Chapter 2- houses garbage and trucks

We have just explored quantitative patterns of aging. Now we begin to explain them with a conceptual model for aging that will guide us throughout the book. It will be a mental framework to organize the huge number of facts about aging, in a way that we can understand and work with.

Our basic assumption is that aging is driven by specific forms of damage. Find them and we have targets to slow down aging.

House and truck metaphor helps to understand the model

To get intuition about the model I like to use a metaphor. This metaphor makes it easy to compute in our mind and see what happens when we intervene in the biology of aging. Our metaphor is a story about houses, garbage and trucks.

A young organism is like a village where each house produces garbage. The village has 100 garbage trucks, more than enough to clear the garbage. Garbage is made and removed, with a low average garbage level.

Every year a new house is built . With time the village grows and eventually becomes a big city that produces a lot of garbage every day.

However, this village was not designed to be so large, so there are still only 100 trucks. But now the trucks are overloaded, and garbage piles up in the streets. Any extra garbage stays around for a long time until the overwhelmed trucks get to it. If there is a truck strike, garbage piles up even higher. Once in a while the trucks go on strike, a kind of noise in the system.

Eventually, when garbage crosses a threshold, the situation becomes incompatible with life.

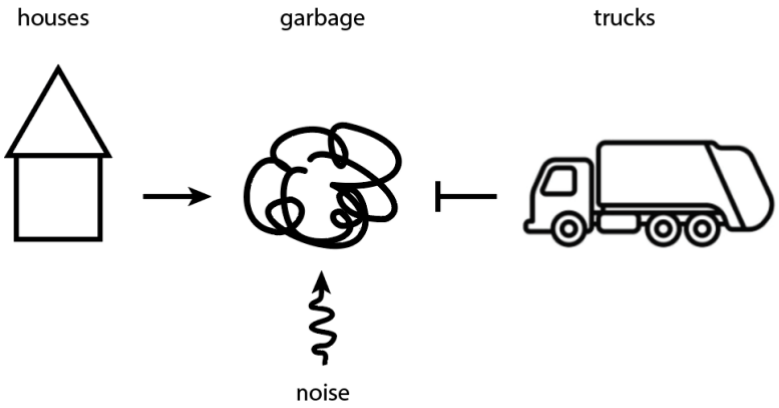


Figure 3.22

Let's interpret the story in terms of aging. The garbage is damage casual for aging, which we call X. Our hypothesis for now for mammals is that X is the total number of senescent cells in the body. These senescent cells secrete factors that cause chronic inflammation and reduced regeneration, leading to disease and decline. Later, when we discuss other organisms, X will represent other forms of damage.

The houses in the story are damage producing units, DPUs, that generate the damage X. They are produced at a constant rate and slowly accumulate in the body over decades. They make 70-year-olds different from 20-year-olds.

Since DPUs are made at a constant rate but are not removed, their number rises linearly with age. Therefore, the production rate of X rises linearly with age.

Our candidate for DPUs in mammals is altered stem cells. More details in the next chapter. The alterations do not harm the stem cell but do affect the differentiated cells it produces. Since alterations are infrequent, and altered stem cells are not removed, the number of altered stem cells rises linearly with age. They produce damaged differentiated cells that become senescent.

Houses, or DPUs, are not removed, but the damage that they create, X, is. It is removed by the trucks, which represent damage-removing processes. For

senescent cells, the trucks are immune cells called NK (natural jKiller) cells and macrophages. The NK cells detect senescent cells by means of special marker proteins that senescent cells display on their surface. They attach to the senescent cells and inject toxic proteins to kill them. Mice without functioning NK cells show accelerated aging and large amounts of senescent cells. Other immune cells, including macrophages, also play a role by swallowing up the remains of the killed cells.

But since biological processes saturate, so does the removal capacity of senescent cells; the trucks become overwhelmed.

Thus, our model has two features: production of damage that rises linearly with age and saturating removal of damage.

This sets up an inevitable catastrophe – when production exceeds maximal removal capacity, the amount of damage X rises sharply.

Indeed, with age, senescent cells pile up. They secrete factors that cause inflammation and reduce regeneration – their impact thus extends to the entire body.

