The damage-independent evolution of ageing by selective destruction

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Article

Keywords: Evolution, Ageing, Mutant selection, Theory, Cancer

Posted Date: December 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1124008/v1

Abstract

Ageing is currently believed to reflect the accumulation of molecular damage due to energetic costs of maintenance, as proposed in disposable soma theory (DST). Here we have used agent-based modelling to describe an alternative theory by which ageing could undergo positive selection independent of energetic costs. We suggest that the selective advantage of fast-growing mutants might necessitate a mechanism of counterselection we name selective destruction, which removes the faster growing cells from the tissue, preventing the threat of morbidity and mortality they pose. As a result, the survival advantage would shift to the slower cells, allowing them to spread, inducing ageing in the form of a metabolic slowdown.

Selective destruction could therefore provide a proximal cause of ageing that is both consistent with the gene expression hallmarks of ageing, and independent of accumulating damage. If true, negligible senescence would acquire a new meaning of increased basal mortality.

Introduction

It is generally considered that ageing is a multifactorial process[1,2]. However, nearly all existing theories ultimately require molecular damage to be the initial cause[1]. In DST for example, Kirkwood[3] posited that investing in maintenance is energetically costly, and therefore molecular damage could be allowed to accumulate if that energy could be better utilised in other processes with greater impact on fitness (e.g. growth and reproduction). While Kirkwood[1] suggested multiple molecular mechanisms were likely to be involved in ageing, including telomere attrition, protein aggregation, and mitochondrial dysfunction, all the suggested mechanisms were based on molecular damage, and ageing would thus reflect “multiple kinds of damage”. However, if that is true then at its core, ageing reflects only one single cause: that organisms do not invest sufficiently in maintenance to prevent damage accumulation. Indeed, few hypotheses suggest how ageing could result without damage accumulation.

The main exceptions are mutation accumulation theory (MAT) and antagonistic pleiotropy (AP). The former suggests that high extrinsic mortality in the wild would allow for ageing-inducing mutations as the forces of selection would decline over an organism’s lifespan[4]. Initially, the idea was attractive because ageing was not believed to occur in the wild[4]. However, it is now accepted that ageing does occur in the wild in multiple species[5]. In contrast to MAT, AP suggests that pro-ageing mutations offer a fitness advantage earlier in life when selection is stronger[6]. As with MAT, there are also multiple SNPs in humans that may contribute to early life fitness at the cost of fitness later in life[7], and predictably, these mutations have detrimental effects significantly earlier. However, while such SNPs may explain the onset of some diseases in some individuals, it is more difficult to identify ancient mutations that are now universal, inducing ageing via a consistent mechanism across species and organisms. As such, these theories currently provide a clearer view of the evolutionary framework that may allow ageing to evolve rather than a comprehensive biological mechanism.

Here, we attempt to outline an antagonistically pleiotropic biological process that could induce ageing independently of damage accumulation and the energetic costs of maintenance. As such, we believe selective destruction theory (SDT) to provide the first comprehensive mechanism that would provide ageing organisms with a fitness advantage over similar organisms undergoing negligible senescence (without dependence on damage accumulation). However, it is not mutually exclusive with damage-centric theories.

Theory outline

Over time, cells can undergo permanent or semi-permanent changes that affect their rate of growth and proliferation. These could reflect mutations or epigenetic changes, but the altered cells will henceforth be referred to as mutants for simplicity. Such mutants are either aberrantly sensitive (AS) or aberrantly resistant (AR) to growth signals compared with wildtype cells. While both AR and AS mutants reduce tissue functionality by responding incorrectly to the environmental cues, AR mutants will grow and proliferate slower than wildtype cells (non-mutants), putting them at a selective disadvantage, whereas AS mutants will grow and proliferate faster, giving them a selective advantage over wildtype cells and AR mutants. AS mutants are therefore a threat to tissue homeostasis in a way that AR mutants are not. If they are not controlled or removed, they will outcompete the wildtype cells and may become the dominant cell type (Figure 1A).

The accelerated growth and division of AS cells would be associated with a faster mutation rate, as can be seen under extreme examples such as RAS and RAF mutations which induce a state of hyperproliferation associated with high levels of DNA damage[8]. While these extreme mutations induce senescence[9], for reasons described below, mutations with milder effects on growth cannot autonomously stop their own growth. Therefore, if left uncontrolled such mutants could undergo successive mutation and transformation.

Fibrosis is a highly metabolic process requiring mTORC1 signalling through the inhibition of eIF4E-binding protein 1 (4E-BP1) to produce the large amounts of extra cellular matrix (ECM) proteins[10]. In some mesenchymal cell types, AS mutants with faster metabolism and more active growth pathways could therefore be expected to increase the risk of fibrosis. For example, insulin-like growth factor-1 (IGF-1) is a key growth factor inducing growth and proliferation, which also stimulates the survival and activation of fibroblasts, causing differentiation to highly fibrotic myofibroblasts[11,12]. AS mutants in IGF-1 signalling would therefore proliferate and activate in response to weaker signals, making it more likely that they would reach the critical mass required for fibrosis[13]. Patients with systemic sclerosis and idiopathic pulmonary fibrosis (IPF) both have high levels of serum IGF-1[14], indicating that these lethal diseases involve increased activation of this pathway which would be associated with AS mutants. Notably, around 45% of all deaths in the developed world are ascribed to chronic fibroproliferative diseases[15].

However, while fibrosis is an important metabolic process, and fibroblasts or pro-fibrotic cells are present across multiple tissues and organs, it is not the only example of cell function that might be upregulated in AS mutants. AS mutants of many cell types are likely to increase their specific functional output as growth and proliferation are usually part of the mechanism to increase long-term production/activity, so the pathways which increase one will increase the other[16]. For example, b cells proliferate and produce insulin when blood glucose is high, and trigger apoptosis and inhibit insulin when glucose is low. The insulin production pathway is therefore intertwined with the growth and proliferation pathways[17] so both can be up or downregulated together in response to stimulus. However, as the human body requires blood glucose to remain at around roughly 5 mM, Karin and Alon[16] identified that mutants sensing blood glucose at an incorrectly high level would quickly cause death if they were allowed to spread. This would happen for two reasons. Firstly, these mutants hypersecreting insulin can lower blood glucose below the point at which the rest of the body's tissues can sustain respiration. Secondly, the wildtype cells sensing the correct level of glucose undergo apoptosis to reduce insulin production, hastening the spread of mutants and the lethal drop in blood glucose. Thus, any cell type that utilises cell division as a mechanism to regulate functional output, and whose functional output relies on keeping metabolites, proteins, and even other cell types within a certain range, is threatened by AS mutants. Even if the outcomes are not lethal, as they are for fibrosis, insulin production, and other pathways such as calcium homeostasis, they will not promote good health or bodily function.

AS mutants will therefore promote morbidity and mortality **through cancer, fibrosis, and overactivity (CFOA)**. From this, we conclude that organisms would gain a fitness advantage from controlling AS mutants, and the stricter the control mechanisms, the longer an organism could hope to retain wildtype functionality and avoid CFOA. There are two possible mechanisms of controlling mutants with a selective advantage, but both require a marker of phenotypic change: as here we are concerned with sensitivity to growth, we have termed this a **growth marker (GM)**. Firstly, cells could autonomously recognise that the absolute concentration of the GM they are expressing is too high and induce their own apoptosis. Oncogenic mutations in RAS, AKT, PI3K, and multiple other mitogenic molecules have been shown to induce senescence and apoptosis depending on cell type and severity[18]. Hypersecreting b cell mutants also undergo apoptosis (termed glucotoxicity)[16].

Importantly, autonomous mechanisms allow both AS and AR mutants to be removed with equal efficiency as cells recognise their own absolute value of GM (Figure 1B). However, such mechanisms are intrinsically dangerous: in the case of b cells, glucotoxicity in hunter gatherers would have removed only dangerous mutant cells producing too much insulin, but modern diets rich in calories and refined sugars are inducing glucotoxicity in wildtype cells which are producing high levels of insulin to remove the high levels of glucose in the blood, thus resulting in pancreatic destruction and type II diabetes[16]. The danger arises because **autonomous mechanisms are incapable of distinguishing between aberrant cells and aberrant conditions**, so any autonomous mechanism must only activate in conditions that are highly unlikely to occur in nature. Consistently, type II diabetes was likely a rare condition before the introduction of refined sugars[19].

Less severe mutants must be controlled by a second non-autonomous method via comparison with the surrounding tissue. For example, tumour cells are distinguished from surrounding tissue by natural killer (NK) cell recognition of MHC class I chain-related protein A (MICA) levels[20]. In the case of b cells, only the most extreme hypersecreting mutants induce glucotoxicity, so there is still a range of mutants which cannot be removed this way, but still have a selective advantage over wildtype cells. Korem Kohanim, et al. [21] suggested a system of immune control called the autoimmune surveillance of hypersecreting mutants (ASHM) for their control: autoimmune T cells would compare the level of pro-insulin expressed by b cells before killing the cell expressing the highest levels. Thus, if a mutation increasing insulin production has occurred in a cell, then it would likely be killed first.

For AS mutants, when correctly calibrated, an ASHM-like mechanism could return the selective advantage to wildtype cells, as shown in Figure 1C (top). However, as also shown in Figure 1C (bottom), such **selective destruction of the fastest growing cells** is incapable of removing the slow growing AR mutants, which will slowly replicate over time until they begin to outnumber wildtype cells in certain areas. At this point, the immune cells would then recognise the AR mutants as wildtype and the wildtype as AS mutants, providing a selective advantage to the AR mutants.

Importantly, in the model of hypersecreting b cells described by Korem Kohanim, et al.[21], the spread of hypo-secreting mutants was unlikely because such cells preferentially induced apoptosis as part of the mechanism to raise blood glucose[22]. Severe AR mutants may also undergo apoptosis due to lack of mitogenic signals, but moderate mutants are likely to persist if the soma uses a system of selective destruction for mutant control. Attempting to remove AR mutants by either autonomous or comparative mechanisms could have serious repercussions: ASHM, for example, has been implicated in type I diabetes through the immune destruction of healthy b cells[21] because chance events such as infection lead to the identification of healthy β cells as hypersecreting mutants, causing the destruction of the pancreas.

