Aging may be a conditional strategic choice and not an inevitable outcome for bacteria

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Communicated by Madhav Gadgil, Indian Institute of Science, Bangalore, India, August 4, 2006 (received for review December 21, 2005)

Aging is known in all organisms that have different somatic and reproductive cells or in unicellular organisms that divide asymmetrically. Bacteria that divide symmetrically were believed to be immune to natural aging. The demonstration of functionally asymmetric division and aging in *Escherichia coli* recently has challenged this belief and led to the suggestion that aging might be inevitable for all life forms. We modeled the effects of symmetric and asymmetric division in bacteria to examine selective advantages of the alternative strategies of division. Aging of cell components was modeled by using a modified Leslie matrix framework. The model suggests that asymmetric division accompanied by aging and death of some cells results in a higher growth rate but a reduced growth yield. Symmetric division with or without gradual replacement of the old components, on the other hand, slows down the growth rate but may increase growth yield over a wide range of conditions. Thus, aging and immortality can be selected under different sets of conditions, and this selection may also lead to a tradeoff between growth rate and growth yield.

Leslie matrix | prokaryotic cell division

Aging has been an important focus of research for many decades, and yet a number of questions remain unanswered. Aging is an inevitable phenomenon in higher organisms in which there is a clear separation between germ-line and somatic cells. Whereas the germ line cells perpetuate indefinitely, the soma undergoes irreversible changes with age and ultimately dies. Bacterial cells that divide morphologically symmetrically were believed to be immortal because each division gave rise to two "young" daughter cells. Thus, as long as the environmental conditions were optimum for growth and division, bacterial cells were believed not to age. Possible exceptions could be bacteria that have a morphological and functional asymmetry in division, such as the stalked versus motile daughter cells of *Caulobacter* sp. *Caulobacter* cells that are known to undergo aging owing to their asymmetric division (1).

Stewart *et al.* (2) showed that growing cells of *Escherichia coli* also undergo aging and death. They demonstrated that although *E. coli* daughter cells looked morphologically identical, there was a functional asymmetry in division. One of the daughter cells received old components and the other daughter cell received the newly formed ones. Cells that inherited old components exhibited a diminished growth rate, decreased offspring production, and an increased probability of death. Stewart *et al.* (2) demonstrated asymmetric cell division by using fluorescence microscopy and showed further that the old pole cells lagged in growth and division cumulatively over generations. The demonstration of aging in *E. coli* led some to suggest that no life strategy was immune to aging and immortality was either impossible or too costly (2–4). Although division in *E. coli* was demonstrated to be functionally asymmetric, it is too early to conclude that symmetrical division and accompanying immortality is absent or impossible in the living world. Because asymmetric division is argued to be responsible for aging, we examine here whether symmetric division and accompanying immortality also could evolve under certain sets of conditions.

Asymmetric division can be viewed as a mechanism by which old components can accumulate in one cell and are ultimately disposed off with the death of the cell (2–4). Oxidatively damaged proteins are shown to be retained selectively in the mother cell in *Saccharomyces cerevisiae* (5). As a result, the mother cell undergoes aging while giving rise to young daughter cells. Alternatively, old components can be distributed symmetrically in the daughter cells. In such a case, they subsequently will get diluted by the new components. Alternatively, they can be repaired or replaced. However, this strategy has a two-fold cost. Presence of old components can reduce the growth rate of the cell, and there will be a cost of repair or replacement. Therefore, it is speculated that the "accumulate and dispose the old" strategy could be better than "repair and reuse" (2–4). However, the possible costs and benefits of the two alternative strategies have not been rigorously examined. It is also necessary to challenge the presumed association of asymmetric division with dispose-off strategy and symmetric division with repair strategy.

We employ a Leslie matrix model, which is commonly used by population biologists to model age structured populations (6), to examine the effects of symmetric and asymmetric division on the dynamics of growth in bacteria. The classical Leslie matrix model depicts the dynamics of individuals in different age classes. We adapt it here to model the dynamics of cell components of varying ages in a growing bacterial population. The distribution of the components in cells is different in symmetric and asymmetric division, and the model is modified accordingly.

