Microbial ageing and longevity

Roy Moger-Reischer and Jay T. Lennon *

Abstract | Longevity reflects the ability to maintain homeostatic conditions necessary for life as an organism ages. A long-lived organism must contend not only with environmental hazards but also with internal entropy and macromolecular damage that result in the loss of fitness during ageing, a phenomenon known as senescence. Although central to many of the core concepts in biology, ageing and longevity have primarily been investigated in sexually reproducing, multicellular organisms. However, growing evidence suggests that microorganisms undergo senescence, and can also exhibit extreme longevity. In this Review, we integrate theoretical and empirical insights to establish a unified perspective on senescence and longevity. We discuss the evolutionary origins, genetic mechanisms and functional consequences of microbial ageing. In addition to having biomedical implications, insights into microbial ageing shed light on the role of ageing in the origin of life and the upper limits to longevity.

Senescence
Decreasing survival and/or reproductive ability of an individual during ageing.

Longevity
The ability of an organism to maintain the homeostatic conditions necessary to remain viable over time.

Life history
The set of adaptive traits, such as size, growth rate, extrinsic mortality rate, age to maturity and age-specific fecundity, that characterize the life course of a typical individual of a particular species.

Replicative ageing
A type of ageing in which age is measured in units of divisions of an individual cell, rather than units of time.

Department of Biology, Indiana University, Bloomington, IN, USA.

**e-mail: lennonj@indiana.edu*

https://doi.org/10.1038/s41579-019-0253-y

Ageing is the increase in age of an individual organism during its life. Understanding ageing, and the related phenomena of senescence and longevity, is central to many areas of biology, and is of practical concern because it influences the quality of human life. As such, substantial research effort has focused on the mechanisms of ageing in multicellular organisms with the goal of understanding what controls lifespan. Biologists have gained insight into the genetics of ageing by studying model organisms including mice, fruit flies and worms[1,2], whereas other plant and animal species such as oak trees, elephants and naked mole rats engender curiosity given their capacity for extended lifespans[3–5].

Despite being the most abundant and diverse forms of life on Earth, microorganisms have largely been overlooked in ageing research. In fact, for numerous reasons, it has long been thought that microorganisms are not subject to ageing[6–9]. The first and foremost reason for this thought is based on the traditional assumption that microorganisms tend to reproduce by symmetrical cell division. By contrast, when macroorganisms reproduce, the mother individual is clearly distinct from the offspring individual. The mother retains all of the old organismal material, whereas the offspring is born or hatched with undamaged, rejuvenated tissue. This mother–offspring asymmetry is a prerequisite for ageing and has an important role in the life history of a species[6,8,10,11]. If reproduction is symmetrical, the mother individual is effectively killed during binary fission to produce two identical daughter cells with no age difference between them (FIG. 1a)[12,13].

To illustrate the problem, first consider a population of *Escherichia coli* cells growing under ideal laboratory conditions. One might surmise that the age of any given individual is equal to the amount of time since it was created by binary fission, which is about 20 min. Yet if division is symmetrical, each daughter is born possessing an equal portion of the mother cell's aged macromolecular cargo, which was already 20 min old at fission. Meanwhile, the mother cell also possessed a portion of macromolecular cargo synthesized by its mother, which carried cargo from its mother, and so forth back to the culture's original inoculum and beyond. With the successive inheritance of both old and new materials, the concept of microbial ageing can seem arbitrary. However, many microorganisms deviate from the simplified assumption of symmetrical division[14,15]. For example, budding yeasts, diatoms and numerous groups of bacteria and archaea grow and divide with high degrees of asymmetry, which allows for replicative ageing, in which longevity is quantified as the number of cell divisions (BOX 1)[12,16–19]. Even in microorganisms that seem to divide symmetrically (for example, *Schizosaccharomyces pombe* and *E. coli*), there can be differential allocation of subcellular components between daughter cells[20–23]. In such cases, the daughter cell inheriting older or more-damaged cellular components effectively becomes the mother whereas the other daughter cell becomes a rejuvenated offspring[10,20,21,24]. As a result, individual microorganisms can occupy defined age classes, giving rise to population age structure and thus allowing for longevity and senescence (BOX 2).

Second, microbial ageing is often dismissed because, even if old individuals can be discerned, they are extremely rare in an exponentially growing population. Let us revisit the scenario of an *E. coli* culture doubling every 20 min in rich medium at 37 °C, but this time assuming that reproduction is sufficiently asymmetrical

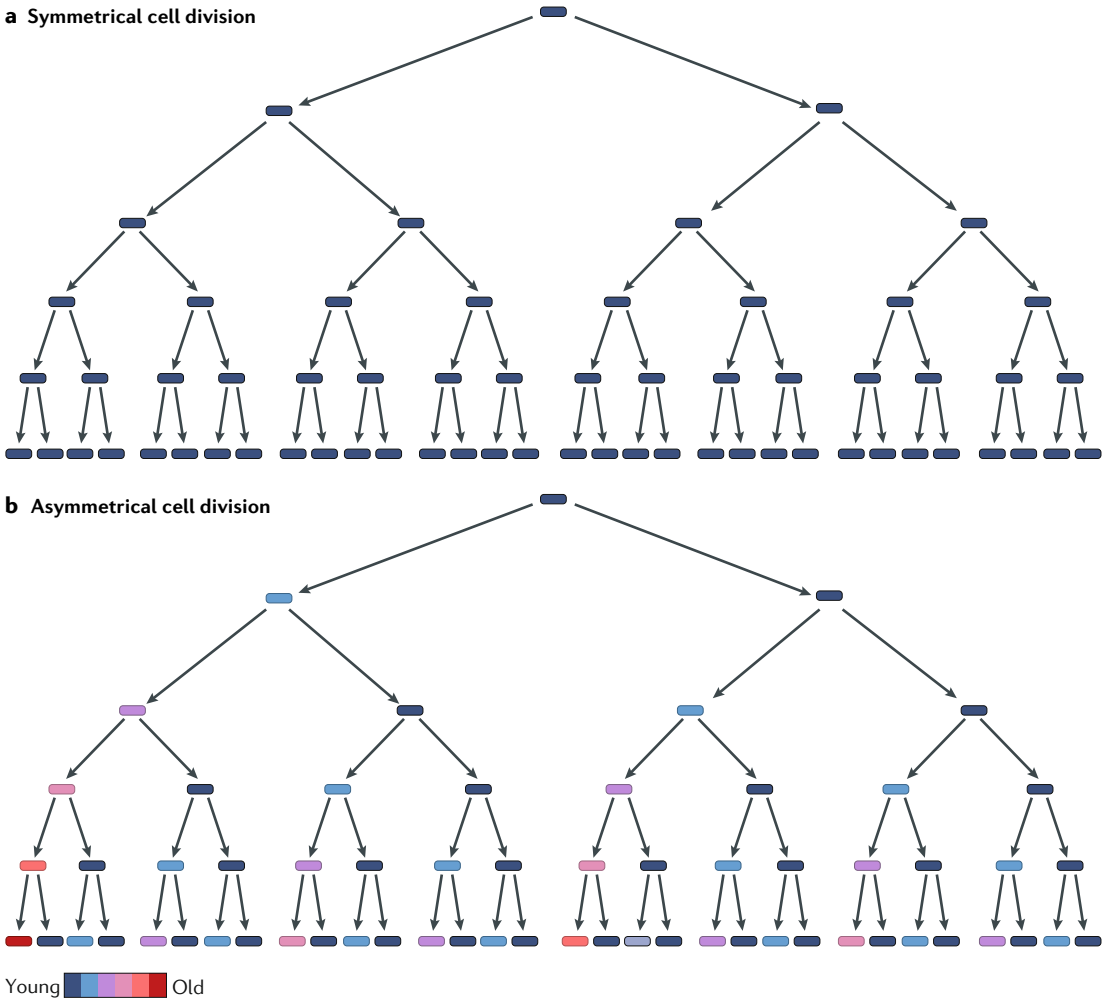


Fig. 1 | **Reproductive asymmetry enables microbial ageing. a** | Ageing does not occur when cells divide by perfectly symmetrical binary fission. Age is arbitrary, because there is no age difference between any two given extant individuals, and no age difference between any extant individual and any progenitor individual. The extant population (bottom row of cells) is homogeneous and there is no age structure. **b** | There is evidence, however, that single-celled organisms experience ageing, owing to asymmetries in reproductive events, which create age differences between mother and offspring individuals at each division. For example, the mother individual may increase in age because it inherits old or damaged cellular material, whereas the offspring inherits newly synthesized material. If this is the case, then the mother individual's age must increase at each cell division. Here, such change in age class is indicated by a change in colour from blue to red, following the left-hand arrow. Each cell division also generates a virgin offspring cell, indicated by a dark-blue right-hand arrow. Thus, asymmetrical cell division enables simultaneous ageing and rejuvenation in the population. During exponential growth, older individuals rapidly become diluted in the population. At equilibrium and assuming no difference in fitness between young and old cells, the proportion of individuals of any given age class is $1/2^{x+1}$, where x is the age class and $x=0$ for virgin cells. Thus, under the null assumptions of uniform fitness and zero mortality, virgin individuals always contribute half of the population's total reproduction and the relative contribution to reproduction from even moderately old individuals becomes vanishingly small. In such a case, evolutionary theory predicts equally vanishingly weak selection for longevity. Of note, in a more realistic population in which senescence occurs as a result of ageing, the rarity of old individuals would likely be even more pronounced, because old individuals may reproduce more slowly than young individuals.

to distinguish mother and offspring cells. If a single individual is transferred into a flask containing fresh medium at 9.00 a.m., then at the end of an 8-h workday the original mother cell will be outnumbered by its progeny by 16 million to 1. Of the nearly 2×10^7 cells in the turbid culture at 5.00 p.m., 87% of the individuals are less than 1 h old whereas older cells generated during the first hour of incubation would only make up 0.0001% of the population (FIG. 1b). This dilution effect is consistent with the theoretical expectation that old individuals contribute minimally to the total reproduction in an expanding population in which births greatly exceed deaths[25,26]. However, outside the confines of the laboratory, conditions are rarely ideal for microbial growth and reproduction. In nature, resources are limited, the environment is stressful and predators are abundant. As a result, doubling times are 2-fold to 50-fold longer in the wild than in the laboratory[27,28]. Similarly, rates of mortality are thought to be considerably higher in natural than in laboratory settings[29]. As such, microbial populations

Box 1 | **Chronological and replicative lifespan of microorganisms**

Asked to define the lifespan for macroorganisms such as plants or animals, one would likely suggest that it is the length of time between the birth and the death of an individual. For microorganisms with asymmetrical reproduction, ageing can also be quantified by the number of cell divisions. Thus, microbial longevity can be expressed either as a chronological lifespan (CLS) or a replicative lifespan (RLS)[17].