Therefore, we hypothesised that no changes to the process of selective destruction could result in a more favourable outcome for the organism. The options would be threefold:

1. Spread of AS mutants resulting from their selective advantage. The result is death from CFOA at an early age.
2. Autonomous control of AR and AS mutants even for low level changes. The result is death from *en masse* apoptosis and senescence when changes in environmental conditions cause wildtype cells to be mistaken for mutants.
3. The **selective destruction (SD)** of AS cells which allows AR mutants to spread. The result is slow functional decline (ageing).

From the three possible outcomes, ageing would provide the greatest fitness advantage, while the rate of ageing would depend on the severity of the SD control mechanism. To address whether this was true in practise we constructed a series of models to address if:

1. SD would prove better at mutant control than **unselective destruction (UD)** i.e. equal attempt to remove both AS and AR mutants.
2. If implementing SD would induce ageing as predicted via the spread of AR mutants.

If SD involved an immune surveillance mechanism similar to that described by Korem Kohanim, et al.[21] for the comparative control of hypersecreting mutants, it would likely work mainly via inducing apoptosis. However, we considered that while such a process might work well for the regulation of the small populations of cells in pancreatic islets, it was unlikely to be the central mechanism across multiple organs and tissues maintained by selective destruction, particularly among simpler creatures with more primitive immune systems that nonetheless still age.

Instead, we hypothesised that selective destruction could be implemented by juxtacrine and paracrine signals from cells within the same tissue. Speculatively, neighbouring cells could communicate their relative level of metabolism by the levels of GM, and influence each other's fate accordingly, with slower cells suppressing the growth potential of faster cells through epigenetic modification, senescence, or apoptosis.

Notch signalling controls juxtacrine communication, and consistently, Notch mutations are associated with the clonal expansion of leukaemia cells[23] as well as skin and lung squamous cell carcinomas[24], which may implicate that these cells are escaping attempts of their neighbours to suppress their clonal advantage, highly consistent with SDT. Indeed, evidence suggests that Notch mutations aid tumour formation via non-autonomous signals from the tumour microenvironment[25], while Notch signalling plays a key role in senescence by mediating juxtacrine signalling[26].

Methods

Netlogo was used to construct a series of cellular automator models on a two-dimensional grid of cells measuring 33 by 33 for a total of 1089 cells of a single cell type. At the beginning, all cells were considered to have wildtype sensitivity to growth and proliferation signals. Then over a series of cycles, the cells could mutate to become either AS or AR mutants as measured on a scale of 0-4: AR mutants had sensitivity values of 0 and 1, the wildtype a value of 2, and AS mutants values of 3 and 4, with 0 being the least sensitive and 4 being the most sensitive. This is considered to represent the viable range of mutations: cells with lower sensitivity would not receive sufficient mitogenic signals to survive, while cells with higher sensitivity could be removed by autonomous mechanisms (as they are sufficiently aberrant that they are unlikely to reflect any natural conditions), as shown in Figure 2.

Random deaths induced by chance probability created gaps in the tissue which could then be filled by dividing cells. To accelerate the model behaviour, mutation had a probability of 0.01 per cell per cycle, with additional chance of mutating during division of 0.02, which could happen before or after division (affecting one or both daughter cells). Cells only divided when there was a gap in the tissue to fill, and the

probability of filling gaps (Pf) was determined by an equation that reflected the relative sensitivity to growth (equation 1).

(1) Pf = Base_Fill + Fill * (S + 1)

The Base_Fill determined the probability of a cell filling a gap independent of sensitivity. The sensitivity of the cell, S (range 0-4 au), was then affected by a multiplier variable, Fill, so that the larger the Fill value the higher the advantage given to AS mutants (and greater disadvantage to AR mutants). Thus, Fill values reflected the viable range of mutations. A Fill value of zero produced a Pf that was unaffected by any mutation, while a Fill value of 10 produced a Pf of 100% for AS mutants with S = 4. We assumed that the actual viable range of mutations would lie somewhere in between. Importantly, each cycle each cell could fill only a single gap, so even at the highest Fill values the AS mutants could not simply expand indefinitely across the tissue.

Each cycle, before potential division, each cell would compare its sensitivity to its neighbours, and potentially be killed by them. Although such a phenomenon is not well documented in the literature, we considered that such juxtacrine regulation of survival would be most efficient (causing the least unnecessary or detrimental cell death) if it advantaged the wildtype cells as much as possible. Therefore, while our model assumes that cells cannot intrinsically know what is the 'correct' (or wildtype) sensitivity, we considered that the initial numerousness of wildtype cells compared to mutants could be used to prevent their deaths if cells with similar sensitivity provided a survival signal. Therefore, each cycle cells would compare the number of cells with the same level of GM to those with different levels, and only if the latter outweighed the former might the cell be killed. Without this protection, it would be considerably harder to prevent the spread of either AS or AR mutants.

The probability of a cell (with more different than similar neighbours) being killed, Pk, was then determined (equation 2).

(2) Pk = Base_Kill + KDiff * Kill

The Base_Kill determined the probability of cells being killed independent of sensitivity. The Kill variable determined the amplitude of the change from Base_Kill that would be applied to cells with different sensitivity. The KDiff variable reflected the sensitivity of the cell relative to its neighbours (equation 3).

(3) $KDiff = S_{(self)} - S_{(Average\ of\ neighbours)}$

We considered there were several reasons that greater difference might result in higher probability of killing: smaller changes were more likely to be obscured by environmental differences, and larger changes could reflect an increasingly aberrant and therefore dangerous cell.

We then used either KDiff or |KDiff| to compare the two different mechanisms of mutant control: SD, which affords protection to slower cells, used KDiff, so that if the cell had less GM than its neighbours KDiff would be negative (subtracted from Base_Kill), while UD utilised |KDiff|, killing slower and faster

cells with equal proficiency. Importantly, the only difference between SD and UD was that SD made it more difficult to kill slower cells, while both models removed faster cells with equal proficiency.

KDiff was then affected by a multiplier variable Kill, which determined the sensitivity-dependent probability of cell death. Kill ranged from zero, producing a killing probability independent of sensitivity (Base_Kill), to 12 which in UD produced a 0.98 probability of cells being killed by neighbours with the greatest sensitivity difference. In SD, higher values of Kill had the same effect on faster cells but made killing slower cells increasingly less likely (0.02 at Kill 12). We could therefore construct a matrix of outcomes for all conditions that ranged from 0% to 100% likelihood for both cell division (Fill) and killing (Kill). An example run is shown in Figure 3. All results were replicated 12 times.

Results

Selective destruction prevents AS mutant takeover

Running the simulations for up to 120,000 cycles demonstrated one of three outcomes depending on the conditions:

- Wildtype cells would retain dominance throughout the simulation
- AS mutants would spread and eventually outnumber wildtype cells
- AR mutants would spread and eventually outnumber wildtype cells

As expected, once an AS or AR mutant had become dominant it would only be superseded by a more extreme mutant (going from 3 to 4 or 1 to 0). Return to wildtype or oscillating from AS to AR never occurred. We could therefore compare the three outcomes between SD and UD as well as the speed at which AS or AR takeover first occurred. As shown in Figure 4A, UD with even the highest values of Kill proved incapable of preventing AS mutant takeover in all but the lowest values of Fill. AS mutants that escaped killing long enough (by chance) to proliferate into a protective niche (where their similarity to each other prevented them being killed) quickly allowed them to become dominant. Whereas SD, shown in Figure 4B, proved a significantly more effective AS mutant control mechanism at several values of Kill as determined by two-tailed T-tests.

As shown by the average sensitivity of cells over the time course (Figure 5), reducing the rates of replication error and mutation (non-replication) can decelerate/prevent takeover within the time limit, while increased rates accelerate takeover, but neither alters the direction of takeover except at such high error levels where no cell can gain any advantage. This suggests that even though we have used high error rates to accelerate the models, the same outcomes will hold true for slower mutation rates in vivo over longer periods.

We concluded that SD provided an important force of counterselection against the faster cells reducing or removing their selective advantage. However, as indicated by the low Fill, high Kill outcomes, if the counterselection was too strong for the range of viable mutations, then SD could cause the spread of AR

mutants. If SDT is correct, this unbalanced counterselection could therefore cause metabolic slowdown, declining tissue function, and inducing ageing as a result.

However, it is equally clear that by balancing the selective and counterselective forces, wildtype cells could retain their dominance with SD. While it is impossible to know whether running these simulations indefinitely would result in mutant takeover, organisms need only delay takeover until they are most likely to have died from extrinsic (or even other intrinsic) causes, and maintaining balance is possible at least for finite periods.

Selective Destruction as an Inducer of Ageing

Initially this appears a strong argument against SDT. If selection and counterselection can be balanced, then there is no need to allow the spread of AR mutants to prevent the spread of AS mutants. However, simply preventing AS cell dominance and takeover may not be sufficient to remove the threat they provide. SDT predicts that AS mutants induce damage and death principally through CFOA. We therefore used the same models to assess whether the conditions of balanced selection and counterselection conferred additional risk through additional persistence of intermittent AS mutants compared with conditions that caused the spread of AR mutants (even if AS mutants were incapable of ever becoming dominant in either state).

We considered that the main determinant of risk of tumour formation was the length of time that individual AS mutants persisted and the number of clones they produced. Only mutants that persisted for at least four cycles were considered to have any risk of transformation. Each cycle after the fourth confers increasing risk of transformation, calculated by x + 0.1 * number of clones, where x is the previous risk coefficient (starting at 1). We then calculated the relative cumulative risk of tumorigenesis from AS mutants over the simulation, as shown in Figure 6. Regression analysis of the highest kill value for negligibly senescing populations and lowest kill value for ageing populations showed a significant increase in cancer risk for negligibly senescing populations ($p < 0.001$, Fill1-3).