Model. We assume that a cell is made up of a finite number of growth-limiting components and each component is subject to aging. In each time unit, every preexisting component passes to the next age class, and all newly synthesized components form the first age class. In the classical Leslie matrix model, the individuals reproduce with age-specific reproductive rates. This assumption may apply to self-replicating cell components, but for other types of components, we need to modify the assumption. In this model, the components have age-specific efficiencies that contribute to growth rate of the cell, and new components are added based on the net rate of cell growth. This dynamics can be represented in a matrix form as follows.

Let C_n be the component of age class n. If there are m discrete age classes of the components in the cells, R_n is the reproductive efficiency of age class n, and P_n is the probability that a component of age class n survives to age class $n + 1$, then the Leslie transition matrix for age class distribution can be given as

$$\begin{bmatrix} R_1 & R_2 & \cdots & R_{m-1} & R_m \\ P_1 & 0 & \ddots & 0 & 0 \\ 0 & P_2 & \ddots & 0 & 0 \\ \vdots & \vdots & \ddots & 0 & 0 \\ 0 & 0 & \cdots & P_{m-1} & 0 \end{bmatrix}. \quad [1]$$

Author contributions: M.W. designed research; S.P., P.J., and S.K. performed research; and M.W. wrote the paper.

The authors declare no conflict of interest.

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After one generation, the number of components of a given class will change as

$$\begin{bmatrix} R_1 & R_2 & \cdots & R_{m-1} & R_m \\ P_1 & 0 & \ddots & 0 & 0 \\ 0 & P_2 & \ddots & 0 & 0 \\ \vdots & \vdots & \ddots & 0 & 0 \\ 0 & 0 & \cdots & P_{m-1} & 0 \end{bmatrix} \times \begin{bmatrix} C_1 \\ C_2 \\ \vdots \\ C_{m-1} \\ C_m \end{bmatrix}_t = \begin{bmatrix} C_1 \\ C_2 \\ \vdots \\ C_{m-1} \\ C_m \end{bmatrix}_{t+1}. \quad [2]$$

We assume that the reproductive efficiency of each component decreases with age linearly or nonlinearly. We use a common expression that gives linear or nonlinear decline at different parameters.

$$R_n = R_1 - an^b, \quad [3]$$

where R_n is the efficiency of the nth class, a is a positive constant deciding the rate of efficiency decline, n is the age class, and b is the power that decides the shape of the curve. At $b = 1$, the decline is linear. At $b > 1$, it is convex, and at $b < 1$, it is concave. We take $R_1 = 1$ throughout. Any negative value of R_n is considered to be zero.

For simplicity, we assume that all of the components of different age classes are carried forward in the next age class, and after the last age class, none of the component survives. Thus,

$$P_n = \begin{cases} 1 & \text{for } n \in 1, 2, 3, \ldots, m-1. \\ 0 & \text{for } n = m. \end{cases} \quad [4]$$

The rate of growth of a cell is assumed to depend on the age class distribution of components in it. A cell with all new components will grow and divide with maximum growth rate of 1, and the growth rate decreases with accumulation of older components in a way described below. We model two limiting conditions of symmetry. In one, the division is completely asymmetric in that all newly synthesized components go to one of the daughter cells, and all older ones to the other. In the other condition, the division is perfectly symmetric in that all new and older components are divided exactly equally between the two daughter cells. The treatment of the Leslie matrix differs in the symmetric and asymmetric model as follows.

Asymmetric Division Model. In this model, a cell elongates by adding new components to one pole such that after division, all components of one cell are new and all those of the other cell are old (Fig. 1). This assumption makes the cell age class and component age class identical. The Leslie matrix therefore can be interpreted as an age class distribution in the population of cells and, thus, is identical to the classical Leslie matrix model. Cells of each age class reproduce by an age-specific rate of reproduction (R_1 to R_m, respectively). The first age class receives cells equal to $C_1R_1 + C_2R_2 + \ldots\ldots C_mR_m$. Because the model assumes growth in a nonlimiting environment, no death is assumed for cells in the intermediate age classes. All cells in the last age class are assumed to die and are removed from the population.