CLS is the length of time that a microbial cell can remain viable in a non-dividing state. Essentially, CLS describes the maximum longevity of a cell between periods of reproduction. For example, a haploid *Saccharomyces* sp. yeast cell will stop reproducing and a *Bacillus* sp. cell will initiate sporulation upon energy starvation. If provided with an energy source a few days later, both individuals will resume reproduction. Wait too long to provide energy, however, and the yeast cell will no longer be viable, as a yeast cell's CLS is on the order of a few weeks or months[17]. By contrast, the CLS of many *Bacillus* species that can form endospores can exceed 10^5 years[161]. In the laboratory, CLS is measured by exposing microorganisms to energy-limited conditions such as spent media or saline, followed by periodically plating a dilution of the culture and counting colony-forming units.

RLS is the number of times that a microbial cell can divide before it senesces and dies, presumably owing to a prohibitive accumulation of cellular damage[17,45]. Because RLS requires tracking and distinguishing individual cells, it is a longstanding method for the budding yeast *Saccharomyces cerevisiae*[16], whose cells are large and divide with a clear mother–offspring dichotomy. Another example of RLS comes from diatoms, a group of eukaryotic algae that are commonly found in freshwater and marine ecosystems. The cell walls of diatoms, known as frustules, are made from two silica valves, one of which (epitheca) is slightly larger than the other (hypotheca). Because the frustules are effectively made of glass, the cell walls cannot expand. Therefore, upon each asexual division, the newly produced offspring valve must fit inside the valve of the mother cell. With each successive division, the diatom gets smaller and smaller, which places a physical constraint on RLS. It is thought that this reduction in cell size serves as a 'sex clock', which favours recombination and the restoration of cell size. For less charismatic microorganisms, in which mother and offspring cells cannot easily be distinguished, specialized cavity slides or microfluidic chambers are typically required for measuring RLS[21,83].

rarely undergo exponential growth and therefore older individuals can potentially persist and accumulate.

Third, one might expect the longevity of microorganisms to be short because of biophysical constraints, which are reflected in the scaling relationship between maximum lifespan and body size[30]. Owing to their ability to function at a more-efficient, slower mass-specific metabolic rate[30], larger organisms live longer than smaller organisms (FIG. 2). For example, the bowhead whale weighing in at nearly 10^5 kg can live for more than 200 years, whereas a typical bacterium weighing 10^{-13} g is predicted to have a maximum lifespan of less than 2 months (FIG. 2). Thus, from a comparative perspective, metabolic theory predicts that microorganisms are the least likely of all taxa to display exceptional longevity. Yet some microorganisms seem to defy first-order scaling expectations. Viable bacteria and archaea have been recovered from ancient materials such as amber, permafrost and halite crystal that are millions of years old[31–33]. Meanwhile, in the 'deep biosphere' below the planetary surface where a large portion of Earth's microorganisms are thought to live, microbial biomass is estimated to turn over less than once a century owing to extreme energy limitation[29,34–36]. Such findings suggest that some microorganisms can overcome some of the energetic forces that are thought to constrain lifespan.

Microbial ageing presents challenges and opportunities for understanding the limits on the longevity of all living systems. In the following sections, we expand upon the conventional view of ageing in animal models with a fresh focus on the mechanistic and evolutionary basis of ageing in microbial systems. We begin with an overview of ageing theory, and discuss a unified perspective for the evolution of ageing in both unicellular and multicellular organisms. We describe some of the genetic and molecular mechanisms involved in microbial longevity and death, which suggest shared evolutionary origins with some of the processes observed in macroorganisms. Finally, we discuss opportunities for predicting microbial evolution, treating disease and understanding ageing as a feature common to nearly all life forms, in the light of microbial ageing.

Theory of ageing

By definition, senescence decreases fitness. The fact that this deleterious trait is pervasive across the tree of life has remained a long-standing challenge in biology[7]. Major contributions during the twentieth century from the fields of population demography (BOX 2)[25,37–40], evolutionary biology[25,37–39,41] and cell biology[16,41–44] led to the prevailing view that senescence is directly caused by the accrual of macromolecular cellular damage[11,45–48], whereas the ubiquity of senescence is often attributable to evolutionary constraints and life-history trade-offs[49–51]. In this section, we briefly review theoretical understanding of the evolution of ageing.

Damage as the cause of senescence. Like any mechanical device, organisms can wear out and break down over time, leading to ever-higher probability of system failure and ultimately death. The root cause of this senescence is that cellular damage accumulates during the lifetime of organisms[11,45–47,52–54]. Structural modifications to macromolecules arise from unavoidable errors during cellular metabolism[45,46,55,56]. This damage can be induced by environmental stressors, but is largely internally generated[11,45,46]. Ageing-associated damage can accrue in DNA owing to mutations[47,57,58], telomere shortening[45,47,59,60] and double-stranded breaks[45,61] along with ectopic recombination and rearrangements[17,61–63]. Meanwhile, ultraviolet radiation, free radicals and other types of oxidative stress cause damage to protein molecules, resulting in their glycation[52], glycoxidation[52], carbonylation[64–66], misfolding[13,66] and aggregation[13,20,66–68]. Damage to other macromolecular components, particularly lipids, can also accumulate during ageing[45,69] (FIG. 3). It is possible to repair damage, and many pathways for DNA and protein repair have evolved[45,70–72]. However, not all damage can be fixed owing to the time and energy costs of repairing the diverse types of damage that accrue in cells[46,55] (FIG. 3).

The evolution of senescence. Senescence is prevalent because it is a difficult trait for natural selection to purge. First, on average, the opportunity to reproduce diminishes with age owing to the cumulative probability of having already died due to random extrinsic mortality (BOX 2). Thus, older individuals comprise a very small proportion of a growing population and contribute minimally to the population's total reproduction, similar to the effect we described above in the example of an exponentially growing *E. coli* population (FIG. 1b).

Trade-offs
Physical or genetic constraints that prevent the simultaneous optimization of multiple traits by natural selection.

Damage
Structural modifications to cellular macromolecules that accumulate during ageing.

Extrinsic mortality
Environment-related causes of death that occur independently of organismal vigour or age.

Box 2 | **Modelling mortality, longevity and ageing**

The lifetime pattern of mortality for a cohort of organisms can be modelled by a survivorship curve, which illustrates the proportion of individuals who survive to any given age. Cumulative survivorship, denoted $l(x)$, is the proportion of individuals remaining alive at a given age x:

$$l(x) = \frac{N(x)}{N_0}$$

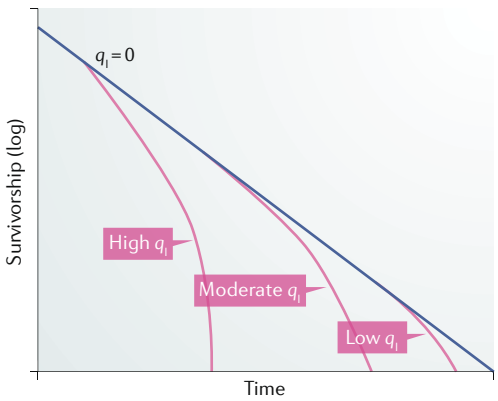
The slope of the survivorship curve is determined by the age-specific mortality[162]. Age-specific mortality is denoted $q(x)$ and is defined as the proportion of individuals alive at age x that die before reaching age $x+1$:

$$q(x) = 1 - \frac{l(x+1)}{l(x)}$$

Thus, $q(x)$ describes the per-unit-time probability of death. As $q(x)$ increases, so does the (negative) magnitude of the slope of the survivorship curve. In the simplest form of survivorship curve, $q(x)$ is constant over time, which generates a negative exponential relationship. Under such conditions, the survivorship curve is a straight line when $q(x)$ is logarithmically transformed, which is expected if all deaths are random mortality events that occur irrespective of individual age[53]. This extrinsic mortality can be estimated by the rate of mortality at the age of peak performance (q_0), when it is assumed there is no intrinsic mortality due to senescence, that is, $q_I = 0$ (REF. [163]).

Intrinsic mortality changes the shape of the survivorship curve. Instead of being a straight line, the log-transformed survivorship curve becomes non-linear, which reflects a change in the per-unit-time probability of intrinsic mortality due to ageing[164]. In contrast to extrinsic mortality, intrinsic mortality is caused by the ageing-associated accumulation of macromolecular damage, leading to physiological failure (that is, senescence)[53,54]. At ages x such that $q(x) > q_0$, intrinsic mortality is inferred to be occurring in addition to extrinsic mortality, represented by q_I. When intrinsic mortality occurs, the shape of the log-survivorship curve becomes a steeper, non-linear decreasing function. A sharply changing curve corresponds to rapid senescence. The rate of senescence is quantified by fitting survivorship data to statistical models, commonly based on the Weibull or Gompertz distribution, that quantify deviation from a null expectation with zero intrinsic mortality[163–165]. In particular, replicative survivorship curves of *Saccharomyces cerevisiae* and *Escherichia coli* have been demonstrated to follow Gompertz mortality dynamics[83,166,167].

Senescence can also manifest itself as decreased reproductive ability with age, called reproductive senescence. In the absence of reproductive senescence, age-specific fecundity, denoted $m(x)$, is constant for all ages x greater than the age of peak performance. Reproductive senescence is inferred when $m(x)$ decreases as x increases[168]. For microorganisms, reproductive senescence is measured as increased doubling time with replicative age[20,24,106]. Combining survivorship and age-specific fecundity data enables calculation of the fitness cost of senescence, a measurement of how much reproduction is lost due to intrinsic mortality, in both multicellular[168] and unicellular[22] organisms.



As a result, selection against deleterious late-life traits, such as senescence, is often weak[25,37,49,73]. Second, trade-offs in life history can constrain longevity. For example, traits that increase reproductive fitness can be under positive selection even when they decrease late-life survival, because reproduction often contributes more to overall fitness than does late-life survival. When trade-offs prevent an organism from maximizing both early-life fitness and late-life fitness, or both reproduction and longevity, theory predicts and empirical evidence shows that senescence evolves[38,41,49,50,74,75].

Because organisms only have a finite amount of time and resources with which to reproduce, the allocation of energy and nutrients represents an important life-history trade-off. One of the most generally applicable trade-off-based theories of senescence is the disposable soma theory of ageing[41,74]. This theory proposes that in multicellular, sexually reproducing organisms, resources can be allocated either to the longevity (that is, to damage repair in somatic tissue) or to the reproduction of the organism (that is, to the germline). Although allocation to longevity is potentially beneficial, it often incurs a hefty fitness cost because it decreases reproductive output. On the other hand, once an organism has reproduced, the soma is no longer critical for fitness. Thus, natural selection can allow the 'disposable' soma to senesce. Meanwhile, the germline lives on through the lineage of the mother organism. The germline–soma distinction is the basis of reproduction–longevity trade-offs that cause senescence to evolve[6,7,41].

