentially increasing hazard rates, $h(t) = h(t_0) e^{b(t-t_0)}$, where $h(t_0)$ is the initial hazard rate and $b$ is the Gompertz aging rate, i.e., the Gompertz law of mortality (1, 16). For wild-type cells, this exponential regime spans from about 13 to 93 hours (Fig. 2A), corresponding to approximately 90% of all cell deaths (see below for the 10% cell deaths outside of the exponential regime), and ranges within at least 100-fold change in hazard rates ($2 \times 10^{-4}$ to $2 \times 10^{-1}$ hour$^{-1}$). Within this exponential regime, the doubling time of hazard rate is $9.4 \pm 0.5$ hours ($N_{\text{total}} = 4744$). In comparison, the doubling time of human mortality hazards is about 8 years (1).

Hazard rates not only define lifespan distributions but also can be thought of as surrogates for system vulnerability, whose dynamics through time reflect the physiological consequence of aging. In this light, the Gompertz law can be interpreted as a dynamic equation
governing the aging process: \( \frac{dh(t)}{dt} = bh(t) \), where \( b \) is the Gompertz aging rate, which can be directly observed in the phase plane of the cumulative hazards \( H(t) \) without any free parameters (Fig. 2B). The fact that the theoretical trajectory (dashed line in Fig. 2B), including the aforementioned linear regime, lies within the confidence intervals (CIs) of almost every state coordinate lends strong statistical credence to the Gompertz law for describing our data. In addition, this dynamic interpretation of Gompertz law should also constrain stochastic models of biochemical decay (fig. S3).

The observed hazard rates deviate from the exponential regime at the beginning (\( t < 15 \) hours, 3% cell deaths) and the end (\( t > 93 \) hours, 7% cell deaths; see Fig. 2B, inset, top x-axis) of the total lifespan. These observations are reminiscent of similar deviations from Gompertz law in human mortality data (17, 18). In our case, the additional mortality at the early age might result from harvesting and transferring exponentially growing cells inside our microfluidic chip. Late-life hazard decelerations are also common phenomena for both model organisms and human populations and are thought not to reflect decelerations of physiological aging on the individual level, but result from changes in composition of heterogeneous populations (18). Despite being genetically and environmentally nondistinguishable, due to the stochastic nature of cellular biochemistry, we think it is reasonable to assume that physiological heterogeneity exists in our single-cell populations for such selection effect to manifest itself. We found that an extension to the Gompertz law with four parameters, named the Gamma-Gompertz-Makeham (GGM) model

\[
h_{\text{ggm}}(t) = \lambda + \frac{bs}{1 + (\beta - 1)e^{-bt}}
\]

could fully model our data satisfactorily across the whole lifespan (see Materials and Methods for parameter explanations and Fig. 3, fig. S4, and tables S1, S2, and S3 for statistics). We find that the GGM model maintains good fit to all of our data and is flexible enough to account for various types of hazard dynamics. For this reason, the GGM model enabled us to evaluate genetic impacts on the aging process, in the next section.

**Modulation of aging rate by the general stress response**

Several nutrient-sensing pathways in both bacteria and metazoans have been shown to control stress resistance and reduce mortality (19–21). Yet, their roles in delaying aging-related damage accumulations are often controversial. After all, it is relatively easy to prolong lifespan and reduce unnecessary mortality in an age-independent way in the laboratory by changing environmental or genetic factors unrelated to aging. Disentangling effects on aging from age-independent components of longevity (22) requires large cohort size and detailed mortality statistical resolution as described in the previous section. Both the Gompertz regime in hazard curves and the fully parametric GGM model can be used to quantify the age-dependent effects as the aging rate \( b \), which is the exponential rate of increase of mortality risk. It can be estimated by examining the slope of the hazard curves on a semi-log scale (Fig. 2A). For example, the original Gompertz law postulation that the risk of death for humans doubles every 8 years (20) is equivalent to \( b_{\text{human}} = \frac{\ln(2)}{8 \text{ years}} = 0.087 \text{ year}^{-1} \).