Symmetric Division Model. In this model, at each cell division, the components of each age class are assumed to be distributed exactly equally in each of the daughter cells. As a result, every cell has identical age class distribution of the components (Fig. 2). Unlike the asymmetric model here, the component age classes are not identical to cell age classes; rather, every cell has an age class distribution of components. The cells are assumed to be immortal because a nonlimiting environment is assumed and each cell has a majority of young components. The components in the last, and therefore lowest, efficiency class are not removed but instead accumulate in this class. However, these components also may be repaired (or replaced) with repair efficiency r, and these repaired molecules are restored to the first age class. As a limiting case, the cost of repair is assumed to be equal to that of synthesizing a new component. Thus, $C_m{}^*r$ is added to the first age class and subtracted from the last age class.

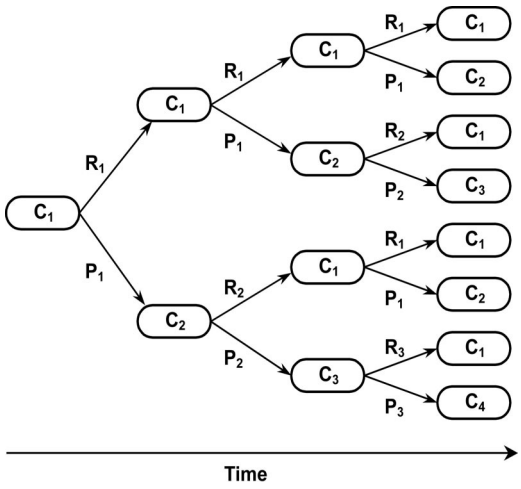

Fig. 1. Diagrammatic representation of asymmetric cell division, where C_n is the component of age class n, R_n is the reproductive efficiency of age class n, and P_n is the probability that a component of age class n survives to age class $n + 1$. In this case, each cell has components of the same age class so all of the cells have a defined age, and the age class distribution of the components is the same as the age class distribution of the cells.

Unlike the asymmetric model, here the intrinsic growth rate of a cell and that of the population are identical, and their value is decided by the component age class distribution. Components decrease their efficiency with age in a manner numerically identical to the asymmetric-division model. We consider two conditions. In the first, the components act independently, and the growth rate of the cell is a weighted average of all R_i. In the second, the least-efficient component is assumed to become rate-limiting in any pathway involving more than one component. In other words, any metabolic pathway proceeds with the speed of the slowest of the components. Components interact randomly irrespective of their age classes. For simplicity, we

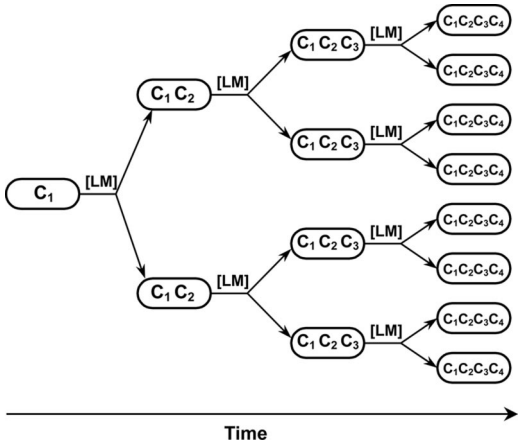

Fig. 2. Diagrammatic representation of the symmetric division model, where C_n is the component of age class n. In this case, cells possess components of different age class, and so in each cell, the components have stable age class distribution given by the Leslie matrix ([LM]).

