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Unraveling the non-senescence phenomenon in *Hydra*

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HIGHLIGHTS

- *Hydra* shows no signs of senescence under laboratory conditions.
- Within-cell repair is imperfect and cannot solely protect against damage accumulation.
- Cellular damage drift leads to accumulation of damaged cell lineages, hence senescence.
- High prevalence and continued division of *Hydra*'s stem cells act against damage drift.
- These features and efficient selection against damaged cells are crucial for non-senescence.

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ABSTRACT

Unlike other metazoans, *Hydra* does not experience the distinctive rise in mortality with age known as senescence, which results from an increasing imbalance between cell damage and cell repair. We propose that the *Hydra* controls damage accumulation mainly through damage-dependent cell selection and cell sloughing. We examine our hypothesis with a model that combines cellular damage with stem cell renewal, differentiation, and elimination. The *Hydra* individual can be seen as a large single pool of three types of stem cells with some features of differentiated cells. This large stem cell community prevents "cellular damage drift," which is inevitable in complex conglomerate (differentiated) metazoans with numerous and generally isolated pools of stem cells. The process of cellular damage drift is based on changes in the distribution of damage among cells due to random events, and is thus similar to Muller's ratchet in asexual populations. Events in the model that are sources of randomness include budding, cellular death, and cellular damage and repair. Our results suggest that non-senescence is possible only in simple *Hydra*-like organisms which have a high proportion and number of stem cells, continuous cell divisions, an effective cell selection mechanism, and stem cells with the ability to undertake some roles of differentiated cells.

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1. Introduction

In most organisms senescence is defined by an increase in mortality and/or a decline in fertility with age as a result of organismal deterioration (but see Jones et al. (2014)). Three main evolutionary theories are invoked to explain this process: mutation accumulation, antagonistic pleiotropy and disposable soma. The first theory predicts that senescence evolved via the accumulation of deleterious mutations acting later in life (Medawar, 1952). Because only a few individuals survive to ages at which these mutations have a deleterious effect, there is a reduced force of selection against those mutations. The second theory proposes that senescence evolved via antagonistic pleiotropy (Williams, 1957). While pleiotropic genes have positive fitness effects at the early life stages, their effects can be disadvantageous later in life; i.e., a mutation acting positively early in life will be rapidly driven to fixation because its fitness value is then higher (Hamilton, 1966). The disposable soma theory assumes the organismal optimization of resource allocation (Kirkwood, 1977): i.e., that organisms have a choice about whether to utilize the available resources for reproduction or for the maintenance of the soma. The disposable soma theory is related to organisms witch distinction between germ and somatic cells: germ line does not accumulate damage during life while soma has to follow the senescence. As aging is a process that leads to the deterioration of the body via the accumulation of damage, an organism is usually unable to repair everything. However, this is not the case for freshwater polyp *Hydra*, a member of the Cnidaria (Hydrozoa) that shows under laboratory conditions no signs of any kind of senescence; i.e., *Hydra* experiences neither an increase in the probability of death nor a decline in budding reproduction with age (Martinez, 1998; Jones et al., 2014).

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Hydra has a body plan consisting of stem cells, a simple nervous system, and two differentiated tissue layers mainly present at the apical edges with a mouth at the top and a foot at the bottom of the animal (Bode, 1996; Steele, 2002). It consists of three active but separated stem cell communities: the ectodermal and endodermal epithelial stem cells, and the interstitial stem cell lineages (Schaible et al., 2014). *Hydra* individuals, called polyps, reproduce mainly clonally by budding, producing genetically identical buds (ramets); or less frequently, sexually by producing new clones (genets). *Hydra* has also no clear distinction between germ and soma cell lines (Bosch and David, 1987). Sexual reproduction of *Hydra* is based only on one cell lineage (interstitial cells), which can also contribute to the somatic cells (Bosch and David, 1987). Although in *Hydra* subpopulations of interstitial stem cells with germ cells-restricted capacities have been found, it seems that those specific cell lineages can be generated at any time from multipotent interstitial stem cells (Nishimiya-Fujisawa and Kobayashi, 2012). Furthermore, maintenance of a separate germ lineage would be difficult with the perspective of constant cell flow in Hydra to tentacles and buds. In the past there was general believe that the lack of senescence should be prescribed to the organisms without distinction to germ and soma lines. However, the lack of germ line sequestration in metazoans (like in *Hydra*) is not decisive to emerge the non-senescence phenotype (Martínez and Levinton, 1992). The stem cells in *Hydra* are always active, with a regular and continuous proliferation and without long periods of inactivity (Bosch and David, 1984). Epithelial stem cells are not completely unspecialized, but are instead tissue-specific, with special functions like forming an epithelial cell layer and providing a polyp its shape and morphogenesis (Technau and Steele, 2011). These stem cells are responsible for the polyp's extraordinary regeneration and survival ability (see Schaible et al. (2014)). Since an individual polyp cannot grow forever, there are three ways it can remove cells from the body: first, after a short migration along the body axis, the cells differentiate at the edges of the cell proliferation zone (at the foot and tentacle regions), and are then lost through death or sloughing (see Steele (2002)); second, the cells all over the body can be removed by apoptosis (Galliot and Ghila, 2010); and third, many cells of the central part of the gastric body can also migrate toward a growing bud (Campbell, 1967) that will later be separated from the mother.

When we look at constant cell proliferation, it is unclear how *Hydra* prevents the accumulation of damage, because it is generally the case that the more divisions a cell undergoes, the more the damage build ups in the cell's biochemical machinery. While it was long assumed that stem cells—and specifically human stem cells—were exempt from aging, in recent years molecular mechanisms have been identified that are associated with stem cell senescence (Beltrami et al., 2011). We should thus expect that *Hydra* would also accumulate damage and undergo senescence. Martinez (1998) and Jones et al. (2014) showed, however, that *Hydra* has a very low mortality level for more than four years, which indicates that the health status of these animals is not negatively affected by the accumulation of mutations or somatic damage with age. Two related questions then arise: how does *Hydra* rid itself of damage (including mutations), and what mechanisms are developed to protect the polyp against the accumulation of damage over time?

There are several processes preventing the accumulation of damage in the body. First, there are processes that prevent various forms of potential damage before they happen; for example, there are cellular defense mechanisms that absorb radical oxygen species. Second, damage can be directly repaired; for example, damaged DNA strands may be repaired, thus preserving the integrity of DNA. Third, damaged structures of the body can be replaced; for example, damaged cells may be removed by programmed cell death. For humans and other complex organisms, the efficient repair of various forms of damage seems to be the main mechanism that prevents damage from accumulating in cells (e.g., Freitas and de Magalhaes, 2011). But over the long term, the repair mechanism alone does not seem to protect an individual human from the aging process, as the efficiency of this mechanism appears to be limited (see Blanpain et al. (2011) and Seluanov et al. (2004)). Consequently, we assume that *Hydra* must have an alternative mechanism that prevents the accumulation of damage and enables it to achieve a long-term constant and low probability of death across all ages; and thus non-senescence.

To provide a qualitative conceptual answer to the question of why *Hydra* does not senesce, we propose a model based on a number of fairly specific assumptions about *Hydra* physiology. This model not only provides new insights into the proximate (cellular physiological) mechanisms that prevent aging, but also gives a rise to new predictions that could be tested empirically in the future. The core of the model is based on two processes that we believe are responsible for the long-term maintenance of a *Hydra*: the continuous and constant production of new cells of all three stem cell populations, and the continuous removal of cells by differentiation, programmed cell death, or budding. It is generally accepted that stem cell proliferation activity is related to the *Hydra's* ability to avoid aging (see review Schaible et al. (2014)). However, as a constant proliferation of stem cell populations could entail the constant accumulation of damage on the cellular level, it is reasonable to assume that cells with accumulated damage should be selectively removed from the body of a *Hydra*. If this process was effective, it would prevent the random accumulation of damaged cell lineages which could eventually undergo fixation in the organism, leading to a significant deterioration in fitness-relevant parameters like reproduction and lifespan. In light of these considerations, we hypothesize that *Hydra* is able to achieve "non-senescence" because of four specific characteristics of its body plan: (i) a large number of stem cells; (ii) the continuous division of stem cells; (iii) a high proportion of dividing cells relative to non-dividing cells within each polyp; and (iv) the constant removal of cells by differentiation, programmed cell death, or budding (see above). The last characteristic provides the basis for an efficient selection against damaged cells, and thus the prevention of the accumulation of damage by the selective elimination of most of the damaged cells within a polyp. Finding sufficient conditions for non-aging in *Hydra*-like animals is helpful for understanding why most animals, including humans, cannot easily avoid aging.