A unified perspective on senescence. At first glance, microorganisms seem to challenge the basic tenets of the disposable soma theory. The entire unicellular organism is devoted to reproduction, not just an isolated germline. Thus, if longevity and reproduction are inextricably coupled, then single-celled organisms should not evolve to exhibit senescence[6–8,38,76]. Senescence can indeed be avoided if damage is low enough or repair is rapid enough that the damage is diluted with each symmetrical cell division[77–80]. In such a scenario, neither daughter is endowed with harmful levels of damage, and there is no senescence[77,81]. Indeed, empirical evidence demonstrates that *E. coli* and *S. pombe*, which both reproduce by binary fission, can avoid senescence when the environment is more favourable[80–84].

Intrinsic mortality
Ageing-related causes of death, due to accumulation of macromolecular damage, which occur independently of the external environment.

Disposable soma theory
Life history-based theory for the evolution of senescence emphasizing constraints of resource allocation. This theory proposes that senescence evolves because it is adaptive to allocate fewer resources to repair in order to allocate more resources to reproduction.

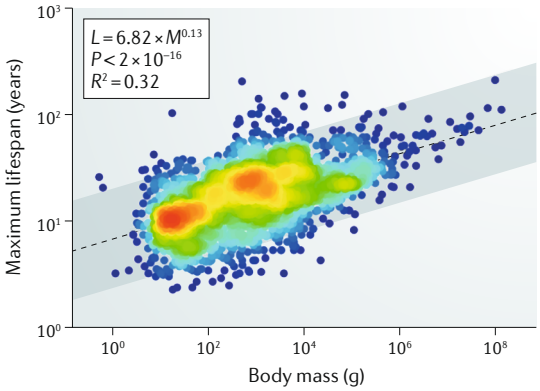


Fig. 2 | **The relationship between body size and lifespan.** A power-law relationship between longevity (*L*) and body mass (*M*) is observed across multicellular vertebrate animals. Larger organisms tend to exhibit longer lifespans. Grey-shaded area represents 95% prediction intervals. Data extracted from the AnAge database[189].

Nevertheless, evidence from bacteria and single-celled fungi demonstrates that microorganisms can and do undergo senescence that decreases the fitness of individuals[17,18,20,22,65]. With slight modification, the disposable soma theory can help to explain the evolution of senescence in microorganisms (Supplementary Box 1). An alternative to repairing and diluting damage is to sequester it into one of the two daughter cells through asymmetrical reproduction[77,79,85]. The damage-inheriting lineage will then senesce like the soma. Meanwhile, the other lineage remains damage-free like the germline, enabling the population to maintain reproductive output even while the damage-inheriting lineage senesces[24,86]. In the same way, the offspring of multicellular organisms begin life rejuvenated and free of cellular damage, whereas the maternal soma senesces. Essentially, the soma–germline distinction that characterizes the disposable soma theory is a special case of the more general phenomenon of reproductive asymmetry that enables senescence[8,11,87]. This observation is consistent with a view that senescence first evolved in single-celled organisms, with the fundamental trade-offs between reproduction and longevity continuing to constrain the evolution of longevity after multicellular organisms evolved a soma–germline separation[10,87].

Microbial ageing: mechanistic framework

Generation of macromolecular damage is a universal problem that all cellular life forms must contend with. Microorganisms have evolved diverse mechanisms of coping with damage. Specifically, they can directly combat cellular damage through three basic processes: repair, prevention or disposal. Another strategy is to sequester damage into one of the two daughter lineages. Although sequestration protects the rejuvenated offspring lineage from damage, it comes at the cost of senescence in the damage-inheriting lineage. When the problem of damage accrual goes unsolved, the cell succumbs and breaks down. In this section, we highlight some of the underlying genetic and physiological mechanisms that microorganisms use to withstand damage, and examine the effects of accumulating damage on the cell.

Damage repair. A cell can diminish the negative effects of damage by simply repairing it. One common cellular target of damage repair is DNA[45]. For example, *E. coli* produces enough base excision repair enzymes to scan the entire chromosome once every 10 min[88]. These base excision repair enzymes repair small DNA lesions and damaged bases, whereas nucleotide excision repair tends to handle bulkier lesions[71]. Meanwhile, homologous recombination, non-homologous end-joining pathways (although we note the latter is absent in the model *E. coli*) repair double-stranded DNA breaks[72,89,90]. Guanine nucleotides are particularly reactive and susceptible to damage. Microorganisms such as *E. coli* have evolved specific mechanisms to repair guanine oxidation and glycation[71,88,91,92]. For example, the *E. coli* proteins Hsp31, YhbO and YajL repair glycated guanine through the catalysis of hydrogen atom migration followed by amidolysis, which results in the formation of 2-hydroxypropanoic acid and a de-glycated guanine[91]. The human guanine glycation repair homologue DJ-1, when expressed in *E. coli*, functions similarly to the endogenous bacterial proteins[91], supporting the view that DNA repair mechanisms are conserved across unicellular and multicellular taxa[71,90]. Microorganisms can also repair damage to other macromolecules, including proteins. For example, molecular chaperones can repair protein damage and aggregation, which are linked to microbial senescence[20,70,93]. Unfoldase chaperones, such as DnaK–DnaJ–GrpE and also GroEL, may refold damaged proteins into their native conformations in an ATP-dependent manner[94–96]. Bacterial holdases, including the conserved chaperones GroEL, IbpA, IbpB and HtpG, prevent damaged proteins from forming aggregates[93,94]. When damaged protein aggregates have already formed, bacteria expend ATP to use the interacting chaperones ClpB and DnaK–DnaJ–GrpE as disaggregase enzymes, which unfold and solubilize aggregates[97–101]. All of these chaperones help to decrease the damage load of a cell and promote its longevity.

Damage prevention. Another way for a cell to reduce damage burden is to decrease the rate at which it is generated. Damage is often generated as a by-product of metabolism[46], for example, due to translational errors or to the production of reactive oxygen species during aerobic metabolism. Microorganisms accrue less damage when they temporarily pause metabolism through processes such as cell cycle arrest, persistence and sporulation. Many such strategies represent forms of dormancy, in which individuals enter a reversible state of reduced metabolic activity that functions as a buffer against environmental stress such as energy scarcity[102]. In the deep biosphere where many microorganisms lie dormant for prolonged periods, it has been estimated that the metabolic flux ranges between 10^{-20} and 10^{-18} W per cell[103,104], which is many orders of magnitude lower than the values of 10^{-12} to 10^{-10} W per cell estimated for *E. coli* growing under typical laboratory conditions[105]. Thus, part of the explanation for extreme chronological

Asymmetrical reproduction
A reproductive event that results in a distinct mother individual, a distinct offspring individual and a difference in age between them.

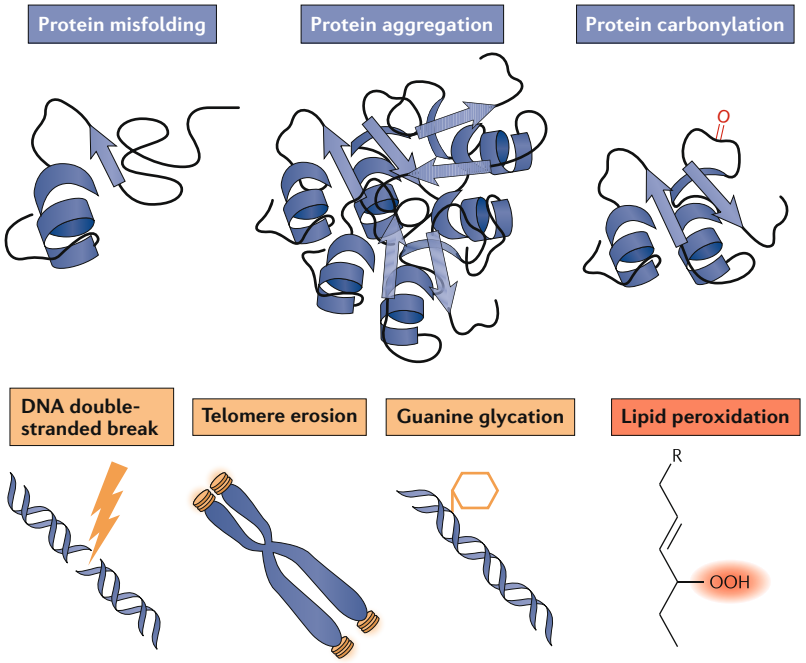


Fig. 3 | **Damage to cellular molecules during ageing.** One of the main reasons why it is difficult to repair all of the damage present in a cell is the great diversity of damage types. Because there are so many types of damage, it becomes inefficient for a cell to attempt to repair them all. Therefore, damage tends to accumulate, increasing the likelihood of ageing and senescence. Common damage types are misfolding, aggregation and carbonylation of proteins, double-stranded DNA breaks, telomere erosion, guanine glycation and lipid peroxidation.

longevity (BOX 1) in the deep biosphere and other energy-limited ecosystems may relate to lower rates of damage generation.

Damage disposal. Microorganisms actively dispose of damaged cellular components. One recently elucidated disposal mechanism is minicell formation[106]. Minicells are spherical miniature cells that contain many cellular macromolecules, but lack a chromosome and are unable to reproduce[107]. Minicell formation occurs by budding at the bacterial cell poles, which is where damaged protein aggregates tend to accumulate[107–109]. Minicell production is more commonly observed under stressful conditions associated with macromolecular damage[106,110], suggesting that one possible function of minicells is to remove damaged or cytotoxic macromolecules from bacterial cells[106]. Minicell formation was observed specifically in cells with a polar protein aggregate. In 95% of the cases in which such a cell formed a minicell, the minicell successfully captured and disposed of the aggregate[106]. Minicell production is regulated by Min proteins, which function to inhibit the divisome protein FtsZ and prevent ectopic cell divisions[111,112]. *ΔminC* mutants, which overproduce minicells, are not susceptible to the antibiotic streptomycin, which interferes with protein synthesis[106]. This suggests that *ΔminC* cells effectively dispose of streptomycin-associated protein damage that inhibits growth[106]. In both *ΔminC* mutants and wild types, minicell production by the mother cell increases the growth rate of subsequent offspring cells, likely because they escape the inheritance of damage[106].

Sequestering damage through asymmetrical reproduction. As an alternative to cellular repair, microorganisms can differentially allocate macromolecular damage between daughter cells through asymmetrical reproduction. Asymmetrical reproduction generates phenotypic variance in offspring fitness, which increases the efficacy of natural selection[10,14,113]. By sequestering damage into one lineage, there may be an overall increase in the population growth rate[10,77,85,114]. However, these benefits come at the cost of causing senescence in the damage-inheriting lineage (see above). But how do microorganisms achieve asymmetrical reproduction? Microbial cells exhibit a range of localized growth and division modes, which are essential for determining the symmetry versus asymmetry of reproduction (FIG. 4)[14]. Here, we explore the mechanisms that underlie asymmetrical reproduction and discuss the consequences for microbial performance.

