Intercellular competition and the inevitability of multicellular aging

Paul Nelson[a,1] and Joanna Masel[a]

[a]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

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Current theories attribute aging to a failure of selection, due to either pleiotropic constraints or declining strength of selection after the onset of reproduction. These theories implicitly leave open the possibility that if senescence-causing alleles could be identified, or if antagonistic pleiotropy could be broken, the effects of aging might be ameliorated or delayed indefinitely. These theories are built on models of selection between multicellular organisms, but a full understanding of aging also requires examining the role of somatic selection within an organism. Selection between somatic cells (i.e., intercellular competition) can delay aging by purging nonfunctioning cells. However, the fitness of a multicellular organism depends not just on how functional its individual cells are but also on how well cells work together. While intercellular competition weeds out nonfunctional cells, it may also select for cells that do not cooperate. Thus, intercellular competition creates an inescapable double bind that makes aging inevitable in multicellular organisms.

negligible senescence | cellular degradation | cellular robustness | cooperation | cancer

Biological aging is defined as a loss in organismal fecundity and/or increase in mortality with age (1). Mutations that increase mortality early in life will have profound fitness consequences and are likely to be purged by selection (1, 2). However, mutations that affect mortality later in life, after reproduction, fall under an ever-darkening "selection shadow" (i.e., progressively relaxed selection) that allows them to fix in a population due to drift (3, 4). Additionally, antagonistic pleiotropy, in which genes that increase fitness components early in life also decrease fitness components later in life, can result in aging as a side effect of selection for early reproduction (5, 6). Both mutation accumulation and antagonistic pleiotropy contain an implicitly optimistic message regarding the potential to ameliorate aging: There exist "longevity genes" for which aging and nonaging alleles are possible. Eliminating aging, according to these two theories, is therefore a practical challenge rather than a fundamental impossibility.

Whereas mutation accumulation and antagonistic pleiotropy theory address the role of organismal selection in aging, we ask here whether aging is a fundamental and intrinsic feature of multicellular life. For an organism to avoid aging, it must overcome or mitigate the consequences of mitotically heritable changes in somatic cells, the vast majority of which are deleterious, and hence best thought of as "damage." Heritable cellular degradation is a product not just of somatic mutations (7) but also of other changes, such as epigenetic drift (8) and the accumulation of misfolded proteins (9). In unicellular organisms, competition between cells can weed out deleterious heritable changes, allowing a population to exist indefinitely despite individual degradation (10). Just as competition between individuals can eliminate deleterious alleles from a unicellular population, competition between cells within a multicellular organism can weed out malfunctioning, slower growing cells within an organism (11–15). Therefore, intercellular competition seems to hold the potential for immortality; by continually eliminating damaged cells, a multicellular organism might persist in perpetuity (16) if only selection to do so were somehow strong enough.

Aging in multicellular organisms occurs at both the cellular and intercellular levels (17). Multicellular organisms, by definition, require a high degree of intercellular cooperation to maintain homeostasis. Often, cellular traits required for producing a viable multicellular phenotype come at a steep cost to individual cells (14, 18, 19). Conversely, many mutant cells that do not invest in holistic organismal fitness have a selective advantage over cells that do. If intercellular competition occurs, such "cheater" or "defector" cells may proliferate and displace "cooperating" cells, with detrimental consequences for the multicellular organism (20, 21). Cancer, a leading cause of death in humans at rates that increase with age, is one obvious manifestation of cheater proliferation (22–24).

The role of intercellular competition in the proliferation of cheater cells is illustrated by organisms that have different levels of intercellular competition in different tissues. Cells of the nematode *Caenorhabditis elegans* are terminally differentiated at birth, precluding intercellular competition, except for germ-line cells. Consequently, cancers in *C. elegans* are limited to the indeterminately dividing germ-line cells, and somatic tissues remain cancer-free (25). Similarly, cells in *Drosophila melanogaster* do not divide after a fly reaches adulthood, save for the gut and germ-line cells. As expected, while larval *D. melanogaster* can develop cancers in several tissues, cancers in adults are relegated to the gut and absent from tissues where cell turnover is limited or nonexistent (26, 27).

Thus, intercellular competition proves to be a double-edged sword; competition can remove damaged cells, but competition can also allow cheating cells to prosper (14). Here, we derive a general model of the effect of somatic evolution on aging and examine the behavior of a related model of discrete genotypes in simple numerical cases. Aging is characterized by the dual, but seemingly contradictory, features of loss of cellular vigor and uncontrolled cell growth (17), and we model the evolution of two

Significance

We lay out the first general model of the interplay between intercellular competition, aging, and cancer. Our model shows that aging is a fundamental feature of multicellular life. Current understanding of the evolution of aging holds that aging is due to the weakness of selection to remove alleles that increase mortality only late in life. Our model, while fully compatible with current theory, makes a stronger statement: Multicellular organisms would age even if selection were perfect. These results inform how we think about the evolution of aging and the role of intercellular competition in senescence and cancer.