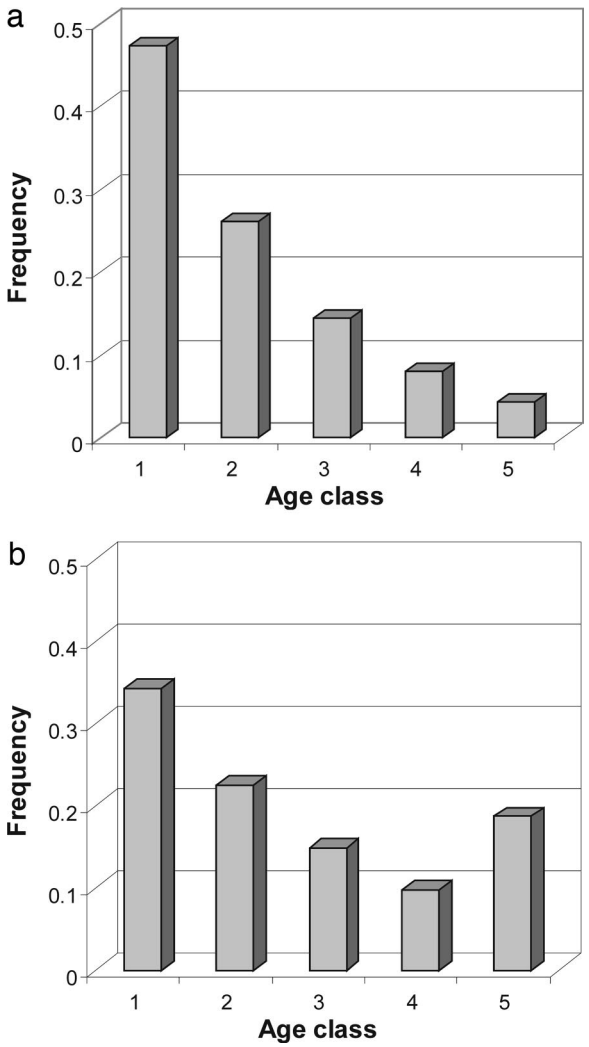

Fig. 3. Typical stable age class distribution of components in the asymmetric (*a*) and symmetric (*b*) division model. In the latter case, the oldest age class has a higher frequency because of the accumulation of components. Simulation results are $a = 0.063$, $b = 2$, $n = 5$, and $r = 0.01$.

assume that the average number of components required for each pathway is two, and we apply basic probability rules to make combinations of components. Therefore, the rate of growth given by a certain age class distribution of components is given by a summation of the product of R_n and the probability that the oldest component in an interaction belongs to the nth class. Thus, the resultant rate of growth is calculated as

$$R_1(C_1^2) + R_2(2C_1C_2 + C_2^2) + \ldots\ldots R_m(2C_1C_m + 2C_2C_m + \ldots\ldots C_m^2), \quad [5]$$

where R_1 to R_m are numerically identical to the asymmetric-division model.

Calculations of Growth Rate and Growth Yield. Numerical simulations were run for 100 generations on both models. After a stable age class distribution was attained, the growth rate and growth yield of the populations were calculated. Growth rate was defined as the increment in population per unit population in unit time, and growth yield was defined as the net increase in the number of living cells or components divided by the number of cells or components synthesized. The denominator included the number of components repaired.

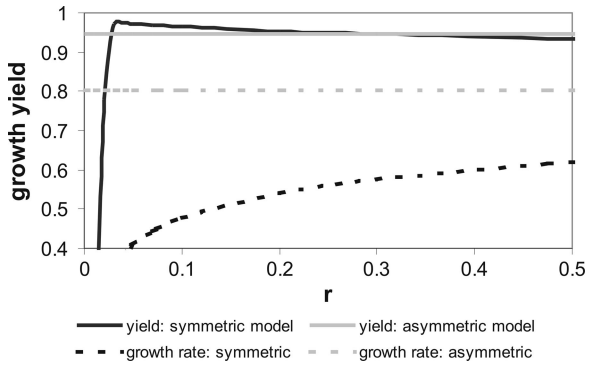

Fig. 4. Growth rate and growth yield, when the efficiency of the last age class is zero. Growth rate of the symmetric model is always less than the asymmetric one, but the growth yield of the symmetric model at optimum repair efficiency is higher than the asymmetric model. Here, $a = 0.03$, $b = 3$, and $n = 5$.