2. Materials and methods

2.1. General description of the algorithm in the biological context

In the model, we consider two kinds of cells: stem cells and differentiated cells. Differentiated cells do not divide, and stem cells divide with some probability in each time unit. Our model cell cycle of stem cells is simplified into two phases: a pre-division phase and a division phase. A cell can undergo one such stage in a unit of time. All of the cells preparing for division or resting are classified as non-dividing cells. The two kinds of cells are assumed to have different rates of damage accumulation and repairs, as we will describe later. In the model, we assume that stem cells are continuously proliferating with the probability of division q in each time unit (Fig. 1). The probability of cell division is explicitly affected by the average damage level of the cells in the organism, and is implicitly affected by resource acquisition dependent on the average damage (Fig. 3). A stem cell can divide into two differentiated cells with the probability p, or it can self-renew by producing two stem cells with the probability $1\text{-}p$ (Fig. 1).

In *Hydra*, the proportion of stem cells is stable and independent of the size of the individual animal (Bode et al., 1973, 1977). In the gastric region (middle part of the body) 80–90% of the epithelial stem cells

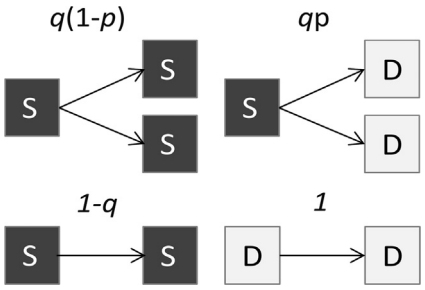

Fig. 1. Cell divisions and differentiation. Only stem cells can divide. The probability of division is given by q and the probability of differentiation is given by p. At the top of each possible case there is its joint probability.

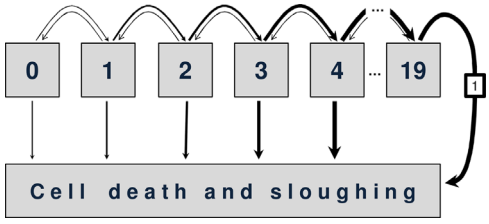

Fig. 2. Damage accumulation, repair, and cell death. There are 20 classes categorizing damage amount. In each unit of time the damage class can be increased due to the accumulation of new damage, or—which is less likely—can be decreased due to repair. The probability of skipping to the next damage class increases with the class number. The probability of the reverse process (called repairs in the model) is independent of the damage class. The probability of death increases with the damage class. The cells that reach the last damage class die during the first attempt at division. The constant fraction of differentiated cells are sloughed, starting with the most damaged class.

are mitotically active (David and Campbell, 1972; Bosch and David, 1984). Many pathways have been identified in *Hydra* that appear to provide signal proteins important for the regulation of cell numbers, as well as for the renewal and differentiation of stem cells (Boettger et al., 2006; Takahashi et al., 2000). We use these observations as assumptions in our model. The constant proportion of stem cells and differentiated cells is controlled by a variable p, which describes the probability of differentiation. This probability is estimated before each time unit (day), taking into account the current proportions of cells, the probability of cellular division q, and the probability of cellular death (see Supplementary materials for details).

The life cycle of a *Hydra* individual can be viewed as a two-phase process which is driven by the same mechanism. Stage 1 is growth, a non-reproductive phase in which the polyp continuously grows, and in which under constant food supply the polyp reaches a constant size M (Otto and Campbell, 1977). Stage 2 is the period of life in which an individual starts forming a new offspring by budding (=asexual reproduction). The bud, which is constructed through cell migration from the mother to the budding zone, is detached when its number of cells is adequate to build a new baby polyp capable of living independently of the mother (Otto and Campbell, 1977). The size of a new polyp is arbitrarily assumed in the model to be 0.2 M. The simulation of each individual starts with the cell number characteristic of a newly detached bud. Whether an individual *Hydra* dies depends on the total number of cells per polyp and on the proportion of differentiated cells that are dedicated to important functions like the ability to catch food. If a *Hydra* individual is dying because of intrinsic forces, there is a strong decline in size and losing of their tentacles until they dissolve completely (see Schaible et al. (2011)). Therefore we assume that an individual polyp will die when the total number of cells drops below 10% of the initial level.

In our model, damage is defined as all kinds of molecular injuries that are hard, costly or impossible to repair in cells, including mutations, DNA damage and mitochondrial damage. We assume that each cell division causes additional damage (Blanpain et al., 2011; Sancar et al., 2004), which means that the damage rate is generally greater in dividing cells than in non-dividing cells (Fig. 2A). After cell division, both new cells inherit the damage from a mother cell (=symmetric cell division). Cells can be categorized into 20 classes, numbered from zero to 19, with linearly increasing amounts of accumulated damage. It is important to note that the fates of particular cells are not followed in the model, and that only the numbers of cells undergoing different stages and different damage classes are studied. Each cell can accumulate damage and undertake repairs (with a constant repair rate for all damage classes) during each unit of time, which is practically realized by the changing damage class affiliation (Fig. 2). Consequently, a cell can stay in the same class or move only to neighboring damage classes in a unit of time (Fig. 2). We assume that the hazard of gaining new damage (modeled in terms of the probability of moving to the next damage class) increases exponentially with already accumulated amounts of damage (modeled as the number of the damage class to which a cell belongs). There is some evidence that justifies this assumption: damaged mitochondria tend to produce more free radicals than intact ones (Arnheim and Cortopassi, 1992; Bandy and Davison, 1990; Kowald and Kirkwood, 2000; Simmons et al., 2005; Sugiyama et al., 1991); and, unrepaired double strand breaks of DNA (DSB) can lead to positive feedback in the deterioration of repair efficiency, increasing the risk of gaining new damage and mutations (Lorat et al., 2012; Orlowski et al., 2011; Rube et al., 2011). DNA damage, including the most hazardous DSB, are important mechanisms underlying age-dependent stem cell decline (Rube et al., 2011).

We assume that the within-cell repair probability (modeled as a probability of moving to the previous damage class) is independent of the amount of damage. This assumption is based on the results of a study showing that the repair efficiency of DSBs does not differ depending on whether radiation doses are high or low (Asaithamby and Chen, 2009). In addition, we assume that a higher repair capacity can be found in dividing than in resting cells, even if the accumulation rate of the damage is greater in the first category. This assumption is based on the observation of more active repair and less error-prone mechanisms and processes during cellular division stages (e.g., homologous recombination HR) (Blanpain et al., 2011). However, the repairs in our model seem to play a minor role, as the probability that repairs will occur is much lower than the probability that damage will accumulate. However, the effect of the repair is indispensable for non-aging in the model, because they prevent a cellular Muller's ratchet (see below); i.e., the mechanism that leads to inevitable aging as cell classes with minimal damage systematically disappear from the cell population. Repairs are an opportunity to reverse the process.

Deleterious damage accumulated in one cell of a *Hydra* may have an impact on the fitness and fate of that cell, but cannot alone cause the death of a whole polyp of many thousands cells. However, through constant cell division, damage present in a single cell could be transferred to many other cells. Thus, not only does the frequency of damaged cells increase, but a specific damage can eventually be fixed in the cell population of a single polyp. Over the longer term, a large amount of different kinds of damage can undergo fixation, leading to the death of the polyp. The process of random damage accumulation and fixation is called “cellular damage drift.” The mechanism is similar to genetic drift and Muller's ratchet in populations of individuals (Kimura and Ohta, 1971).

The damage-dependent cell death is based on differentiating the probability of death for various damage classes. The probability that a cell will die during cell division increases linearly with increasing damage class (Fig. 3). This approach to the modeling of selective death is based on the results of several studies. Research on primary human fibroblasts (Rothkamm and Lobrich, 2003), lymphocytes (Torudd et al., 2005), and fibrosarcoma (Asaithamby and Chen, 2009) has shown that