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[1]To whom correspondence should be addressed. Email: pgnelson@email.arizona.edu.

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corresponding cellular traits. First, we use the term "vigor" to reflect general cellular function or metabolic activity. Cells that lose vigor due to cellular damage are "senescent." Second, we use the term "cooperation" to represent investment in traits that are costly to the cell but beneficial for the organism as a whole; one manifestation of loss of cooperation is an increased propensity toward cancer. We show that intercellular competition produces a double bind resulting in inevitably declining organismal vitality with age in multicellular organisms.

Methods

Multicellular vitality (i.e., the general health and overall condition of an organism) is a function of both the fraction of cellular output devoted to sustaining the multicellular organism (cellular cooperation, c) and the total amount of output a cell can muster (cellular vigor, v). A central assumption of our work is that the distinction between c and v represents a natural categorization of cellular traits; that is, somatic mutations or other cellular degradation events tend to primarily affect only one of the two traits: cellular cooperation in the case of mutations to tumor suppressors and oncogenes and vigor in the case of basic cellular metabolism and other internal housekeeping functions. Cells with low vigor are a small burden on the organism (e.g., refs. 28, 29) but can be eliminated via intercellular competition. Cells with low cooperation, which decline to invest in traits that are costly to the cell but beneficial to the multicellular organism, have an advantage during intercellular competition but do not contribute to multicellular vitality.

We use the probability distribution of effects of somatic mutations and other cellular degradation events to construct a coordinate system for describing cell (epi) genotypes (Fig. 1). Each cell is identified by its cellular competitive potential, f (described in more specific detail below), and contribution to organism vitality, z (compatible with a variety of quantitative interpretations). The probability distribution of the effects of a new somatic mutation or other heritable change is assumed to be a function of a cell's current values of f and z. We use the expected effect conditional on the sign of change in f and z to define a pair of vector fields capturing the path of degradation:

$$\vec{v}(f,z) = E_{f,z}[(f'-f, z'-z) | f' < f, z' < z],$$

$$\vec{c}(f,z) = E_{f,z}[(f'-f, z'-z) | f' > f, z' < z].$$

An $\{f,z\} \rightarrow \{v,c\}$ map can be defined over the entire space of genotypes $\{f,z\}$, provided that these vector fields vary in a smooth and consistent manner ($\nabla \vec{v} \cdot \vec{c} = \nabla \vec{c} \cdot \vec{v}$). The existence and construction of such a map follow from two well-known mathematical theorems: the Picard–Lindelöf theorem (30) and Frobenius' theorem (31).

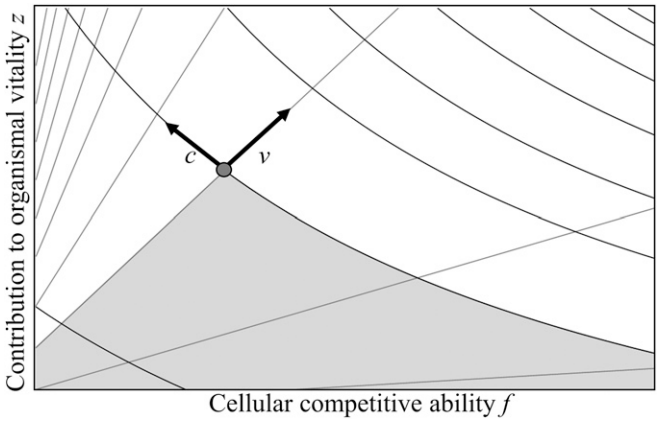

Fig. 1. Illustrative example of a $\{f,z\} \rightarrow \{v,c\}$ mapping, showing contour lines of equal v in black and equal c in gray, with arrows showing the direction of positive change. A degradation event of average size, and affecting only v or only c, moves a genotype down by exactly one contour line. Robustness to senescence and cancer is illustrated by lines that are more closely spaced at the beginning of the degradation process than at the end. Robustness to cancer is also illustrated by curvature in the contour lines showing changing c with constant v. Our argument assumes that mutations tend to affect only v or only c, and the map is constructed in such a way as to maximize the extent to which this is true. We also assume the absence of strong positive degradational covariance. From the genotype shown with a dot, degradation events in the gray region contribute to such covariance (as do those in the sector above, in which far fewer mutations are found).