Results

A stable age class distribution always was achieved as expected in most Leslie matrix models. In the symmetric model, the distribution was biased to the oldest age class as compared with the asymmetric model (Fig. 3). In the asymmetric model, the frequency declined monotonically with age class as in a typical Leslie matrix model. However, because we assumed no death in the symmetric model, the old components accumulated in the last and, therefore, the least efficient age class. The accumulation depended on the repair efficiency r. At $r = 1$, the distribution was identical to that of the asymmetric division model and at lower values of r, the last age class had a substantially higher frequency. It is important to note that even if we assumed no repair and that all old components were allowed to accumulate, this process did not always result in declining fitness of the entire population. If the efficiency of the intermediate age classes was sufficiently high, growth continued, and the older components were simply diluted out by growth. This process led to a balance between dilution and accumulation such that molecules in the oldest age class reached a stable proportion.

With higher r in the symmetric model, there was faster replacement of old components. An increase in r always resulted in an increased growth rate because repair was assumed to regenerate young components and increased proportion of young components resulted in faster growth rate. On the other hand, the effect of r on growth yield depended on the age-efficiency curve. For slowly declining curves (i.e., small values of a and b), the growth yield decreased monotonically with r, whereas for larger a and/or b, it peaked at an optimum r and then declined slowly (Fig. 4). The optimum r largely was decided by the growth to repair ratio. Growth added to both the numerator and denominator in yield calculations, whereas repair added only to the denominator. Thus, the process of repair increased the proportion of younger components but did not add to biomass. Growth, on the other hand, added to biomass and younger components. A greater proportion of young components, in turn, increased growth. Thus, growth and young components were related by a positive feedback cycle. If the cells had accumulated old components, repair could generate young components and initiate the positive feedback cycle. Therefore, the growth rate and yield increased with r. However, if the positive feedback cycle already was operative, further increase in r did not benefit the cell proportionately, giving rise to an optimum value of r where the yield was maximized.

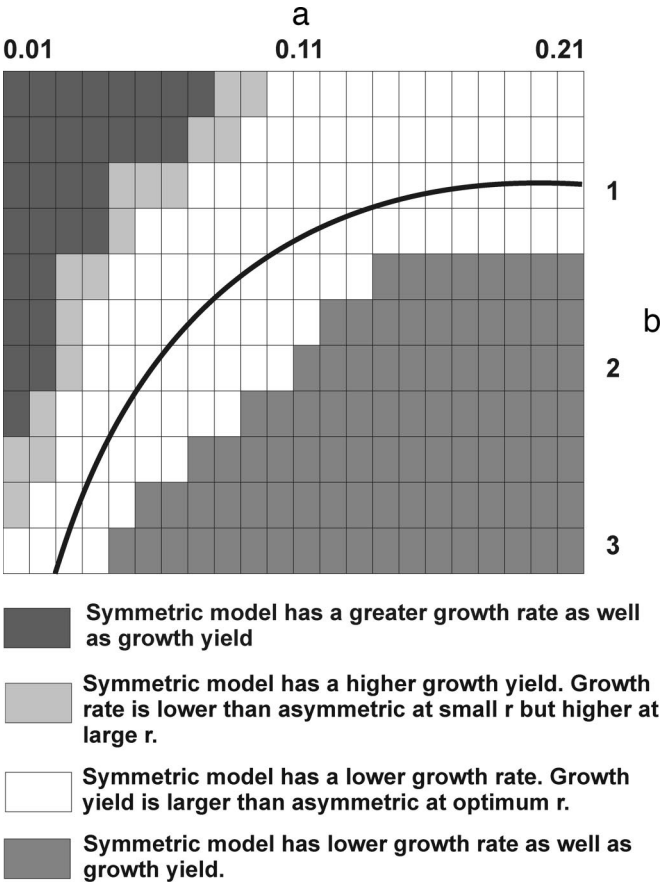

Fig. 5. Parameter areas of differential advantages to the symmetric and asymmetric models of growth: The curve indicates the combination of *a* and *b*, where efficiency of the last age class becomes zero ($n = 5$).