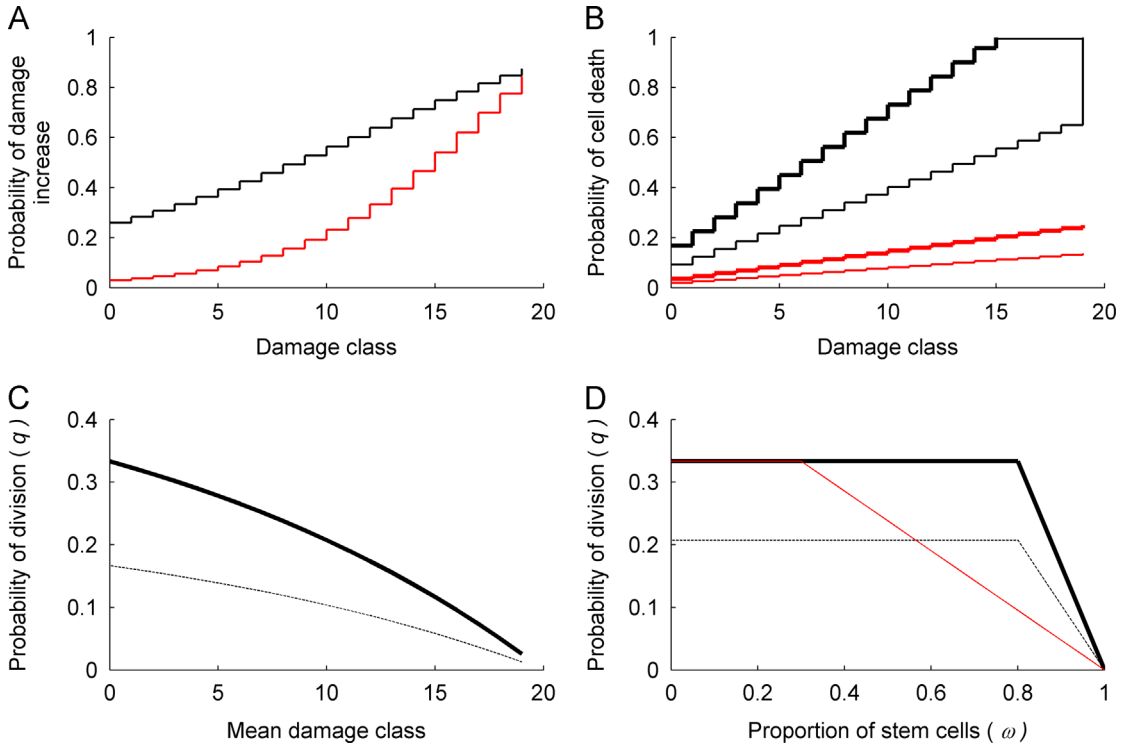

Fig. 3. Graphical representation of the main assumptions of the model for exemplary parameters. (A) Probability of damage increase (moving to the higher damage class) for dividing stem cells (upper black line) and non-dividing cells (bottom red line) as the function of belonging to a given damage class; a in Eq. (1) equals to 0.3 for dividing stem cells and 0.03 for non-dividing cells. (B) Probability of cell death for dividing stem cells (two black upper lines) and non-dividing cells (two red bottom lines); $\alpha=1.5$ for bold lines and 0.825 for thin lines. (C) Probability of division q as a function of the mean number of damage represented as mean damage class number; bold line: $\omega \in [0, 0.8]$ thin dotted line: $\omega=0.9$. (D) Probability of division q as a function of the proportion of stem cells ω; two cases for hydra free of damage, with ω^* equals to 0.8 (thick black line) and 0.3 (thin crossing red line); one case for *Hydra* with damaged cells: thin dotted black line, average damage class equals to 10, ω^* equals to 0.8; ω^* is the proportion of stem cells at which probability of cell division starts decreasing linearly.

double-strand breaks increase linearly (or, in the first case, linearly on the log–log scale) with a dose of irradiation (see Singh et al. (2001) for a comparison of the accumulation of damage due to irradiation with the accumulation due to aging). It has also been suggested that apoptosis incidence increases linearly or semi-logarithmically with the size of the dose (Feinendegen, 2005; Feinendegen et al., 2007; Henriquez-Hernandez et al., 2011; Pinar et al., 2010). It follows from these two linear functions that the dependence of cellular death on damage level should also be linear or quasi-linear.

If a cell reaches the last, most damage-loaded class, it inevitably dies during the next attempt at division. Other cells die during attempts at division, with the probability of dying increasing with the damage class number. Stem cells in the pre-division stage have the same probability of dying as the differentiated cells (which do not divide), but their probability of dying is lower than that of the dividing stem cells. As differentiated cells accumulate damage without dividing, the oldest differentiated cells have the highest level of damage. These cells die by sloughing with a constant probability. Similarly to the selection of genotypes that overcomes genetic drift (e.g. Kimura nad Ohta, 1971) in a macro-scale-, damage-dependent selection may overcome "cellular damage drift."

One iteration of the algorithm (one time unit) consists of five stages: (i) cell deaths and sloughing, (ii) cell divisions according to the calculated division/differentiation probabilities (q and p, respectively), (iii) damage accumulation/repair, (iv) possible reproduction, and (v) estimation of the probabilities p and q for the next iteration. The iterations are repeated until a polyp dies, or up to 10^6 iterations (almost 2740 years if one iteration is treated as a day).

Even if *Hydra* can be considered a non-senescing organism, these animals can still die in their natural environment due to high extrinsic hazards like accidents, diseases, or predation. To analyze whether extrinsic mortality affects the rate of damage accumulation—and, consequently, the lifespan of an individual polyp—we consider in our model two different scenarios. In the first scenario, the individual lifespan is studied without extrinsic (ecological) mortality. This model can be called "*Hydra* in the lab." Budding is important in this model only as a mechanism for decreasing the mother-*Hydra* size. In the second population model *Hydra* is under the pressure of ecological mortality. We assume that population of *Hydra* is leaving in a constant environment regulated via density dependence acting uniformly on fertility or on survival before maturity. In such a case appropriate measure of fitness is lifetime bud production (Mylius and Diekmann, 1995). The assumption about density dependence is supported by Łomnicki and Slobodkin (1966), who observed that populations of *Hydra* are regulated by migration of young individuals. This kind of population regulation is equivalent to the density dependence acting on survival before maturity. This model can be called "*Hydra* in the field." Budding is important in this model not only as a mechanism for decreasing the mother-*Hydra* size, but also as a significant factor affecting fitness.

2.2. "Hydra in the lab" model

2.2.1. Cell divisions

The probability of a stem cell division q (see the equation (A2) in Supplementary materials) is a function of the mean amount of damage in the body ($\overline{\psi}_t$) and the proportion of the stem cells (ω_t) at age t. The more the cells of the body are damaged, the lower the productivity of the organism is (in terms of its capacity for biosynthesis). Thus, the organism's ability to accumulate, process, and distribute resources throughout the body is diminished. This compromised productivity leads in turn to reduced cell division and budding rate. In addition, if the proportion of the stem cells is too high, there is a shortage of the differentiated cells needed to maintain

somatic functions that are crucial for survival and reproduction; e.g., there may be too few cells dedicated to catching external food. On the other hand, having too few stem cells compromises the regenerative and maintenance potential of whole body parts. The dependence of q on the mean amount of damage $\overline{\psi}_t$ (the average damage class the cells belong to) is shown in Fig. 3C, and the dependence of q on the proportion of stem cells among all cells is shown in Fig. 3D. Below the threshold proportion ω^* division probability is constant, and above the threshold it declines linearly to zero because a shortage of differentiated cells suppresses the cell division rate (Fig. 3D). Two ω^*'s were assumed in simulations: 0.3 and 0.8.

2.2.2. Damage accumulation

At the beginning of life, all cells are free of damage. The damage accumulation rate depends on (i) whether a cell is in a division or a non-division phase, and (ii) the number of the damage class to which a cell is assigned. We assume that the damage rate k increases exponentially with damage class ψ

$$k(\psi) = ae^{b\psi} \quad (1)$$

where a and b are normalization and shape parameters. The damage rate k is converted to the probability that a cell will move to the higher damage class according to the following equation:

$$P_D(\psi) = 1 - e^{-k(\psi)} \quad (2)$$

Since most damage and mutations occur during cell divisions, the parameter a in Eq. (1) is set to 0.3 throughout the paper for dividing stem cells and to 0.03 for differentiated and non-dividing stem cells. The graphical representation of Eq. (2) is shown in Fig. 3A. Having a limited number of damage classes, we assume that the P_D in the last damage class equals to $z=0.9$. P_D in the last damage class defines the probability of death as there are no further classes that cell can move to. The parameter b is calculated directly from a and z ($b=0.217$ for non-dividing and $b=0.102$ for dividing cells, see Supplementary materials for details).

In the model, there is also a reverse process to the damage accumulation that can be interpreted as repairs or back mutations: a cell can move to a neighboring class with a smaller amount of damage. We assume that this process is independent of damage, and that it happens with a probability of 0.002 for dividing cells, and a half that for non-dividing cells.

2.2.3. Probability of differentiation during cell division

The body of a *Hydra* consists of two different cell types: stem cells and differentiated cells. Bode et al. (1973) have shown that a constant proportion of stem cells and differentiated cells is an important characteristic for the body plan of a polyp. If the proportion of the stem cells is too high, the limited number of differentiated cells could lead to a deterioration of the somatic functions that are crucial for survival and reproduction; e.g., the forming of tentacles for catching food. On the other hand, having too few stem cells might undermine the regenerative and maintenance potential of parts of the body (e.g., tentacles) and of the whole body.

We assume that *Hydra* maintains roughly constant proportions of stem cells and differentiated cells ω_0 (Bode et al., 1973). In the "*Hydra* in the lab" model, different pairs ω_0 and ω^* were studied, but the results are shown only for two pairs ($\omega_0 = 0.8$, $\omega^* = 0.8$ and $\omega_0 = 0.64$, $\omega^* = 0.3$). Please note that in the latter case the optimal proportion is greater than the threshold. These proportions ω_0 maximize fitness in the "*Hydra* in the field" model under given ω^*, independent of assumed extrinsic mortality. In order to preserve the assumed ω_0's, an individual adjusts the probability of differentiation during division (differentiation factor p, see Fig. 1), which is calculated according to the algorithm described in the Supplementary materials.

