Ageing in *Escherichia coli* requires damage by an extrinsic agent

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Evidence for ageing in symmetrically dividing bacteria such as *Escherichia coli* has historically been conflicting. Early work found weak or no evidence. More recent studies found convincing evidence, but negative results are still encountered. Because bacterial ageing is believed to result from non-genetic (e.g. oxidative) damage, we tested the possibility that the negative outcomes resulted from the lack of an extrinsic damage agent. We found that streptomycin, which produces mistranslated proteins that are more vulnerable to oxidation, was able to induce both damage and ageing in bacterial populations. A dosage effect relating the level of damage to the concentration of streptomycin was observed. Our results explain the previous inconsistencies, because all studies that failed to find evidence for bacterial ageing did not use a damage agent. However, all studies that succeeded in finding evidence utilized fluorescent proteins as a visual marker. We suggest that ageing in those studies was induced by the harmful effects of an extrinsic factor, such as the proteins themselves or the excitation light. Thus, all of the earlier studies can be reconciled and bacterial ageing is a real phenomenon. However, the study and observation of bacterial ageing require the addition of an extrinsic damage agent.

INTRODUCTION

Recent studies demonstrating ageing or the deterioration of function with time in bacteria recall past debates on immortality, longevity, programmed processes, and the concept of ageing itself (Chao, 2010; Ferber, 2005; Ksiażek, 2010; Lele et al., 2011; Lindner et al., 2008; Nystroém, 2002; Rang et al., 2011; Stewart et al., 2005; Turke, 2008; Veening et al., 2008; Wang et al., 2010; Williams, 1957). Discussions of what is biological ageing are often confused because of the existence of several definitions, which we classify as cellular, individual and lineage ageing. Cellular ageing is manifested by the cessation of mitosis in untransformed cells after 40–50 divisions (Hayflick, 1965). Also known as replicative senescence or the Hayflick’s limit, cellular ageing results from a genetic programme and is a characteristic of metazoans. Although debated, replicative senescence has been suggested to explain ageing and to have evolved to suppress tumours (Campisi, 2005). It is generally agreed that cellular ageing does not apply to single-celled organisms such as bacteria (Nystroém, 2002). The second definition of ageing attributes the deterioration to the wear and tear incurred by individual organisms. Individuals challenged by oxidation, toxins, heat or other stressful agents will age more quickly, and evolution by natural selection should favour adaptations that better resist the challenges. Because bacterial cells can be ecologically and evolutionarily equivalent to individuals, they are susceptible to individual ageing. The final and third definition comes from evolutionary biologists, who see evolution as having accelerated individual ageing by a tradeoff (Kirkwood & Melov, 2011; Partridge & Barton, 1993; Turke, 2008; Williams, 1957). Accelerated ageing itself is not an adaptation favoured by selection, but rather the price paid for another trait that is favoured by selection. For example, ageing could be accelerated by a pleiotropic mutation that increases both fecundity rates at early life stages and death rates at later life stages (Williams, 1957). Because natural selection generally favours early fecundity, the increased death rate evolves as the tradeoff. Recent studies of ageing in *Escherichia coli* also demonstrate both the acceleration of ageing and the tradeoff (Lindner et al., 2008; Rang et al., 2011; Stewart et al., 2005; Veening et al., 2008). If a mother *E. coli* cell has non-genetic damage that cannot be repaired, her lineage has a higher evolutionary fitness if she allocates asymmetrically more of the damage to one daughter, rather than symmetrically. The asymmetry provides an advantage by generating variation and increasing the efficiency of natural selection (Chao, 2010). The tradeoff is that while the daughter receiving less damage is rejuvenated, the one receiving more is aged. Thus, lineage ageing corresponds to the amount accelerated by the tradeoff.
Because bacteria do not have long-lived multicellular life stages, it is reasonable that they should not manifest replicative ageing. Because they may frequently face extreme and stressful environments, it is not surprising that bacteria show individual ageing. Host defence systems deploying reactive oxidative species or antibiotics can be viewed as killing by subjecting bacterial cells to ageing (Doke et al., 1996; Lambeth, 2004). However, the existence of lineage ageing in bacteria such as \textit{E. coli} was not anticipated on theoretical grounds (Partridge & Barton, 1993; Rose, 1991; Turke, 2008; Williams, 1957). Lineage ageing had been demonstrated in yeast (Mortimer & Johnston, 1959) and \textit{Caulobacter crescentus} (Ackermann et al., 2003), but cell division in these microbes produces morphologically distinct mother and daughter cells. In both microbes, the smaller daughter could be viewed as a juvenile state (Stewart et al., 2005). On the other hand, in \textit{E. coli} and other bacteria that divide by symmetrical binary fission, the two daughters are morphologically indistinguishable. If the daughters were also physiologically identical, lineage ageing would not be possible. Thus, the dogma was that lineages of bacteria dividing by symmetrical binary fission did not age.