If the components were assumed to act independently and the growth rate of the cell was assumed to be a weighted average of R_n, the two division models became numerically identical at $r = 0$. The growth yield of the symmetric division model was higher because there was no loss of cells or components. Therefore, independent component model offered no advantage to asymmetric division. Under this assumption, asymmetric growth never should evolve. However, asymmetric growth has been demonstrated in *E. coli* (2), suggesting that this assumption may be unrealistic.

With the growth-limiting step assumption, at very small values of *a* and *b*, the growth rate as well as growth yield of the symmetric model was greater than the asymmetric model. Lower *a* and *b* resulted in small decrements in the efficiencies with age, and the last age class contributed substantially to the growth rate. Under these conditions, the cost of death was relatively high and, therefore, asymmetric division did not result into better fitness.

When *a* and *b* were moderate, so as to result into zero or near zero efficiencies in the oldest age class, the asymmetric model had higher growth rate. However, at optimum *r*, the growth yield of the symmetric model was greater than that of the asymmetric model. When the age-efficiency curve was very steep so as to hit zero efficiency much before the last age class, the asymmetric model outperformed the symmetric model in both growth rate and yield.

Fig. 5 shows the parameter areas over which the selective advantages to the alternative strategies are distributed. When reduction in efficiency was small and concave, symmetric division had a clear advantage in both growth yield and growth rate. On the other hand, when this reduction was large and highly convex, asymmetric division had an advantage in both the parameters. However, at intermediate levels, the symmetric division gave better growth yield after optimizing repair efficiency but a lower growth rate.

The qualitative conclusions of the model were robust toward change in *n* and the average number of components that interact in a metabolic pathway. Increases in either or both variables made the model more complex, but the results were similar. Reported here are only the results with $n = 5$ and the number of interacting components as two.

To examine the sensitivity of the model to the assumptions, we relaxed the critical assumptions one by one. To break the association between asymmetric division and death versus symmetric division and repair, we introduced repair in the asymmetric division model. This change, however, did not increase the growth rate of asymmetrically dividing cells substantially but reduced the growth yield further. This was so because in a cell with majority of old components, replacement of a fraction of the components affected the growth rate only marginally because older components continued to be growth limiting. On the other hand, the cost of repair reduced the growth yield further (results not shown). Asymmetric division with repair therefore did not offer any selective advantage.

We have assumed that in a symmetric division model, the cost of repair of a component is reflected in terms of growth yield alone and does not reflect on growth rate. With relaxation of this assumption, asymmetric division model gave even lower growth rates. We also assumed the cost of repair of a component to be equal to resynthesis of the entire component. In reality, a component may be made up of many molecules, and replacing some of them could be sufficient. If the cost of repair was assumed to be less than resynthesis, the symmetric division model resulted into a further higher yield. Thus, relaxing any of the above assumptions did not change the direction of difference between symmetric and asymmetric division models, although there were changes in the magnitude.

Discussion

The model accommodates different shapes and slopes of the age-efficiency curve. Currently, we do not have sufficient empirical data on this curve to describe it precisely. In experiments with *E. coli*, the decline appeared to be linear for seven generations (2), whereas in yeast, it was convex when measured over a longer period (7). However, the precise relationship does not appear to be crucial to the model. The outcome is largely decided by whether the oldest and least efficient class had efficiency close to zero. This situation would seem logically to be the only realistic one in the case of asymmetric division. If the last class still has a high efficiency, it would be improper to assume death after this class. If, on the other hand, near zero efficiency was reached earlier, then that itself should be considered the last age class. Therefore, only the combinations of *a* and *b* that converge close to zero in the last age class should be considered realistic for a given *n*. Under this condition, there appears to be a unique and simple result. Asymmetric division consistently gave higher growth rate, whereas symmetric division gave higher growth yield on optimization of the repair rate. It is possible, therefore, that symmetric and asymmetric division may be favored in different ecological niches.