Many bacteria species regulate the level of cellular maintenance through a genetic pathway called general stress response (GSR), controlled by the master transcriptional regulator \( rpoS \) that is activated by nutrient deprivation among other signals (Fig. 3A) (23). Evolutionary theories of aging predicted the existence of aging-modulating mechanisms and their activation by nutrient limitation (24). To empirically test this idea, we measured and compared the full lifespan distributions of two GSR mutants, \( \Delta rpoS \) and \( \Delta rssB \), with that of the
The GSR of *E. coli* modulates aging rate. (A) A scheme representing relevant regulatory features of the GSR and, in particular, the functions of the genes *rpoS* and *rssB*. (B) Experimental and GGM model survivorship. The lifespan distributions for the wild-type (wt), Δ*rpoS* (lacking GSR), and Δ*rssB* (overexpressing the GSR) strains are measured multiple times by independent microfluidic experiments. Representing the experimental survivorship, color bands are the 95% CI of the Kaplan-Meier estimators. Colored dashed lines are GGM models whose parameters are estimated from maximum-likelihood (ML) methods. (C) Hazard rates estimated using only cell deaths within discrete time intervals (error bar markers), and GGM hazard models estimated from the whole dataset using ML methods. Error bars are similar to those in Fig. 2A. Parametric comparisons and GLM-based models (see "Experimental design and experimental variation analysis" section) indicate that Δ*rssB* prolongs longevity entirely by reducing aging rate and that Δ*rpoS* both accelerates aging and increases basal mortality rate. (D) Aging rates for each strain, estimated by three independent experiments and GLMs. Error bars represent 95% CI.

wild-type strain. Δ*rpoS* is devoid of GSR, while Δ*rssB* displays an elevated GSR due to increased RpoS stability (25).

Consistent with the well-documented role of GSR in stress resistance (23), we observed that a higher GSR level promotes longevity in the microfluidic experiments, whereas the absence of GSR results in shortened longevity (Fig. 3B and fig. S5A). In contrast to similar studies, where only the average lifespan or the survival curve was measured, our microfluidic experiments can measure the hazard dynamics (Fig. 3C) of each strain and extract the rates of aging and not just longevity.

Remarkably, we found that increased GSR reduces the rate of aging (Fig. 3D and fig. S5C). Given how well the GGM model fitted for all three strains, the impact of genotypes on aging parameters such as aging rates could be extracted using generalized linear models (GLMs). With enhanced GSR, Δ*rssB* cells double their mortality risk every 14.1 hours, with a 95% CI ranging from 13.1 to 15.3 hours, compared to 9.4 hours for the wild-type (CI, 8.9 to 9.9 hours) and 7.3 hours for the null strain Δ*rpoS* (CI, 6.8 to 7.7 hours). We assessed the variability of aging rate measurements using three independent experimental cohorts for each strain (fig. S4). We visualized nonparametrically the overall experimental variations (fig. S5, A and B), and also tested parametric differences in aging rates among experimental repeats using GLMs and the Akaike information criterion (AIC). We confirmed that experimental repeats shared similar aging rates (*H*<sub>0</sub>), same aging rate for experimental repeats. Difference in degrees of freedom: *H*<sub>0</sub> − *H*<sub>1</sub> = −1; ΔAIC<sub>ΔrpoS</sub> = −2.56; ΔAIC<sub>ΔrssB</sub> = −2.26; ΔAIC<sub>ΔrpoS</sub> = −2.30; *N*<sub>ΔrpoS</sub> = 6867, *N*<sub>ΔrssB</sub> = 6969, *N*<sub>ΔrpoS</sub> = 4793; for details, see tables S1 to S4).

The systematic increase in vulnerability of *E. coli* in our experiments is likely driven by the catabolism of preexisting macromolecules and dissipation of biomass (26), which is necessary to provide energy to express housekeeping genes and maintain physiological homeostasis (12, 27). Our finding that GSR modulates aging rate suggests that optimizing this maintenance energy requirement is likely one of the physiological functions of the RpoS regulon (see fig. S3).

**Evolutionary trade-offs mediated by the GSR**

Is slower aging an adaptive life-history trait? This was one of original questions raised by Sir Peter Medawar (28) that motivated much of later aging research. Optimal life-history theory suggested that trade-offs and constraints among fitness components shape metazoan aging rates (7). The possibility of modulating aging rate through GSR offers us the opportunity to test these ideas in a fast-evolving organism as *E. coli*.

The relatively well-understood GSR pathway provides a clear molecular mechanism for a trade-off between growth and maintenance. The master regulator *rpoS* encodes the RNA polymerase (RNAP) sigma subunit σ^70^, which competes with the other sigma factors including the vegetative σ^′^ to recruit the core RNAP and direct the transcription and translation machinery toward the RpoS regulon (Fig. 4A). By titrating protein synthesis activity away from metabolic and ribosomal genes controlled by σ^′^, RpoS activity inhibits growth and nutrient assimilation (29). We measured quantitatively the growth impact of modulating GSR levels and modeled its effect using a simple coarse-grained model of proteome sectors (30). We found that the proportion of protein synthesis devoted to the RpoS regulon increases the time scale of growth (top axis in Fig. 4B).