In this study, we use the Price equation to describe in a general way, within a $\{v,c\}$ coordinate system, the effects of intercellular competition over an organismal life span. We then generate illustrative numerical results using a related system of discrete genotypes.

Cellular Fitness. Let $g(t)$ be the growth per developmental time step of the number of cells, $N(t)$, in an organism. We assume that this is developmentally programmed independently from the set of cellular genotypes. When $g(t) = 1$, the number of cells is not changing; a constant value of $g(t) > 1$ indicates that cells are reproducing exponentially. We divide somatic cells into two groups: a fraction $\alpha(t)$ undergoing genotype-dependent turnover at time t, and therefore subject to natural selection between somatic cells, and the remaining fraction $1 - \alpha(t)$ whose growth or decline $g(t)$ is independent of cellular genotype. Genotype dependence of turnover can result from either differences in growth, as with rapidly dividing cancer cells, or differences in survival, as with cells that avoid entering apoptosis. The value of $\alpha(t)$ may change over time, for example, in response to organismal development $g(t)$ or to changes in cellular genotypes, and may be different in different tissues.

Following one developmental time step of somatic growth and selection (but not yet somatic mutation or other heritable changes), the number of cells, n^*_{ij}, of vigor state i and cooperation state j is:

$$n^*_{ij}(t+1) = g(t)\left(\alpha(t)\frac{f_{ij}}{\bar{f}} n_{ij}(t) + (1-\alpha(t)) n_{ij}(t)\right), \quad [1]$$

where $\bar{f} = 1/N \sum_{ij} n_{ij} f_{ij}$ is the mean cellular competitive ability of cells in an organism. We define cellular absolute fitness, w_{ij}, as the expected contribution of one cell of genotype $\{i,j\}$ to the next generation due to organismal growth and somatic selection:

$$w_{ij} = \frac{n^*_{ij}(t+1)}{n_{ij}(t)} = g(t)\left(\alpha(t)\left(\frac{f_{ij}}{\bar{f}} - 1\right) + 1\right), \quad [2.1]$$

which yields mean cellular absolute fitness:

$$\bar{w} = \frac{1}{N}\sum_{ij} n_{ij}(t) w_{ij} = g(t). \quad [2.2]$$

Somatic Evolution of Traits. The expected change per developmental time step in organismal mean values of v and c that stems from spontaneous changes in cellular genotype, given that the number of cells that can potentially degrade after selection is $n_{ij}w_{ij}$, is then:

$$\overline{w\Delta v} = \frac{1}{N}\sum_{ij} n_{ij} w_{ij} \sum_{pq} (v_p - v_i)\mu_{ij\rightarrow pq}, \quad [3.1]$$

$$\overline{w\Delta c} = \frac{1}{N}\sum_{ij} n_{ij} w_{ij} \sum_{pq} (c_q - c_j)\mu_{ij\rightarrow pq}, \quad [3.2]$$

where $\mu_{ij\rightarrow pq}$ is the probability that a cell of genotype $\{i,j\}$ switches to genotype $\{p,q\}$ during one developmental time step, assuming cells switch genotype independently. This $\mu_{ij\rightarrow pq}$ includes somatically heritable changes due to somatic mutation, accumulation of misfolded proteins, and/or epigenetic drift occurring with or without cell division.

The Price equation gives the change $\Delta\bar{z}$ in the mean value of a trait z as a function of the absolute fitness, w_a, of cells of genotype a: $\bar{w}\Delta\bar{z} = \mathrm{cov}(z_a, w_a) + \overline{w\Delta z}$ (32). The rightmost term, $\overline{w\Delta z}$, incorporates any factors that may affect the change in trait z other than direct selection, which is mutation in our case. Here, we use the Price equation to track the changes in mean value of trait values between somatic cells within an individual, as opposed to between multicellular organisms. Somatic changes are not inherited by offspring; therefore, our model does not require multilevel selection of the kind detailed by Frank (33).