The most conspicuous examples of reproductive asymmetry are characterized by morphological differences between mother and offspring cells. Morphologically asymmetrical reproduction is commonly achieved through a complex and closely regulated cell developmental cycle. The classic example of a microorganism that divides morphologically asymmetrically is the budding yeast *Saccharomyces cerevisiae*. Here, the mother cell's cytoskeleton as well as organelles establish cellular polarity that enables asymmetrical retention of ageing-associated protein aggregates, leading to a systematic difference in levels of damaged cargo between mother and offspring through an active damage segregation mechanism[115–117]. Phenomena of morphological asymmetry also occur in bacteria, perhaps best studied in the α-proteobacterium *Caulobacter crescentus*[118]. A mature *C. crescentus* cell has a polar stalk. At cell division, the stalked *C. crescentus* individual forms two morphologically distinct cells. The old-pole mother cell remains a reproductively capable stalked cell, whereas the new-pole offspring cell becomes a reproductively immature swarmer cell with a flagellum, capable of dispersal (FIG. 4d). To create one cell of each type at division, the flagellum must be synthesized at the new pole, opposite the stalk (FIG. 4d). Correct positioning of the flagellum depends on the protein PflI[119]. Deletion or overexpression of PflI causes ectopic flagellum placement without affecting other aspects of cell polarity[119]. PflI localization depends on c-di-GMP-activated TipF, a protein that is also required for flagellum assembly and polar localization of flagellar switch proteins (FliG and FliM)[120]. TipF localization depends on TipN[121], which localizes asymmetrically to the new pole of stalked cells by recognizing the site of FtsZ-induced cell constriction[121,122], causing the flagellum to be synthesized opposite the stalk. Thus, the new-pole offspring cell has the flagellum and disperses, whereas the old-pole mother maintains its stalk and increases in replicative age.

The mechanisms of reproductive asymmetry have also been identified in microorganisms with less discernible forms of cell division. For example, the rod-shaped bacterium *E. coli* undergoes dispersed elongation and divides in a morphologically symmetrical

fashion[14] (FIG. 4a). This is achieved with the help of the actin-like protein MreB. MreB filaments localize to the midcell and orient themselves based on cell-wall curvature[123,124]. There, MreB promotes the addition of new peptidoglycan to the cell sidewall, whereas the cell poles remain inert[124,125]. Thus, despite symmetrical growth, upon division each daughter cell has a young pole (former actively growing cell centre) and an old pole (former inert cell pole). This effect compounds over generations to create age and fitness heterogeneity among individuals, especially when specific functional macromolecules are associated with a specific pole[14,113]. For example, the *E. coli* multidrug efflux pump AcrAB–TolC complex segregates preferentially to the old pole, conferring a drug resistance advantage[113]. Among rod-shaped archaea, a different actin-like protein called crenactin has roles similar to MreB[126], suggesting that features of cryptic reproductive asymmetry occur across domains of microbial life.

Importantly, reproductive asymmetry enables some microorganisms to experience ageing and senescence, despite the fact that they lack a developmental cycle, asymmetrical growth or morphologically distinct offspring[14,21]. For example, senescence in *E. coli* cells has been observed[20,21] and connected to the asymmetrical polar allocation of protein aggregates as a putative damage factor[20]. The amount of protein aggregates is correlated with reproductive senescence (increased doubling time)[20] and survival senescence (decreased survival)[67]. Protein aggregates are assembled in a manner that depends on ATP and the chaperones DnaK and DnaJ[127,128]. To preferentially allocate damage to a single daughter by asymmetrical reproduction, protein aggregates must be localized to cell poles[108]. Polar localization is mainly driven by diffusion and nucleoid occlusion[108,109,128,129] The observation that the degree of asymmetrical damage segregation increases with the size of the aggregate is also consistent with a passive segregation mechanism in *E. coli*[114]. Whether localization also requires energy-dependent cellular activity might depend on aggregate size and cytoplasm crowding[128,130]. Thus, although protein aggregates tend to localize to the old pole[20], aggregate localization is also driven by random processes. Stochastic and deterministic allocation of damage to one or the other daughter cell are both sufficient for bacterial senescence to evolve[85]. The extent to which deterministic allocation is favoured over random allocation to one pole depends on the tendency of aggregates to remain tethered at the old pole[85].

Breakdown of a cell. If an individual belongs to a damage-inheriting lineage or is otherwise unable to contend with the accumulation of cellular damage, it breaks down. The best studied model for cellular breakdown is *S. cerevisiae*. As a budding yeast individual ages, the plasma membrane ATPase Vma1p starts to accumulate[131], which pumps protons out of the cell and causes the cytosolic pH to rise[131]. The lack of cytosolic protons prevents proper acidification of the vacuole, the function of which declines with age[131,132]. Specifically, an increase in pH disrupts the storage and transport of neutral amino acids in the vacuole, which leads to mitochondrial depolarization[133]. This depolarization likely leads to mitochondrial fragmentation and dysfunction[133–135], which has consequences for genomic instability in the form of excessive recombination[63,134], loss of heterozygosity[63,134]

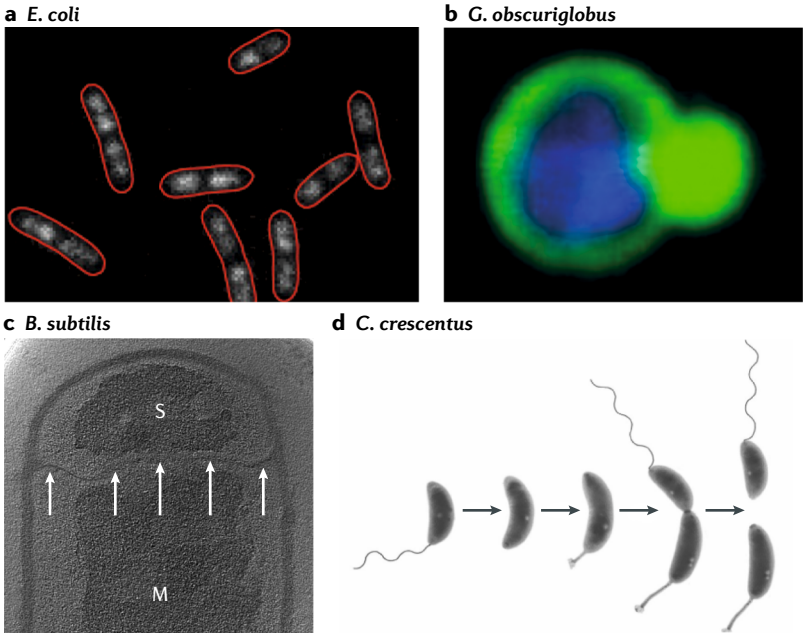


Fig. 4 | **Asymmetrical reproduction in different microorganisms.** Asymmetrical reproduction is a prerequisite for ageing. Although some single-celled organisms can reproduce symmetrically, microorganisms exhibit great diversity of cell division modes and mechanisms. **a** | Perhaps a default expectation for microbial cell division is symmetrical binary fission, with no systematic differences in morphology or age between the two daughter cells. Here, *Escherichia coli* cells divide by binary fission, yielding two apparently equivalent daughter cells. However, it is still possible for such daughter cells to inherit subcellular materials that differ in their age or level of damage. In the image, cell nucleoids stained with SYTOX orange appear in grey. **b** | Initiation of asymmetrical cell division by budding in the planctomycete *Gemmata obscuriglobus*. After budding initiates, the bud (right) will grow as new cellular material is added to it. When the bud reaches the same mature size of the mother cell (left) after about 12 h of growth, the mother cell completes dividing and the offspring cell separates. In the image, nucleoids stained with DAPI appear blue; internal membranes stained with $DiOC_6$ appear green. Note that the bud initially contains no nucleoid. **c** | Some microorganisms such as *Bacillus subtilis* may undergo a morphological change into a resistant endospore when faced with environmental challenges such as starvation. Endosporulation occurs by an asymmetrical cell division during which the mother cell (labelled M) forms a septum and deposits a spore coat around an intracellular prespore (labelled S). When the spore is mature, it is released through lysis of the mother cell. White arrows point to the peptidoglycan barrier separating the endospore from the mother cell. **d** | The life cycle of *Caulobacter crescentus* is characterized by asymmetrical cell division. Before reproducing for the first time, a cell loses its flagellum and synthesizes a stalk and holdfast. In the mature, sessile cell, cell division and flagellar synthesis occur at the pole opposite to the holdfast and stalk. New-pole offspring cells with a flagellum disperse in search of resources before reaching maturity, losing the flagellum, synthesizing stalk and holdfast, and beginning cell division of their own. The figure shows transmission electron micrographs of *C. crescentus* cells at different stages of the cell cycle. Note that the penultimate, provisional cell was separated into two cells in Photoshop to illustrate cell separation and the darkness of the flagellum was enhanced in Photoshop for illustration purposes. Part **a** is from REF.[190]: *Applied and Environmental Microbiology*, 2014, **80**, 4977–4986, https://doi.org/10.1128/AEM.00989-14, reproduced with permission from American Society for Microbiology. Part **b** is reproduced from REF.[191], CC-BY-2.0, https://creativecommons.org/licenses/by/2.0/. Part **c** is reproduced with permission from REF.[192], Wiley-VCH. Part **d** is courtesy of Yves Brun, Université de Montréal, and from REF.[193]: ©2000 American Society for Microbiology. Modified with permission. No further reproduction or distribution is permitted without the prior written permission of American Society for Microbiology.

Programmed cell death (PCD) Cell death that is caused by factors encoded in the genome of the organism.

and the formation of toxic extrachromosomal ribosomal DNA circles[136,137]. Other types of genomic instability include mutations, histone loss, deterioration of transcriptional control and double-stranded DNA breaks[58,61]. Meanwhile, the amount of reactive oxygen species, especially in the dysfunctioning mitochondria, increases in the ageing cell[138]. Cellular proteins accumulate oxidative damage[65,139] and form damaged protein aggregates[68] that are retained in the older mother upon cell division[115,116]. Although some of the specific mechanisms involved in cellular breakdown vary among species (extrachromosomal ribosomal DNA circles, for example[136,140]), many of the features documented in *S. cerevisiae* have been observed in ageing cells in a diverse array of organisms ranging from bacteria to mammals[20,45,57,66,67,141,142].

Perspectives and implications

We close with some perspectives on the consequences of microbial ageing for broader disciplines in the biological sciences. Microbial ageing lends insight not only into microbial evolution and clinical microbiology, but also into the ageing process of humans at the cellular level. We finally discuss the ramifications of the evidence that the trait of senescence is commonly exhibited by organisms from all known domains of life.

Microbial evolution. Longevity can influence the ability of microbial populations to evolve. The rate of evolution is related to a population's generation time given that the majority of mutations occur during DNA replication. Intuitively, longevity should reduce the rate of evolution by lengthening the generation time. The slowing of population genetic change is particularly strong when longevity is accompanied by a reduced ability to reproduce, as occurs during dormancy[143]. Empirical support for reduced rates of evolution in long-lived populations comes from metagenomic and single-cell sequencing of microorganisms in the deep biosphere[36]. Population genetic methods based on the genomic distribution of polymorphisms indicate that adaptive evolution in the deep biosphere effectively comes to a halt, implying that the microorganisms are likely pre-adapted to the stressful and energy-poor conditions that are characteristic of the Earth's subsurface[28,36].