To assess the life-history optimality of different aging rates, we integrated numerically the experimentally derived growth and mortality rates into fitness, defined as the long-term population growth rates. In contrast to metazoans, for whom fertility and mortality schedules are connected through fixed age structures, our model of *E. coli* life history consisted of alternating environmental episodes of varying durations, in which *E. coli* populations either grow (“feast”) or decline (“famine”) (Fig. 4B). These feast-or-famine cycles were parameterized by two transition rates, controlling respectively the average lengths of feast and famine episodes. To identify selective pressure for aging rates, we directly compared the fitness of the three strains with different GSR phenotypes, each representing a different strategic position in the growth-maintenance trade-off. We identified the environmental regimes selecting for faster or slower aging. We characterized the boundaries between these regimes along two axes (Fig. 4C): lifestyle ratio, defined as the ratio of time spent in famine over feast; versus average duration of famine episodes.

There are two necessary conditions for selecting slower aging strategies represented by Δ*rssB*. First, populations have to spend much more time in famine rather than feast so that, over the long term, populations decline rather than grow. Second, but no less important, given the same lifestyle ratio, famines should consist of longer episodes rather than short but more frequent ones. This condition is necessary due to the exponential mortality dynamics described by Gompertz law: Investing
that attenuate or knock out RpoS activity are among the first to arise do not pass through prolonged growth-arresting bottlenecks, mutations o u rp r e d i c t i o n s. I nc o n t i n u o s c u l t u r e so f strategies, as is observed in experimental evolution (et al Yang et al Sci. Adv. 2019;5). In contrast to the isolated populations under constant environmental...4. Trade-offs between growth and maintenance mediated by the rpoS pathway and its fitness consequences. (A) Scheme of the ecological processes (dashed line) and regulatory relationships (solid line) involved in the trade-offs mediated by rpoS. (B) Experimentally measured fitness of ΔrpoS (red), wild-type (blue), and ΔrssB (green) strains as functions of time spent in feast (top) and famine (bottom) conditions. Fitness is defined as the logarithmic change of population sizes (for measurements, see Materials and Methods). Ranges of environmental conditions could be modeled by two transition rates between feast and famine, k1 and k2. (C) Fitness simulation results identifying environmental regimes favoring faster and slower aging strategies, exemplified by the three strains measured in (B). The color-coded regions identify environmental conditions under which one strain dominates over the other two. Environmental conditions are parameterized by lifestyle ratio k1/k2, the ratio between time spent in famine and feast conditions, and the average duration of famine episodes 1/k2 (see Materials and Methods for simulation details).

in cellular maintenance only becomes beneficial at old age when the exponentially increasing benefits of slower aging eventually overcome the more immediate cost on growth. It is the typical time scale of famine that provides the selective pressure for aging rates.

By representing the complex regulations of GSR with two mutants, we can now understand the ecological role of GSR activation and its adaptive consequences. Activated by declining nutrient availability, GSR directs resources toward internal maintenance to counter the adverse conditions, whose lengths determine the optimal activation level. Previous observations from experimental evolution of E. coli support our predictions. In continuous cultures of E. coli where the populations do not pass through prolonged growth-arresting bottlenecks, mutations that attenuate or knock out RpoS activity are among the first to arise (31). In contrast to the isolated populations under constant environmental conditions in our chip experiments, E. coli populations, in nature, influence their environments and also interact with each other. These interactions may give rise to frequency-dependent selection and evolutionary game dynamics between slower and faster aging strategies, as is observed in experimental evolution (32).

DISCUSSION

We demonstrated that aging of growth-arrested E. coli follows the Gompertz law of mortality. Moreover, by regulating the GSR, E. coli could modulate its aging rate and rescale the lifespan distribution temporally. We further articulated, in a demographic model, the trade-offs and selective pressure driving the evolution of aging rates.

To rescale the lifespan distribution without changing the shape of hazard functions, GSR has to orchestrate a coordinated response to manage various damages that accumulate on different time scales (2). RpoS regulates hundreds of genes conferring resistance to various internal and external stresses (23). For this reason, the aging rate, in our case, reflects the general level of macromolecular catabolism. We hypothesize that the maintenance energy (14), i.e., the energetic cost of homeostasis, is paid by the loss of biomass, leading to the gradual increase in the probability of death. It is thus the rate of living (33) that correlates with the aging rate in our system (fig. S3).