Incorporating cellular fitness w_{ij} (Eq. **2.1**) and spontaneous change in cell genotype $\overline{w\Delta v}$ and $\overline{w\Delta c}$ (Eqs. **3.1** and **3.2**) into the Price equation, after some simplification, yields the expected changes in mean organismal cellular vigor $\bar{v}$ and cell cooperation $\bar{c}$:

$$\Delta\bar{v} = \alpha(t)\frac{1}{\bar{f}}\mathrm{cov}(v_i, f_{ij}) + \frac{1}{g(t)}\overline{w\Delta v}, \quad [4.1]$$

$$\Delta\bar{c} = \alpha(t)\frac{1}{\bar{f}}\mathrm{cov}(c_j, f_{ij}) + \frac{1}{g(t)}\overline{w\Delta c}. \quad [4.2]$$

Organismal Fitness. In populations with overlapping generations, the fitness of an organismal genotype corresponds to the Malthusian growth rate, r, of the population of that genotype; this captures the declining force of selection later in life, known as the selection shadow (2). Given organismal fecundity $b(t)$ at time t, and survival $s(t)$ to time t, which are monotonic functions of the values of z for each cell in the organism, the Malthusian fitness, r_x, of an organismal genotype x is obtained by solving (2):

$$1 = \sum_{t=0}^{\infty} e^{-r_x t} l b(t) s(t), \quad [5]$$

where l is a constant that scales the absolute number of offspring born to the time step size of cellular degradation and turnover. For a given developmental time step length, high l indicates rapidly reproducing organisms and discounts the effect of late life cellular degradation on organismal fitness, while low l allows organisms' late life vitality to contribute more to fitness. It is important to note that we calculate the Malthusian fitness r in the absence of any density-dependent (but potentially organismal genotype-independent) ecological feedbacks. In other words, a positive or negative r is not meant to be taken as an assertion that a population is increasing or decreasing exponentially; instead, it is merely a tool to compare the fitness of one organismal genotype with that of another.

Numerical Model. We refer to cells that have lost general metabolic function or vigor v as senescent and cells that have lost intercellular cooperation c as "cancerous." Because multiple mutations are usually required for the initiation of cancer (34) or loss of cellular function, we compare a one-hit model, in which cells become cancerous or senescent after a single degradation event, with a two-hit model, in which two mutations are required for a cell to lose function of that trait. We assume that cells only degrade one genotype class at a time, and movement into lower vigor and cooperation classes occur at rates μ_v and μ_c, respectively, for all genotypes $\{i,j\}$ that are not yet completely degraded. Each mutation affects only one of the two traits (an extreme case of our general assumption of low pleiotropy between v and c). Cells that are functional in both traits have $z_{ij} = 1$, while cells that have lost function in either trait have $z_{ij} = 0$. This makes $\mu_{i,j\to i+1,j} = \mu_v$ and $\mu_{i,j\to i,j+1} = \mu_c$.

We simplify our model by assuming that organisms are not growing ($g = 1$). Organismal fecundity is:

$$b(t) = \frac{1}{N}\sum_{ij} n_{ij}(t) z_{ij}. \quad [6]$$

Death is assumed to occur [$s(t)$ is set to 0] once $b(t)$ falls below a threshold value. This procedure assumes that somatic evolution has a deterministic effect on fertility, making Eq. **5** convenient to use, but the same principles also apply to cases where the fraction of functional cells affects mortality rates, either deterministically or stochastically.

Parameter values were chosen to yield reasonable organismal Malthusian fitness values ($-1 < r < 1$). Unless otherwise specified, we use $k = 0.5$, $l = 0.001$ (one offspring expected per 1,000 developmental time steps), $\mu_v = 10^{-3}$, following the observation that ~1% of human genes affect cancer risk (35), $\mu_c = 10^{-5}$. An exploration of rates of cellular degradation is provided in Rates of Cellular Degradation. When an organism is born, all cells are assumed to be fully functional.

We simulate a life span according to Eqs. **1** and **6**, and numerically solve Eq. **5** for r (available at https://github.com/pgnelson/Intercellular-Competition-and-aging). We assume a constant value of α for all developmental times t and numerically find values of α that maximize r.

Results

Universality of Multicellular Aging. In our model, loss of cellular functionality z and, hence, aging over time can arise from two sources, loss of cellular vigor v and loss of intercellular cooperation c, as described by Eqs. **4.1** and **4.2**, respectively. Because most spontaneous changes are deleterious to the organism, and because most load either primarily on v or primarily on c, it follows that $\overline{\Delta v}$ and $\overline{\Delta c}$ are negative, as are $\overline{w\Delta v}$ and $\overline{w\Delta c}$. Therefore, whenever $\alpha(t) = 0$, Eq. **4.1** yields $\Delta\bar{v} < 0$, and whenever $\alpha(t) > 0$ and $\mathrm{cov}(c_j, f(v_i,c_j)) < 0$, Eq. **4.2** yields $\Delta\bar{c} < 0$. Because cellular competitive ability f is, by construction, a monotonically decreasing function of c, the only way to get cov $(c_j, f(v_i,c_j)) > 0$, and hence $\Delta\bar{c} > 0$, is via strong positive covariance between v and c. Otherwise, whenever $\alpha(t)$ is positive and there is variance in c, any decrease in c is permanent.

