Mutation-rate plasticity and the germline of unicells

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Mutation is the ultimate source of genetic variation and the mutation rate therefore is a central parameter in evolutionary genetics. Recently, mutation rates of unicellular organisms were found to be strongly reduced at high density by a hitherto unknown mechanism. We show that increased asymmetry in DNA-template strand inheritance when growth shifts from exponential to linear at high density may be this mechanism. In yeast, recent studies show that under high-density conditions, senescing cells start to act like stem-cell lineages that continue budding rejuvenated non-dividing offspring cells for the remainder of their replicative lifespan. We show that this shift from symmetrical to asymmetrical division would result in a strong *increase* in the number of mutant cells if template and copied DNA strands were inherited randomly by senescing and rejuvenated cells, diametrically opposite to the observed reduction. However, if senescing cells retain the template DNA strands so that rejuvenated cells receive the copied strands, the number of mutant cells is reduced, consistent with the empirical evidence. We provide various additional lines of support for this model from a variety of microbes and also from certain mammalian stem cells, which have been found to have template strand retention at high density. This model of density-dependent asymmetrical cell division and asymmetrical DNA-strand inheritance of unicellular organisms fundamentally changes our understanding of mutation rates and the distinction between germline and soma.

Mutation rates are typically minimized, as far as population genetic constraints allow[1]. However, mutation rates can vary, not only between organisms but also with environmental conditions. A recent study identifies a completely unexpected kind of mutation-rate plasticity in response to population density[2], which is dependent on quorum sensing[3]. Across a wide range of unicellular organisms, both eukaryotic and prokaryotic, the mutation rate consistently was found to decrease with increasing population density, with up to 23-fold lower mutation rates at high density than at low density. We propose a model that explains how reduced mutation rate at high density can be attributed to increased asymmetry in mutation acquisition between senescing 'stem-cell' lineages and rejuvenated 'offspring' cells, and discuss recent experimental studies that support this model.

It was long believed that unicellular organisms potentially do not age, thus exhibiting functional immortality. However, the last two decades have seen increasing evidence for asymmetrical cell division leading to differential cell fates, even in organisms with morphologically symmetrical division, such as *Escherichia coli* and fission yeast[4,5]. An asymmetrical cell division results in a senescing 'mother' cell and a rejuvenated 'daughter' cell, and fecundity of the mother cell decreases with each division as damaged proteins and cell components accumulate. Asymmetrical division can generate cells specialising in 'somatic' functions such as antibiotic production and cells retaining high viability[6]. Theoretical studies demonstrate that asymmetrical division can lead to higher viability of a culture[7,8].

Recently, the degree of asymmetrical cell division leading to senescence was experimentally found to be higher at high density, in two independent studies, for budding yeast[9] and for *E. coli*[10]. We reason that those two independent findings, on one hand reduced mutation rate under high-density conditions[2] and on the other hand increased asymmetry under those conditions[9,10], are functionally linked. This requires that the increased asymmetry between cells not only manifests itself in physiological and morphological cell characteristics, but also in the acquisition of new mutations. Such asymmetries have been shown to occur during cell division in yeast[11,12] and in *E. coli*[13]. Furthermore, there is increasing evidence from a broad range of organisms that cells distribute their DNA asymmetrically during each division, in a way supporting the 'Immortal Strand Hypothesis', originally proposed for mammalian stem cells[14-19]. According to this hypothesis, adult stem cells have 'Template Strand Co-segregation' (TSC[14,15]), where the daughter cell maintaining the stem-cell function retains specific 'master' templates of the DNA strands of each chromosome (the parental strands[20]) at each division, while the differentiating daughter cell receives the new, 'copy' strands. Assuming that most mutations occur in the newly synthesized DNA strands and fewer in the template strands, this asymmetrical distribution reduces the mutation rate in the stem cells[14]. Interestingly, for muscles it has been demonstrated that the asymmetry of strand segregation can increase when stem cells are seeded at higher cell densities[21].