In order to have such systemic effects, the number of senescent cells does not have to be very large. For example, the entire body is affected by hormones like cortisol secreted by a 10g gland, and since we weigh on the order of 100kg, they make up ~ 0.01% of the body's cells. One out of 10,000 cells. Similarly, it may be enough that senescent cells are only one out of a hundred or a thousand cells for their secreted factors to affect physiology at large.

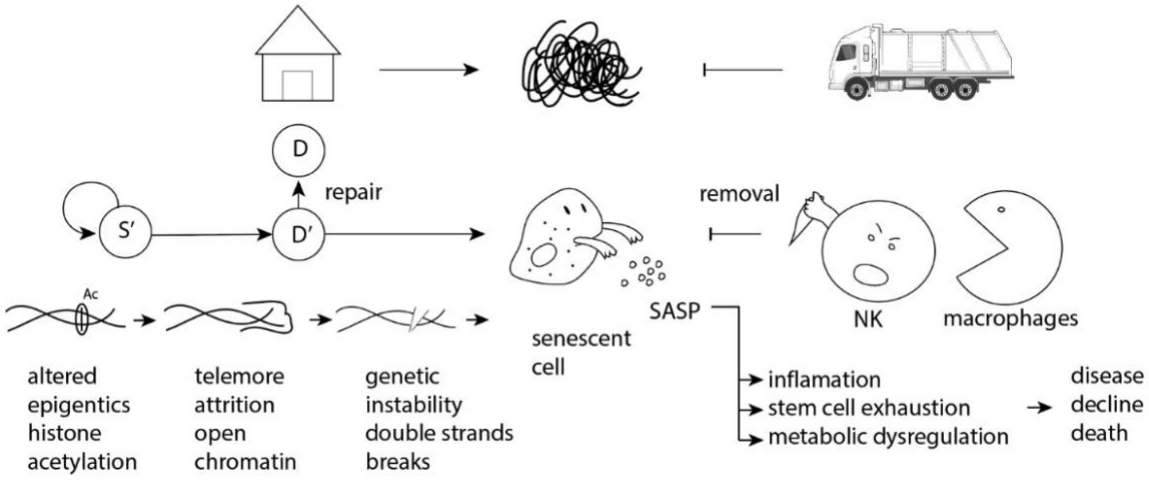
The saturation of the immune cell "garbage trucks" contributes to decline in another important way: saturation hampers their other tasks, including fighting infection and cancer. Together, these systemic changes increase the risk of illness and organ dysfunction.

Noise in the model, the truck strikes, is essential to explain why genetically identical organisms in the same conditions die at different times.

An essential point in the theory is separation of timescales. Just as garbage is made and removed daily, much faster than the rate at which houses are built, so is damage X made and removed much faster than the slow accumulation of damage-producing units. Senescent-cell half-life is days to weeks, whereas their production rate, given by the number of altered stem cells, rises over decades.

Why doesn't the number of trucks rise with age? We can think about this from the point of view of natural selection. Damage removal capacity, the number of trucks, is selected for the young. The amount of NK cells and macrophages is designed to allow young organisms to fight infection and recover from injury. We are not designed to be old – natural selection does not support a mechanism to sufficiently increase removal capacity at old age since most individuals in the wild never reach old age. Indeed, the number of NK cells does not increase strongly with age, they just seem more exhausted (Brauning et al. 2022).
Another explanation is that too many trucks cause collateral damage- immune overactibotynis the cause of many diseases. Maybe the number of trucks is limited by the need to protect us from
Self/attack by the immune system.

More precisely, we can discount as our X any form of damage whose repair mechanism rises with age in a way sufficient to effectively remove X. Damage can't rise to cause aging. Damage casual for aging in this theory needs to saturate its trucks when damage levels are excessive.



It is likely that each organism has several types of houses, generating several types of garbage, each with its own kind of truck. Perhaps altered stem cells and senescent cells are the first to accumulate; if they were to be eliminated, we might expose the next set of houses and garbage, perhaps damaged neurons, and so on.

In a nutshell, our theory for aging derives from three “laws of physiology”:

• All cells come from cells – Stem cells produce differentiated cells.