In other words, when $\alpha > 0$, Eq. **4.2** acts as a ratchet to decrease $\bar{c}$. This will translate into decreases in organismal fitness $\bar{z}$ unless increases in $\bar{v}$ are so large as to compensate. Compensation is impossible if, for a given value of $\Delta f > 0$, there are more mutations available that achieve this increase by decreasing c than there are mutations that achieve it by increasing v. Given a history of multicellular selection that has already favored high v and high c, this assumption is biologically reasonable; it is easier to break the adaptive product of selection than to improve upon it.

If cells do not compete ($\alpha = 0$), as in organisms with nondividing adult somatic cells, senescent cells are not replaced and organismal vitality declines with age because Eq. **4.1** yields $\Delta\bar{v} < 0$. If cells compete ($\alpha > 0$), senescent cells can be replaced but noncooperative cells can proliferate and the organismal vitality declines with age because of loss of intercellular cooperation (i.e., cancer).

A core assumption in this argument is that there is little or no positive covariance between vigor v and cooperation c. A positive covariance means an overrepresentation of cells that are both cancerous and senescent, which would allow intercellular competition to increase both mean cell cooperation and vigor in a synergistic manner. Covariance between vigor and cooperation can accumulate either due to "degradational covariance" (the equivalent of mutational covariance, introduced by heritable somatic mutations and other degradation events) or to covariance that is driven by somatic selection.

A build-up of negative covariance is an inherent feature of intercellular competition (i.e., somatic selection) from a uniform and high-functioning starting point. Given that most mutations affect either v or c but not both, single degradation events initially lead to either senescent/cooperative cells or vigorous/cancerous cells, and therefore create small amounts of negative covariance. Intercellular competition then affects the trajectory of the negative covariance initially created by single mutations. Senescent/cooperative cells are removed by competition, and so any negative covariance they create is short-lived. However, competition causes vigorous/cancerous cells to proliferate, amplifying the initially trivially small amount of negative covariance created by mutation. Double mutants (senescent/cancerous cells), which create positive covariance and might cancel this effect out, can also proliferate under intercellular competition. However, selection favoring senescent/cancerous cells is weaker than selection favoring vigorous/cancerous cells; thus, amplification of positive covariance due to selection is less than any amplification of negative covariance, leading to a net negative covariance between v and c.

This selective force promoting negative covariance between v and c could, in principle, be countered by sufficiently large and positive degradational (mutational) covariance. Recall that we have constructed v and c as the paths close to which most mutations fall. By construction, a mutation falling exactly on this path contributes nothing to covariance. Positive covariance arises when there are more mutations between these two vectors than there are outside them (Fig. 1). While the distribution of effects of somatic mutations is generally unknown, there is no a priori reason to expect a sufficiently large overrepresentation of mutations contributing positively to covariance to overwhelm the selective force. However, at a higher level of organization, cell stratification may temporarily create large and positive degradational covariance (we return to this in *Discussion*).

Note that the degree $\alpha(t)$ to which cells compete might not be constant. For example, intercellular competition may be higher during periods of rapid growth [high $g(t)$], such as the expansion in cell number from zygote to adult. Changing values of intercellular competition over time (or between tissues) may evolve as an adaptation to delay aging. If cancer is already present and poses a greater short-term risk than senescent cells [i.e., if $|\mathrm{cov}(c_i, f(v_i,c_i))| >> \mathrm{cov}(v_i, f(v_i,c_i))$], decreasing intercellular competition may slow their proliferation, extend life span, and increase fitness. Conversely, when cancerous cells are absent from a tissue, increasing intercellular competition is a safe way to purge senescent cells. However, as described above, any tissue at any point in time must always suffer a decline in either cellular vigor or cooperation; facultatively changing the level of intercellular competition can, at most, slow the loss. Note that $\alpha(t)$ might also change for nonadaptive reasons; with cancer, some cells go beyond simple loss of cooperation and actively alter their extracellular environment to facilitate their own growth (36).

Numerical Illustration. We use a simple model of four somatically heritable cell states (healthy, senescent, cancerous, and both cancerous and senescent) to numerically illustrate the double bind. Without intercellular competition and with the rate of cancer-promoting changes set to zero (Fig. 2*A*), senescent cells accumulate (gray), depleting the population of functional cells (black). Intercellular competition (Fig. 2*B*) effectively purges these senescent cells, yielding a mutation-selection equilibrium that maintains a population of functional cells indefinitely. Conversely, with the rate of mutations promoting cell senescence set to zero, cancer-causing somatic changes have limited impact in the absence of intercellular competition (Fig. 2*C*, black diagonal stripes) but cancerous cells can proliferate when cells compete, eventually depleting the pool of functional cells (Fig. 2*D*). When organisms are subject to both senescence and cancer-causing somatic changes, senescent cells accumulate when cells do not compete (Fig. 2*E*, gray), while intercellular competition slows the loss of functional somatic cells (Fig. 2*F*, black) at the cost of allowing cancer cells to proliferate later in life. By purging senescent cells early in life, intercellular competition slows the loss of functional cells (Fig. 2*F*, black to the right of the dotted white line), increasing organismal fitness.