We propose a model that connects reduced mutation rate at high density to increased asymmetrical division under that condition (Figure 1). Consider two cultures of unicellular organisms with different nutritional levels. Initially, when nutrition is not limiting yet, growth will be maximal and exponential in both cases[22]. A difference in density will thus only arise after the low-nutrient culture approaches the stationary phase, and the high-nutrient culture is still increasing in population size, but increasingly non-exponentially (Figure 1a). As recent studies indicate, at increasing density, cell division becomes more asymmetrical[9,10,21]. As has been shown for yeast under high-nutrient conditions, senescing cells start to act like stem-cell lineages that continue budding off rejuvenated offspring cells for their entire replicative lifespan or for the remainder of it, while the rejuvenated offspring cells are quiescent and do not divide further[23] (Figure 1b). In contrast, at low nutrition, the transition to non-exponential growth is less strictly associated with differentiation between stem cells and rejuvenated offspring[9,10,21].

Crucially, at high density we assume that TSC occurs, where the senescing stem cells receive the template strands, and the offspring cells the newly synthesised DNA strands (Figure 2). If mutations mostly occur in the newly synthesised strands, this inheritance pattern will reduce the final number of mutant cells, since mutant cells do not divide further. Consider the example of Figure 1 and assume asymmetrical division where a mother cell can bud off 31 rejuvenated daughter cells when asymmetrical cell division starts. Furthermore, assume that on average a single mutation occurs during the production of those 32 cells. Random distribution of the DNA strands (random strand segregation; RSS) would then be expected to yield an average of 8.5 mutant cells, while TSC in the mother cell would yield only a single mutant cell (see Methods and Figure 2 for the calculation). The latter number is also lower than the number expected with symmetrical growth, where a single mutation in one of five subsequent rounds of divisions will yield an average of 2.6 mutant cells among the 32 resulting cells (Table I).

In Figure 3, the expected number of mutant cells for a single mutational event as a function of the number of past cell division is given for the four possible combinations of division and strand segregation. As can be seen, for asymmetrical growth with TSC the expected number of mutant cells is one, irrespectively of the number of cell divisions. In contrast, for symmetrical growth, the expected number of mutant cells increases with the number of cell divisions and this is irrespective of whether template strand co-segregation occurs or random strand segregation. For asymmetrical growth with RSS, the number of mutant cells increases even stronger with the number of cell divisions. For microorganisms where the maximal replicative lifespan has been determined at 30 cell divisions[24], a 2.6-fold reduction in the expected number of mutant cells compared to symmetrical division leading to the same number of cells seems the maximum.

So our model can account for a significant reduction in mutation rate estimates, although not sufficient to account for the density-dependent mutation-rate plasticity recently reported[2]. However, there is another catch when growth shifts from exponential to linear. Estimates of the mutation rate assume exponential growth[25]. The fluctuation test takes into account the probability that a mutation occurs at an early growth stage, in which case a large proportion of the population will have the mutation (a so-called 'jackpot'). The model proposed here, with linear growth by division from a stem-cell mother that retains the template strands will never yield a 'jackpot', since mutations in the non-exponential phase always occur in terminal branches. This implies that the mutation rate will be systematically underestimated, which may account for the remaining difference. Furthermore, if asymmetrical growth occurs in the later stages of both low-density and high-density conditions, but TSC only at high density, the difference in mutation rate between low and high density will further increase (Figure 3, grey line).

The apparent universality of density-associated mutation-rate plasticity begs for a general mechanism. Given the independent evidence for a link between the degree of asymmetrical cell division and density in widely divergent organisms as bacteria[10], single-celled eukaryotes[9] and stem cells of multicellular eukaryotes[21], it seems plausible that this mechanism is based on asymmetrical cell division. The model proposed here is best supported for yeast. In yeast, at high density, a larger fraction of the cells becomes quiescent, being arrested in the G_0 phase of the cell cycle[9,10], and consisting almost exclusively of rejuvenated quiescent daughter cells with a high capacity to grow when conditions improve[23]. The remaining cells are heterogeneous and show senescence. In support of a role for TSC, in yeast asymmetries in kinetochore inheritance have been shown[11], and one study found support for asymmetrical strand segregation[12,26], although another study did not[12,26]. However, the latter study used a low population density, which may account for this difference.