\textit{In vivo} studies have confounded the problem. Pioneering microscopy studies that anticipated lineage ageing sought for but found either no or only weak evidence for an asymmetry in the elongation rate of \textit{E. coli} daughters descending from the same mother (Powell & Errington, 1963; Schaechter et al., 1962). It was only recently that lineage ageing was re-examined in \textit{E. coli} (Stewart et al., 2005). With larger sample sizes, Stewart and colleagues were able to document that a daughter cell receiving the old pole from the mother had a slower elongation rate than a daughter receiving the new pole (Fig. 1). The slower elongation rate was attributed to the asymmetrical allocation of damage because inclusion bodies harbouring aberrant proteins were also associated with the old maternal pole (Lindner et al., 2008). A second study soon countered those results by showing that \textit{E. coli} cells trapped in micro-fluidic devices sustained robust growth with no lineage ageing (Wang et al., 2010). By reinterpreting the data of Stewart and colleagues and Wang and colleagues from the perspective of a population genetic model, we were able to reconcile the differences and show that both studies together provided strong support for lineage ageing in \textit{E. coli} (Rang et al., 2011). However, a more recent paper that tested for lineage ageing in \textit{E. coli} under high and low glucose concentrations again reported no asymmetry between the growth rates of daughters (Lele et al., 2011).

We have encountered equally conflicting results in our recent attempts to quantify lineage ageing in \textit{E. coli}. Because we relied on phase contrast microscopy, our bacteria were not labelled with fluorescent proteins, as had been done by the Stewart and the Wang groups. Because fluorescent proteins are known to harm bacteria by reducing growth rates (Rang et al., 2003), we hypothesized that the failure of our and earlier studies (Lele et al., 2011; Powell & Errington, 1963; Schaechter et al., 1962) to find lineage ageing resulted from the absence of fluorescent proteins or other damage agents (such as the excitation light used to visualize the fluorescence). Although both the Stewart and the Wang groups had labelled their bacteria with yellow fluorescent protein (YFP), most assumed that damage rates under standard aerobic culture conditions were sufficient to trigger lineage ageing. If damage rates were negligible and fluorescent proteins the actual agent of damage in the studies of the Stewart and Wang groups, the failure of our study and others to find lineage ageing could be explained. Without a damage agent, daughters cannot be distinguished on the basis of growth rates. With the aim of testing our hypothesis, we examined the effect of low but increasing levels of a known damage agent on lineage ageing in \textit{E. coli}. Because a second aim was to develop an agent that could be dosed in future studies, we chose not to use YFP or the excitation light. Fluorescent proteins are more difficult to dose and excitation light is more difficult to quantify and standardize between equipment. We chose instead to use streptomycin as the agent because it is known to induce non-genetic damage (Lindner et al., 2008) and its dosage relationship for concentration and growth rate is well characterized for \textit{E. coli} (Regoes et al., 2004). Streptomycin causes mistranslated proteins (Edelmann & Gallant, 1977), which are then more sensitive to oxidative damage in the studies of the Stewart and Wang groups, the failure of our study and others to find lineage ageing could be explained. Without a damage agent, daughters cannot be distinguished on the basis of growth rates. With the aim of testing our hypothesis, we examined the effect of low but increasing levels of a known damage agent on lineage ageing in \textit{E. coli}. Because a second aim was to develop an agent that could be dosed in future studies, we chose not to use YFP or the excitation light. Fluorescent proteins are more difficult to dose and excitation light is more difficult to quantify and standardize between equipment. We chose instead to use streptomycin as the agent because it is known to induce non-genetic damage (Lindner et al., 2008) and its dosage relationship for concentration and growth rate is well characterized for \textit{E. coli} (Regoes et al., 2004). Streptomycin causes mistranslated proteins (Edelmann & Gallant, 1977), which are then more sensitive to oxidative damage.
damage (Dukan et al., 2000). At levels below 10 μg streptomycin ml⁻¹, *E. coli* is still able to grow and divide. Because we will be focusing only on lineage ageing, the word ageing will be used hereafter to denote exclusively this third definition of ageing.