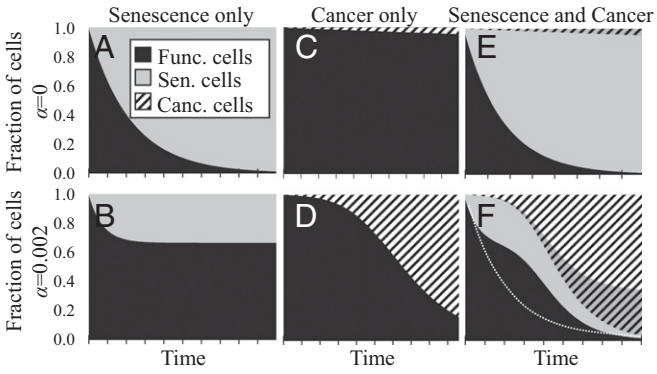

Fig. 2. Intercellular competition prevents accumulation of senescent cells at the cost of allowing cancerous cells to proliferate. (*A* and *B*) Cellular changes causing senescence ($\mu_v = 10^{-3}$, $\mu_c = 0$) cause senescent cells (gray) to accumulate without intercellular competition ($\alpha = 0$, top row), depleting functional cells (black), but are purged when cells compete ($\alpha = 0.002$, bottom row). Cellular changes causing cancer ($\mu_v = 0$, $\mu_c = 10^{-5}$) lead to a small population of cancerous cells (black diagonal stripes) that are prevented from proliferating without intercellular competition (*C*) but can spread when cells compete (*D*). When cells are subject to both senescence- and cancer-causing changes, senescent cells accumulate without intercellular competition (*E*) and cancerous cells proliferate when cells compete (*F*). In *F*, a portion of cancerous cells acquire senescent changes, resulting in a class of cells that are both cancerous and senescent (gray with diagonal stripes). The white dashed line in *F* indicates functional cells from *E* to illustrate the extent to which intercellular competition delays (but does not prevent) the loss of functional cells.

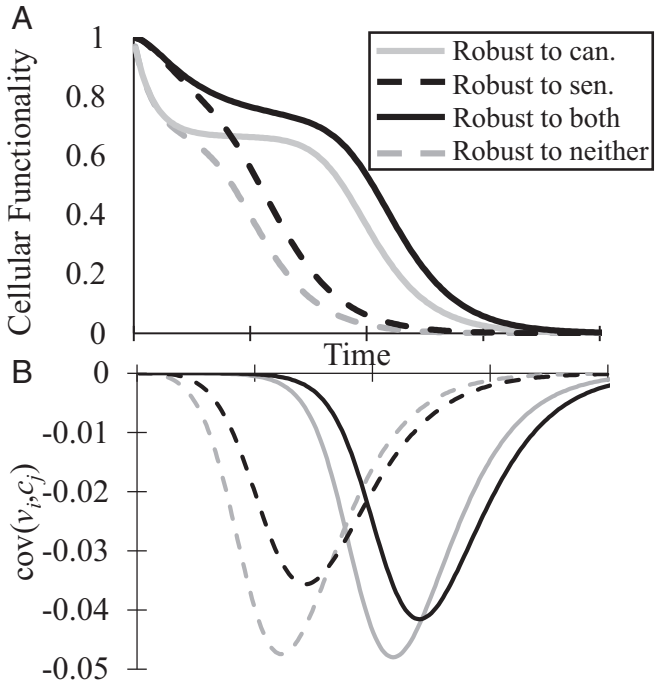

Fig. 3. (*A*) Robustness to somatic changes delays, but does not halt, loss of functional cells. (*B*) Failure to halt aging is a result of negative covariance between cellular cancer (can.) *c* and senescence (sen.) *v*, and is negative under all conditions. While the magnitude of negative covariance decreases later in life, this decrease is due to a depletion of total variance of cell types (most cells are either senescent or cancerous) and occurs long after a multicellular organism would have died (fraction of functional cells ~0.1). $\alpha = 0.002$ throughout.