Longevity can also facilitate adaptive evolution because it allows individuals to outlive the duration of environmental challenges such as feast–famine cycles. When challenged by a long-term stationary phase, 99% or more of the individuals in an *E. coli* culture will typically perish and the small proportion of survivors exhibit a growth-advantage-in-stationary-phase (GASP) phenotype[144]. GASP mutants remain long-lived in subsequent stationary phases, and furthermore can outcompete wild-type lineages even in fresh medium[144,145]. GASP mutants carry distinct, recurring genetic changes, consistent with the view that their evolution of longevity is adaptive[146,147]. Similarly, long-lived *E. coli* individuals who survive exposure to the antibiotic ciprofloxacin exhibit high numbers of mutations[148]. This genetic diversity is found to promote the rapid evolution of genetically based resistance, which is adaptive in environments where antibiotics are encountered[148].

Biomedicine. By understanding microbial longevity and senescence, clinicians may be able to better treat disease. One straightforward application is through the use of compounds that trigger programmed cell death (PCD)[149,150] (BOX 3). Natural oligopeptides that induce PCD in bacteria have been isolated from the supernatants of bacterial cultures, and may be subsequently developed as antibiotics[149,151]. Longevity and senescence can also guide the treatment of persister cells. Persister cells are long-lived microorganisms that use a form of shallow dormancy that allows a population to outlast a course of antibiotic treatment. The increased frequency of antibiotic-tolerant cells contributes to chronic infections and can facilitate the evolution of genetically based antibiotic resistance[102,148,152,153]. Some mechanisms underlying microbial persistence and longevity appear to share similarities. For example, persister cells in *S. cerevisiae* are able to survive exposure to the antifungal drug fluphenazine by overexpressing the chaperone protein Hsp12 (REF.[154]). Thus, chaperones, which are a key component of cellular repair, which promotes microbial longevity, are also implicated in the persister

Box 3 | Programmed cell death: increasing intrinsic mortality

A somewhat puzzling way by which microorganisms can modulate intrinsic mortality is through genetic pathways devoted to killing the cell in which they are expressed. Whereas programmed cell death (PCD) of eukaryotic cells has long been known to be indispensable for development and health, it constitutes suicide for unicellular organisms. In microorganisms, PCD kills both the soma and the germline. Thus, this type of intrinsic mortality has no payoff in reproductive fitness, rendering it difficult to imagine how microbial PCD could evolve due to reproduction–longevity trade-offs. One possible explanation is that microbial PCD could evolve by kin selection, when the fitness effect of an allele at the level of the lineage, in addition to the individual level, is considered[169–171]. If the action of a 'suicide gene' can increase the success of other individuals bearing the same suicide allele, the allele could have an evolutionary advantage.

Altruistic traits like PCD evolve most readily when recipients of altruism tend to be highly related[171,172]. In the ecological contexts in which unicellular PCD is commonly observed, individuals are indeed often highly related[169]. For example, during biofilm development in *Pseudomonas aeruginosa*, the accumulation of reactive oxygen species seems to induce a prophage in the *P. aeruginosa* genome that kills a large fraction of the cells, but allows a subpopulation of survivors to benefit owing to increased dispersal and reproductive capability[173]. Another potential benefit of PCD is that dead cells release extracellular DNA, which can be used by surviving cells for construction of the biofilm matrix[174–177]. In addition, altruistic suicide has been documented in nutrient-limited populations of budding yeast and cyanobacteria, where PCD provides a benefit to neighbouring cells by releasing nutrients that may be used for regrowth of a subpopulation[178–181]. Thus, intrinsic mortality of some individuals increases the longevity and reproduction of other related individuals.

Although some microbial PCD systems, such as the MazEF toxin–antitoxin system characterized in *Escherichia coli*[182,183], are unique to bacteria, some types of microbial PCD appear related to eukaryotic PCD. In budding yeast, altruistic suicide occurs by *bona fide* eukaryotic apoptosis[178,179]. In *ΔmazEF* cells of *E. coli*, treatment with DNA-damaging agents induces PCD through the action of RecA and LexA[184,185]. In particular, RecA–LexA-mediated death displays many key characteristics of eukaryotic apoptosis, including chromosome condensation, membrane depolarization, elevated •OH radicals and DNA fragmentation[184,186]. *E. coli* RecA shares substrate specificity with eukaryotic caspases involved in apoptosis[186], further emphasizing surprising evolutionary conservation of this PCD pathway. Overall, the mechanistic similarities suggest a shared evolutionary origin of some PCD systems, with a likely bacterial origin. In addition, the evolution of multicellularity itself likely occurred in populations of cooperating single-celled organisms in which kin selection favoured altruistic traits such as PCD[187,188].

phenotype. Another line of evidence connecting cellular damage and repair to persister cells is the recent finding that the depth of the *E. coli* persister state increases with the amount of protein aggregation[155]. Furthermore, the ability of *E. coli* persister cells to recover after treatment with ampicillin depends on the disaggregase activity of the chaperones DnaK and ClpB[155]. Thus, cellular repair seems to be necessary for enabling microbial persistence in several domains of life.

The principles of microbial ageing may also be important for treating non-communicable diseases, such as neurodegenerative disease and other diseases associated with ageing in humans. Just as for microbial individuals, single cells in a multicellular organism accumulate damage over time and endow this damage to daughter cells when they divide. Asymmetrical inheritance of damaged protein aggregates was recently shown to occur during mitosis in animal cells[141,142,156,157]. Preliminary evidence indicates that the cell that inherits a damaged protein aggregate during mitosis also has a decreased division rate, analogous to reproductive senescence in microorganisms[142]. Inheritance of damage is also involved in the two distinct cell fates of the offspring of neural stem cell (NSC) divisions[141,158]. When NSCs divide, one daughter remains a multipotent stem cell while the other daughter differentiates to become an oligodendrocyte, astrocyte or non-dividing neuron. During division of mouse NSCs, damaged proteins are segregated to neuron daughter cells[158]. By segregating damage to the non-reproductive neuron, the multipotent stem cell remains free of damage and escapes senescence[158], much like the rejuvenated offspring cell in asymmetrical microbial reproduction. The smaller the amount of damaged protein received by the daughter stem cell, the more quickly it is able to divide again[158]. The degree of damage asymmetry decreases in NSCs isolated from older animals[158]. This breakdown of appropriate asymmetrical allocation of damage away from NSCs during ageing could point to an underlying factor in neurodegeneration. In addition, there are mechanistic aspects of damage inheritance that are conserved from single-celled eukaryotes to mammals[141,142]. In both yeast and mouse cells, asymmetrical inheritance relies on the filament protein vimentin and the degradation centre for damaged proteins called the juxtanuclear quality control compartment[142,158]. Given the numerous similarities, research in microbial ageing and damage allocation has the potential to yield further insights into the underlying causes of non-communicable disease associated with ageing.

Senescence as a nearly universal feature of life. The presence of conserved lifespan-modulating pathways among diverse microbial lineages suggests that longevity and ageing are ancient biological phenomena that evolved perhaps billions of years ago[10,87]. In particular, the recurrent patterns and mechanisms of senescence across taxa on Earth suggest that senescence, in addition to longevity, was also present at the origin of life. Although it can lead to senescence, microbial asymmetrical division can confer a reproductive advantage, especially when the rate of damage is high[10,77,79,80,114,159] (Supplementary Box 1). In multicellular organisms, trade-offs between longevity and reproduction tend to be found wherever they have been sought[50]. The intimate connection between the life-history traits of reproduction and death is rooted in chemical physics. The maximum reproductive rate of any self-replicating entity is limited by the capacity for longevity of this entity[160]. That is, entities with greater reproductive potential wear out more quickly[160]. Therefore, as natural selection is universal in populations of imperfectly self-replicating entities and fitness always depends on reproduction, we expect senescence to be common anywhere in the Universe that life has evolved.

Published online: 18 September 2019

1. Kapahi, P., Kaeberlein, M. & Hansen, M. Dietary restriction and lifespan: lessons from invertebrate models. *Ageing Res. Rev.* **39**, 3–14 (2017).
2. Yuan, R., Peters, L. L. & Paigen, B. Mice as a mammalian model for research on the genetics of aging. *ILAR J.* **52**, 4–15 (2011).
3. Plomion, C. et al. Oak genome reveals facets of long lifespan. *Nat. Plants* **4**, 440–452 (2018).
4. Vazquez, J. M., Sulak, M., Chigurupati, S. & Lynch, V. J. A zombie *LIF* gene in elephants is upregulated by TP53 to induce apoptosis in response to DNA damage. *Cell Rep.* **24**, 1765–1776 (2018).
5. Ruby, J. G., Smith, M. & Buffenstein, R. Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age. *eLife* **7**, e31157 (2018).
6. Partridge, L. & Barton, N. H. Optimality, mutation and the evolution of ageing. *Nature* **362**, 305–311 (1993).
7. Kirkwood, T. B. & Austad, S. N. Why do we age? *Nature* **408**, 233–238 (2000).
8. Kirkwood, T. B. L. Asymmetry and the origins of ageing. *Mech. Ageing Dev.* **126**, 533–534 (2005).
9. Bell, G. Evolutionary and nonevolutionary theories of senescence. *Am. Nat.* **124**, 600–603 (1984).
10. Ackermann, M., Chao, L., Bergstrom, C. T. & Doebeli, M. On the evolutionary origin of aging. *Aging Cell* **6**, 235–244 (2007).
This article presents one of the first theoretical and quantitative models for the evolution of ageing and asymmetrical reproduction in single-celled organisms.
11. Kirkwood, T. B. L. Understanding the odd science of aging. *Cell* **120**, 437–447 (2005).
12. Johnson, L. R. & Mangel, M. Life histories and the evolution of aging in bacteria and other single-celled organisms. *Mech. Ageing Dev.* **127**, 786–793 (2006).
13. Tyedmers, J., Mogk, A. & Bukau, B. Cellular strategies for controlling protein aggregation. *Nat. Rev. Mol. Cell Biol.* **11**, 777–788 (2010).
14. Kysela, D. T., Brown, P. J. B., Huang, K. C. & Brun, Y. V. Biological consequences and advantages of asymmetric bacterial growth. *Annu. Rev. Microbiol.* **67**, 417–435 (2013).
15. Angert, E. R. Alternatives to binary fission in bacteria. *Nat. Rev. Microbiol.* **3**, 214–224 (2005).
16. Mortimer, R. K. & Johnston, J. R. Life span of individual yeast cells. *Nature* **183**, 1751–1752 (1959).
This article presents one of the first studies documenting ageing in a microorganism.
17. Longo, V. D., Shadel, G. S., Kaeberlein, M. & Kennedy, B. Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metab.* **16**, 18–31 (2012).
18. Ackermann, M., Stearns, S. C. & Jenal, U. Senescence in a bacterium with asymmetric division. *Science* **300**, 1920–1920 (2003).
19. Laney, S. R., Olson, R. J. & Sosik, H. M. Diatoms favor their younger daughters. *Limnol. Oceanogr.* **57**, 1572–1578 (2012).
20. Lindner, A. B., Madden, R., Demarez, A., Stewart, E. J. & Taddei, F. Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl Acad. Sci. USA* **105**, 3076–3081 (2008).
This work shows that damaged protein aggregates are associated with reproductive senescence in *E. coli*.
21. Stewart, E. J., Madden, R., Paul, G. & Taddei, F. Aging and death in an organism that reproduces by morphologically symmetric division. *PLOS Biol.* **3**, e45 (2005).
This article is one of the first studies to document ageing in a microorganism that reproduces by binary fission.
22. Boehm, A. et al. Genetic manipulation of glycogen allocation affects replicative lifespan in *E. coli*. *PLOS Genet.* **12**, e1005974 (2016).
23. Coelho, M., Lade, S. J., Alberti, S., Gross, T. & Tolić, I. M. Fusion of protein aggregates facilitates asymmetric damage segregation. *PLOS Biol.* **12**, e1001886 (2014).
24. Proenca, A. M., Rang, C. U., Buetz, C., Shi, C. & Chao, L. Age structure landscapes emerge from the equilibrium between aging and rejuvenation in bacterial populations. *Nat. Commun.* **9**, 3722 (2018).
25. Hamilton, W. D. The moulding of senescence by natural selection. *J. Theor. Biol.* **12**, 12–45 (1966).
26. Caswell, H. Matrix Population Models: Construction, Analysis, Interpretation (Sinauer Associates, 2001).
27. Gibson, B., Wilson, D. J., Feil, E. & Eyre-Walker, A. The distribution of bacterial doubling times in the wild. *Proc. R. Soc. B Biol. Sci.* **285**, 20180789 (2018).
28. Orsi, W. D. Ecology and evolution of seafloor and subseafloor microbial communities. *Nat. Rev. Microbiol.* **16**, 671–683 (2018).
29. Jørgensen, B. B. & Marshall, I. P. G. Slow microbial life in the seabed. *Annu. Rev. Mar. Sci.* **8**, 311–332 (2016).
This review examines patterns of microbial ageing and longevity in the deep biosphere environment.

30. Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789 (2004).
31. Johnson, S. S. et al. Ancient bacteria show evidence of DNA repair. *Proc. Natl Acad. Sci. USA* **104**, 14401–14405 (2007).
32. Jaakkola, S. T. et al. The complete genome of a viable archaeum isolated from 123-million-year-old rock salt: genome sequence of *Halobacterium hubeiense*. *Environ. Microbiol.* **18**, 565–579 (2016).
33. Greenblatt, C. L. et al. *Micrococcus luteus*—survival in amber. *Microb. Ecol.* **48**, 120–127 (2004).
34. Trembath-Reichert, E. et al. Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds. *Proc. Natl Acad. Sci. USA* **114**, E9206–E9215 (2017).
35. Braun, S. et al. Microbial turnover times in the deep seabed studied by amino acid racemization modelling. *Sci. Rep.* **7**, 5680 (2017).
36. Starnawski, P. et al. Microbial community assembly and evolution in subseafloor sediment. *Proc. Natl Acad. Sci. USA* **114**, 2940–2945 (2017).
This study uses metagenomics and single-cell genomics to show that population genetic patterns among deep biosphere microorganisms are consistent with preadaptation to the low-energy environment, rather than ongoing adaptive evolution.
37. Medawar, P. *An Unsolved Problem of Biology* (H. K. Lewis, 1952).
38. Williams, G. C. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**, 398–411 (1957).
39. Haldane, J. B. S. *New Paths in Genetics* (Allen & Unwin, 1941).
40. Caswell, H. A general formula for the sensitivity of population growth rate to changes in life history parameters. *Theor. Popul. Biol.* **14**, 215–230 (1978).
41. Kirkwood, T. B. L. Evolution of ageing. *Nature* **270**, 301–304 (1977).
This paper proposes the disposable soma theory of ageing.
42. Kennedy, B. K., Austriaco, N. R. & Guarente, L. Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life span. *J. Cell Biol.* **127**, 1985–1993 (1994).
43. Szilard, L. On the nature of the aging process. *Proc. Natl Acad. Sci. USA* **45**, 30–45 (1959).
44. Jazwinski, S. M. Aging and senescence of the budding yeast *Saccharomyces cerevisiae*. *Mol. Microbiol.* **4**, 337–343 (1990).
45. Ogrodnik, M., Salmonowicz, H. & Gladyshev, V. N. Integrating cellular senescence with the concept of damage accumulation in aging: relevance for clearance of senescent cells. *Aging Cell* **8**, e12841 (2019).
46. Gladyshev, V. N. The origin of aging: imperfectness-driven non-random damage defines the aging process and control of lifespan. *Trends Genet.* **29**, 506–512 (2013).
47. Maynard, S., Fang, E. F., Scheibye-Knudsen, M., Croteau, D. L. & Bohr, V. A. DNA damage, DNA repair, aging, and neurodegeneration. *Cold Spring Harb. Perspect. Med.* **5**, a025130 (2015).
48. Kirkwood, T. B. L. Evolution of ageing. *Mech. Ageing Dev.* **123**, 737–745 (2002).
49. Flatt, T. & Partridge, L. Horizons in the evolution of aging. *BMC Biol.* **16**, 93 (2018).
50. Austad, S. N. & Hoffman, J. M. Is antagonistic pleiotropy ubiquitous in aging biology? *Evol. Med. Public Health* **2018**, 287–294 (2018).
51. Ricklefs, R. E. Insights from comparative analyses of aging in birds and mammals. *Aging Cell* **9**, 273–284 (2010).
52. Sadowska-Bartosz, I. & Bartosz, G. Effect of glycation inhibitors on aging and age-related diseases. *Mech. Ageing Dev.* **160**, 1–18 (2016).
53. Ricklefs, R. E. Intrinsic aging-related mortality in birds. *J. Avian Biol.* **31**, 103–111 (2000).
54. Ricklefs, R. E. The evolution of senescence from a comparative perspective. *Funct. Ecol.* **22**, 379–392 (2008).
55. Gladyshev, V. N. On the cause of aging and control of lifespan: heterogeneity leads to inevitable damage accumulation, causing aging; control of damage composition and rate of accumulation define lifespan. *BioEssays* **34**, 925–929 (2012).
56. Voit, M. & Meyer-Ortmanns, H. How aging may be an unavoidable fate of dynamical systems. *New J. Phys.* **21**, 043045 (2019).
57. Risques, R. A. & Kennedy, S. R. Aging and the rise of somatic cancer-associated mutations in normal tissues. *PLOS Genet.* **14**, e1007108 (2018).
58. Lee, M. B. et al. Defining the impact of mutation accumulation on replicative lifespan in yeast using cancer-associated mutator phenotypes. *Proc. Natl Acad. Sci. USA* **116**, 3062–3071 (2019).
59. Carneiro, M. C., de Castro, I. P. & Ferreira, M. G. Telomeres in aging and disease: lessons from zebrafish. *Dis. Model. Mech.* **9**, 737–748 (2016).
60. Blackburn, E. H., Epel, E. S. & Lin, J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. *Science* **350**, 1193–1198 (2015).
61. Hu, Z. et al. Nucleosome loss leads to global transcriptional up-regulation and genomic instability during yeast aging. *Genes Dev.* **28**, 396–408 (2014).
62. Zhang, K., Zheng, D.-Q., Sui, Y., Qi, L. & Petes, T. D. Genome-wide analysis of genomic alterations induced by oxidative DNA damage in yeast. *Nucleic Acids Res.* **47**, 3521–3535 (2019).
63. McMurray, M. A. & Gottschling, D. E. An age-induced switch to a hyper-recombinational state. *Science* **301**, 1908–1911 (2003).
64. Baraibar, M. A., Ladouce, R. & Friguet, B. Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging. *J. Proteom.* **92**, 63–70 (2013).
65. Reverter-Branchat, G., Cabiscol, E., Tamarit, J. & Ros, J. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction. *J. Biol. Chem.* **279**, 31983–31989 (2004).
66. Dasgupta, A., Zheng, J. & Bizzozero, O. A. Protein carbonylation and aggregation precede neuronal apoptosis induced by partial glutathione depletion. *ASN Neuro* **4**, AN20110064 (2012).
67. Maisonneuve, E., Ezraty, B. & Dukan, S. Protein aggregates: an aging factor involved in cell death. *J. Bacteriol.* **190**, 6070–6075 (2008).
This study shows that damaged protein aggregates are associated with survival senescence in *E. coli*.
68. Erjavec, N., Larsson, L., Grantham, J. & Nyström, T. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Dev.* **21**, 2410–2421 (2007).
69. Vágási, C. I. et al. Longevity and life history coevolve with oxidative stress in birds. *Funct. Ecol.* **33**, 152–161 (2019).
70. Hartl, F. U., Bracher, A. & Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. *Nature* **475**, 324–332 (2011).
71. Shafirovich, V. & Geacintov, N. E. Removal of oxidatively generated DNA damage by overlapping repair pathways. *Free. Radic. Biol. Med.* **107**, 53–61 (2017).
72. Wigley, D. B. Bacterial DNA repair: recent insights into the mechanism of RecBCD, AddAB and AdnAB. *Nat. Rev. Microbiol.* **11**, 9–13 (2013).
73. Wensink, M. J., Caswell, H. & Baudisch, A. The rarity of survival to old age does not drive the evolution of senescence. *Evol. Biol.* **44**, 5–10 (2017).
74. Kirkwood, T. B. L. & Holliday, R. The evolution of ageing and longevity. *Proc. R. Soc. B Biol. Sci.* **205**, 531–546 (1979).
75. Partridge, L. Evolutionary theories of ageing applied to long-lived organisms. *Exp. Gerontol.* **36**, 641–650 (2001).
76. Zwaan, B. J. The evolutionary genetics of ageing and longevity. *Heredity* **82**, 589–597 (1999).
77. Chao, L. A model for damage load and its implications for the evolution of bacterial aging. *PLOS Genet.* **6**, e1001076 (2010).
78. Clegg, R. J., Dyson, R. J. & Kreft, J.-U. Repair rather than segregation of damage is the optimal unicellular aging strategy. *BMC Biol.* **12**, 52 (2014).
79. Lin, J., Min, J. & Amir, A. Optimal segregation of proteins: phase transitions and symmetry breaking. *Phys. Rev. Lett.* **122**, 068101 (2019).
This study defines the parameter space in which symmetrical or asymmetrical allocation of beneficial or deleterious proteins is most adaptive.
80. Rang, C. U., Peng, A. Y., Poon, A. F. & Chao, L. Ageing in *Escherichia coli* requires damage by an extrinsic agent. *Microbiology* **158**, 1553–1559 (2012).
81. Coelho, M. et al. Fission yeast does not age under favorable conditions, but does so after stress. *Curr. Biol.* **23**, 1844–1852 (2013).
82. Wang, P. et al. Robust growth of *Escherichia coli*. *Curr. Biol.* **20**, 1099–1103 (2010).
83. Spivey, E. C., Jones, S. K., Rybarski, J. R., Saifuddin, F. A. & Finkelstein, I. J. An aging-independent replicative lifespan in a symmetrically dividing eukaryote. *eLife* **6**, e20340 (2017).
84. Nakaoka, H. & Wakamoto, Y. Aging, mortality, and the fast growth trade-off of *Schizosaccharomyces pombe*. *PLOS Biol.* **15**, e2001109 (2017).
85. Chao, L., Rang, C. U., Proenca, A. M. & Chao, J. U. Asymmetrical damage partitioning in bacteria: a model for the evolution of stochasticity, determinism, and genetic assimilation. *PLOS Comput. Biol.* **12**, e1004700 (2016).
86. Rang, C. U., Peng, A. Y. & Chao, L. Temporal dynamics of bacterial aging and rejuvenation. *Curr. Biol.* **21**, 1813–1816 (2011).
87. Kirkwood, T. B. Understanding ageing from an evolutionary perspective. *J. Intern. Med.* **263**, 117–127 (2008).
88. Lee, A. J. & Wallace, S. S. Hide and seek: how do DNA glycosylases locate oxidatively damaged DNA bases amidst a sea of undamaged bases? *Free. Radic. Biol. Med.* **107**, 170–178 (2017).
89. Shuman, S. & Glickman, M. S. Bacterial DNA repair by non-homologous end joining. *Nat. Rev. Microbiol.* **5**, 852–861 (2007).
90. Lieber, M. R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* **79**, 181–211 (2010).
91. Richarme, G. et al. Guanine glycation repair by DJ-1/Park7 and its bacterial homologs. *Science* **357**, 208–211 (2017).
92. Foti, J. J., Devadoss, B., Winkler, J. A., Collins, J. J. & Walker, G. C. Oxidation of the guanine nucleotide pool underlies cell death by bactericidal antibiotics. *Science* **336**, 315–319 (2012).
93. Mattoo, R. U. H. & Goloubinoff, P. Molecular chaperones are nanomachines that catalytically unfold misfolded and alternatively folded proteins. *Cell. Mol. Life Sci.* **71**, 3311–3325 (2014).
94. Veinger, L., Diamant, S., Buchner, J. & Goloubinoff, P. The small heat-shock protein IbpB from *Escherichia coli* stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. *J. Biol. Chem.* **273**, 11032–11037 (1998).
95. Priya, S. et al. GroEL and CCT are catalytic unfoldases mediating out-of-cage polypeptide refolding without ATP. *Proc. Natl Acad. Sci. USA* **110**, 7199–7204 (2013).
96. Sharma, S. K., De Los Rios, P., Christen, P., Lustig, A. & Goloubinoff, P. The kinetic parameters and energy cost of the Hsp70 chaperone as a polypeptide unfoldase. *Nat. Chem. Biol.* **6**, 914–920 (2010).
97. Fernández-Higuero, J. A., Aguado, A., Perales-Calvo, J., Moro, F. & Muga, A. Activation of the DnaK–ClpB complex is regulated by the properties of the bound substrate. *Sci. Rep.* **8**, 5796 (2018).
98. Doyle, S. M. et al. Interplay between *E. coli* DnaK, ClpB and GrpE during protein disaggregation. *J. Mol. Biol.* **427**, 312–327 (2015).
99. Seyffer, F. et al. Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces. *Nat. Struct. Mol. Biol.* **19**, 1347–1355 (2012).
100. Diamant, S., Ben-Zvi, A. P., Bukau, B. & Goloubinoff, P. Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery. *J. Biol. Chem.* **275**, 21107–21113 (2000).
101. Goloubinoff, P., Mogk, A., Zvi, A. P. B., Tomoyasu, T. & Bukau, B. Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl Acad. Sci. USA* **96**, 13732–13737 (1999).
102. Lennon, J. T. & Jones, S. E. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* **9**, 119–130 (2011).
103. Bradley, J. A., Amend, J. P. & LaRowe, D. E. Survival of the fewest: microbial dormancy and maintenance in marine sediments through deep time. *Geobiology* **17**, 43–59 (2019).
104. LaRowe, D. E. & Amend, J. P. Power limits for microbial life. *Front. Microbiol.* **6**, 718 (2015).
105. Milo, R. & Phillips, R. *Cell Biology by the Numbers* (Garland Science, 2016).
106. Rang, C. U., Proenca, A., Buetz, C., Shi, C. & Chao, L. Minicells as a damage disposal mechanism in *Escherichia coli*. *mSphere* **3**, e00428–18 (2018).
This study shows how disposal of damaged protein aggregates through minicell formation can help *E. coli* to avoid senescence.
107. Farley, M. M., Hu, B., Margolin, W. & Liu, J. Minicells, back in fashion. *J. Bacteriol.* **198**, 1186–1195 (2016).
108. Winkler, J. et al. Quantitative and spatio-temporal features of protein aggregation in *Escherichia coli* and consequences on protein quality control and cellular ageing. *EMBO J.* **29**, 910–923 (2010).