**METHODS**

**Strains and culture conditions.** The K-12 *E. coli* MG1655 (Blattner et al., 1997) wild-type strain was used throughout the study. All overnight bacterial cultures were grown in modified Luria–Bertani medium (LC broth; per litre: 10 g tryptone, 5 g yeast extract, 5 g NaCl) at 37 °C with aeration.

**Microscopy, slide preparation and streptomycin treatment.** Images were captured with an Olympus BX60 microscope with a ×100 phase contrast objective equipped with an Olympus C5050 camera. Preparation of bacteria and slides for microscopy was as described by Stewart et al. (2005). Bacteria were placed on 1.5% agarose pads made with filter-sterilized LC broth placed on 13 mm depression slides. Pads were made by placing into the depression 8 μl of agarose melted by microwaving, which was allowed to solidify to a flat-topped surface under a coverslip. After removing the coverslip and allowing the pad to dry for 1 min, 1 μl of bacteria from a 1:1000 dilution of an overnight culture was added and the pad was dried for an additional 3 min. The agar pad was covered with a new coverslip and sealed with petroleum jelly and Parafilm to avoid desiccation. The chosen dilution for the overnight yields approximately 1–15 cells in the microscope field. Inoculated slides were kept at 37 °C and sealed with petroleum jelly and Parafilm to avoid desiccation. An additional 3 min. The agar pad was covered with a new coverslip and sealed with petroleum jelly and Parafilm to avoid desiccation. Streptomycin (Sigma-Aldrich) at concentrations of 0, 1, 2 and 3 μg ml⁻¹ was added directly to the 1.5% agarose solution that had been cooled to approximately 50 °C. Time-lapse images were taken at 1 min intervals with the commercial software Pine Tree Computing Camera Controller on a desktop computer.

**Notation of old and new poles and daughters.** Rod-shaped bacteria such as *E. coli* reproduce by cleaving their long axis with the division plane (Fig. 1). Because two new poles are formed at the plate, poles distal to the plate are the old poles. All bacteria, including mother and daughter bacteria, have a new and an old pole. However, whenever a mother bacterium divides, one daughter receives the maternal old pole and the other receives the maternal new pole. Thus, following the notation of Stewart et al. (2005), we denote the former as the old daughter and the latter as the new daughter.