We provide rare experimental support for antagonistic pleiotropy (6), a key concept in evolutionary theories of aging, despite bacteria’s vast differences from animals. Sigma factor competition, a well-understood mechanism in the physiology of bacterial stress response, provides a molecular basis for a trade-off between growth and maintenance. Activated by nutritional deprivation and now shown to decrease the aging rate and prolong lifespan, GSR has immediate analogies in calorie restriction–induced longevity. Whereas many metazoan studies have only shown that calorie restriction promotes longevity, our demographic data indicate that GSR changes the rate of aging in addition to longevity promotion. It provides not only resistance against stress but also insurance against prolonged growth arrests, or more generally, protection against withering through time.

In our work, the proximate and ultimate causes of aging are integrated and applied to the iconic model organism, E. coli. The proximate cause explains aging as the stochastic and inevitable erosion of organismal order and biochemical redundancy. The ultimate cause views aging as a component of the optimized life-history strategy. In bacteria, aging and mortality processes are indeed stochastic but still could be characterized quantitatively by the Gompertz law of mortality in terms of lifespan distributions. Regulatory and selective forces do not modulate age-specific mortalities independently, as traditionally modeled (34), they rescale the whole distributions. Our empirical observations and integrated perspective in bacteria call for similar approaches in understanding aging as a general phenomenon in living systems.

MATERIALS AND METHODS

Experimental methods

Microfluidic chip fabrication

Our polydimethylsiloxane (PDMS) lab-on-a-chip system consists of two layers, containing the flow channel and the array of cell-sized
of the Keio
lized by dry heat. Carbon-free minimum medium mentioned below
organ contamination during autoclaving, and glassware was steri-
Yang et al (diameter, 1.2
channel, SU8 3050 photoresist (MicroChem, MA, USA) was patterned
on a silicon wafer using photolithography. SU8 3050 was spin-coated
on a silicon wafer at 4000 rpm for 30 s, baked at 95°C for 15 min, and
then subjected to ultraviolet exposure (25 s, 10 mW/cm²). After post-
exposure baking (95°C for 5 min), the master was developed using SU8
developer (MicroChem, USA), rinsed with isopropanol, and dried with
filtered nitrogen.

The negative master for the layer with cell-sized chambers was fab-
ricated using reactive ion etching (R.I.E.) technology on a silicon wafer.
The mask was patterned on a silicon wafer using photoreist
AZ5214 (MicroChem, USA), and a 100-nm layer of nickel was sput-
tered onto the substrate. A lift-off procedure was applied to remove
the photoreist layer yielding the metal mask for the R.I.E. process.
By adjusting the R.I.E. parameters [SF6, 4 sccm (standard cubic centi-
meter per minute); CHF3, 16 sccm; pressure, 10 mTorr; power, 30 W], we
managed to achieve a large array of micropillars with high aspect ratio
diameter, 1.2 μm; height, 6 μm).

To form the device, PDMS mixtures (RTV615; Momentive Per-
f ormance Materials Inc., Waterford, NY) were poured (flow channel)
and spin-coated (array) onto the masters to a thickness of 5 mm (main
channel) and 80 μm (array), respectively. Heat curing initially formed
solid PDMS layers with patterned surfaces. After drilling inlets and out-
lets through the flow channel layer, and mounting the array layers onto
cover glasses, the two layers were then bonded together using oxygen
plasma (90 s, 1000 mTorr). Last, the assemblies were cured at 80°C
overnight to produce the integrated microfluidic chips. On the day of
use, the wetted surfaces of the PDMS chip were first activated by expo-
sure to oxygen plasma (90 s, 250 mTorr), immediately followed by in-
fusion of 20% (v/v) polyethylene glycol 400 (PEG400) solution to
prevent bacterial adhesion and biofilm formation.

**Medium preparation**
All equipment used for medium preparation, sterilization, and infusion
were made of nonleaching materials (glass, polytetrafluoroethylene i.e.,
PTFE, or similar perfluoropolymer material) to avoid contamination
with trace level carbon sources from leachable plastic additives (table S5 for details).
Medium was filter-sterilized (0.2 μm) to avoid volatile organic contamination during autoclaving, and glassware was steri-
lized by dry heat. Carbon-free minimum medium mentioned below
refer to those prepared in this fashion.