2.2.4. Cell death and sloughing

Cell death is an important factor in the elimination of damage in the model. We assume in the model that most cellular death is due to damage-dependent cell death. Dividing cells may die, and the probability of dying depends on the amount of damage. To model this probability, we choose the simplest assumptions; namely, that (i) it increases linearly with damage class ψ, (ii) it cannot exceed one, and (iii) in the last class it is set to one, although this value can be reached in a lower class (Fig. 3B; see also Eq. A1 in the Supplementary materials). Two parameters determine the slope of the linear increase in the probability of dying with the number of damage class: α, which models the sensitivity of cell death to cell damage (called damage sensitivity hereafter); and λ, which is set to 0.75 throughout the paper, and which models the accuracy of a damage-dependent cell death (and thus modulates the selectivity of the process). The parameter λ determines both the slope and intercept of the relationship between damage class and probability of cell death (see Supplementary materials for details). When $\lambda = 1$ the slope of the function is proportional to α and the intercept equals to zero; when $\lambda = 0$ then the slope is equal to zero and the intercept is proportional to α. In other words, the lower the λ the less selective cell death is, and the closer the shape of the function is to the horizontal line. At the assigned value of λ, both the slope and the intercept of the probability function are high at high α (Fig. 3B). We assume that α is five times lower for non-dividing stem cells and differentiated cells than for dividing stem cells.

Differentiated cells can also die by sloughing. We assume that the fraction of cells dying due to sloughing is constant and is set to 0.05. For the sake of simplicity, we assume that the most damaged cells are sloughed first, as they are on average the oldest ones. This scenario is analogous to the sloughing of the most distant cells situated at the tentacles or foot. The loss of too many cells due to cell death and sloughing is the only source of death for the individual polyp in the "*Hydra* in the lab" model.

2.2.5. Growth, reproduction, and body size

Since the performance in growth and asexual reproduction depends on the size of a polyp, we test two different sizes at maturity M (25,000 or 50,000 cells). A mature polyp builds a new bud with a size of 0.2 M. After a bud detaches, the parental polyp must regrow to again reach size M. Thus, the body size of fully grown *Hydra* fluctuates between 0.8 M and M, unless the polyp shrinks due to accumulated damage. The proportion of cell types (differentiated and stem cells) in the bud is the same as in the body of the parental polyp. The bud formation is a random process, which means that each cell of the mother has the same probability of migrating to a bud, regardless of the level of the damage.

The more the cells of the body are damaged, the lower the productivity of the organism is (in terms of its capacity for biosynthesis), which depends on gaining, processing and distributing resources throughout the body. The limited productivity leads in turn to diminished cell division and consequently to the reduced growth and budding rate.

2.2.6. Sources of within-clone stochasticity

Stochasticity plays a crucial role in the biological process, and it is also an important part of our model. Without the introduction of randomness, we would not, for example, be able to observe the effect of cellular damage drift or analyze the demography of simulated *Hydra* clones. The potential sources of stochasticity are the probabilities of (i) cellular death, (ii) damage class increase or decrease, (iii) cell division, and cell differentiation. The model was run with the same set of parameter values (for the same clone) multiple times in order to quantify the expected outcomes and the variation based on

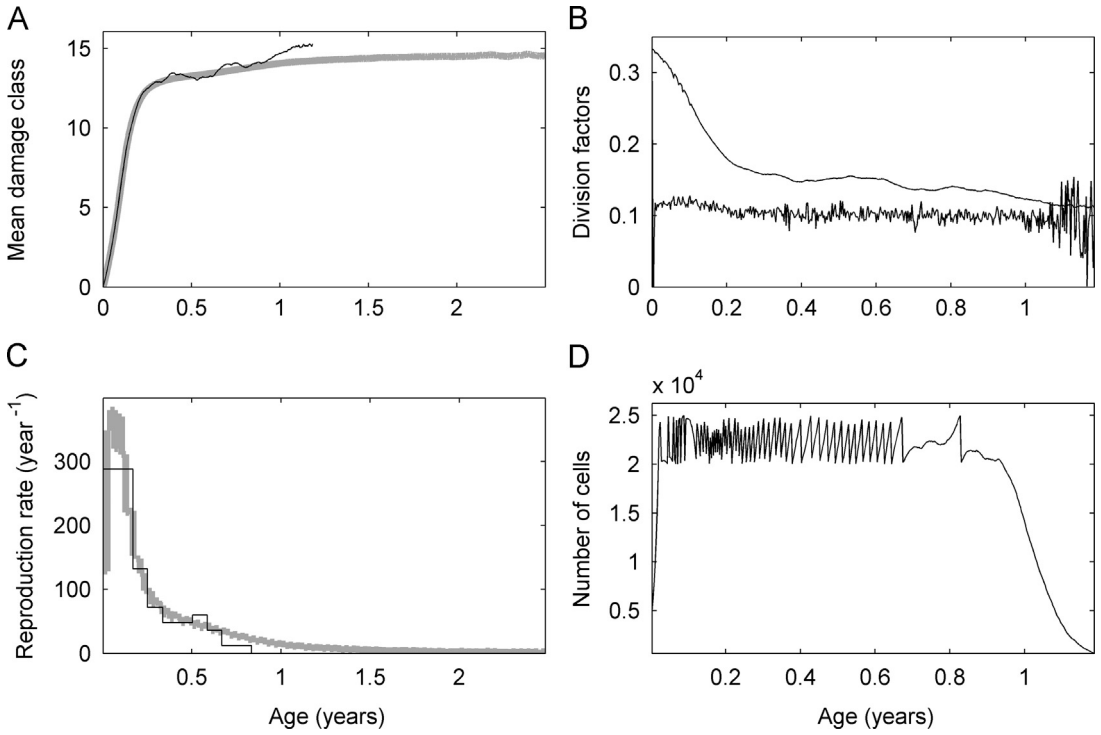

Fig. 4. Exemplary run without selection of damaged cells. Exemplary run (thin, black lines) and average runs for the whole clone (thick gray lines in (A) and (C)) with the parameters: $\alpha=0$ (damage dependent cell death limited only to the last class), $\omega_0 = 0.8$, $\omega^* = 0.8$, $M=25000$. (A) The dynamics of the mean damage class. (B) The dynamics of division factors q (probability of division; upper line) and p (probability of differentiation during division; bottom line). (C) The dynamics of the reproduction rate, measured as the number of buds per year. (D) The dynamics of body size, measured as the number of cells per polyp. In (C), the reproduction rate was calculated for month intervals in an exemplary individual, and daily for an average individual.

those values. Repetition was at least 20-fold (for the longest living clone), and was sometimes several thousand-fold.

2.3. "Hydra in the field" model

2.3.1. Extrinsic mortality

This part of the method is important only for the "*Hydra* in the field" model because estimating the expected lifetime offspring production (fitness measure) is necessary and possible then. In order to trace the life history of this model of *Hydra*, we need information on the demography of one clone "in the lab" (a set of individuals calculated for the same set of parameters): its survivability and bud production under both intrinsic (damage-dependent cell death) and extrinsic (ecological; e.g., predation) sources of mortality. A clone is defined by the set of parameters for the "*Hydra* in the lab" model, and consists of members which differ as a result of the stochastic cellular processes within their bodies. In the "*Hydra* in the lab" model, we simulated hundreds or thousands of individuals within each clone. One option for studying the population level would be to draw the age of death caused by ecological factors separately for each individual. Such a procedure would introduce enormous stochastic noise at the population level, which is not the subject of our studies. Instead, we calculated expected lifetime bud production at birth for a single *Hydra* individual, taking into account the possibility that it could die due to ecological reasons at any time. We repeated these calculations for several hundred individuals, and then averaged the results for all of the individuals of the same clone. Such a procedure is equivalent to studying an infinite number of individuals representing a finite number of classes (each clone member is a representative of a single class). The algorithm of adding extrinsic mortality is described in the Supplementary materials.

The time horizon for the calculation of the expected lifetime bud production is limited by the physiological lifespan calculated in the "*Hydra* in the lab" model. Some individuals which do not die because of aging in 10^6 iterations are considered dead at this age. This right censoring does not bias the results, because the probability of surviving to such an old age is negligible under ecological mortality.

3. Results

We tested in our model various combinations for size (=cell number) at maturity (M: 25,000 or 50,000), extrinsic mortality (μ: 0, 1/12, 1/6 and 1/3 per year), sensitivity of damage-dependent cell death α in the range from zero to two, two values of stem cell threshold proportions below which cell division drops ($\omega*$: 0.3 and 0.8), and the proportion of stem cells in the total number of cells (ω_0: 0.64 and 0.8).

3.1. "Hydra in the lab" model

Fig. 4 shows the results of an exemplary run and the average results for the clone with no selection against damaged cells in all of the damage classes except the last one ($\alpha = 0$; all of the cells die during division when they reach the last damage class, and/or the most damaged differentiated cells die by sloughing). Fig. 5 shows the results for the clone with moderate selection against damaged cells ($\alpha = 0.825$; more damaged cells have a higher risk of dying). The oscillations in size (Figs. 4D and 5D) are due to the budding and re-growing processes. However, the mean size measured as the cell number does not change across most parts of life in both cases.