We considered that the main determinant for risk of fibrosis was the clustering of AS mutants, as wounding/inflammation at those clusters would be more likely to trigger cascades that led to self-sustaining populations of fibrotic cells. Risk at each cycle was therefore calculated as the sum of the squares of the size of the clusters. In both cases additional weight was applied to mutants with sensitivity 4 over those with sensitivity 3. Regression analysis of the highest kill value for negligibly senescing populations and lowest kill value for ageing populations showed a significant increase in fibrosis risk for negligibly senescing populations ($p < 0.001$, Fill1-3). As overactivity is cell-type specific, we did not include it here.

The results clearly demonstrate that increasing Kill values reduces the size of AS mutant clusters and the lifespan and clonal expansion of individual mutants. Fitness advantage in the form of reduced potential

for early life carcinogenesis and fibrosis could therefore be obtained by raising Kill values high enough to cause spread of AR mutants, and thus induce ageing as a result.

Positive Selection of Ageing by Selective Destruction

To assess whether genes that induced increasingly strong SD would spread through a population even if they induced a fitness cost in the form of ageing, we constructed an additional Netlogo model of an agent population with three genes for mutant control, each with two alleles that either strengthened SD (S alleles) or relied on UD (U alleles). The outcomes of the different allelic combinations are shown in Table 1.

Risk is separated into intrinsic and extrinsic mortality. Extrinsic mortality was not considered to be affected by risk of CFOA, which would mainly cause acute intrinsic mortality. To maintain a stable population, extrinsic mortality increased with population size. More importantly, it was also affected by ageing, increasing linearly with age after a specified (*Peak*) age. As shown in Table 1 and Figure 7, organisms with less than two S alleles did not age at all, maintaining basal extrinsic mortality throughout life, while organisms with two S alleles aged, and organisms with three S alleles aged twice as fast, as specified by the variable AgeRate (AR).

Intrinsic mortality was considered to reflect mainly the induction of CFOA which would occur as a result of AS mutants. In organisms with three U alleles (AS mutant dominance), intrinsic mortality increased with age as the AS mutants accumulated. In organisms with two U alleles or less, where AS mutants would arise and be cleared, intrinsic risk did not increase with age, but decreased from two to one to zero U alleles, reflecting the decreased risk of CFOA with stronger SD. For the sake of completeness, we also considered that at an age greater than the Peak age (where extrinsic mortality begins to increase as a result of ageing), there would also be a point where the intrinsic mortality begins to increase as a result of ageing, determined by the variable Rapid_Decline (RD), summarised in Figure 7.

Simulations began with all organisms having three U alleles, while S and U alleles could arise by mutation during reproduction (example in Figure 8A). Organisms had to be at the mating age to reproduce and then meet another organism (also at mating age) to produce offspring. To accelerate the simulation, offspring had a one in a hundred chance of mutation, which could be in any one of the three genes.

As shown in Figure 8B and C, when looking at the average percentage of individuals with each genotype over a 100,000 cycle time course, for a range of extrinsic and intrinsic mortality values that go from 100% lethal to risk low enough to allow exponential population increase, the results suggest conditions where immortality, ageing, and rapid ageing phenotypes produce the greatest fitness. This was also true across a range of combinations of peak fitness age and age of sexual maturity (tested across different intrinsic and extrinsic survivability values, Figure 8D-F), and importantly was unaffected if the population started with three S alleles rather than three U alleles (i.e. a rapid ageing population, Figure 8C), suggesting this

was the result of positive selection rather than drift. There were two causes of differences between populations starting with UUU or SSS across the spectrum:

- In conditions of high intrinsic mortality populations starting with UUU often did not survive long enough to evolve and select SSS genotypes.
- Conditions of very low extrinsic risk allowed exponential growth (shown with black border), so the starting allelic combination was always dominant.

Lowering intrinsic risk never allowed exponential growth as extrinsic risk was the more pertinent factor; however, at very low intrinsic risk values the persistence of individuals with UUU indicates the negligible impact of CFOA on risk in these populations.

Thus, ageing via SD could be expected to spread in multiple environments, particularly as intrinsic risk (from CFOA) increases, and this occurs despite the presence of individuals with declining fitness due to ageing within the populations (Figure 8F), with a fraction of some populations even reaching the age of rapid decline where ageing affects intrinsic as well as extrinsic mortality.

Unexpectedly, it was the individuals with higher extrinsic mortality that benefitted most from immortality: it is widely accepted that lower extrinsic mortality should result in slower ageing, as organisms that live longer can produce more offspring if not killed by extrinsic causes[27,28]. Contrarily, these data suggest that if ageing reflects, in part, overactive SD, higher extrinsic risk selects for slower ageing, as organisms in functional decline will be more likely to be killed due to their reduced fitness compared with younger individuals. Therefore, a reduction in extrinsic mortality alone would not allow any fitness benefit from a slower rate of ageing, unless the organisms also have reduced intrinsic mortality. This could explain why ageing varies between but not within species as suggested by the invariant rate of ageing hypothesis[29,30], as the main factors altered within populations of the same species are environmental[29], which would not affect the intrinsic mortality from CFOA, and would therefore be incapable of producing large changes in the rate of ageing. Importantly, if ageing could be retarded solely by increasing investment in maintenance (or making any other antagonistically pleiotropic trade that would not increase intrinsic risk, then reductions in extrinsic mortality should lead to straightforward fitness gains from doing so, and rate of ageing should differ between populations of different species. Thus, SDT may help explain the invariant rate of ageing in a way that DST does not.

Selective Destruction and Molecular Damage

It might be argued that because the generation of AS and AR mutants involves molecular damage that such damage could be prevented by the upregulation of maintenance, in line with DST. However, the level of damage required to induce a change in growth sensitivity could be as simple as a single point mutation – multiple different point mutations in RAS are associated with tumorigenesis[31]. Thus, selective destruction does not require damage *accumulation*, and predicts that upregulating maintenance will only

extend lifespan to the extent that it can significantly slow single base mutations in the DNA. Plausibly, upregulating maintenance mechanisms to such an extent would delay the cell cycle and transcriptional machinery to a degree that would reduce fitness even aside from the associated energetic costs. Therefore, while molecular damage likely plays a significant role in selective destruction, damage accumulation need not.

In addition, while we have been referring to changes in growth signalling as mutations, they are likely to reflect both genetic and epigenetic changes. Epigenetic changes are heritable and semi-permanent, so could provide long lasting changes in growth signalling. In our previous models we have assumed that SD works primarily by killing the surrounding cells. However, this mechanism is obviously inefficient. We therefore considered a scenario where a certain percentage of neighbours identifying more sensitive cells would induce epigenetic changes to slow them down (rather than killing them). Slower cells would still be removed as before.

As shown in Figure 9, 50% and 100% conversion to one or two sensitivity levels below the current level were significantly and increasingly more effective at controlling AS mutants. The incontrovertible conclusion is that an epigenetic program of metabolic slowdown could evolve, undergo positive selection, and induce ageing across the vast majority of species. The rate of ageing would be determined both by the strength of the SD and the rate of conversion. The only remaining question is whether this is actually what is happening.

Discussion

In *Ending Aging*, De Grey[32] suggested the “age-related shifts in gene expression seen in studies of tissues are not the cause of ageing, but are an adaptive (and sometimes maladaptive) response” because they are co-ordinated, while “mutations and epimutations, by contrast, would affect one gene in one cell and a different gene in the next cell.” However, this reasoning is circular because it pre-assumes that random molecular damage is the cause, rather than changes in gene expression, and ignores both the forces of selection, which will push cells toward a faster metabolism, and selective destruction, which will cause the reverse. As the result of selective and counterselective pressures, changes that appear like co-ordinated responses could in fact be inducers of functional decline.

A recent review by Frenk and Houseley[33] examined a collection of meta-analyses and systematic reviews of gene expression changes with age, identifying six hallmarks reflecting consistent changes across multiple species including worms, flies, mice, and humans, as well as different organs within these species. The first and second most common hallmarks were the downregulation of mitochondrial genes and the downregulation of protein synthesis machinery, particularly ribosomal proteins and biogenesis factors, which is highly consistent with a metabolic slowdown resulting from SD. Another hallmark was the reduction in growth factor signalling. The reviewed studies demonstrated reduced expression of genes associated with cell growth in human and worm muscle[34,35]; reduced expression of IGF-1 and GH pathway genes in mouse liver[36]; and DNA methylation of cell cycle genes in β cells[37]. All these changes

are highly consistent with SDT. Equally, studies suggest that the rate of cell division in humans declines with age[38], which has been proposed to explain the reduced rates of cancer in the oldest old[39]. Interestingly, the same is not observed in mice, which showed small non-significant drops in proliferation across the three tested tissues[38]. Considering that mice have higher rates of cancers than humans, both these observations appear consistent with SDT: mice having less effective mutant control do not suffer the same metabolic slowdown but are instead more susceptible to CFOA.

As this might suggest, a role for SD in ageing would have important consequences for therapies. Unlike treatments designed to remove accumulated molecular damage, which might be expected to have little downside, treatments that slow ageing by weakening SD could be expected to increase the risk of early death by CFOA. We therefore predicted that there would be some longevity treatments that induced a degree of early deaths, and sure enough multiple calorie restriction (CR) studies in mice[40–43] showed Kaplan-Meier curves with early dips in survival in the CR groups compared with control, as did the Kaplan-Meier curves presented by Mattison, et al.[44] for the two main primate studies. This occurred even though the survivors of CR went on to live longer and/or healthier lives than controls. Therefore, a key impact of SDT is that curing ageing need not produce immortality, and instead negligible senescence may simply reflect a higher basal risk of death.