**Strain information**
All lifespan distributions described above were measured for strains
of the Keio E. coli BW25113 strain (‘wild-type’) single-gene knockout
collection (35). For the knockout strains, the presence and location of
genomic inserts were verified by kanamycin resistance and polymerase
chain reaction amplification. The GSR phenotypes of ΔpoS and ΔrssB
were verified using the catalase test, where ΔpoS correctly showed
minimal catalase activity and ΔrssB greatly increased catalase activity
compared to wild-type (36). In addition, in developing the microfluidic
device and validation of our method, we used an MG1655-derived
E. coli strain with a chromosomally integrated cyan fluorescent protein
under the P2rnmB constitutive promoter (Fig. 1B).

**Cell culture and loading**
Single isolated colonies of the bacterial strains E. coli wild type, ΔpoS,
and ΔrssB were grown overnight in minimal medium (1× M9 salts,
2 mM MgSO4, and 0.1 mM CaCl2) supplemented with 20% (w/v)
glucose (final concentration of 0.4%). The following day, the overnight
cultures were diluted 200-fold in 50 ml of fresh medium in 250-ml
Erlenmeyer flasks and grown to early exponential phase [optical density
at 600 nm (OD600) = 0.2]. This growth phase was chosen to guarantee
that the variation of birth time among cells was less than one cell cycle
and thus to minimize the uncertainty of lifespan measurement. Cells
were concentrated by centrifugation (4000 rpm for 15 min at 37°C)
and washed by three cycles of gentle resuspension with carbon-free
minimal medium and centrifugation before injection into the micro-
fluidic channels. Cells were then trapped into the dead-ended wells by
centrifugation at 2000 rpm for 15 min at 37°C with a surface density up
to 6.25 × 10^4 cells/mm^2. The main channels were then thoroughly
washed with carbon-free minimal medium.

**Experimental setup and microscopy**
A constant flow of carbon-free M9 minimal medium at 20 μl per hour
was provided to the microchannels using a high-precision syringe
pump (Harvard Apparatus PHD 2000 Programmable) and Hamilton
GC-grade glass/PTFE syringes (Gastight 1000 Series). PTFE tubing was
used to connect the syringes to the microfluidic chip. The medium
was supplemented with 1.5% (v/v) PEG400 to prevent unspecific ad-
herence of cells to the channels, and PI (5 μg/ml), as a fluorescent
indicator of cell viability, was added. Cell viability was monitored
using temperature-controlled (37°C) automatic time-lapse microscopy
(Zeiss AX10, 63× oil-immersion objective, controlled with MetaMorph
software). Focus was maintained by a Z-scanning maximum-contrast
procedure using phase-contrast illumination so that dead ends of the
microwells are imaged. Focus was automatically readjusted before each
imaging cycle for every position and maintained within a Z range of
0.2 μm around the maximum-contrast Z-position. Phase-contrast
and fluorescence images (PI signal excitation, 546/12 nm; emission,
605/75 nm) were acquired for every stage position once every hour for
up to 150 hours.

**Growth phenotypes**
Growth phenotypes of aforementioned E. coli strains on selected carbon
sources in minimal media were measured in the 96-well format using a
TECAN Spark microplate reader. To induce the appropriate metabolic
enzymes before growth curves could be measured, strains were first grown
for 24 hours in minimal medium (M9, as in those used for microfluidic
experiments) supplemented with the assayed carbon sources.
The optimal densities of these cultures were determined and diluted into
fresh medium identical to those used for the overnight cultures. The
dilution ratios were chosen so that all experimental cell cultures have
an initial optimal density of OD600 of 0.002. For each experimental
well on the microplates, 50 μl of mineral oil was added to 100 μl of cell
culture to prevent evaporation during the experiments. The microplates
were then maintained at 37°C and shaken constantly in double-orbital
motion at 150 rpm by the plate reader. Microplates with flat-bottomed
wells were used to maximize agitation. OD600 readings were taken every
10 min. The growth phenotypes used in Fig. 4 are based on minimum
medium culture supplemented with 60 mM acetate as the carbon source.

**Statistical and computational methods**

**Image analysis**
The cells in our experiments were trapped in an evenly spaced 2D grid.
The fluorescence signals of every cell throughout their whole lifespan
could be extracted at fixed positions, once images in the time-lapse stack
were properly registered. A simple registration procedure might mis-
identify one cell for another because the cells were vertically imaged and
looked very similar to each other if only local features were considered.
To register the images based on global features, such as the presence/absence of cells at individual grid positions, we devised a two-pass, coarse-to-fine registration strategy. Cells were first identified and segmented within the images using the point spread function (37). These segmented binary image stacks containing global information were registered in a coarse pass using least-square minimization. The obtained 2D translations were applied to the original images. A second fine registration pass was executed on these preregistered original images using the Pyramid Approach (38). After registration, the salient positions of cells were detected on the Z-projected images of the whole time-lapse stacks, and fluorescence time series was extracted from these positions.