In the first few months of life, we can observe the rapid increase in the mean number of damage class per cell, but this number levels off into quasi-equilibrium (Fig. 4A) or equilibrium (Fig. 5A). This phase lasts longer than growth in size. After reaching maturity, the reproduction rate initially decreases because of rapid damage accumulation (Figs. 4C and 5C), and then continues to decrease (Fig. 4C) or reaches equilibrium (Fig. 5C). Individual trajectories of damage and reproduction (thin black

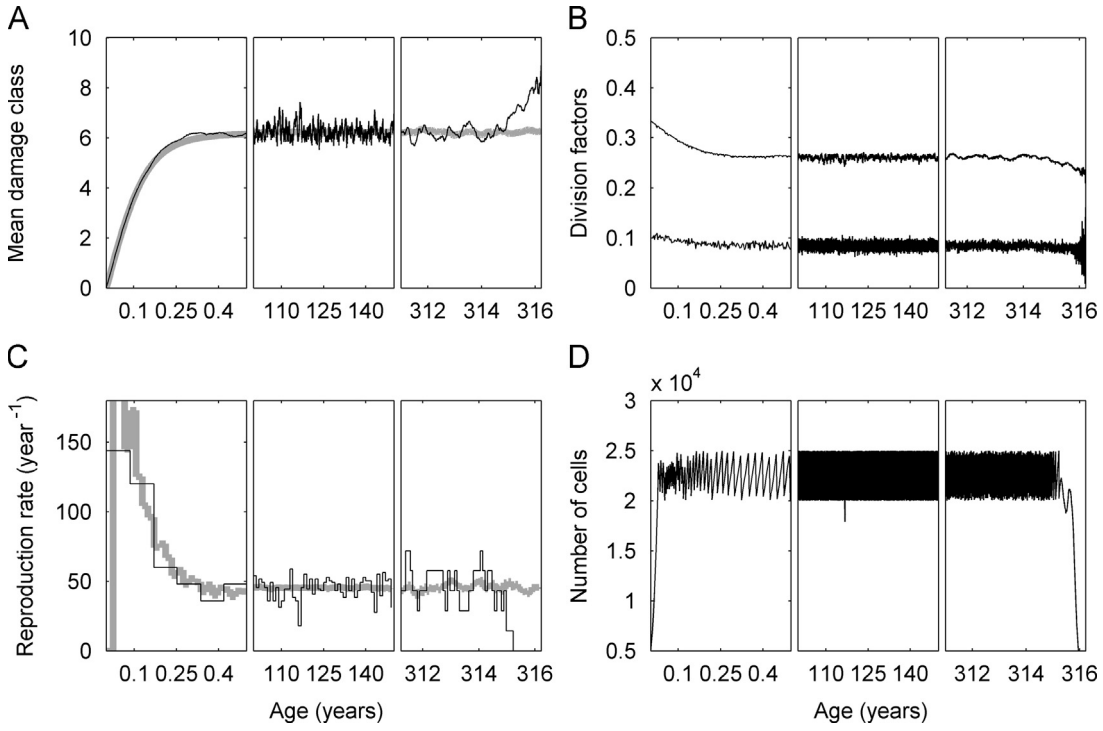

Fig. 5. Exemplary run with selection of damaged cells. Exemplary run (thin, black lines) and average runs for the whole clone (bold gray lines in (A) and (C)) with the parameters: $\alpha=0.825$, $\omega_0=0.8$, $\omega^*=0.8$, $M=25\,000$. (A) The dynamics of the mean damage class. (B) The dynamics of division factors q (probability of division; upper line) and p (probability of differentiation during division; bottom line). (C) The dynamics of the reproduction rate, measured as the number of buds per year. (D) The dynamics of body size measured as the number of cells; regular oscillations reflect the budding process and then regrowth. In (C), the reproduction rate was calculated for month intervals in the left and right panels and in year intervals in the middle panel for an exemplary individual, and daily for an average individual.

lines) are close to averages for the clone (gray bold lines). The increase in damage at the beginning of life causes a decline in the probability of stem cell division (Figs. 4B and 5B); however, the probability of differentiation stays at roughly the same level almost to the end of life (Figs. 4B and 5B).

The differences between these two examples are striking, albeit mainly from a quantitative perspective. In the scenario in which the selection against damaged cells is limited only to the highest damage class, the level of damage reached is much higher (Figs. 4A vs. 5A), while the reproduction rate drops to zero. This *Hydra* not only reproduces less, it also has a shorter life. When the *Hydra* is less than one year old, the number of cells drops dramatically (Fig. 4D), reproduction stops, and, as assumed, the polyp dies when its size falls below 10% of the initial size. In this case, we also observe a slightly higher reproduction rate in the initial months of life relative to the case with moderate selection against damaged cells, as fewer cells are killed and thus more cells can build new buds than in the second case (Figs. 4C vs. 5C). The exemplary *Hydra* that can efficiently remove damaged cells lives hundreds of years, with damage levels at an equilibrium (Fig. 5A). Only at such an advanced age does senescence begin and the polyp finally dies (Fig. 5). The point of entry into the senescence path is random: the timing may differ substantially between individuals simulated with the same values of all parameters. It is clear that even a moderately selective death of damaged cells enormously increases life span.

Let us now consider the average fates of individuals belonging to three clones, each of which has a different set of parameters (Fig. 6). The clones differ in terms of size at maturity M (25,000 or 50,000 cells) and the sensitivity of cell death to cell damage level α (0.750 or 0.825). It is important to recall that only the internal sources of mortality are taken into account ("*Hydra* in the lab" model). The average damage level, measured as an average damage class $\overline{\psi}$, stays constant after a rapid increase at the beginning of life (Fig. 6D). Consequently, the hazard rates are flat, but their levels depend on the parameters: the level is the lowest if the *Hydra* is large and the sensitivity of cell death to damage is high, and the level is the highest when the *Hydra* is small and the sensitivity of cell death to damage is low (Fig. 6A). In the latter case, the probability of survival drops to 0.003 at about 100 years, whereas it is still high at this age in two other clones (Fig. 6B). The reproduction rate also stays constant (Fig. 6C). Only the first 100 years are shown in Fig. 6, but for two clones the lifespans exceeded 2700 years (the limit of simulations). Thus, none of the three clones ages (they have a flat mortality rate), and two of them are practically immortal (they have, on average, a mortality rate so low that living thousands years in a lab is likely).

The role of the sensitivity of cell death to damage level (α) is shown in more detail in Fig. 7. The mean damage level decreases monotonically with this sensitivity (Fig. 7B), but the lifespan is longer than 10 years (Fig. 7A)—which is a feasible duration for a lab experiment, but only for a limited range of α. Below this range aging occurs because of the quick accumulation of damage, and above this range the mortality rate increases because too many cells die. Having a large size at maturity (M) decreases the hazard rate (Fig. 6C), and thus increases the lifespan of the *Hydra* (Fig. 7A) at the same values of α, although the mean level of damage is hardly affected (Figs. 6D and 7B).

We also tested the effect of the threshold proportion of the stem cells ω^*, which defines the costs of stem cells maintenance (Fig. 3D). A high value for this parameter means that a high proportion of the stem cells can be maintained without effort (i.e., that there is no reduction in the probability of division) by a small fraction of differentiated cells. We show the results for two cases, with $\omega^*=0.8$ (Fig. 7A and B) and $\omega^*=0.3$ (Fig. 7C and D). The proportions ω_0 of stem cells which give the highest average offspring production were assumed to be 0.8 for the first case and 0.64 for the second case. When there are high costs associated with maintenance of the stem cells, a short lifespan results from the whole range of damage sensitivities. For this case, the longest