**Image analysis and data collection.** Lengths of individual bacterial cells were extracted manually from recorded time-lapse images with the free software ImageJ (NIH). From lengths compiled over time, the elongation rate *l* was estimated as the slope of a linear regression of log(length) over time. A log transformation was used because elongation rates are known to be exponential (Stewart et al., 2005). All lengths were measured immediately after division and prior to the next division. Doubling times were derived as *t* = log(2)/*l*. For any given mother cell, the trio values of her doubling time (denoted *t*₂) and the doubling times of her new and old daughters (*t*₁ and *t*₂, respectively) were collected. Sample sizes presented always correspond to the number of mother cells or trios sampled. To ensure that the recorded cells were out of the lag phase, growing exponentially and aerobically, and not starved for resources, we only analysed images from growing micro-colonies of a size between 100 and 400 cells. The upper cut-off of 400 cells under these conditions is conservative, because *E. coli* in micro-colonies of less than 600 cells is known to be growing under aerobic and non-limiting conditions (Stewart et al., 2005). Additionally, we verified the absence of limitation in our experimental system by monitoring cell size and growth rates (both signs of oxygen and resource starvation; Neidhardt et al., 1990), both of which did not decrease in our colony size window.

**Data analysis and parameter estimation.** The doubling times of mothers and their respective old and new daughters collected for a given concentration of streptomycin were analysed by a best fit to a population genetic model of bacterial ageing. This model, which was originally constructed to study the evolution of bacterial ageing (Chao, 2010), assumes that (i) cells need to achieve a target to divide, (ii) the ability of a cell in meeting the target is negatively affected by the amount of damage it possesses, (iii) the damage to a cell equals the amount it received from its mother plus the new damage that accrues at a rate *λ*, and (iv) upon dividing the cell allocates a fraction *a* of its damage to the new daughter and a fraction *(1−a)* to the old daughter. If the asymmetry coefficient *a* equals ½, the two daughters receive equal amounts. Besides *λ* and *a*, the model has a third parameter *π*, which represents the doubling time achieved by the most fit and damage-free bacterium. The model effectively provides a function *f*, which is able to predict the values of *t*₁ and *t*₂ given an input value of *t*₀ and the parameters *λ*, *π* and *a*, i.e. *t*=*f*(*t*₀, *λ*, *π*, *a*) and *t*=*f*(*t*₀, *λ*, *π*, 1−*a*). Because the data consisted of a set of observed *t*₀, *t*₁ and *t*₂ trios, the best fit was initiated by taking a trio and using the observed *t*₀ as an input, obtaining the squared difference between the model’s predicted output *t*₁ and *t*₂ values with the observed *t*₁ and *t*₂ values, repeating the process for all trios, determining the mean squared deviation for the entire set, and searching for the combination of *λ*, *π* and *a* values that minimized the mean squared deviation. The best fit estimates of *λ*, *π* and *a* were taken as the values that minimized the deviation. Confidence intervals for the best fit estimates were derived by an additional bootstrap of the fit of the dataset to the model. The bootstrap resampled the dataset with replacement while preserving the sample size. The 2.5% tails of the distribution obtained by 1000 bootstraps were discarded to obtain the 95% confidence intervals.

**RESULTS**

Following the methods described, we generated time-lapse images of dividing *E. coli*, measured cell elongation rates, converted the rates to doubling times, and analysed the results by fitting them to our model of bacterial ageing. Following the notation described above, we denote the daughter receiving the old maternal pole as the old daughter and the one receiving the new maternal pole as the new daughters (Fig. 1). As indicated, Stewart et al. (2005) had shown that the old daughter receives more damage and has a longer doubling time. A fit of our data to the model provided estimates of three parameters key to bacterial ageing, which are *λ*, *π* and *a*. The damage rate *λ* measures the amount of damage that a bacterial cell incurs per time unit (min⁻¹). The parameter *π* represents in minutes the doubling time of the fittest, most damage-free bacterium. The asymmetry coefficient *a* represents the amount of damage a mother cell allocates to her old daughter. The value of *a* ranges from 0 ≤*a*<½. If *a*=½ there is no ageing because the allocation of damage is symmetrical and both daughters receive the same amount. A value of *a*=0 represents extreme asymmetry, in which the new daughter receives none of the mother’s damage. The model has been used successfully to estimate the mean and confidence intervals of these three parameters (Chao,
2010). It has also been used to make testable predictions (Rang et al., 2011) that reconcile the differences between the results of Stewart et al. (2005) and Wang et al. (2010). The parameters $a$ and $\lambda$ are key for the present study because they quantify the hypothesized effect of streptomycin on bacterial ageing.