109. Coquel, A.-S. et al. Localization of protein aggregation in *Escherichia coli* is governed by diffusion and nucleoid macromolecular crowding effect. *PLOS Comput. Biol.* **9**, e1003038 (2013).
110. Hoffman, H. & Frank, M. E. Time-lapse photomicrography of the formation of a free spherical granule in an *Escherichia coli* cell end. *J. Bacteriol.* **86**, 1075–1078 (1963).
111. Jamroskovic, J., Pavlendová, N., Muchová, K., Wilkinson, A. J. & Barák, I. An oscillating Min system in *Bacillus subtilis* influences asymmetrical septation during sporulation. *Microbiol. Read. Engl.* **158**, 1972–1981 (2012).
112. Patrick, J. E. & Kearns, D. B. MinJ (YvjD) is a topological determinant of cell division in *Bacillus subtilis*. *Mol. Microbiol.* **70**, 1166–1179 (2008).
113. Bergmiller, T. et al. Biased partitioning of the multidrug efflux pump AcrAB–TolC underlies long-lived phenotypic heterogeneity. *Science* **356**, 311–315 (2017).
114. Vedel, S., Nunns, H., Košmrlj, A., Semsey, S. & Trusina, A. Asymmetric damage segregation constitutes an emergent population-level stress response. *Cell Syst.* **3**, 187–198 (2016).
115. Liu, B. et al. The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell* **140**, 257–267 (2010).
116. Spokoini, R. et al. Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast. *Cell Rep.* **2**, 738–747 (2012).
This study reveals important mechanisms in the active segregation of damage at cell division in the budding yeast *S. cerevisiae*.
117. Coelho, M. & Tolić, I. M. Asymmetric damage segregation at cell division via protein aggregate fusion and attachment to organelles. *BioEssays* **37**, 740–747 (2015).
118. Curtis, P. D. & Brun, Y. V. Getting in the loop: regulation of development in *Caulobacter crescentus*. *Microbiol. Mol. Biol. Rev.* **74**, 13–41 (2010).
119. Obuchowski, P. L. & Jacobs-Wagner, C. PflI, a protein involved in flagellar positioning in *Caulobacter crescentus*. *J. Bacteriol.* **190**, 1718–1729 (2008).
120. Davis, N. J. et al. De- and repolarization mechanism of flagellar morphogenesis during a bacterial cell cycle. *Genes Dev.* **27**, 2049–2062 (2013).
121. Huitema, E., Pritchard, S., Matteson, D., Radhakrishnan, S. K. & Viollier, P. H. Bacterial birth scar proteins mark future flagellum assembly site. *Cell* **124**, 1025–1037 (2006).
122. Lam, H., Schofield, W. B. & Jacobs-Wagner, C. A landmark protein essential for establishing and perpetuating the polarity of a bacterial cell. *Cell* **124**, 1011–1023 (2006).
123. Hussain, S. et al. MreB filaments align along greatest principal membrane curvature to orient cell wall synthesis. *eLife* **7**, e32471 (2018).
124. Ursell, T. S. et al. Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. *Proc. Natl Acad. Sci. USA* **111**, E1025–E1034 (2014).
125. de Pedro, M. A., Quintela, J. C., Höltje, J. V. & Schwarz, H. Murein segregation in. *Escherichia coli. J. Bacteriol.* **179**, 2823–2834 (1997).
126. Ettema, T. J. G., Lindås, A.-C. & Bernander, R. An actin-based cytoskeleton in archaea. *Mol. Microbiol.* **80**, 1052–1061 (2011).
127. Rokney, A. et al. *E. coli* transports aggregated proteins to the poles by a specific and energy-dependent process. *J. Mol. Biol.* **392**, 589–601 (2009).
128. Govers, S. K., Dutre, P. & Aertsen, A. *In vivo* disassembly and reassembly of protein aggregates in. *Escherichia coli. J. Bacteriol.* **196**, 2325–2332 (2014).
129. Neeli-Venkata, R. et al. Robustness of the process of nucleoid exclusion of protein aggregates in. *Escherichia coli. J. Bacteriol.* **198**, 898–906 (2016).
130. Parry, B. R. et al. The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. *Cell* **156**, 183–194 (2014).
131. Henderson, K. A., Hughes, A. L. & Gottschling, D. E. Mother–daughter asymmetry of pH underlies aging and rejuvenation in yeast. *eLife* **3**, e03504 (2014).
This study elucidates the role of cytosolic pH in *S. cerevisiae* ageing.
132. Aufschnaiter, A. & Büttner, S. The vacuolar shapes of ageing: from function to morphology. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 957–970 (2019).
133. Hughes, A. L. & Gottschling, D. E. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* **492**, 261–265 (2012).
This study demonstrates that ageing-associated changes in vacuolar pH causes senescence in *S. cerevisiae*.
134. Veatch, J. R., McMurray, M. A., Nelson, Z. W. & Gottschling, D. E. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell* **137**, 1247–1258 (2009).
135. Scheckhuber, C. Q. et al. Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. *Nat. Cell Biol.* **9**, 99–105 (2007).
136. Sinclair, D. A. & Guarente, L. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* **91**, 1033–1042 (1997).
One of the first studies to specifically identify a type of ageing-associated molecular damage that causes senescence of a cell.
137. Kaeberlein, M., McVey, M. & Guarente, L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* **13**, 2570–2580 (1999).
138. Laun, P. et al. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. *Mol. Microbiol.* **39**, 1166–1173 (2001).
139. Aguilaniu, H., Gustafsson, L., Rigoulet, M. & Thomas, N. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* **299**, 1751–1753 (2003).
140. Longo, V. D. & Kennedy, B. K. Sirtuins in aging and age-related disease. *Cell* **126**, 257–268 (2006).
141. Moore, D. L. & Jessberger, S. Creating age asymmetry: consequences of inheriting damaged goods in mammalian cells. *Trends Cell Biol.* **27**, 82–92 (2017).
This paper summarizes recent findings that asymmetrical cell divisions are associated with ageing and senescence in multicellular organisms.
142. Ogrodnik, M. et al. Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. *Proc. Natl Acad. Sci. USA* **111**, 8049–8054 (2014).
143. Shoemaker, W. R. & Lennon, J. T. Evolution with a seed bank: the population genetic consequences of microbial dormancy. *Evol. Appl.* **11**, 60–75 (2018).
144. Finkel, S. E. Long-term survival during stationary phase: evolution and the GASP phenotype. *Nat. Rev. Microbiol.* **4**, 113–120 (2006).
145. Finkel, S. E. & Kolter, R. Evolution of microbial diversity during prolonged starvation. *Proc. Natl Acad. Sci. USA* **96**, 4023–4027 (1999).
146. Kram, K. E. et al. Adaptation of *Escherichia coli* to long-term serial passage in complex medium: evidence of parallel evolution. *mSystems* **2**, e00192–16 (2017).
147. Zinser, E. R. & Kolter, R. *Escherichia coli* evolution during stationary phase. *Res. Microbiol.* **155**, 328–336 (2004).
148. Windels, E. M. et al. Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates. *ISME J.* **13**, 1239–1251 (2019).
149. Kumar, S. & Engelberg-Kulka, H. Quorum sensing peptides mediating interspecies bacterial cell death as a novel class of antimicrobial agents. *Curr. Opin. Microbiol.* **21**, 22–27 (2014).
150. Chan, W. T., Balsa, D. & Espinosa, M. One cannot rule them all: are bacterial toxins–antitoxins druggable? *FEMS Microbiol. Rev.* **39**, 522–540 (2015).
151. Kumar, S., Kolodkin-Gal, I. & Engelberg-Kulka, H. Novel quorum-sensing peptides mediating interspecies bacterial cell death. *mBio* **4**, e00314–13 (2013).
152. Ayrapetyan, M., Williams, T. & Oliver, J. D. The relationship between the viable but nonculturable state and antibiotic persister cells. *J. Bacteriol.* **200**, e00249–18 (2018).
153. Levin-Reisman, I. et al. Antibiotic tolerance facilitates the evolution of resistance. *Science* **355**, 826–830 (2017).
154. Yaakov, G., Lerner, D., Bentele, K., Steinberger, J. & Barkai, N. Coupling phenotypic persistence to DNA damage increases genetic diversity in severe stress. *Nat. Ecol. Evol.* **1**, 0016 (2017).
155. Pu, Y. et al. ATP-dependent dynamic protein aggregation regulates bacterial dormancy depth critical for antibiotic tolerance. *Mol. Cell* **73**, 143–156.e4 (2019).
156. Fuentealba, L. C., Eivers, E., Geissert, D., Taelman, V. & De Robertis, E. M. Asymmetric mitosis: unequal segregation of proteins destined for degradation. *Proc. Natl Acad. Sci. USA* **105**, 7732–7737 (2008).
157. Rujano, M. A. et al. Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes. *PLOS Biol.* **4**, e417 (2006).
158. Moore, D. L., Pilz, G. A., Arauzo-Bravo, M. J., Barral, Y. & Jessberger, S. A mechanism for the segregation of age in mammalian neural stem cells. *Science* **349**, 1334–1338 (2015).
This study demonstrates that asymmetrical allocation of damage can promote the proliferation of stem cells in the brain of a mammal.
159. Koleva, K. Z. & Hellweger, F. L. From protein damage to cell aging to population fitness in *E. coli*: insights from a multi-level agent-based model. *Ecol. Model.* **301**, 62–71 (2015).
160. England, J. L. Statistical physics of self-replication. *J. Chem. Phys.* **139**, 121923 (2013).
161. Christner, B. C., Mosley-Thompson, E., Thompson, L. G. & Reeve, J. N. Bacterial recovery from ancient glacial ice. *Environ. Microbiol.* **5**, 433–436 (2003).
162. Carey, J. R. Insect biodemography. *Annu. Rev. Entomol.* **46**, 79–110 (2001).
163. Ricklefs, R. E. Life-history connections to rates of aging in terrestrial vertebrates. *Proc. Natl Acad. Sci. USA* **107**, 10314–10319 (2010).
164. Baudisch, A. The pace and shape of ageing. *Methods Ecol. Evol.* **2**, 375–382 (2011).
165. Jones, O. R. et al. Diversity of ageing across the tree of life. *Nature* **505**, 169–173 (2014).
166. Yang, Y. et al. Temporal scaling of aging as an adaptive strategy of *Escherichia coli*. *Sci. Adv.* **5**, eaaw2069 (2019).
167. Jo, M. C., Liu, W., Gu, L., Dang, W. & Qin, L. High-throughput analysis of yeast replicative aging using a microfluidic system. *Proc. Natl Acad. Sci. USA* **112**, 9364–9369 (2015).
168. Bouwhuis, S., Choquet, R., Sheldon, B. C. & Verhulst, S. The forms and fitness cost of senescence: age-specific recapture, survival, reproduction, and reproductive value in a wild bird population. *Am. Nat.* **179**, E15–E27 (2012).
169. Nedelcu, A. M., Driscoll, W. W., Durand, P. M., Herron, M. D. & Rashidi, A. On the paradigm of altruistic suicide in the unicellular world. *Evolution* **65**, 3–20 (2011).
170. Longo, V. D., Mitteldorf, J. & Skulachev, V. P. Programmed and altruistic ageing. *Nat. Rev. Genet.* **6**, 866–872 (2005).
171. Hamilton, W. D. The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**, 1–16 (1964).
172. West, S. A., Griffin, A. S. & Gardner, A. Social semantics: how useful has group selection been? *J. Evol. Biol.* **21**, 374–385 (2008).
173. Webb, J. S. et al. Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* **185**, 4585–4592 (2003).
174. Rice, K. C. et al. The *cidA* murein hydrolase regulator contributes to DNA release and biofilm development in *Staphylococcus aureus*. *Proc. Natl Acad. Sci. USA* **104**, 8113–8118 (2007).
175. Zetzmann, M. et al. DNase-sensitive and -resistant modes of biofilm formation by *Listeria monocytogenes*. *Front. Microbiol.* **6**, 1428 (2015).
176. Okshevsky, M. & Meyer, R. L. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. *Crit. Rev. Microbiol.* **41**, 341–352 (2015).
177. Thomas, V. C., Thurlow, L. R., Boyle, D. & Hancock, L. E. Regulation of autolysis-dependent extracellular DNA release by *Enterococcus faecalis* extracellular proteases influences biofilm development. *J. Bacteriol.* **190**, 5690–5698 (2008).
178. Fabrizio, P. et al. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J. Cell Biol.* **166**, 1055–1067 (2004).
179. Herker, E. et al. Chronological aging leads to apoptosis in yeast. *J. Cell Biol.* **164**, 501–507 (2004).
180. Fabrizio, P. & Longo, V. D. Chronological aging-induced apoptosis in yeast. *Biochim. Biophys. Acta Mol. Cell Res.* **1783**, 1280–1285 (2008).
181. Bar-Zeev, E., Avishay, I., Bidle, K. D. & Berman-Frank, I. Programmed cell death in the marine cyanobacterium *Trichodesmium* mediates carbon and nitrogen export. *ISME J.* **7**, 2340–2348 (2013).
182. Aizenman, E., Engelberg-Kulka, H. & Glaser, G. An *Escherichia coli* chromosomal 'addiction module' regulated by guanosine [corrected] 3′,5′-bispyrophosphate: a model for programmed bacterial cell death. *Proc. Natl Acad. Sci. USA* **93**, 6059–6063 (1996).
183. Engelberg-Kulka, H., Hazan, R. & Amitai, S. mazEF: a chromosomal toxin–antitoxin module that triggers