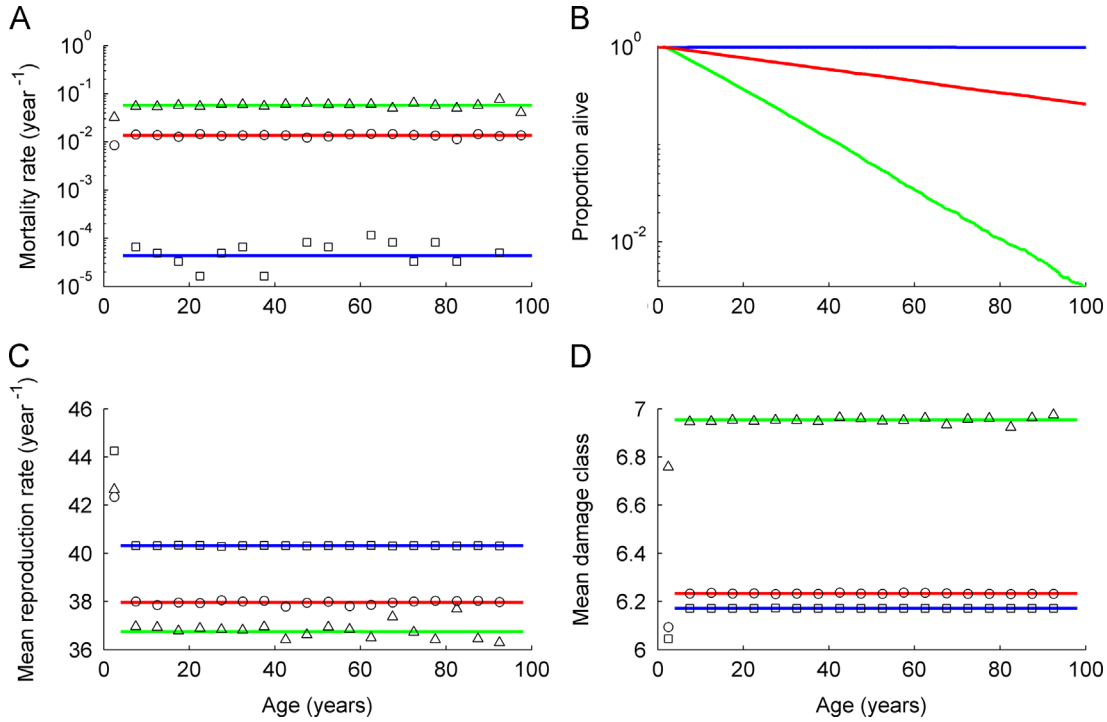

Fig. 6. Demographic parameters of three different hydra clones. Mortality rate (A), survivorship (B), the reproduction rate as the number of buds per year (C) and the mean damage class (D) for the first 100 years in three clones of *Hydra*. All clones had $\omega_0=0.8$, $\omega^*=0.8$. Symbols indicate five-year averages and lines indicate the fitting. I. Blue lines and squares: mature size $M=50{,}000$, damage sensitivity $\alpha=0.825$; II. Red lines and circles: $M=25{,}000$, $\alpha=0.825$; III. Green lines and triangles: $M=25{,}000$, $\alpha=0.75$. In (A), (C) and (D) annual data were fitted by constant and linear functions ((A) – maximum likelihood method, (C) and (D) – linear regression) and then model selection was performed ((A): likelihood ratio test, (B) and (C): *t*-test). In all cases slopes were not significant (*p*-Values for I, II and III in (A): 0.633,0.312, and 0.115; in (C): 0.64, 0.383, and 0.1318; in (D): 0.604, 0.406, and 0.165). Only the fitted constant functions are shown. Note that measures have not reached plateau in the first 5-year interval which was omitted in the model-fits.

lifespan, which is in the range of a few years, is achieved at the lowest damage sensitivities (Fig. 7C). When, by contrast, maintaining stem cells is cheap, the longest lifespan is achieved at intermediate values of damage sensitivity (Fig. 7A).

3.2. "Hydra in the field" model

So far we have considered only the intrinsic sources of mortality, and we have completely ignored the extrinsic death causes. However, *Hydra* evolved in an environment full of predators and other external death hazards. Environmental mortality is considered in the model as a constant factor independent of age and the level of damage. When extrinsic mortality is introduced, we can look for optimal values of damage-dependent cell death sensitivity α and the proportion of stem cells ω_0 which maximize fitness (Fig. 8). It is obvious that the higher the extrinsic mortality, the lower the expected production of offspring and the shorter the expected lifespan. There are, however, two interesting patterns. First, larger individuals of *Hydra* ($M=50{,}000$) not only have higher fitness levels than the smaller individuals ($M=25{,}000$), but their maximum fitness level appears at lower values of α; similarly, larger Hydra approach the maximum expected lifespan at lower α. Second, optimal α (which maximizes fitness that is measured as the lifetime offspring production) not only depends on M, but also decreases with increasing extrinsic mortality, as is analyzed in more detail in Fig. 9.

Up to now we have tested only one proportion of stem cells: $\omega_0=0.8$. In Fig. 9 we show lifespan and fitness under different levels of external mortality and different values of α and ω_0. With increasing levels of extrinsic hazard the optimal sensitivity of programmed death α decreases, but the optimal proportion of stem cells ω_0 does not change (Fig. 9, lower row). It seems that the optimal strategy is always at $\omega_0=0.8$, independent of extrinsic mortality. It is important to note that the value 0.8 is also a boundary proportion of stem cells; exceeding this value leads to the decrease in the division rate of the stem cells (see Methods and Fig. 3). Additionally, the range of plausible α is limited to a very narrow range – roughly between 0.6 and 0.9; outside of this range fitness is very low or even equal to zero. Suboptimal strategies can exist in a relatively broad range of ω_0 if α is low, but in a very narrow range of ω_0 if α is high.

The lifespan landscape has such a broad plateau at the top that it was not possible to identify values of parameters ω_0 and α that maximize the length of life (Fig. 9, upper row). These maximum values appear to be in the range of α 0.95–1.5 and ω_0 0.72–0.84. The maximum fitness level lies below this flat area for all of the extrinsic mortalities studies; that is, at lower values of α.The effects of other parameters on the model results are presented in the Supplementary materials.

4. Discussion

4.1. The non-senescence phenotype of Hydra

According to evolutionary theories of aging based on soma deterioration with age, a non-aging pattern can occur when maintenance mechanisms are able to prevent any kind of progressive accumulation of deleterious damage, or to keep damage at least at a long-term equilibrium (e.g., Cichoń, 1997; Kirkwood, 1977). Such efficient and constant maintenance mechanisms could lead to a constant mortality rate over the whole individual lifetime, and hence to a non-senescence pattern. Because *Hydra* polyps do not age, the question of how they prevent or remove any accumulation of damage or mutations with age arises.

In general, the damage response pathways (e.g., the DNA repair systems) seem to be very complex, and the differences between such pathways could contribute to known differences in longevity

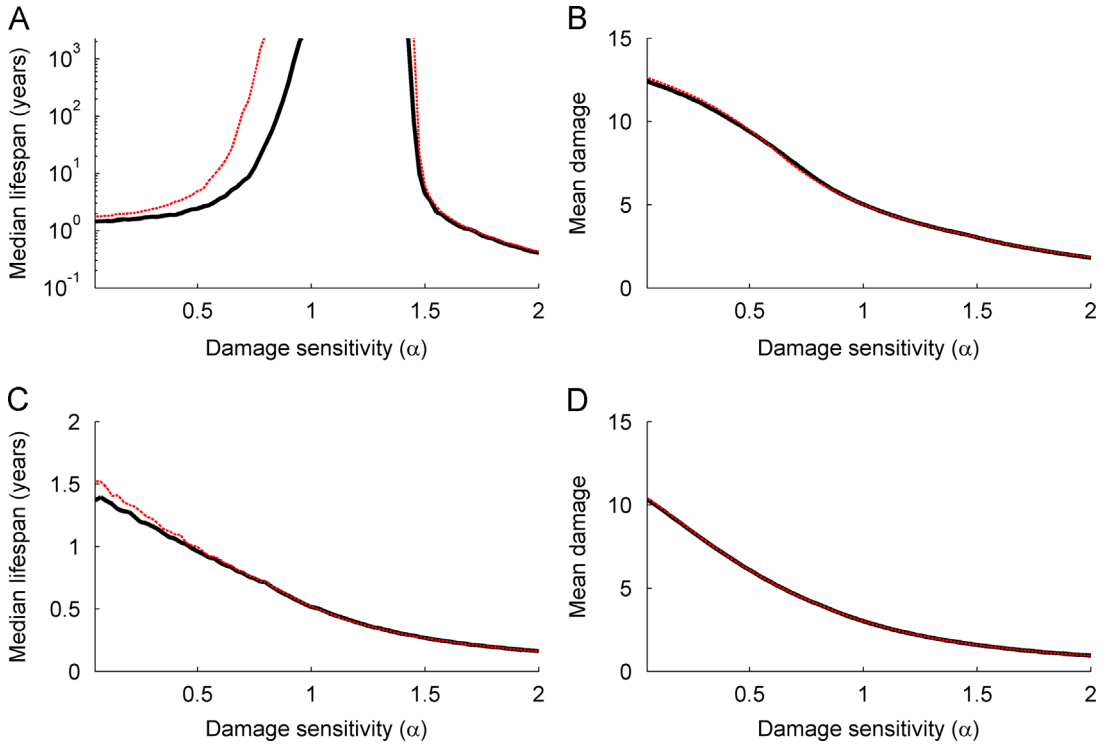

Fig. 7. Effect of damage sensitivity on median lifespan and mean damage. The effect of size at maturity (M) and damage sensitivity (α) on median life span ((A) and (C)) and the average damage class during life ((B) and (D)). Black solid thick lines: hydra with $M=25{,}000$ cells; red dotted lines: hydra with $M=50{,}000$ cells; (A) and (B): $\omega_0=0.8$ and $\omega^*=0.8$; (C) and (D): $\omega_0=0.64$ and $\omega^*=0.3$. In (A) the peaks for black and red lines lopped off because lifespan reached the limit of our simulation study being truncated at year 2740.