**Regularized estimation of PI signal**

To determine the true PI signal of each individual cell and to remove the noisy effects of focusing fluctuations, we designed and implemented a correction algorithm to the raw fluorescence intensity time series (fig. S7). Our three-step algorithm estimated the focusing noises of each imaging position and deduced them from the raw time series to arrive at the true PI signals of each cell. The first step of our algorithm took advantage of the fact that focusing fluctuations should change synchronously the intensities of all fluorescent objects within an imaging position, while the true PI signal from the cells should move independently of each other. By averaging the fold changes in intensities over all cells within a given time-lapse image stack, the focusing noise was enhanced while cell-specific signals were spread over hundreds of independent time series. In the second step, the averaged fold changes were decomposed into focusing noises and population-wide PI trends. This was possible because noise from focusing should have very quick fluctuations (small auto-focusing noises and population-wide PI trends. This was possible because cells within a given time-lapse image stack, the focusing noise was enhanced independently of each other. By averaging the fold changes in intensities over all cells within a given time-lapse image stack, the focusing noise was enhanced while cell-specific signals were spread over hundreds of independent time series. In the second step, the averaged fold changes were decomposed into focusing noises and population-wide PI trends. This was possible because noise from focusing should have very quick fluctuations (small auto-correlation time on the order of imaging cycles) yet no long-term trends (focusing was maintained with a narrow Z range of 0.2 μm). We applied a total variation regularization algorithm (39) to effectively denoise the average fold changes to produce the population-wide long-term trends. In the last step, the population-wide PI trends were combined with the cellspecific signals to recover the true PI signals used to determine the times of death. See fig. S7 for the details and the effects of the algorithm.

**Time-of-death determination**

To establish the time of death or time of censoring, we used a thresholding method (fig. S2). A threshold specific to each cell was defined as the half-way point between peak PI signal of each cell and background fluorescence. The first time that PI signal exceeded the threshold was considered to be the time of death. Our method was designed to capture the mortality events of cells trapped at the ends of cell-sized chambers. Throughout our experiments, the focusing plane of the microscope was maintained at the bottom ends of these 6-μm-long chambers. Because the cells trapped there were in focus, their deaths would generate the biggest PI upticks in their respective PI time series. Thus, it would be most likely the deaths of these cells that take the PI signal across the half-way threshold between peak and background.

In cases where no PI signal declines were observed before the end of the experiments when alcohol was injected, we could not establish for the cells in question reliable peak intensities for the PI time series nor times of death. Only lower bounds of both quantities could be established. In the language of survival analysis, these cells were considered to be censored at the time that their PI signals cross the half-way point between the observed PI maximum and the background. Censoring here referred to a technical procedure in survival analysis, indicating that their survival up to the censoring time still contributed to the overall likelihood of the model, while their precise time of death were not considered. The average proportions of censorship for each of three strains were 0.0% (ΔArpsS), 2.7% (wild-type), and 28.4% (ΔrssB).

**Nonparametric estimation of hazard functions**

The conventional method of estimating cohort hazard rate is to estimate the cumulative hazard \( H(t) = \int_0^t h(t') dt' \) using the Nelson-Aalen (N-A) estimator \( \hat{H}_{NA}(t) \) (40). This is because \( H(t) \) can be estimated independent of binning, avoiding unnecessary reductions in statistical power. However, in most of our experiments, the sizes of cohorts allowed us to estimate \( h(t) \) directly, by binning mortality events within discrete time intervals \((t_i, t_i + 1)\). \( \hat{h}_i \), the hazard rate within \((t_i, t_i + 1)\), could be estimated with binomial error \( \hat{h}_i \sim d/Y_0/\Delta t_0 \) where \( Y_0 \) is the number of individuals at risk at time \( t_0 \), and \( d_i \) is the number of cell deaths within \((t_i, t_i + 1)\).

Because of the exponential nature of Gompertz mortality, we could observe the Gompertz law nonparametrically without any free parameters. The instantaneous hazard rate \( h(t) \) is related to the state of the whole population before time \( t \) with the state of the whole population before time \( t \) [because \( H(t) = -\ln(S(t)) \), where \( S(t) \) is the survival function]. Thus, when the two independent hazard estimates were plotted against each other in Fig. 2B, the x-axis coordinates \( \hat{h}_i \) were only determined by events occurred within each time interval \((t_i, t_i + 1)\), while the cumulative hazard estimate \( \hat{H}_{NA}(t_i) \) was only determined by events occurred before \( t_i \). Given the independence of these two estimates [\( \hat{H}_{NA}(t_i), \hat{h}_i \)], the linear regime in Fig. 2B lent strong statistical evidence to the Gompertz law.