Perhaps the main challenge for SDT is that in mainly post-mitotic organisms such as C. elegans and Drosophila, a mechanism of ageing that relies mainly on cell proliferation is much less likely to play a role. However, we could also speculate that the opposite is plausible: these organisms have evolved to become post-mitotic to escape the dangers of AS mutants. As such, the post-mitotic life history trait would be an extreme form of selective destruction, essentially removing every proliferating cell. Organisms that chose this strategy of SD would totally prevent mutant spread at the cost of their ability to remove damaged cells and replace them with new ones, potentially inducing mechanisms of ageing which were avoided in their single-celled ancestors like yeast, which essentially divide away the damage (although this is conventionally referred to as ageing) and need not occur in organisms that maintained or re-evolved mitotic somas. If SDT is correct, we would predict that molecular damage accumulation may prove less relevant to ageing in mitotic than post-mitotic organisms.

Importantly, the widespread failure of anti-ageing treatments aimed at removing molecular damage may reflect the presence of a second mechanism. If ageing is, even in part, a metabolic slowdown from SD, then even if this causes molecular damage to accumulate at late stages, removing this damage will have short term effects at best, as it is the altered functionality of cells which causes them to produce misfolded proteins and not remove them, for example. The accumulated damage would not reflect the slow build-up over time, but a shift in homeostasis from clearance to accumulation, suggesting it will quickly return to previous levels when treatments are ceased. Conversely, activities such as smoking which increase molecular damage would be expected mainly to affect mortality while they persist, as has been shown to be the case[45]. This is consistent with the observation that smoking increases clonal

expansion of cells with driver mutations (causing cancer), which are cleared once individuals cease smoking[46].

We believe that SDT provides the first theory which could explain both the evolution and mechanism of ageing independent of molecular damage accumulation and the associated energetic costs, which would also undergo positive selection. The necessity of studies to frame their results within the sphere of accumulating damage has created a myopic view of ageing, so a second hypothesis could be a huge boon to the field. That said, we accept that many of the concepts put forward here are in their nascent stages. The idea of neighbouring cells controlling each other's growth rate is currently hypothetical. We have suggested Notch signalling may be a key part of this process and shown that mutations in this network are clearly linked with loss of mutant control and cancer, but there is still considerable experimental work to be done here, and the relevance of SD to ageing will come down in large part to the results of these experiments. While mutant control must exist in some forms, it is possible that AS mutants requiring non-autonomous control do not occur in sufficient quantities to merit a system of SD. The rates of mutation affecting growth sensitivity used in our simulations were artificially high for the sake of expediency, although both mutations and epimutations could generate AS mutants, and the latter may be orders of magnitude more frequent than the former[47]. Indeed, the very idea of SD through epigenetic conversion may require redefinition of epimutation. Initially defined by Holliday as "aberrant patterns of DNA methylation that cause the silencing of a normally expressed gene," or the contrary aberrant "ectopic expression"[48], within the context of SD this should at least be subdivided into those that are adverse reflecting chance conditions, and those that are deliberately induced to slow the metabolism of potentially dangerous cells (or otherwise spread by the force of this counterselection). If SD is correct, the latter kind likely produce the epigenetic clocks that predict both chronological and biological age, while the former will represent the increasing epigenetic noise.

Evidence that clonal expansion occurs with age and may contribute to cancer is continually increasing, recently reviewed by Kakiuchi and Ogawa[49], who described the spread of clonal mutants across multiple tissues, including one study where "virtually all endometrial glands were replaced by one or more driver-mutated clones" in women by the age of 50[50].

To conclude, we have shown that SD provides a more effective mutant control mechanism than UD, and given evidence that reducing the risk of cancer, fibrosis, and overactivity (CFOA) could be adequate to explain why organisms might strengthen SD to the point that it begins to allow the spread of AR mutants, while epigenetic growth suppression may also contribute to the metabolic slowdown. SD could therefore provide the proximal cause of ageing.

Declarations

Acknowledgements

We would like to acknowledge Professor Tom Kirkwood for developmental input. Images were created using BioRender.com. This work was funded by the Novo Nordisk Fonden Challenge Programme: Harnessing the Power of Big Data to Address the Societal Challenge of Aging NNF17OC0027812.

Author contributions

Author contributions: James Wordsworth developed the theory and models, and wrote the manuscript with input and supervision from Daryl Shanley. Hannah O'Keefe contributed to model development and Peter Clark helped with cluster work.

Competing Interests

We have no competing interests to disclose.

References

1. Kirkwood, T. B. Understanding the odd science of aging. *Cell* **120**, 437–447, doi:10.1016/j.cell.2005.01.027 (2005).
2. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194–1217, doi:10.1016/j.cell.2013.05.039 (2013).
3. Kirkwood, T. B. L. Evolution of ageing. *Nature* **270**, 301–304, doi:10.1038/270301a0 (1977).
4. Medawar, P. B. *An Unsolved Problem of Biology*. (H. K. Lewis, 1952).
5. Nussey, D. H., Froy, H., Lemaitre, J.-F., Gaillard, J.-M. & Austad, S. N. Senescence in natural populations of animals: widespread evidence and its implications for bio-gerontology. *Ageing Res Rev* **12**, 214–225, doi:10.1016/j.arr.2012.07.004 (2013).
6. Williams, G. C. Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution* **11**, 398–411, doi:10.2307/2406060 (1957).
7. Rodríguez, J. A. *et al.* Antagonistic pleiotropy and mutation accumulation influence human senescence and disease. *Nat Ecol Evol* **1**, 55, doi:10.1038/s41559-016-0055 (2017).
8. Janda, E., Litos, G., Grünert, S., Downward, J. & Beug, H. Oncogenic Ras/Her-2 mediate hyperproliferation of polarized epithelial cells in 3D cultures and rapid tumor growth via the PI3K pathway. *Oncogene* **21**, 5148–5159, doi:10.1038/sj.onc.1205661 (2002).
9. Di Micco, R. *et al.* Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* **444**, 638–642, doi:10.1038/nature05327 (2006).
10. Woodcock, H. V. *et al.* The mTORC1/4E-BP1 axis represents a critical signaling node during fibrogenesis. *Nature Communications* **10**, 6, doi:10.1038/s41467-018-07858-8 (2019).
11. Hung, C. F., Rohani, M. G., Lee, S.-s., Chen, P. & Schnapp, L. M. Role of IGF-1 pathway in lung fibroblast activation. *Respiratory Research* **14**, 102, doi:10.1186/1465-9921-14-102 (2013).
12. Choi, J. E. *et al.* Insulin-like growth factor-I receptor blockade improves outcome in mouse model of lung injury. *Am J Respir Crit Care Med* **179**, 212–219, doi:10.1164/rccm.200802-228OC (2009).

13. Adler, M. *et al.* Principles of Cell Circuits for Tissue Repair and Fibrosis. *iScience* **23**, 100841-100841, doi:10.1016/j.isci.2020.100841 (2020).
14. Hamaguchi, Y. *et al.* Elevated serum insulin-like growth factor (IGF-1) and IGF binding protein-3 levels in patients with systemic sclerosis: possible role in development of fibrosis. *J Rheumatol* **35**, 2363–2371, doi:10.3899/jrheum.080340 (2008).
15. Wynn, T. A. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *The Journal of clinical investigation* **117**, 524–529, doi:10.1172/JCI31487 (2007).
16. Karin, O. & Alon, U. Biphasic response as a mechanism against mutant takeover in tissue homeostasis circuits. *Mol Syst Biol* **13**, 933, doi:10.15252/msb.20177599 (2017).
17. Rhodes, C. J. & White, M. F. Molecular insights into insulin action and secretion. *Eur J Clin Invest* **32 Suppl 3**, 3–13, doi:10.1046/j.1365-2362.32.s3.2.x (2002).
18. Liu, X.-L., Ding, J. & Meng, L.-H. Oncogene-induced senescence: a double edged sword in cancer. *Acta pharmacologica Sinica* **39**, 1553–1558, doi:10.1038/aps.2017.198 (2018).
19. Johnson, R. J., Sánchez-Lozada, L. G., Andrews, P. & Lanaspa, M. A. Perspective: A Historical and Scientific Perspective of Sugar and Its Relation with Obesity and Diabetes. *Advances in Nutrition* **8**, 412–422, doi:10.3945/an.116.014654 (2017).
20. Møller, S. H. *et al.* Cytoplasmic Citrate Flux Modulates the Immune Stimulatory NKG2D Ligand MICA in Cancer Cells. *Frontiers in Immunology* **11**, doi:10.3389/fimmu.2020.01968 (2020).
21. Korem Kohanim, Y., Tendler, A., Mayo, A., Friedman, N. & Alon, U. Endocrine Autoimmune Disease as a Fragility of Immune Surveillance against Hypersecreting Mutants. *Immunity* **52**, 872-884.e875, doi:https://doi.org/10.1016/j.immuni.2020.04.022 (2020).
22. Topp, B., Promislow, K., Devries, G., Miura, R. M. & Finegood, D. T. A Model of β -Cell Mass, Insulin, and Glucose Kinetics: Pathways to Diabetes. *Journal of Theoretical Biology* **206**, 605–619, doi:https://doi.org/10.1006/jtbi.2000.2150 (2000).
23. Kimura, S. *et al.* NOTCH1 pathway activating mutations and clonal evolution in pediatric T-cell acute lymphoblastic leukemia. *Cancer Sci* **110**, 784–794, doi:10.1111/cas.13859 (2019).
24. Wang, N. J. *et al.* Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. *Proceedings of the National Academy of Sciences* **108**, 17761-17766, doi:10.1073/pnas.1114669108 (2011).
25. Demehri, S., Turkoz, A. & Kopan, R. Epidermal Notch1 loss promotes skin tumorigenesis by impacting the stromal microenvironment. *Cancer Cell* **16**, 55–66, doi:10.1016/j.ccr.2009.05.016 (2009).
26. Hoare, M. *et al.* NOTCH1 mediates a switch between two distinct secretomes during senescence. *Nat Cell Biol* **18**, 979–992, doi:10.1038/ncb3397 (2016).
27. Austad, S. N. Retarded senescence in an insular population of Virginia opossums (Didelphis virginiana). *Journal of Zoology* **229**, 695–708, doi:https://doi.org/10.1111/j.1469-7998.1993.tb02665.x (1993).