We present first the results of treatment with 0 µg streptomycin ml$^{-1}$. At this concentration, a plot of the doubling time of the new and old daughters ($t_1$ and $t_2$) as a function of the doubling time of the mother ($t_0$) revealed little difference between the two daughters (Fig. 2). The absence of difference indicated no ageing. This outcome was supported by a fit of the results to the model (Table 1). The estimated value of $\lambda$ was supported by a fit of the results to the model (Table 1). At 1 µg ml$^{-1}$, as we had observed at 0 µg ml$^{-1}$, the concentration of streptomycin was too weak to generate damage, and the estimates for $\lambda$ and $a$ were not significantly different from zero and $1/2$. However, at 2 and 3 µg ml$^{-1}$, the damage caused by streptomycin was now sufficiently large that the estimated values of $\lambda$ were significantly greater than zero. Additionally, the estimated values of $a$ were significantly less than $1/2$, although the difference from $1/2$ was slight. Thus, although the partitioning of damage in E. coli is asymmetrical, it is slight and not extreme.

We also examined whether the three parameters were plastic and exhibited a dosage response relative to streptomycin concentration. We recognized that the test would be weak because there were only four streptomycin concentrations but wondered whether there could be sufficient power in the data. A linear regression of the estimated $\pi$, $\lambda$ and $a$ values onto streptomycin concentrations was not significant for both $\pi$ and $a$ ($P=0.29$ and 0.42); however, the regression of $\lambda$ was significant ($P=0.024$; Fig. 4). Thus, of the three parameters, $\lambda$ was the only one that was plastic and responded to the increase in streptomycin.

**DISCUSSION**

The main conclusion derived from our study is that ageing in E. coli requires the input of damage by an extrinsic factor such as streptomycin under standard laboratory culture conditions. Our results establish the requirements for documenting and studying the phenomenon of bacterial ageing. The fact that streptomycin induces damage and ageing reveals that the asymmetrical partitioning of damage can help bacteria resist the harmful effects of antibiotics. Although the concentrations used were minimal, asymmetry can potentially also help bacteria survive much higher concentrations and levels of damage (Chao, 2010).

Our results also explain why earlier reports, all of which did not have an extrinsic damage agent, did not find evidence for ageing in bacteria (Lele et al., 2011; Powell & Errington, 1963; Schaechter et al., 1962). Without a significant level of damage, it was not possible to identify ageing on the basis of growth rate differences, regardless of whether the mother bacterium was partitioning macromolecules (damaged or not) in an asymmetrical manner to her daughters. The lack of evidence in the first studies had a big impact. Ageing in symmetrically dividing bacteria ceased to be a topic of research until Stewart and colleagues resurrected it with their publication in 2005, nearly half a century later. Stewart and colleagues were motivated to re-examine the phenomenon by evidence that old daughter cells were harbouring older copies of some macromolecules, e.g. cell wall components that did not turn over quickly. While it is easy to attribute the failure to identify bacterial ageing of the first studies to poorer optics and a lack of computer-assisted microscopy,
our results suggest that an equally compelling reason is the absence of an extrinsic damage factor.

