**The plastic mutation rate and the constant germline of unicells**

Duur K. Aanen & Alfons J.M. Debets

*The mutation rate of unicellular organisms recently was found to be strongly reduced at high density, which we reason can be attributed to increased asymmetry in mutation acquisition between senescent and non-senescent daughter cells.*

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*). 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. We reason that the reduced mutation rate at high density can be attributed to increased asymmetry in mutation acquisition between senescent and non-senescent daughter cells, with senescent cells receiving the bulk of new mutations. Consistent with this hypothesis, 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 (*3*) and for *Escherichia coli* (*4*). By varying the degree of asymmetrical division in response to population density, unicells achieve a fairly constant mutation rate per space and time, remarkably analogously to the germline of multicellular organisms.

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, even in organisms with morphologically symmetrical division (*5, 6*). An asymmetrical cell division results in a senescent ‘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 (*7*). Theoretical studies demonstrate that asymmetrical division can lead to higher viability of a culture (*8, 9*).

Recently, the nutrient concentration of the growth medium was found to influence the degree of asymmetry between cells, presumably also via cell density (*3, 4*). We reason that those two independent findings, reduced mutation rate under high-density conditions (*2*) and increased asymmetry under those conditions (*3, 4*), 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. In budding yeast such an asymmetry in the number of mutations between rejuvenated and senescent cells has indeed been found, and this difference is higher under nutrient-rich conditions (*3, 10*).

If senescent cells have a higher probability to acquire new mutations than rejuvenated cells, this reduces the population-wide mutation rate for two reasons. Most importantly, the senescent fraction contributes less to the total population due to increased cell death and reduced rates of mitotic division. Additionally, concentrating mutations in a subset of cells will increase the variance among cells in the number of mutations. Since the methods used to estimate the mutation rate assumes maximally a single mutation per cell, this increased skew will reduce the estimated mutation rate.

The difference in mutation rate and in the degree of asymmetrical cell division between a low-density and a high-density culture likely arises in the latter stages of growth (Figure 1). Consider two cultures of unicells with different nutritional levels. Initially, when nutrition is not limiting yet, growth will be maximal in both cases (*11*). A difference in density will thus only arise after the low-nutrient culture approaches the stationary phase, and the high-nutrient culture is still exponentially increasing in population size (Figure 1a). A higher degree of asymmetrical division at high density influences senescence in two different ways, either via the relatively faster-dividing aging cells in yeast, or via the slowly-dividing aging cells in *E. coli*. In yeast at high density, a larger fraction of the cells become quiescent, being arrested in the G0 phase of the cell cycle, and consisting almost exclusively of rejuvenated daughter cells with a high capacity to grow when conditions improve (*12*). The remaining cells are heterogeneous and show senescence (Figure 1b). In contrast, in *E. coli* rejuvenation of the culture at high density occurs because senescent cells divide more slowly than rejuvenated cells (Figure 1c; *9, 13*).

Many questions remain to be answered. First, what is the adaptive significance of increased asymmetry and ‘dumping’ mutations into the senescent cells at high density? Asymmetrical division can generate cells specialising in ‘somatic’ functions such as antibiotic production and cells retaining high viability. Sacrifice of a fraction of the cells via asymmetric cell division is a form of altruism, which can be selected if increased mortality of a focal senescent cell increases survival of its rejuvenated daughter, sufficiently to outweigh the survival probability of the two cells if they were non-senescent. The latter depends on external mortality, which will be higher at high density due to increased competition.

Second, what is the mechanism of asymmetrical mutation probability? By using specific knock-out mutants, Krasovec et al. could show that density-associated mutation plasticity is based on plasticity in mutation avoidance and not on plasticity in mutation repair (Krasovec et al., 2017). This implies that the asymmetry we hypothesise underlies a difference in mutation rate between cells must be related to mutation avoidance. Thus, under high-density conditions, with more asymmetrical cell division, the rejuvenated cells avoid mutations more than the senescent cells. This could be due to either a higher activity of mutation avoidance genes, or to an unequal distribution of the scavenger proteins across cells.

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 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 mutational events 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 (*16*). In plant meristems, the stem cells from which reproductive organs will develop undergo a minimal number of divisions during plant growth (*17*). Also, the number of cell divisions separating axilliary meristems from the central meristem is minimized (*18*). Similarly, in a long-lived fungus 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 (*15, 19*). 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 (*20*).

The realisation that also unicellular organisms 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 (*21*). Leo Buss challenged Weismann’s doctrine, noticing that an early germline sequestration as seen in vertebrates is rare among multicellular organisms (*22*). The recent findings discussed in this perspective, however, revive Weismann’s doctrine. A picture emerges that the germline-soma distinction is not limited to some animals, but also occurs in plants, fungi and even unicellular organisms. Furthermore, those convergent cases have in common that germline cells have mechanisms to reduce the mutation rate, supporting the hypothesis that reduced mutation rate is the primary function of an early germline-soma differentiation.

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| a. | b. | c. |
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| Figure 1. How asymmetrical cell division can lead to a difference in mutation rate. 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 in the exponential phase. The difference in mutation rate between both conditions therefore can thus mostly be attributed to differences in the later stages of growth when densities start to differ and the level of asymmetrical division starts to differ between cultures. There are two ways how a higher degree of asymmetrical division under high-density conditions influences rejuvenation, either via the more-slowly dividing cells, in yeast (b) or via the faster-dividing cells, in *E. coli* (c). The remaining cells sampled at high density are indicated. In the lower panels, the difference in asymmetrical cell division and its consequences for senescence between yeast and *E. coli* are schematically illustrated. The light cells indicate rejuvenated cells with fewest mutations. | | |

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