It seems paradoxical that the senescing cell retains the template DNA strands, and thus acquires the fewest mutations, while the rejuvenated offspring cells receive the copied strands, and thus any mutant cells. However, as explained above this inheritance pattern reduces the number of mutants among the rejuvenated cells. Perhaps the strongest argument in favour of the model proposed in this article is that the mutation rate will be strongly *increased* and not decreased if DNA strands were inherited randomly when cell division becomes asymmetrical. Even for the

production of 16 rejuvenated cells by a mother cell, asymmetrical cell division with RSS would yield 4.5 times more mutant cells than asymmetrical cell division with TSC, and still over two times more than symmetrical division (Figure 3). The finding of on one hand, a reduction in the mutation rate at high density[2] and on the other hand, an increase in asymmetrical division at high density[9,23], therefore makes it plausible that template-strand co-segregation must occur. However, direct evidence for our model remains to be provided. Recent improvements in the detection of mutations in single cells may make it feasible to test our hypothesis directly[27,28]. An intriguing question is whether our model also applies to density-associated mutation rate plasticity found in viruses[2]. Since viruses are dependent on their host for genome replication, in the experiments used to measure the mutation rates at various density, virus density may correspond to host density, in which case our model may also apply to viral replication.

The plasticity in mutation rate in response to population density implies that numbers of mutational events per space and time vary much less with final population size than expected from a fixed mutation rate per cell division. In other words, the total number of cells with mutations occurring in a high-density and a low-density culture of unicells are more similar than expected based on the number of cell divisions that have occurred. This buffered number of mutant cells per space and time fits remarkably well in an emerging picture that the mutation rate of organisms is reduced by specific aspects of their growth mode, not only for vertebrate animals, which set aside germ cells early in development, but also for organisms that do not. For example, taller, long-lived plants have been found to have lower rates of molecular evolution per unit time than small plants, implying that the mutation rates per generation are more similar[29]. In plant meristems, the stem cells from which reproductive organs will develop undergo a minimal number of divisions during plant growth[30]. Also, the number of cell divisions separating axilliary meristems from the central meristem is minimized[31]. Similarly, in a fungus with an estimated age of more than 1,500 years the number of mutations was much lower than expected, presumably due to an unknown mechanism to reduce the number of mitotic divisions of cells at the growth front[32,33]. In ciliates, a transcriptionally silent germline nucleus is present, whose mutation rate per cell division is more than an order of magnitude lower than that of other eukaryotes, but, converted to a per-sexual generation mutation rate, is remarkably similar to that of multicellular eukaryotes with a similar genome size[34].

The realisation that unicellular organisms also have mechanisms to reduce the mutation rate makes the germline-soma distinction more general than once believed. August Weismann was the first to distinguish an immortal germline from a disposable soma and argued that variations within individuals cannot be transmitted to the germline[35]. Leo Buss challenged Weismann's doctrine, noticing that an early germline sequestration as seen in vertebrates is rare among multicellular organisms[36]. The recent findings discussed in this perspective, however, revive part of Weismann's doctrine. A picture emerges that germline sequestration is not limited to some animals, but also occurs in plants, fungi and even unicellular organisms, although the timing of sequestration may vary between organism groups and with ecological conditions such as population density.

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Author contributions

DKA designed the study and wrote the manuscript with input from AJMD.

Methods

Calculating the number of mutant cells resulting from a single mutational event.

We first consider the number of mutant cells resulting from a single mutational event for four different ways to get fours cells from a single ancestor: symmetrical cell division with random strand segregation (RSS), symmetrical cell division with template strand co-segregation (TSC), asymmetrical cell division with RSS, and asymmetrical cell division with TSC (Figure 2). In all cases, we assume that mutations only occur in a copied strand, and not in a template strand.

For symmetrical growth with RSS, the mutation can occur during the first division for which there are two routes or the second division, for which there are four routes. In the first case, two mutant cells will result, in the second case a single mutant cell. So we get 2+2+1+1+1+1=8 possibilities to get mutant cells and we have six routes for those to occur, so on average this yields 8/6=1.33 mutant cells. For symmetrical cell division with TSC, the expected number of mutant cells is the same. For *k* rounds of symmetrical division (with RSS or TSC), the number of mutant cells produced by a single mutational event can be calculated as: $\frac{k*2^k}{\sum_{n=1}^{n=k} 2^n}$.