Loss of cellular function often requires multiple mutations (34), many of which may be selectively neutral (37). To incorporate cellular robustness to single mutations, we compare robust (two-hit) and nonrobust (one-hit) versions of our model (an analysis of the effects and evolution of partial robustness is given in Partial Robustness). Robustness to both cancer and senescence delays, but does not prevent, the loss of functional cells and increases organismal fitness (Fig. 3*A*). The effect of robustness to senescence is largest early in life, while the effect of robustness to cancer is most profound later in life.

Covariance between cellular cooperation and vigor is negative under intercellular competition (Fig. 3*B*). Note that the return of covariance toward zero occurs only when the pool of functional cells is depleted, resulting in a loss of overall genetic variance.

Maximum Malthusian age-adjusted organismal fitness (from Eq. **5**) occurs at intermediate levels of intercellular competition. Robustness to senescence (Fig. S2, solid lines) shifts the optimal level of intercellular competition lower, while robustness to cancer (Fig. S2, black lines) results in higher optimal intercellular competition. Furthermore, when cells are robust to cancer, fitness around the optimum is relatively flat, indicating that organisms are fairly insensitive to the precise level of intercellular competition.

Discussion

To conclude that all multicellular organisms must age may seem trivial. Aging is so intrinsic to the human condition that the English language lacks separate words for the mere increase of years versus the march toward decrepitude (1). Indeed, aging is not unique to multicellular organisms; unicellular organisms, such as

Escherichia coli and *Saccharomyces cerevisiae*, also accumulate damage over time (38, 39). The rate of aging varies widely across different multicellular species, including some that age exceptionally slowly (40). Several taxa, such as turtles and naked mole rats, have even been purported to lack evidence of aging (41), although recent observations suggest that even these long-lived taxa show age-related decline (42, 43). Absence of aging, or negligible senescence, is not without theoretical justification; classical theories of aging implicitly assume that aging is due to the failure of selection to maintain an otherwise immortal genotype (1, 2, 44). Theory positing the evolution of negligible senescence shows that, under certain developmental schemes or gene network structures, selection is sufficiently strong to maintain an ageless genotype (45, 46). The apparent conflict between our model, which concludes that aging is an inescapable feature of multicellularity, and those that suggest that aging can be avoided (e.g., refs. 45, 46) can be resolved by examining three critical assumptions used in our model: a tendency toward degradation of cellular traits, independence of multicellular vitality from developmental programming, and lack of positive covariance between degradation events affecting cellular cooperation and those affecting vigor.

First, we assume that somatic degradation is nonzero. Kogan et al. (46) point out that gene networks can be indefinitely stable if cellular repair mechanisms are sufficiently effective, raising the possibility that cellular degradation may occur at a rate of zero were selection on repair mechanisms somehow made sufficiently strong. However, as the ability to avoid or repair cellular damage is itself a trait subject to degradation, the rate of degradation will likely increase with age as the genetic factors that constrain errors are themselves degraded (47, 48). Thus, the rate of cellular degradation may accelerate through time, precluding indefinite stability.

Second, developmental programming, represented as $g(t)$ in Eq. **1** and controlling organismal size, can have profound effects on survivability (49). Because mortality often decreases with body size, and body size tends to increase with age, some have argued that senescence, or aging, may remain flat or even decrease over a lifetime (45). Despite coming to seemingly opposite conclusions, with our model showing that aging is inevitable and Vaupel et al. (45) showing that aging is optional, the two approaches are complementary. Vaupel et al. (45) use a model of antagonistic pleiotropy, similar to that of Williams (44), to argue that increasing body size over time can decrease mortality and increase reproduction, such that selection favors investment in longevity, and thereby negligible senescence. Our model focuses on somatic evolution to show that, regardless of the number of cells, either cell function or cooperation will eventually break down. Combining the two approaches suggests that organisms may experience a temporary decrease in mortality due to increasing body size, but there will come a point when cellular dynamics result in the breakdown of the multicellular state.

Third, a positive correlation between vigor and cooperation among cells in an organism may extend life span. While our results show selection driving a build-up of negative covariance between vigor and cooperation, more complicated systems may temporarily engender a positive correlation. For example, T lymphocyte precursors replicating in the thymus degrade more rapidly than precursor stem cells in the bone marrow (50). Cellular migration from the bone marrow to the thymus therefore creates a stratified population of cells; this stratification is responsible for positive covariance between cellular vigor and cooperation, allowing competition to purge cells with a greater history of degradation, which are both poorly growing and cancer-prone. Cellular stratification is not, however, a permanent solution to age-related decline. This scenario requires competition between nondegraded cells and cells that are both senescent and cancerous, and is therefore contingent on a supply of nondegraded cells. As stem cell reserves divide, they degrade or are depleted (51, 52). Without a continuous influx of nondegraded cells, positive covariance, and thereby the efficacy of intercellular competition to maintain functional cells, erodes. Hence, selection upon a stratified population of cells only delays, but does not solve, aging.