**Parametric models of hazard functions and lifespan distributions**

We used the GGM model, \( h_{gm}(t) = \lambda + \mu \frac{b t}{1 + e^{-bt}} \), for four parameters to fully model our data across the whole lifespan. In addition to the Gompertz law parameters, the aging rate \( b \) and \( \mu \), we added two parameters to account for deviations from the Gompertz regime at the beginning and the end of the lifespan.

The additional mortality at the early age might result from harvesting and transferring exponentially growing cells from batch culture directly to growth-arresting conditions inside our microfluidic chip, in a way analogous to infant mortality in human mortality data. The standard model of this is the Gompertz-Makeham model, by adding an age-independent component \( \lambda \) to the hazard function: \( h_{gm}(t) = \lambda + \mu \frac{b t}{1 + e^{-bt}} \), where \( \lambda \) controls the level of frailty heterogeneity: \( h_{rg}(t) = \mu \frac{b t}{1 + e^{-bt}} \), where \( \lambda \) is the survival function of Gamma-Gompertz, and other parameters are as before.

**Survival analysis**

The lifespan data of each cohort were fitted parametrically using the family of lifespan distributions described above. Maximum-likelihood (ML) estimators of the model parameters and their CIs were obtained using the R package “flexsurv.” We tested the two-, three-, or four-parameter hazard models mentioned in the previous section, with the
four-parameter GGM model being the most general, and chose the best among these candidate models according to the AIC at their ML parameters.

Goodness of fit of the ML models were tested using the one-sample version of sup(|Y(t)|), a Kolmogorov-Smirnov (K-S) type statistic adapted to account for right censorship (42), which occurs in our experiments for cells whose PI peaks were observed after alcohol injection. In the one-sample case of goodness-of-fit testing, Y(t) is the normalized K-S distance between the empirical survivorship and the survival function of the fitted model, i.e., the fitting residues. The best models and their fitting residues Y(t) were plotted in Fig. S4. For comparison and visual inspection, we also plotted alongside the Kaplan-Meier estimators for survivorship, N-A estimators for cumulative hazards, and their respective 95% CI.

**Experimental design and experimental variation analysis**

Populations within the same microfluidic channels were followed at multiple imaging positions, distributed evenly throughout the flow channel. We tested the statistical consistency and homogeneity of lifespan distributions among subpopulations at different imaging positions within the same channel (see figs. S5A and S8 for examples of these subpopulations). The PI time series data and lifespan distributions from each imaging position were visualized in the style of Fig. 1D (fig. S8A). Statistically, we tested for nonparametric differences between subpopulation lifespan distributions using a two-sample K-S statistic sup(|Y(t)|), i.e., the supremum of empirical distribution distances |Y(t)| normalized to account for censorship (fig. S8B) (42). After passing the consistency test, the subpopulations from the same channel were merged to form the experimental cohorts.

Three independent experimental cohorts were tracked on separate dates for each strain. Two statistical methodologies were used to assess the level of experimental variability and test for significant differences in lifespan distribution and its parameters among the strains. Overall, variations in lifespan distribution were analyzed using hierarchical clustering. The two-sample K-S statistic sup(|Y(t)|) (2, 42) mentioned in the previous section was used as a distance measure in complete linkage clustering. Preferential aggregation of experimental cohorts of the same genotype within the lower branches of the clustering tree was taken as evidence that lifespan distributions of the strains were significantly different.

Because the GGM model adequately described our data, we also analyzed variability of its parameters and the sources of the said variability. GLMs with GGM as the probability distribution were built to examine the variability of its parameters and the sources of the said variability. GLMs with GGM as the probability distribution were built to examine the variability of its parameters and the sources of the said variability. GLMs with GGM as the probability distribution were built to examine the variability of its parameters and the sources of the said variability. GLMs with GGM as the probability distribution were built to examine the variability of its parameters and the sources of the said variability.