Why then did Stewart and colleagues and some of the recent studies succeed in finding bacterial ageing (Lindner et al., 2008; Rang et al., 2011; Stewart et al., 2005; Veening et al., 2008; Wang et al., 2010)? We note that they all utilized fluorescent proteins (GFP and YFP) for cell imaging. Expression of gfp has been shown to be toxic in the absence of excitation and to reduce the rate of population growth in many bacterial species, including E. coli (Rang et al., 2003). Additionally, the excitation light used to visualize the fluorescent proteins could also have induced damaged macromolecules. If fluorescent proteins or the additional damaged molecules are harmful, bacteria may treat them both as non-genetic damage. They would be partitioned asymmetrically and ageing would be induced. We find this to be the most parsimonious explanation, given that all previous studies utilizing fluorescent proteins have reported evidence for ageing and all those not using them have found none. We note that Stewart et al. (2005), Lindner et al. (2008) and Wang et al. (2010) all used the E. coli strain MG1655, as we did, in their studies. Thus, a key difference between their experiments and our 0 μg streptomycin ml⁻¹ treatment was the inclusion of a fluorescent protein. It is ironic that Stewart and colleagues had chosen to re-examine bacterial ageing in what they described as a ‘protected environment without external causes of cell mortality’. While they did not deliberately add any external causes of damage, the yfp genes that they inserted were not original components of the genome of wild-type E. coli. Had Stewart and colleagues not fortuitously included YFP as a visual aid, their groundbreaking study might not

**Table 1.** Estimated parameter values and confidence intervals for the damage-free doubling time \( \pi \), the damage rate \( \lambda \), and the asymmetry coefficient \( a \) as functions of increasing streptomycin concentration

Notation: \( n \), sample size; NS, not significant at \( P<0.05 \). Sample size corresponds to the number of mother cells examined and is the same for \( \pi \), \( \lambda \) and \( a \) for any one concentration of streptomycin. Significance was determined by whether a test value (0 or 0.5) resided in the 95% confidence interval for the estimated parameter values. Estimates of parameter values are best fit values obtained by fitting recorded data to a bacterial ageing model (Chao, 2010). Confidence intervals were derived by a bootstrap resampling of the fit of the data to the model.

<table>
<thead>
<tr>
<th>Streptomycin concn (μg ml⁻¹)</th>
<th>( \pi ) (min)</th>
<th>95% confidence interval</th>
<th>( n )</th>
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<tbody>
<tr>
<td>0</td>
<td>28.53</td>
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<tr>
<td>1</td>
<td>23.34</td>
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<tr>
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<td>23.84</td>
<td>20.92–27.65</td>
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<tr>
<th>Streptomycin concn (μg ml⁻¹)</th>
<th>( \lambda ) (min⁻¹)</th>
<th>95% confidence interval</th>
<th>Estimate greater than 0</th>
</tr>
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<tbody>
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<td>−0.00333</td>
<td>−0.00568–0.00333</td>
<td>NS</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.00428–0.00780</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>0.00662</td>
<td>0.00425–0.00813</td>
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<table>
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<th>Streptomycin concn (μg ml⁻¹)</th>
<th>( a )</th>
<th>95% confidence interval</th>
<th>Estimate less than 0.5</th>
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<td>0.456</td>
<td>0.418–0.473</td>
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<tr>
<td>3</td>
<td>0.464</td>
<td>0.412–0.484</td>
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* Significant at \( P<0.05 \)

**Fig. 3.** Doubling times of old and new daughters (\( t_1 \) and \( t_2 \)) as a function of the doubling time of their mother (\( t_0 \)) at 2 μg streptomycin ml⁻¹, \( t_1 \) (○); \( t_2 \) (●). Solid lines denote relationships predicted by a model (Chao, 2010) using best fit parameters obtained from the data (Table 1). Upper solid line, \( t_2 \); lower solid line, \( t_1 \).
Fig. 4. Dosage effect of streptomycin on damage rate, \( \lambda \). Values presented are estimated values from Table 1, where confidence intervals are also provided. The solid line was generated by linear regression with the y intercept forced through the origin (\( P=0.024 \) for regression slope being greater than zero).

have been possible and ageing in symmetrically dividing bacteria would have been pushed again beyond the horizon of microbial research.