More broadly, any adaptation that may prevent or delay cancer can itself become the target of a selfish trait. Telomeres, for example, are the repetitive regions of DNA at the end of chromosomes that can limit cell division. Telomeres tend to get shorter with every division of somatic cells, leading to a limit on the number of divisions of normal somatic cells, termed the Hayflick limit (53). The enzyme telomerase can indefinitely repair telomeres, and is normally only expressed in immortal germline cells (54). Limiting the number of somatic replications limits the overall chance that a mutation leading to cancer will arise; this mechanism, like the mechanism of intercellular competition studied here, mediates a tradeoff between cancer and cellular senescence (55–57). Somatic cells that anomalously produce telomerase reap a fitness benefit; thus, the existence of a Hayflick limit creates the opportunity for a selfish trait to evolve in the presence of intercellular competition.

Apoptosis, or programmed celled death, is another mechanism by which cellular proliferation, cooperation, and disease are regulated (19, 58). Apoptosis is often initiated by signals from neighboring cells (59), and comes with an obvious fitness cost to the cell: Cellular fitness becomes zero, making apoptosis a cooperative trait. Cells that can ignore such signals, and avoid entering apoptosis, enjoy a fitness benefit over more cooperative cells. Accordingly, alteration of pathways associated with apoptosis is a characteristic of many precancerous and cancerous cells (60).

The past evolution of distinct cooperative traits, such as telomerase suppression and sensitivity to apoptosis in multicellular species, provides support for another of the key assumptions of our model, namely, that somatic mutations tend to primarily affect either c or v, but less often both. Note that we have shown the assumptions used in this model (both the most important assumptions discussed above as well as more minor ones within the main text) to be sufficient but that, in most cases, we have not shown them to be necessary to conclude that aging is inevitable.

Mechanisms such as immune function, redundancy of cancer suppression genes, and tissue structure affect the rate at which cell cooperation degrades (61, 62). This can be captured in our numerical model either as change in the rates of degradation μ_v and μ_c or as a shift from a one- or two-hit model to a multihit model, and in our analytical model as either the magnitude of mutational effects on each of the two traits or in the shape of the $\{z,f\} \rightarrow \{v,c\}$ map and resulting coordinate system. Such mechanisms might significantly slow aging, perhaps even to negligible rates. Our model does not inform the magnitude of age-related decline, only its inevitability.

Defense mechanisms, such as telomere shortening and apoptosis, limit the rate at which cancer arises but provide little defense once cancer evolves. Other mechanisms, such as a rigid extracellular matrix, may provide longer lasting protection to more dangerous (metastatic) forms of cancer. For example, some plant tissue structures can provide near-immunity to metastatic cancer by limiting cellular mobility of cancerous cells (63, 64). Perhaps due to this reduced organismal harm from cancer/loss of cooperation, plant meristem cells are arranged to maximize intercellular competition, thereby increasing longevity (65).

If these results show that multicellular aging and death are inevitable, how then are multicellular lineages immortal? Compounding this problem, gametic cells also replicate, introducing loss-of-function errors (66). A solution to lineage immortality may come from the alternation of life history stages in which most multicellular organisms engage. Alternation between unicellular and multicellular stages always passes through a unicellular bottleneck; selection at the bottlenecked stage (e.g., sperm or pollen competition) helps to efficiently purge mutations that impair basic cellular metabolism or other unicellular housekeeping functions, while selection among multicellular individuals purges

transgenerationally inherited mutations that impair cooperation among the cells of a multicellular organism.

Conclusion

Given most organisms' capacity to grow and regenerate, aging does not seem, at first glance, inevitable (7). Consequently, many have argued that aging is an accident of imperfect selection (1, 2, 67), where selection fails to purge deleterious, age-related mutations from an otherwise potentially immortal genotype. We have shown that even if selection against aging could be made more powerful, aging would remain an inescapable facet of multicellular life. As our model addresses the role of somatic evolution in aging, it should be seen as complementary, rather than contradictory, to models of aging via evolution by natural selection of multicellular individuals (e.g., refs. 2, 44, 45). Our model points to intercellular competition as a key factor in navigating the double bind of cellular degradation and cancer. It suggests that research programs focusing on quantifying the degree of intercellular competition and making comparisons across taxa, among individuals in the same population, among tissues of the same individual, and across developmental time, may be key to understanding the evolution and progress of aging.

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