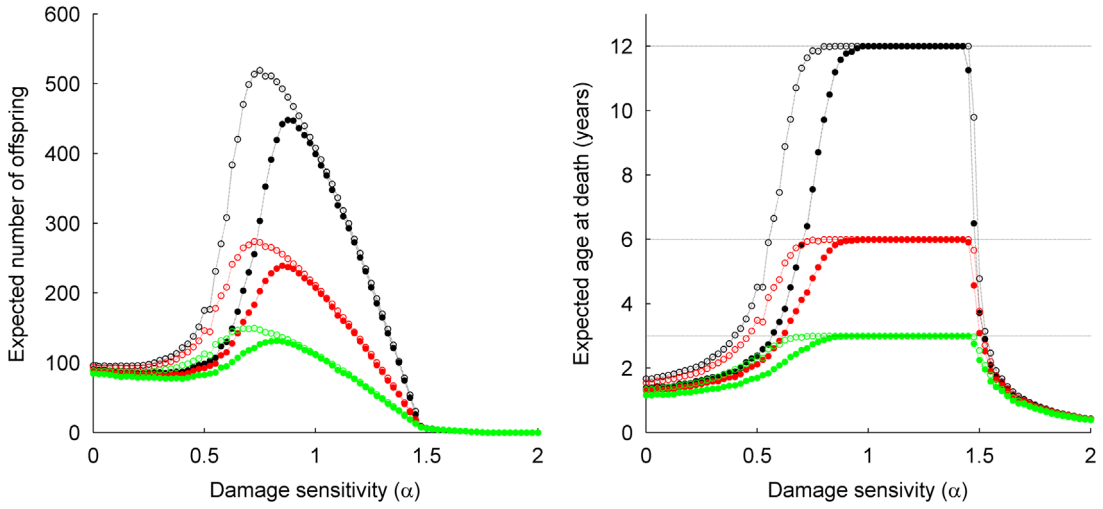

Fig. 8. Effect of damage sensitivity on fitness and age at death. Fitness (left panel) and expected age at death (right panel) in "*Hydra* in the field" model, taking into account extrinsic mortality, as the function of damage sensitivity α. Lines with open circles are for adult body size $M=50{,}000$, and lines with filled circles for $M=25{,}000$. External mortality rate equals 1/12 (year^{-1}) for black lines, 1/6 for red lines, and 1/3 for green lines. The three dotted horizontal lines in the right panel represent expected age at death for the case when only extrinsic mortality would operate. $\omega_0=0.8$ and $\omega^*=0.8$ for all lines.

and aging between mammal species (Freitas and de Magalhaes, 2011). As the authors noted, a very efficient DNA repair mechanism is necessary to avoid damage accumulation in cells and to preserve DNA integrity. However, even if such efficient repair pathways could postpone aging (see Freitas and de Magalhaes (2011)), it is not likely that within-cell damage repair optimization is involved in non-senescence pattern. As these kinds of mechanisms have been shown to have limited efficiency (Blanpain et al., 2011; Seluanov et al., 2004), the accumulation of damage would be inevitable within cells. Furthermore, efficient within-cell repair in *Hydra* may be not beneficial if we take into account the extraordinary cell dynamics of *Hydra*. These cell dynamics, combined with other unique features of *Hydra*, allow them to deal efficiently with damage on a higher level using the mechanisms of cell selection. Indeed, these unique characteristics of the simple body plan of a *Hydra* forms the basis of our model assumptions; including the high proportion and number of stem cells per polyp, the continuous division of stem cells, and the continuous removal of cells from the polyp. The results of our model confirm our expectations about the importance of the selective removal of damaged cells as an

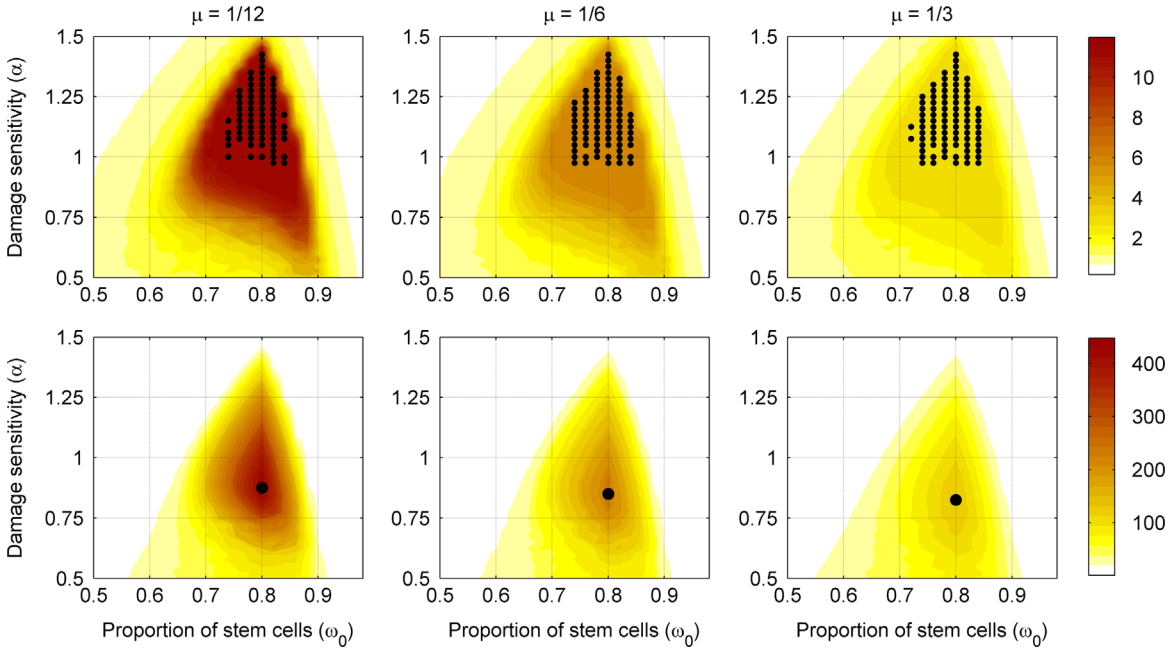

Fig. 9. Lifespan and fitness landscapes. The expected age at death (upper row) and fitness (bottom row) landscapes for extrinsic mortalities $\mu = 1/12, 1/6$, and $1/3$ (year^{-1}), as the functions of damage sensitivity α and the proportion of stem cells ω_0. The black dots in the bottom row represent the position of maximum fitness. The landscape for the expected age at death (upper row) is so flat at the top that many combinations of α and ω_0 give numerically undistinguishable values; sets of drops represent the region with maximum lifespan. Other parameters: $M = 25{,}000$, $\omega^* = 0.8$.

alternative mechanism to within-cell repairs for repairing and preventing long-term damage accumulation and cellular damage drift. We believe that this is the only possible mechanism that can lead an individual *Hydra* to achieve a very low and constant mortality rate with age, as well as a constant rate of reproduction.

4.1.1. *Selective removal of damaged cells as an alternative mechanism to prevent damage accumulation*

The damage occurring in one cell is likely to be transferred to many other cells through constant cell division. As a result, the number of damaged cells may increase, and the damage may eventually become fixed in the cell population of a single polyp in a process of cellular damage drift. Such a scenario is possible because most of *Hydra* cells divide constantly and frequently (for interstitial stem cells see David (2012); for epithelial cells see Bosch and David (1984)), and because at each subsequent cell division further damage may accumulate. The idea of cellular damage drift has support from empirical research. Recent lineage-tracing studies have shown that in several tissues with high cell turnover, the balance between proliferation and differentiation is achieved by frequent and stochastic stem cell loss and replacement. In such cases, the lifespan of the individual stem cells is not defined, which leads to the neutral drift of a number of stem cells per clone (Doupe et al., 2010; Klein and Simons, 2011). The mechanism of this process is analogous to the processes of genetic drift and Muller's ratchet. This cellular analog of neutral drift can to some extent be controlled by selection processes that remove deteriorated cells. Without these processes, we would expect to see random accumulation of damaged cell lineages in the organism. Indeed, the results of our model support our suggestion, and clearly show the importance of selective cell removal for the non-senescence phenotype (compare Figs. 4 and 5). There is a clear relationship between cell death sensitivity and the amount of accumulated damage (Fig. 7B and D). Insufficient selection leads to a pattern characterized by damage accumulation and senescence (Fig. 4). On the other hand, if cell death due to this kind of selection is higher than the creation of new cells through cell division, then a polyp shrinks (its cell number decreases), its reproduction rate declines, and it eventually dies. We have noted that the optimization of the selection level (the damage sensitivity) allows for such a long life in laboratory conditions that we can speak of practical "immortality". The selection of the cells can only suppress the drift of damaged cells. Random processes are, however, unavoidable. As a result, we observe an exit from the damage-selection equilibrium that it is followed by the entry of individuals into the senescence path, as exemplified by the thin lines in Fig. 5. It must be stressed that this exit from a state of equilibrium is a random and age-independent process: the exemplary individual died at the age of roughly 315 years, but some modeled individuals were still alive at the age of a thousand years.