The model was constructed by assuming that all types of cellular components aged at the same rate, but this assumption is unlikely to be realistic. In an asymmetric model, a cell will die when one of the vital components is damaged beyond a threshold. However, many still-functional components will be wasted along with each cell death. This wastefulness is unlikely to happen in a symmetric model, which therefore should be more

advantageous from the growth yield point of view than what the model predicts.

The assumption that less efficient components are rate-limiting is most critical for the model. If the components acted independently, the two types of divisions had no difference in the growth rates. In such a case, the "dispose off" strategy has nothing to gain at the cost of death of some of the cells, resulting into lower growth yields. Therefore, asymmetric division is unlikely to evolve if the components acted independently.

For simplicity, we assumed that there is no death of cells up to $(m - 1)$th age class and all individuals of the last age class die in the asymmetric model, whereas cells with symmetric division are immortal. Stewart *et al.* (2) argued on the other hand that the probability of death increased with cell age. Incorporating increasing death probabilities in the model complicates the model and poses problems in keeping similar conditions for the two types of divisions. We can make the probability of death a function of the cell age in the asymmetric model, but the symmetric model is a mixture of different age class components. We avoided this complexity because there is no *a priori* reason to believe that a continuous change in death probability will make a qualitative difference in the results.

It is possible that natural selection favors high growth rates and growth yields under different sets of conditions. Most laboratory studies of selection on bacteria have focused on growth rate alone. However, slow growing organisms with high efficiency of biomass conversion are abundant in natural environments (8). Therefore, selection must be favoring high yielding slow growers under some set of conditions. It has been suggested that competition for shared resource leads to the selection for high but inefficient ATP production, even though slow but efficient ATP production would be more beneficial to all users of the resource (9–12). Thus, in a competitive environment, there will be selection for higher rate than yield, whereas in noncompetitive environments, there will be selection for low rate but high yield. In nutrient-rich environments, bacteria with high growth rates are likely to be favored, whereas in nutrient-poor environments, there is evidence for selection of slow-growing bacteria with higher biomass conversion efficiency (8). This phenomenon is a microbial equivalent of the *r* and *K* selection dichotomy (8). The question whether and under what conditions high yields would get selected can be addressed empirically, and the model may stimulate experiments in this direction.

It is likely therefore that symmetrical division may exist in nature, and one might look at oligophilic bacteria for possible candidates. Prosthecate bacteria such as *Caulobacter* sp. are generally oligophilic. However, they have asymmetric division owing to a dimorphic cell cycle that consists of alternating mobile and stalked phase (1). There are many nonprosthecate oligophilic bacteria in which symmetric division is very likely to have evolved (8), owing to selection for high-growth yield.

We modeled here perfectly symmetric and perfectly asymmetric division. These strategies should be viewed as two extreme ends of a continuum rather than distinct compartments. Because each strategy has a distinct advantage, some optimization of symmetry may be achieved. The optimum could be different for different organisms depending on the selective forces in their environments. It therefore is too early to assume that immortality is inevitable. We need to look at many organisms evolved under different environments before reaching a firm conclusion. There is suggestion for a growth rate-growth yield tradeoff in bacteria, and possible mechanisms have been postulated (9–12). Symmetry of division can be another possible reason for such a tradeoff. This tradeoff may have wider implications, and symmetry of division may give a new dimension to the problem of the evolution of aging, not only in prokaryotes, but cutting across all taxa of the living world.

We thank Richard Lenski for his critical comments on an earlier draft of the manuscript and Neelesh Dahanukar for help in preparation of a revised manuscript.

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