In the analysis of our results, we used the data to estimate values of \( \lambda \), \( \pi \) and \( a \) (Table 1), three parameters in our model for bacterial ageing. When estimated values of all three parameters were regressed against streptomycin concentration, only \( \lambda \) was found to have a significant dependence (Fig. 4). Although the value of \( \pi \) appears to be higher at 0 \( \mu \)g streptomycin ml\(^{-1}\) (Table 1), the data do not have the power to show a significant effect of concentration. The higher \( \pi \) may be spurious and the result of the fact that the model is not effective when the damage rates are low. The model relies on a difference between the old and new daughters, and the difference may be too small when there is little or no damage. This possibility is supported by the fact that the estimated values for \( a \) were also more variable at the lower concentrations of streptomycin (Table 1). The significant regression of \( \lambda \) onto the concentration of streptomycin indicates that the parameter is plastic and responds to the level of damage operating in the system. Thus, although we were limited to only four treatment concentrations of streptomycin and more would be desirable, the model is effective for quantifying the rate of damage that drives ageing in bacterial lineages.

Our current estimated values of \( \lambda \), \( \pi \) and \( a \) at 2 and 3 \( \mu \)g streptomycin ml\(^{-1}\) (Table 1) are similar to the only other available estimates of 0.007737 min\(^{-1}\), 18.95 min and 0.4836, respectively (Chao, 2010). The earlier estimates came from a fit of the data of Stewart and colleagues to our ageing model. The earlier estimate of \( \lambda \) suggests that the harm caused by YFP or light excitation in the study of Stewart and colleagues was comparable with the damage inflicted by a concentration of 2 or 3 \( \mu \)g streptomycin ml\(^{-1}\). The earlier estimate of \( \pi \) is smaller, possibly because of differences in culture conditions. However, it is noteworthy that the earlier estimate of \( a \) was so close to \( \frac{1}{2} \), as were our current estimates. Values of \( a \) so close to \( \frac{1}{2} \) and indicating near symmetry were initially unexpected on the basis of our evolutionary models (Ackermann et al., 2007; Chao, 2010). The models had predicted that natural selection should favour extreme asymmetry near values of \( a=0 \). However, an inspection of the fitness landscape generated by the model revealed that fitness returns increased with a diminishing rate after a value of \( a=0.475 \) (Chao, 2010). In other words, fitness as a function of \( a \) increases rapidly from \( a=\frac{1}{2} \) to \( a=0.475 \), but the rate increases by less and less as \( a \) goes below 0.475. If asymmetry incurs a cost, which is a reasonable assumption not yet incorporated into the models, it could be overcome by the fitness gain from \( a=\frac{1}{2} \) to \( a=0.475 \), but not from \( a=0.475 \) to \( a=0 \). Thus, with a cost, asymmetry in a bacterial population can evolve from \( \frac{1}{2} \) to 0.475 but not beyond. The fact that our current and independent estimates of \( a \) are again in the vicinity of 0.475 reinforces the possibility that the estimates and the landscape interpretation are valid.

Credence for the phenomenon of bacterial ageing resolves one of the paradoxes that has troubled researchers. An initial reason for the notion that bacteria did not age was that they lacked soma (Rose, 1991; Turke, 2008). Some of the models for the evolution of ageing required a tradeoff between the germline and the soma (Kirkwood & Holliday, 1979; Kirkwood & Melov, 2011). The fitness advantage gained by mother bacteria that allocate non-genetic damage asymmetrically to their daughters resolves the paradox. If the daughter that receives more damage (the old daughter) is regarded as a continuation of the mother cell, then that larger damage fraction can be interpreted to be equivalent to soma. It is a non-genetic component that is kept by the mother and not transmitted to the new daughter. Thus, the pre-existence of soma is not needed for the evolution of ageing. Rather, soma and ageing arose simultaneously as the consequences of the evolution of the asymmetrical allocation of non-genetic damage by a mother bacterium.

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REFERENCES


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