28. Kirkwood, T. B. L. Evolution of ageing. *Mechanisms of Ageing and Development* **123**, 737–745, doi:https://doi.org/10.1016/S0047-6374(01)00419-5 (2002).
29. Colchero, F. *et al.* The long lives of primates and the 'invariant rate of ageing' hypothesis. *Nat Commun* **12**, 3666, doi:10.1038/s41467-021-23894-3 (2021).
30. Finch, C. E., Pike, M. C. & Witten, M. Slow mortality rate accelerations during aging in some animals approximate that of humans. *Science* **249**, 902–905, doi:doi:10.1126/science.2392680 (1990).
31. Miller, M. & Miller, L. RAS mutations and oncogenesis: not all RAS mutations are created equally. *Frontiers in Genetics* **2**, 100, doi:10.3389/fgene.2011.00100 (2012).
32. De Grey, A. *Ending aging: the rejuvenation breakthroughs that could reverse human aging in our lifetime*. (St. Martin's Press, 2007).
33. Frenk, S. & Houseley, J. Gene expression hallmarks of cellular ageing. *Biogerontology* **19**, 547–566, doi:10.1007/s10522-018-9750-z (2018).
34. Ma, X. *et al.* Analysis of C. elegans muscle transcriptome using trans-splicing-based RNA tagging (SRT). *Nucleic Acids Res* **44**, e156, doi:10.1093/nar/gkw734 (2016).
35. Zahn, J. M. *et al.* Transcriptional profiling of aging in human muscle reveals a common aging signature. *PLOS Genetics* **2**, e115, doi:10.1371/journal.pgen.0020115 (2006).
36. Schumacher, B. *et al.* Delayed and accelerated aging share common longevity assurance mechanisms. *PLOS Genetics* **4**, e1000161, doi:10.1371/journal.pgen.1000161 (2008).
37. Avrahami, D. *et al.* Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. *Cell metabolism* **22**, 619–632, doi:10.1016/j.cmet.2015.07.025 (2015).
38. Tomasetti, C. *et al.* Cell division rates decrease with age, providing a potential explanation for the age-dependent deceleration in cancer incidence. *Proceedings of the National Academy of Sciences* **116**, 20482-20488, doi:10.1073/pnas.1905722116 (2019).
39. Hanson, H. A., Smith, K. R., Stroup, A. M. & Harrell, C. J. An age-period-cohort analysis of cancer incidence among the oldest old, Utah 1973-2002. *Popul Stud (Camb)* **69**, 7–22, doi:10.1080/00324728.2014.958192 (2015).
40. Cameron, K. M., Miwa, S., Walker, C. & von Zglinicki, T. Male mice retain a metabolic memory of improved glucose tolerance induced during adult onset, short-term dietary restriction. *Longev Healthspan* **1**, 3–3, doi:10.1186/2046-2395-1-3 (2012).
41. Mitchell, S. J. *et al.* Effects of sex, strain, and energy intake on hallmarks of aging in mice. *Cell Metab* **23**, 1093–1112, doi:10.1016/j.cmet.2016.05.027 (2016).
42. Koopman, J. J. *et al.* Measuring aging rates of mice subjected to caloric restriction and genetic disruption of growth hormone signaling. *Aging (Albany NY)* **8**, 539–546, doi:10.18632/aging.100919 (2016).
43. Yu, D. *et al.* Calorie-restriction-induced insulin sensitivity is mediated by adipose mTORC2 and not required for lifespan extension. *Cell Rep* **29**, 236-248.e233, doi:10.1016/j.celrep.2019.08.084 (2019).

44. Mattison, J. A. *et al.* Caloric restriction improves health and survival of rhesus monkeys. *Nature Communications* **8**, 14063, doi:10.1038/ncomms14063 (2017).
45. Alberg, A. J., Brock, M. V., Ford, J. G., Samet, J. M. & Spivack, S. D. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* **143**, e1S-e29S, doi:10.1378/chest.12-2345 (2013).
46. Yoshida, K. *et al.* Tobacco smoking and somatic mutations in human bronchial epithelium. *Nature* **578**, 266–272, doi:10.1038/s41586-020-1961-1 (2020).
47. van der Graaf, A. *et al.* Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proceedings of the National Academy of Sciences* **112**, 6676-6681, doi:10.1073/pnas.1424254112 (2015).
48. Holliday, R., Holliday, R., Monk, M. & Pugh, J. E. DNA methylation and epigenetic inheritance. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* **326**, 329–338, doi:doi:10.1098/rstb.1990.0015 (1990).
49. Kakiuchi, N. & Ogawa, S. Clonal expansion in non-cancer tissues. *Nature Reviews Cancer* **21**, 239–256, doi:10.1038/s41568-021-00335-3 (2021).
50. Moore, L. *et al.* The mutational landscape of normal human endometrial epithelium. *Nature* **580**, 640–646, doi:10.1038/s41586-020-2214-z (2020).

Table

Table 1 | Allele combinations and outcomes for evolutionary simulation of SD and UD in a population of agents. Orange rows show effects on ***extrinsic mortality (1/X)****, and blue rows* ***intrinsic mortality (1/Y)****. X is determined by the variable ExMort, and Y by the variable IntMort. Both are stochastic reflecting chance conditions and occurrences, thus* ***X'*** *and* ***Y'*** *refer to a randomly selected number between 0 and X or Y.* ***RD, Rapid_Decline*** *is a variable determining the age at which ageing begins to have significant effect directly on intrinsic mortality. The* ***Peak*** *variable determines the age after which extrinsic fitness begins to decline. AgR, is the rate of ageing determined by the number of S alleles. AT, AgeTot is the number of U alleles. AS, aberrently sensitive to growth; AR, aberrantly resistant to growth; CFOA, cancer fibrosis and overactivity.*

Alleles	AgeTot (AT)	AgeRate (AgR)	Dominance	Outcome	Mortality Functions	Function summary
UUU	3	0	AS mutant	Increasing CFOA risk	Die if $X' < 1$	Constant extrinsic mortality
					Die if $Y' < AT +$ Age	Intrinsic mortality increases with age from birth
SUU	2	0	Wildtype	Immortal (with constant CFOA risk)	Die if $X' < 1$	Constant extrinsic mortality
					Die if $Y' < AT$	Constant intrinsic mortality
SSU	1	1	AR mutant	Ageing	if Age >= Peak: $X_{(t+1)} = (X_t - AgR)$ Die if $X' < 1$	Extrinsic mortality begins increasing after peak age
					if Age < RD / AR: Die if $Y' < AT$ **else** die if $Y' < AT$ + (Age - RD / AgR)	Intrinsic mortality begins increasing at older age (RD)
SSS	0	2	AR mutant	Rapid ageing	if Age >= Peak: $X_{(t+1)} = X_t - AgR$ Die if $X' < 1$	Extrinsic mortality begins steeply increasing after peak age
					if Age < RD / AR: Die if $Y' < AT$ **else** die if $Y' < AT$ + (Age - RD / AgR)	Intrinsic mortality begins increasing at older age (RD/2)

Figures

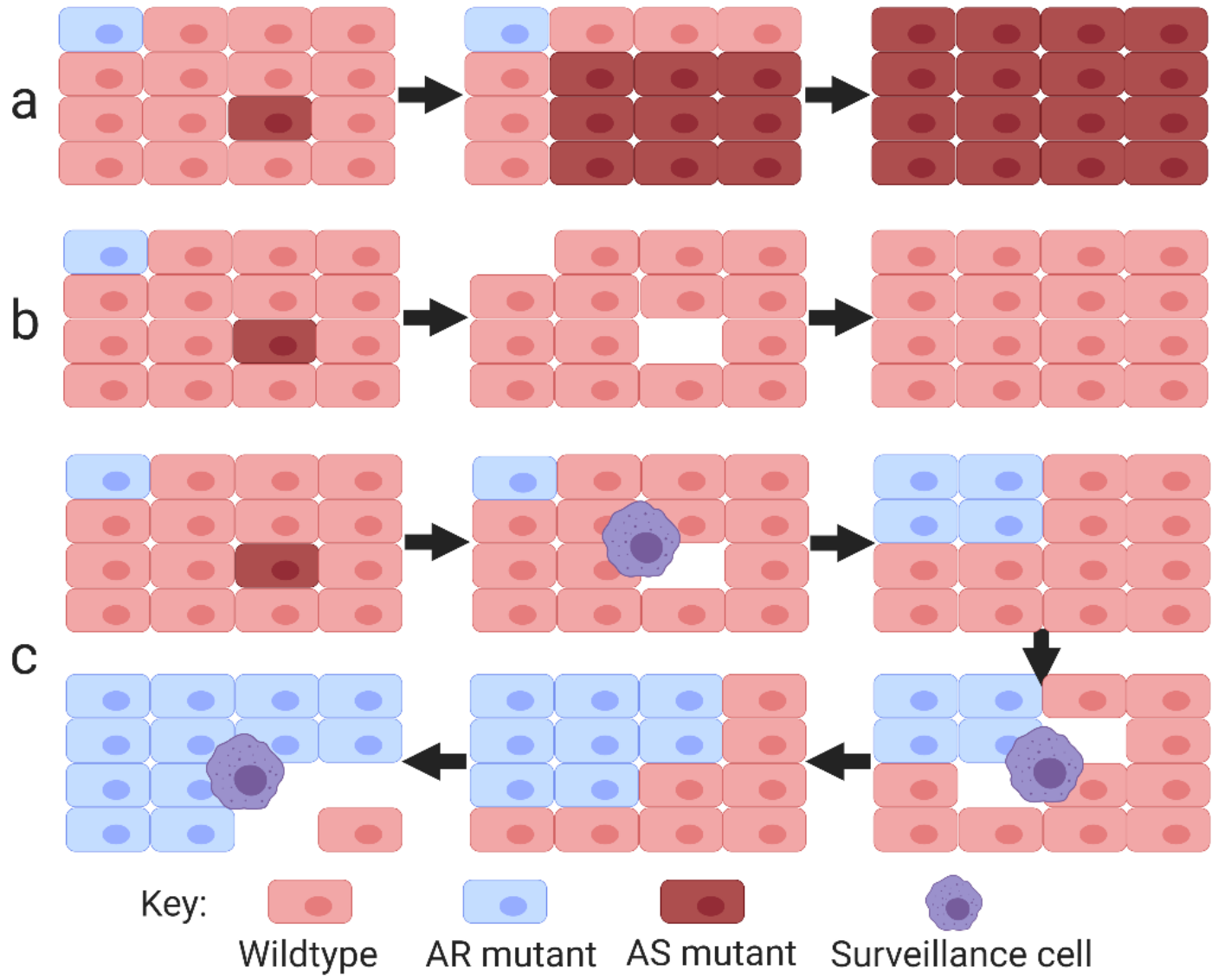

Figure 1

Tissue development without the control of growth sensitive mutants (A); with autonomous control (B); and control by selective destruction (C). AR, aberrantly resistant; AS, aberrantly sensitive.