**Fitn ess mo dels**

Fitness is defined over a time period from t1 to t2 as $f(s, t_1, t_2) = \Delta \ln \left( \frac{t_2}{t_1} \right)$, where s denotes one of the three strains. We calculated fitness based on time spent in feast or famine episodes. These fitness functions were extrapolated from experimental data. For famine episodes, changes in logarithmic population size were simply the negatives of cumulative hazards $H(t_1) - H(t_2)$, so that $f_{\text{famine}}(s, t_1, t_2) = [H(t_1) - H(t_2)]/(t_2 - t_1)$. Extrapolations from experimental data were done using the fitted GGM models. For feast episodes, $f_{\text{feast}}(s, t_1, t_2) = \frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \mu_s(t) \, dt$. Specific growth rates $\mu_s(t)$ and maximum growth rates $\mu_s^{\text{max}}$ were determined from the experimental growth curves smoothed by cubic B-splines. For feast episodes longer than the time at which $\mu_s^{\text{max}}$ was reached, we assumed exponential growth at $\mu_s^{\text{max}}$.

Overall fitness $f(s, k_1, k_2)$ of each strain s at a given set of ecological rates $k_1$ and $k_2$ was calculated by averaging the episodic fitness $f_{\text{famine}}$ and $f_{\text{feast}}$ over 5000 episodes of both feast $t_{\text{feast}}$ and famine $t_{\text{famine}}$, where $i = 1, \ldots, 5000$. Episode lengths $t_{\text{feast}}$ and $t_{\text{famine}}$ were independently drawn from exponential distributions parameterized by the two ecological rates, $k_1$ and $k_2$, as shown in Fig. 4B. In summary, the formula for over fitness is $f(s, k_1, k_2) = \frac{1}{k_2} \sum_{i=1}^{k_1} \mu_i(t_{\text{feast}} - t_{\text{famine}}(t_i))$.

To generate the color map in Fig. 4C for visual comparison of fitness, for each set of environmental parameters $k_1$ and $k_2$, the fitness of the three strains $f(s, k_1, k_2)$ were converted into RGB color values using the softmax function: $rac{e^{s}}{e^{s} + \ldots + e^{s}}$, where $s = \Delta \text{rpoS}$, wild-type, or $\Delta \text{rpoS}$ B. The parameter $T$ was immaterial to the model conclusions as it did not affect the boundaries between different environmental regimes. It was used for visualizing the sharpness of transitions among these boundaries (for Fig. 4C, in particular, $T = 200$ hours).

**Programming codes and data availability**

The image analysis procedure described above was implemented in Java as an ImageJ plugin. Fitness modeling, plotting, and time series analyses including noise correction and time-of-death determination were implemented in Python2. We relied on R package “flexsurv” (v1.1) for the core algorithm of survival analysis and GLM, and Python package “py2” (v2.8.6) was used for interoperability between the Python and R codes and custom Python codes for other statistical analysis. All source codes and data are made available through GitHub (https://github.com/y1Hanyang/coliTemporalScaling).

**Supplementary Materials**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/5/eaa0069/DC1

**Fig. 1A.** The feast-and-famine life cycle of E. coli.

**Fig. 2.** Time-of-death determination from PI fluorescence time series.

**Fig. 3.** Gompertz law of mortality informs and constrains biochemical models of aging and mortality in growth-arrested E. coli cells.

**Fig. 4.** Mortality statistics and goodness-of-fit of three independent experimental replicates for the three strains under study.

**Fig. S1.** The feast-and-famine life cycle of E. coli.

**Fig. S2.** Time-of-death determination from PI fluorescence time series.

**Fig. S3.** Gompertz law of mortality informs and constrains biochemical models of aging and mortality in growth-arrested E. coli cells.

**Fig. S4.** Mortality statistics and goodness-of-fit of three independent experimental replicates for the three strains under study.

**Fig. S5.** Experimental repeatability and variances.

**Fig. S6.** Fabrication and Implementation of the two-layer microfluidic chip.

**Fig. S7.** Noise removal from focusing fluctuations using regularized estimation of PI signal.

**Fig. S8.** Visualization and nonparametric statistics of variability among imaging positions within one experimental cohort.

**Table S1.** AIC-based model selection among GLMs for the three experimental replication cohorts ($i = 1, 2, 3$) of wild-type strain.

**Table S2.** AIC-based model selection among GLMs for the three experimental replication cohorts ($i = 1, 2, 3$) of rpoS strain.

**Table S3.** AIC-based model selection among GLMs for the three experimental replication cohorts ($i = 1, 2, 3$) of rpoS strain.

**Table S4.** Testing for aging rate differences among the three strains using AIC and GLM.

**Table S5.** List of plasticwires that leach carbon-supplying contaminants and their nonleaching replacements.

**References and Notes**


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Temporal scaling of aging as an adaptive strategy of *Escherichia coli*
Yifan Yang, Ana L. Santos, Luping Xu, Chantal Lotton, François Taddei and Ariel B. Lindner

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