programmed cell death in bacteria. *J. Cell Sci.* **118**, 4327–4332 (2005).
184. Erental, A., Kalderon, Z., Saada, A., Smith, Y. & Engelberg-Kulka, H. Apoptosis-like death, an extreme SOS response in *Escherichia coli*. *mBio* **5**, e01426–14 (2014).
185. Erental, A., Sharon, I. & Engelberg-Kulka, H. Two programmed cell death systems in *Escherichia coli*: an apoptotic-like death Is inhibited by the mazEF-mediated death pathway. *PLOS Biol.* **10**, e1001281 (2012).
186. Dwyer, D. J., Camacho, D. M., Kohanski, M. A., Callura, J. M. & Collins, J. J. Antibiotic-induced bacterial cell death exhibits physiological and biochemical hallmarks of apoptosis. *Mol. Cell* **46**, 561–572 (2012).
187. Engelberg-Kulka, H., Amitai, S., Kolodkin-Gal, I. & Hazan, R. Bacterial programmed cell death and multicellular behavior in bacteria. *PLOS Genet.* **2**, e135 (2006).
188. Szathmáry, E. & Smith, J. M. The major evolutionary transitions. *Nature* **374**, 227–232 (1995).
189. de Magalhães, J. P. & Costa, J. A database of vertebrate longevity records and their relation to other life-history traits. *J. Evol. Biol.* **22**, 1770–1774 (2009).
190. Bakshi, S. et al. Nonperturbative imaging of nucleoid morphology in live bacterial cells during an antimicrobial peptide attack. *Appl. Environ. Microbiol.* **80**, 4977–4986 (2014).
191. Lee, K.-C., Webb, R. I. & Fuerst, J. A. The cell cycle of the planctomycete *Gemmata obscuriglobus* with respect to cell compartmentalization. *BMC Cell Biol.* **10**, 4 (2009).
192. Tocheva, E. I. et al. Peptidoglycan transformations during *Bacillus subtilis* sporulation. *Mol. Microbiol.* **88**, 673–686 (2013).
193. Brun, Y. V. & Janakiraman R. in *Prokaryotic Development*. (eds Brun, Y. V. & Shimkets, L. J.) 297–317 (ASM Press, 2000)

Acknowledgements
The authors acknowledge D. A. Schwartz (Indiana University), N. I. Wisnoski (Indiana University), M. Coelho (Harvard University), A. Amir (Harvard University), J. Lin (Harvard University) and J. Min (Harvard University) for critical feedback on earlier versions of this manuscript along with the National Science Foundation (1442246, J.T.L.) and a US Army Research Office Grant (W911NF-14-1-0411, J.T.L.) for financial support.

Author contributions
R.M.-R. and J.T.L. conceived and wrote the manuscript.

Competing interests
The authors declare no competing interests

Peer review information
Nature Reviews Microbiology thanks A. Amir, together with J. Lin and J. Min, and M. Coelho for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/s41579-019-0253-y.

RELATED LINKS
Available code and data: https://github.com/LennonLab/MicroLong