Having an extremely long lifespan cannot be an adaptation limited to tiny organisms like the *Hydra*, which is prone to ecological risks of death. It is rather a side effect of special features of *Hydra* that offers this organism a different way of dealing with damage. Nevertheless, environmental factors to some extent shape the non-senescence phenotype, by, for example, modulating the optimal sensitivity for the selection of damaged cells. Our results from the "*Hydra* in the field" model support such a conclusion. The lower panel in Fig. 9 shows that the optimal damage sensitivity of cell death drops with the increase in the mortality rate. This is reasonable because in a highly hazardous environment it is better to allocate more resources to reproduction—or budding in the case of the *Hydra*—at the cost of keeping the soma in good shape (e.g., Cichoń, 1997). While it is obvious that the average age at death decreases as mortality increases, a plateau at this age is reached at the same damage sensitivity, independent of the external mortality level (Fig. 8, right panel). The upper panel in Fig. 9 shows that the maximum of the average age at death does not move on the damage sensitivity proportion of the stem cells plane if the external mortality changes.

4.1.2. *The number and the proportion of stem cells within a polyp*

The cellular damage drift, like its genetic equivalent, is a random process that strongly depends on the population number (here, the number of cells per individual (Figs. 7 and 8)). If the cell number is too small, the effect of cellular damage drift is larger; whereas if the cell number is very large, the effect of the drift is smaller. This assumption is supported by three main results: the

higher levels of survivability and expected offspring production, as well as the lower optimal level of selection against the damaged cells in larger *Hydra*. Interestingly, the mean damage class is almost independent of the size at maturity (Figs. 6D, 7B, and D). This means that *Hydra* of two different sizes reach similar plateaus of a mean amount of damage in cells (Fig. 6D) even if the mortality plateau (Fig. 6A) is very different. This is reasonable, as the selection processes and the accumulation of damage are independent of the cell number, and only the rate of damage fixation can be affected.

We simulate the dependency of cellular damage drift and stem cell population size using one polyp with a small stem cell population size and another polyp with a large stem cell population size (M). The results support the hypothesis: the polyp with larger number of stem cells had higher survivability and expected offspring production, as well as a lower optimal level of selection against damaged cells. We should note here that the offspring size is assumed to be proportional to the maturity size, so we can easily determine the effect of cellular-damage drift on *Hydra* of different sizes. Interestingly, the amount of damage in cells is independent of the size of a polyp (Figs. 6D, 7B and D), even if the mortality rate is very different (Fig. 6A). The higher mortality level for smaller *Hydra* indicates the importance of an efficient selection against damaged cells, because the smaller the size of the polyp the higher the chances of the accumulation of damage through drift and the entry into the senescence path.

In our model the non-senescence phenotype was possible only for the narrow range of *Hydra* with high proportions of stem cells (Fig. 9). This high proportion of stem cells in *Hydra* is possible because *Hydra* stem cells can play additional roles in the organism that are originally reserved to differentiated cells; thus, the maintenance conducted by additional cells is not necessary, and it appears that *Hydra's* stem cells are at least partially self-supporting. Without these special stem cell functions, the costs of supporting these large populations would be too expensive and consequently would negatively affect fitness. Our results suggest that if the cost of stem cell maintenance increases, the potential "immortality" cannot be reached, as there are not enough stem cells in a body to prevent cellular damage drift (Figs. 3D and 7).

4.2. Why is Hydra able to avoid senescence, while other organisms are not?

In the paper we proposed a model that is specific to *Hydra*, because *Hydra* is one of very few species in which a non-senescence pattern—i.e., no change in the probability of death and in the fertility rate with age—is credibly validated (Jones et al., 2014; Martinez, 1998). Although Jones et al. (2014), in their study of aging patterns across the tree of life, have given other examples of non-aging species, all of these species have constant and relatively high age-specific mortality rates which result in short lifespans. These high mortality rates are likely to come from background (field) sources of death, and thus mask original intrinsic patterns related to senescence.

First, *Hydra* has a simple body plan, which, with respect to cell dynamics, can be seen as a "flow" system. In *Hydra*, divisions of multifunctional stem cells lead to a doubling of cell populations in around 20 days (Hobmayer et al., 2012). Most *Hydra* epithelial cells proliferate and migrate along the body axis, and are eventually removed by cellular death, differentiation, and budding. If a stem cell population behaved this way in humans, we would observe uncontrolled cell agglomeration, which would compromise organ function. Even in a constantly growing organism (e.g., indeterminate growers) both the proliferation and removal of cells can be structurally limited. This is especially true for organs built with many highly specialized and terminally differentiated cells (e.g., the brain). The physical structure of such organs limits the possibility of the presence of a high proportion of continuously dividing cells, as there is simply no place for them or their activities. These organs must rely on a small number of quiescent stem cells that work on demand, when there is a need for more differentiated cells.

Second, a whole polyp is made up of three continuously proliferating stem cell populations characterized by a very short or no quiescent state, and which consequently have a unique functionality. The behavior of stem cells is very different in non-growing organisms, and is closely related to their niche. In *Hydra* it is easy to delineate a niche for interstitial stem cells (David, 2012), but the determination of the niche's dimension or composition seems very difficult for epithelial cells (Hobmayer et al., 2012). However, Bosch et al. (2010) suggested that the whole body column of the polyp may be considered a single large stem cell niche. Within the niche, stem cells are grouped around the mesoglea, an extracellular matrix, and the whole stem cell community is regulated by the common signals (e.g., Boettger et al., 2006; Takahashi et al., 2000). The capacity for continuous stem cell renewal within the niche and for escaping cellular damage drift due to a high proportion and number of cooperating stem cells guarantees the tissue homeostasis, growth, and budding of the whole polyp. But among mammals—including among those with high levels of cell division—stem cells occur in small numbers in well-defined niches, and mostly remain in a quiescent state (Alonso and Fuchs, 2003; Arai and Suda, 2008; Casali and Batlle, 2009; Morrison and Spradling, 2008). In such a case, the differentiation and regeneration of the somatic tissues is realized exclusively by differentiating the progenitor/transit-amplifying cells with limited numbers of divisions (e.g., Doupe et al., 2010; Rando, 2006), but not by the stem cells themselves.

Third, while *Hydra* can have an extremely high proportion of stem cells (Bode et al., 1973), this share is impossible to achieve in other organisms. The high proportion of stem cells is possible because huge part of stem cells in *Hydra* (i.e. epithelial stem cells) are less specialized than they are in more complex organisms, and are capable of performing several physiological or structural functions, like forming an epithelial cell layer and determining the polyp's shape and morphogenesis (e.g., Technau and Steele, 2011). Such functions in complex organisms (e.g., humans) are reserved for differentiated cells. Moreover, the stem cell numbers must be small in complex organisms because supporting a large population would be too expensive and have a negative effect on fitness. The multifunctionality of many *Hydra* stem cells decreases the costs of their maintenance as the presence of many additional specialized cells is no longer necessary.

Furthermore, the proportion and the number of stem cells are limited in the complex organism by the complexity itself. In vertebrates, the functions undertaken by *Hydra's* stem cells beyond supplying new cells are spread between different tissues. Complex organisms are equipped with many heterogenic tissues in which the differences in the stem cell turnover rates are huge (Rando, 2006). Rando (2006) classified human tissues by the rate of cell turnover and regenerative potential. He distinguished three non-strict classes for mammals: (i) tissues with high stem cell turnover and high regenerative potential; e.g., blood, skin, and gut; (ii) tissues with low stem cell turnover, but still high regenerative potential; e.g., skeletal muscle and liver; and (iii) tissues with low stem cell turnover and limited regenerative potential, e.g., brain, heart, and kidney. With its capacity for continuous proliferation, constant migration across the body, and regeneration, a *Hydra* stem cell system is most similar to that of the blood, skin, and gut stem cell systems of mammals. Nevertheless, all of these tissues, unlike those of the *Hydra*, undergo senescence.

Fourth, continuous cell proliferation, migration, differentiation, and death are prerequisites for a successful mechanism for preventing damage accumulation by the selective removal of damaged cells. In complex organisms with many different and heterogeneous tissues, there must be different ways of dealing

with damaged cells and different abilities to self-renew. In tissues of limited regenerative potential (e.g., the brain) cell divisions are rare, and the cells mostly stay in the quiescence stage. The cell selection mechanisms in these tissues appear to be ineffective, this is likely because an intensive selection for damaged cells would be very risky, as it might compromise tissue function. Even tissues with a high regenerative potential and a high cell turnover rate (e.g., skin, blood, and gut) are at risk of cellular damage drift because the stem cell populations localized in the specific niches are still small in number; and in many cases, when the cells are exhausted or heavily damaged, they have rather limited support from other niches (see reviews by Goodell (2003), Herzog et al. (2003), and Wagers and Weissman (2004)). In addition to these physical limitations, it may be the case that in the more complex organisms the possibility of the migration of different stem cells between niches is costly from the perspective of the survival of the whole body because the same mechanism may be used by tumor cells to chemo-attract them to different organs (e.g., lymph nodes, lungs, liver, or bones; see Kucia et al. (2005) for review).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2015.06.043.

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