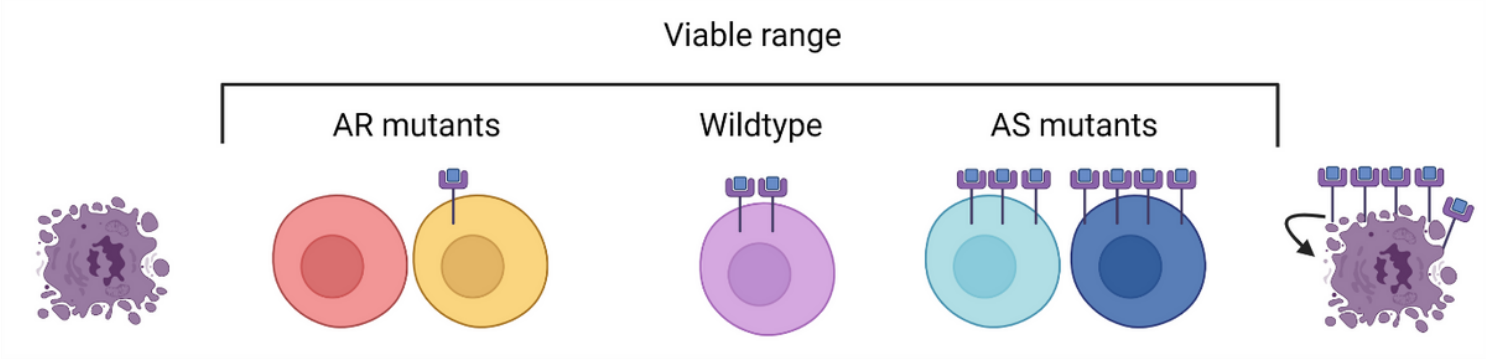

Figure 2

Schematic of hypothetical viable range of mutations conferring different sensitivity to growth and proliferation stimuli, with changes outside this range inducing death. Cell colours and number of receptors indicate the relative production of the GM to reflect the cell's sensitivity and allow comparison between neighbouring cells. AR, aberrantly resistant; AS, aberrantly sensitive.

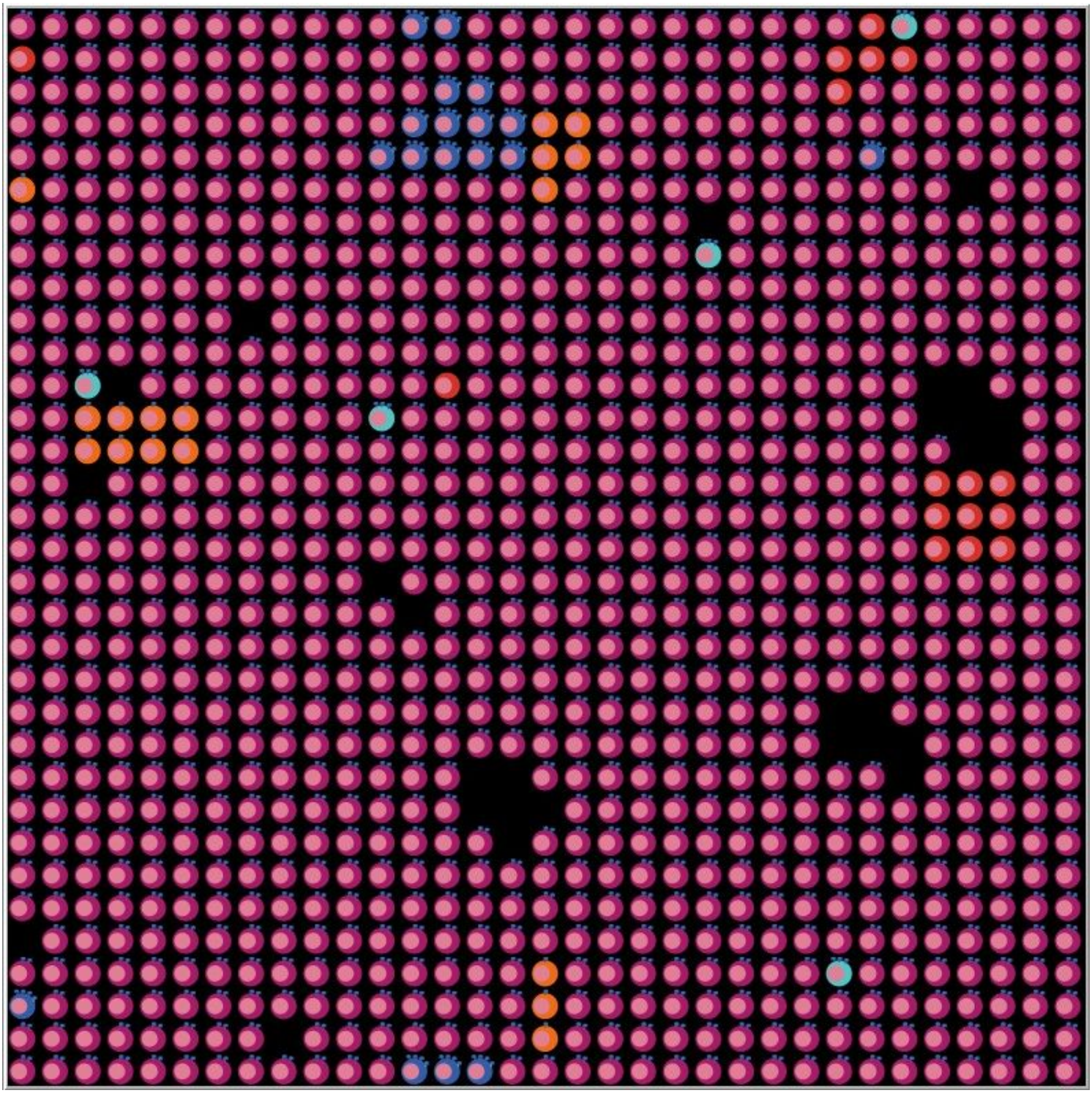

Figure 3

Example run. Magenta cells are wildtype S2, Cyan and Blue are AS mutants with S3 and S4, respectively. Orange and red cells are AR mutants with S1 and S0, respectively.

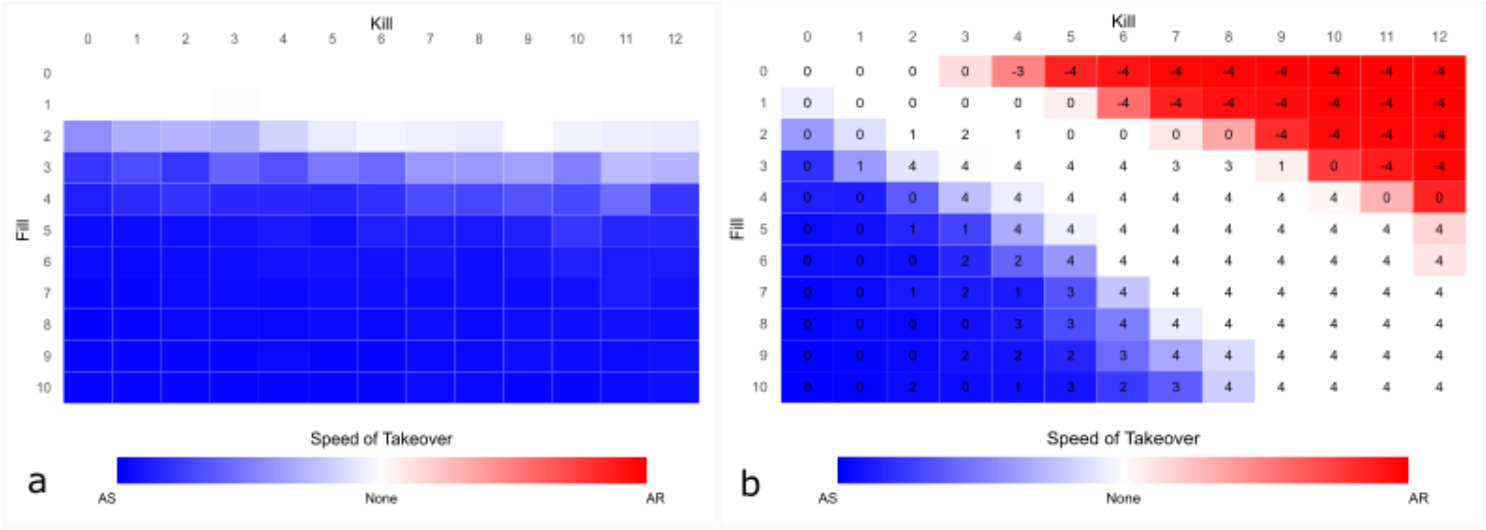

Figure 4

Heatmaps showing speed and direction of mutant takeover for UD (A) and SD (B). Number 0 indicates SD was not significantly different to UD; 1 indicates $p < 0.05$; 2 indicates $p < 0.01$; 3 indicates $p < 0.001$; 4 indicates $p < 0.0001$. Numbers are negative if takeover occurred earlier than in UD.