For asymmetrical growth with RSS, a single mutation either ends up in a daughter cell (left branch), or in the mother cell (right branch). In the first case it will yield a single mutant cell only. If the mutation ends in the mother cell, it can either yield three mutant cells (when the mutation occurs in the first division), two mutant cells (when the mutation occurs in the second division) or a single mutant cell (when the mutation occurs in the third division). The average number of mutant cells if the mutation occurs in the mother cell is thus two. So the average number of mutant cells resulting from a single mutational event if cells division is asymmetrical and strands segregate randomly is (1+2)/2=1.5. More generally, for *n* cells formed with asymmetrical cell division, the expected number of mutant cells resulting from a single mutational event is 0.5+0.25*n*.

For asymmetrical cell division with template-strand co-segregation, the mutation will always end in a non-dividing daughter cell, and thus only yield a single mutant cell.

In Table I, we have summarised the average number of mutant cells for varying numbers of cells under various division schemes, and in Figure 3 those data are presented in a graph. As can be seen, the number of mutant cells resulting from asymmetrical growth with TSC is one, and independent of the number of cell divisions, but both for symmetrical growth, and

asymmetrical growth with RSS the average number of mutant cells is an increasing function of the number of past cell divisions.

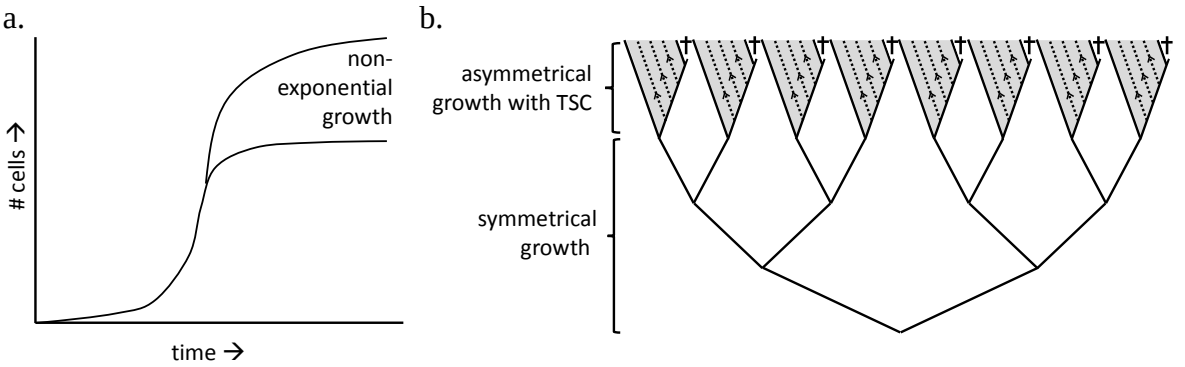

Figure 1. How growth changes from symmetrical exponential to asymmetrical with TSC at high density. **a.** Two cultures at low and high nutrition. Initially, when nutrition is not limiting yet, exponential growth occurs in both cases. A difference in density will thus only arise after the low-nutrient culture approaches the stationary phase, and the high-nutrient culture is still growing, be it increasingly non-exponentially. **b.** Schematic representation of the shift from exponential to linear growth. In the high-nutrition culture, at high density, cells increasingly start to divide asymmetrically. According to the model proposed, a senescing 'mother' cell, acting as a stem-cell, continues to bud off rejuvenated 'offspring' cells, which stop dividing. If the mother cell inherits the template DNA strands (Template Strand Co-segregation; TSC) and if mutations primarily occur in the newly synthesised strand, new mutations will mostly not be copied further and be confined to terminal branches, thus limiting the number of mutant cells. In contrast, with symmetrical cell division, if a mutation occurs early, many cells in the culture will inherit the mutation (see Methods for a detailed explanation and calculation).

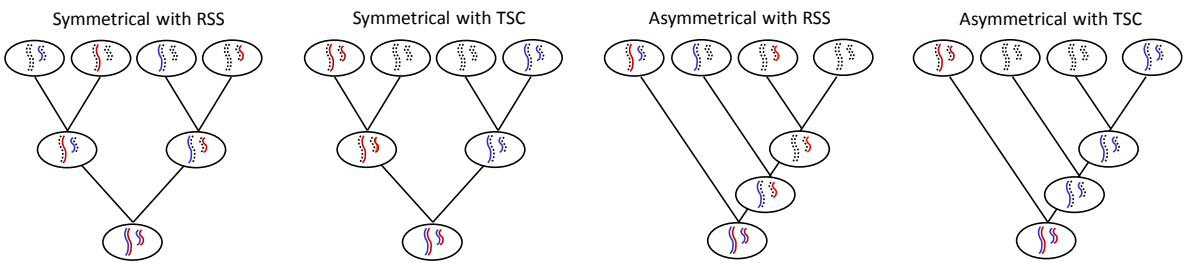

Figure 2. A comparison between symmetrical cell division with random strand segregation (RSS; left), symmetrical cell division with template strand co-segregation (TSC; second left), asymmetrical cell division with RSS (second right) and asymmetrical cell division with TSC (right). Following DNA replication, an asymmetrical cell division results in two daughters, one of which becomes a new 'stem cell', and the other of which is a rejuvenated cell that stops dividing. According to the 'immortal strand hypothesis' the sister chromatids containing the older strands (blue non-dashed) are retained in the continually dividing mother cell.

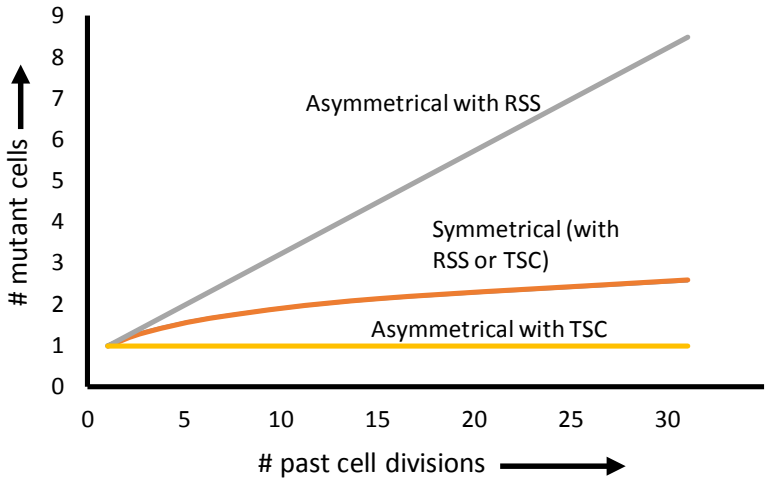

Figure 3. The expected number of mutant cells resulting from a single mutational event as a function of the number of past cell divisions under symmetrical cell division (with TSC or RSS; orange line; this number can be calculated as $\frac{k*2^k}{\sum_{n=1}^{n=k} 2^n}$, with k number of rounds of cell division with symmetrical growth), asymmetrical cell division with RSS (grey line; this number can be calculated as 0.5+0.25*n*, with *n* number of cells formed) and asymmetrical division with TSC (yellow line; always 1, irrespective of the number of cell divisions). The number of mutant cells under symmetrical division and asymmetrical division with RSS are both increasing functions of the number of cell divisions and both yield a higher number of mutant cells than asymmetrical division with TSC, which always yields only a single mutant cell, irrespectively of the number of cell divisions.

Table I. The expected numbers of mutant cells if one mutational event occurs during the formation of *n* cells under symmetrical and asymmetrical cell division, and with either RSS or TSC.

symmetrical cell division						asymmetrical cell division			
				RSS	**TSC**			**RSS**	**TSC**
rounds of cell division	#cells	#past cell divisions	branch length in the tree	**# expected mutations**		# cells	#past cell divisions	**# expected mutations**	
0	1	0	1	**0**	**0**	1	0	**0**	**0**
1	2	1	2	**1**	**1**	2	1	**1**	**1**
2	4	3	6	**1.33**	**1.33**	4	3	**1.5**	**1**
3	8	7	14	**1.71**	**1.71**	8	7	**2.5**	**1**
4	16	15	30	**2.13**	**2.13**	16	15	**4.5**	**1**
5	32	31	62	**2.58**	**2.58**	32	31	**8.5**	**1**
6	64	63	126	**3.05**	**3.05**	64	63	**16.5**	**1**
7	128	127	254	**3.53**	**3.53**	128	127	**32.5**	**1**
8	256	255	510	**4.02**	**4.02**	256	255	**64.5**	**1**
9	512	511	1022	**4.51**	**4.51**	512	511	**128.5**	**1**
10	1024	1023	2046	**5.00**	**5.00**	1024	1023	**256.5**	**1**

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