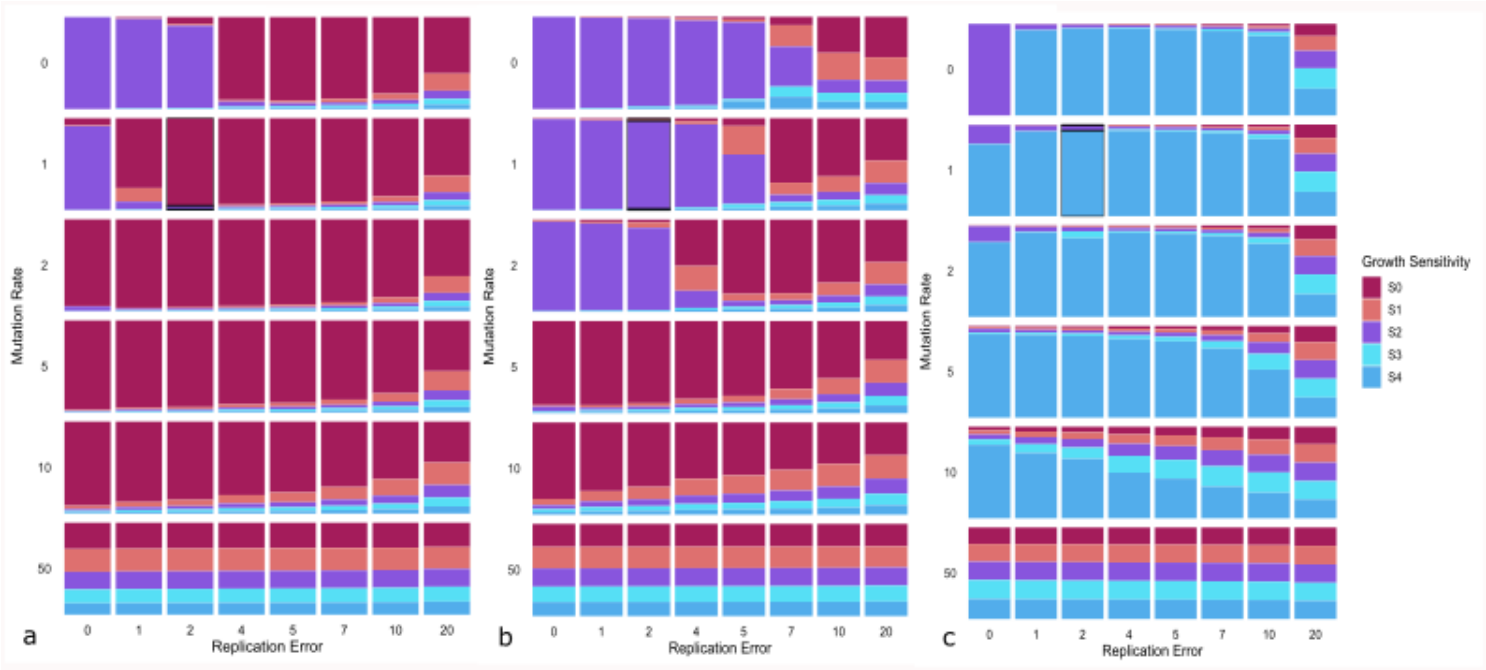

Figure 5

Effect of varying replication error and mutation rate on the percentage cells with different sensitivities over the time course. Three different Kill and Fill combinations were used corresponding to: (A) AR mutant takeover (Fill 2 Kill 11); (B) Wildtype dominance (Fill 5 Kill 8); and (C) AS mutant takeover (Fill 8 Kill2) at mutation rate 1 and replication error rate 2, which have black borders to indicate the values used in other simulations.

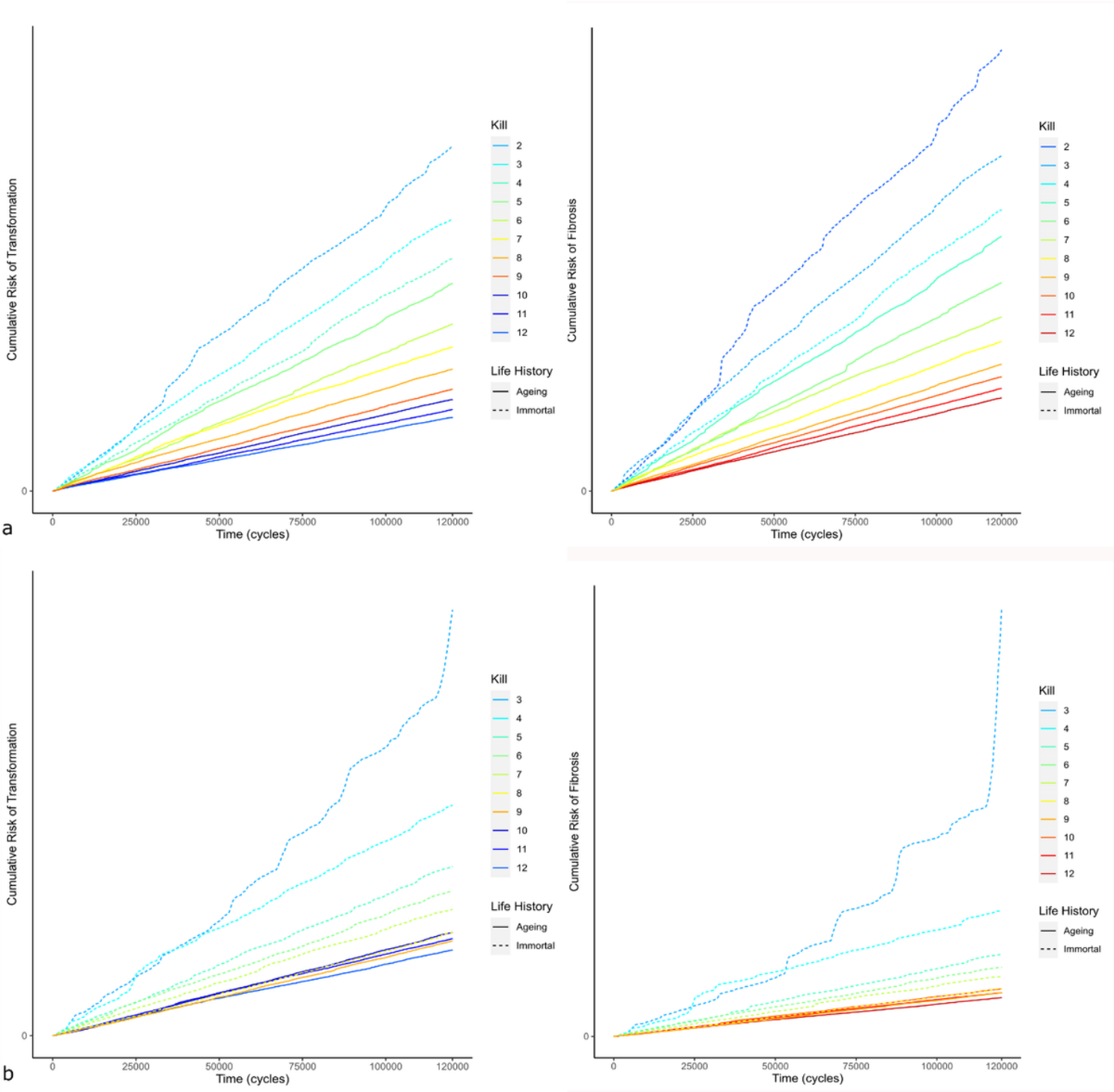

Figure 6

Relative cumulative risk of cell transformation (left) and fibrosis (right) for simulations with Fill values 1 (A) and 3 (B). The Kill values which correspond to AR dominance (and cause ageing) have solid lines, while the Kill values which correspond to Wildtype dominance (and allow for negligible senescence) have dotted lines.

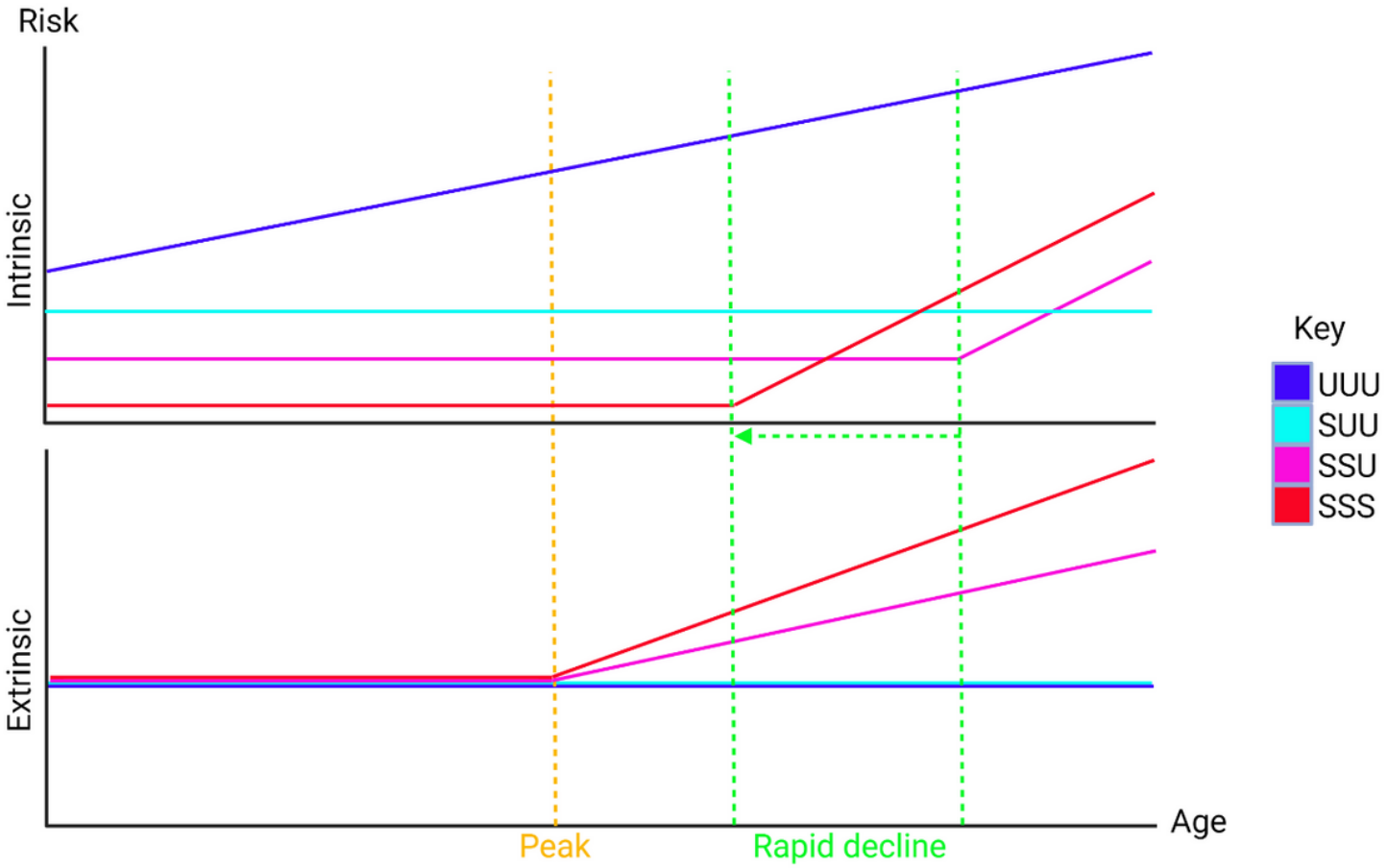

Figure 7

Relative risks for different allelic combinations over time.

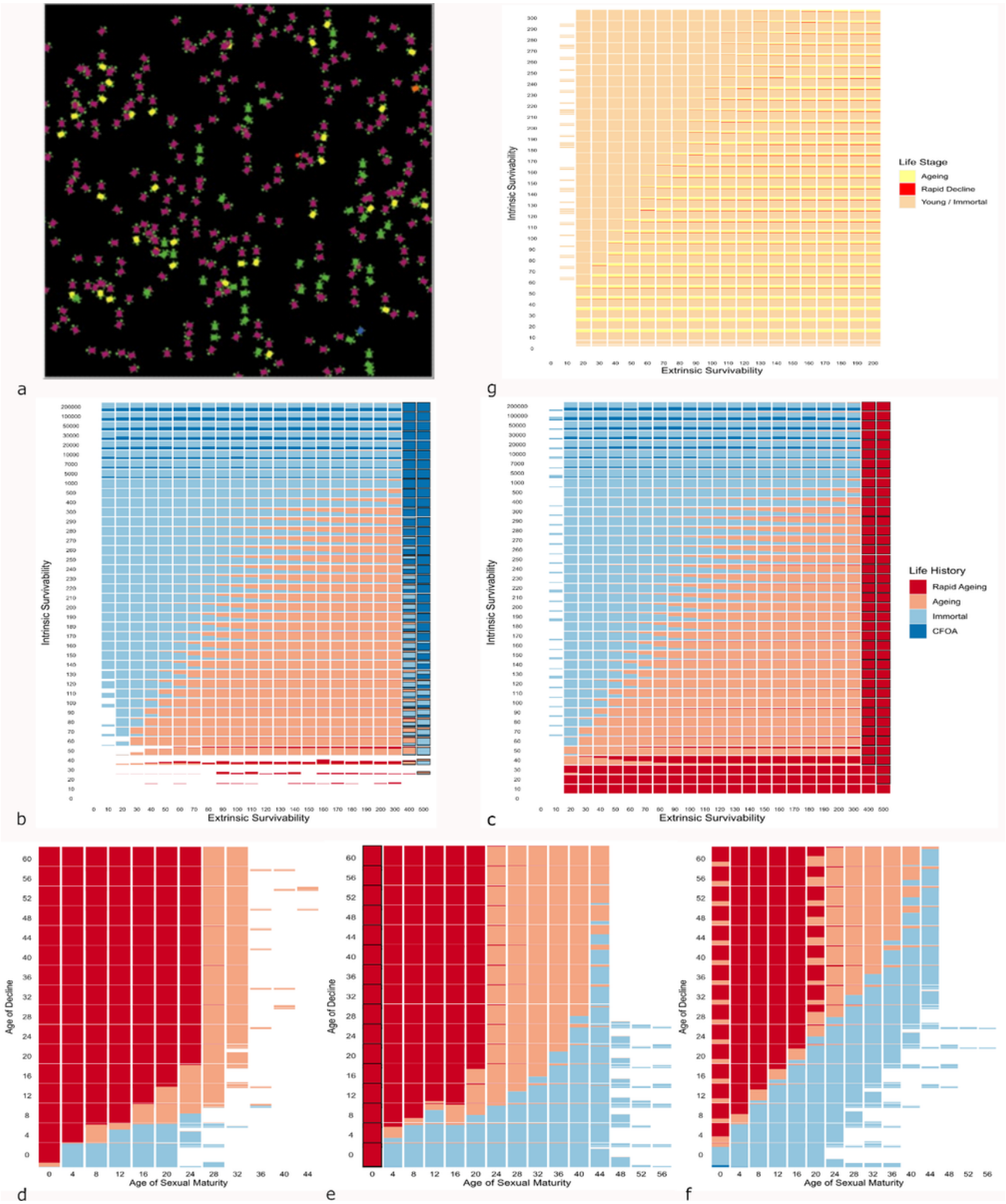

Figure 8

(A) Example run. Blue agents have UUU alleles, green agents have SUU, magenta, yellow and orange agents have SSU or SSS alleles. Yellow agents are past the peak age and orange agents are in rapid decline. (B and C) Mean abundance of agents with different allelic combinations across a range of extrinsic and intrinsic mortality values (inverted to survivability for ease of interpretation). (B) Starting populations are uniform UUU alleles (for UD). (C) Starting populations are uniform SSS alleles (for rapid

ageing under SD). (D, E and F) Mean abundance of agents with different allelic combinations across a range of peak ages (before ageing begins to reduce extrinsic fitness) and age of sexual maturity (where meeting another agent results in offspring). (D) Intrinsic survivability 50 and extrinsic survivability 50 (close to rapid ageing dominance in B and C). (E) Intrinsic survivability 150 and extrinsic survivability 150 (Ageing dominance). (F) Intrinsic survivability 250 and extrinsic survivability 50 (Immortality dominance). NB: In B and C, the age of sexual maturity and peak fitness were both 10. (G) Mean abundance of agents in peak fitness (both pre-ageing and immortal) ageing (decreasing extrinsic fitness) and rapid decline (decreasing intrinsic and extrinsic fitness) across a range of extrinsic and intrinsic mortality values. White space indicates populations went extinct, black borders indicate populations underwent exponential increase and simulations were ended at 10,000 agents. Units are all arbitrary units.

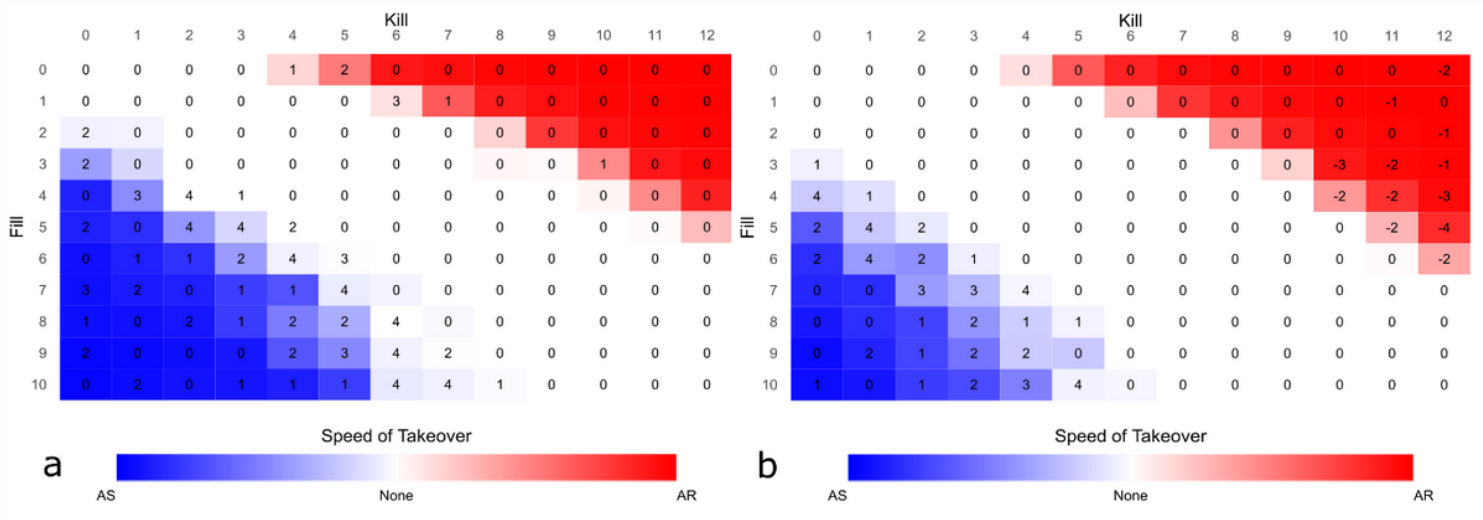

Figure 9

Heatmaps showing speed and direction of mutant takeover for: (A) SD with 50% conversion of faster cells; and (B) SD with 100% conversion of slower cells. Number 0 indicates no significant change (from SD0 to SD50 in (A) and SD50 to SD100 in (B)); 1 indicates $p < 0.05$; 2 indicates $p < 0.01$; 3 indicates $p < 0.001$; 4 indicates $p < 0.0001$. Numbers are negative if takeover occurred earlier with higher percentage conversion.