• Cells mutate – Epigenetically altered stem cells increase linearly with age, and produce damaged and senescent cells.

• Biological processes saturate – the removal processes of these damaged and senescent cells eventually reach their maximal capacity and saturate. Damaged/senescent cell levels rise sharply leading to inflammaging and decline.

Natural selection is the driving force because damage removal capacity was selected for the young, not the old, and hence does not increase with age. A useful physiological process in the young, production of senescent cells that cause inflammation as part of normal injury repair, is pushed beyond its design specifications to cause inflammaging.

We are now ready to see how stochasticity (randomness) can lead to the Gompertz law and more.

Time for a nice deep sigh of relief

The SR model explains the Gompertz law

Here is our first test for the model - how does risk of death rise with age? Recall that human hazard rises exponentially and doubles every 8 years.

In the SR model, at first there are few houses, and garbage is cleared by trucks so there is a low steady-garbage level. When trucks go on strike (noise) the garbage rises a bit. When the strike is over, the garbage returns to baseline

.Suppose that strikes occur at random, like flipping a coin. Suppose it takes ten strikes in a row (10 flips to land heads) for garbage to cross the death threshold. Ten heads in a row is rare - a chance of 1/1024 .

Now after 8 years more houses are built , so more garabage is produced, and garbage is now higher on average . It only takes 9 coin flips to land on heads to reach death, which is one in 512. The risk of death has doubled in 8 years.

In another 8 years there are more houses and now only 8 coin flips are needed - the chance of death doubles every eight years. We have the Gompertz law.

A linear rise in damage producing units (houses) creates an exponentially rising risk of death.

the linear rise of houses is essential. If the houses rose like t^2, the risk of death would go like e^T^2, instead of e^t.

The SR model explains slowdown of risk at very old ages

Recall that the exponential rise of hazard begins to slow down around age 90. Simulations of the model show this clearly.

Around age 90, a special thing happens- the numerous houses makes
So much garbage that it is very close to the maximal Removal Capacity of the trucks. Garbage is very high at steady state.In our houses and trucks model, very old individuals have garbage close to the threshold, and are saved by the noise.

The dynamics is now dominated by noise - like a random Walk. The chance of dying is basically the chance to flip heads once (eg a truck strike that takes garbage over the threshold). That means a constant chance of death per unit time in this age range.

Mathematically, this occurs close to the age where production equals maximal truck removal. Eta t=beta, t=beat/eta =110 years. Near this age, noise dominates (production and remote cancel out), and like a random walk, the chance of crossing the threshold are approximately constant .

At super-old ages that are way past 100 (not seen in real life but can be simulated on the computer) production rises above maximal removal (eta t >beta). A wind of production exceeds removal and pushes X over the cliff. hazard is predicted to rise as t^2. We will see experimental evidence for this when we discuss aging in a single cell organism, yeast.

The model explains rapid effect of some longevity interventions at middle and old age

Recall that shifting flies to a life-extending diet caused a rapid drop in hazard to the better curve. The Sins of the past are forgotten.
A longevity diet like caloric restriction causes the flies to delay growth and reproduction, and turn on repairp, so that they can live to see a day when food is plentiful again so that their progeny have a chance to survive.
The effect on the cells is to reduce, reuse and recycle - making fewer new proteins, recycling damaged proteins and mitochondria and reusing their building blocks. This amounts to the houses making less garbage per unit time. The trucks are fast and so the steady-state garbage drops on the fast timescale of the trucks. Any shift in midlife that makes the houses produce less garbage, or increases the trucks, causes a rapid shift to longer fly lifespan.

But not all interventions do that. Other interventions, such as lowering temperature, make flies live longer, but only change the slope of the hazard curve. The sins of the past are not forgotten.

Pair up: how does this change of slope happen in the houses and trucks model?

Answer: if you slow the rate of building new houses, but they still make as much garbage per house per unit time, you reduce the slope of the hazard curve. This is because you still have all the old houses, and just change the rate of building new ones.

This is how we work as physicists. We try to challenge our favorite model. The more tests it passes the more confidence we have in the model. We can never prove it.

The SR model was derived from three damage datasets that give the same best fit model

The SR model was built from data on damage dynamics- longitudinal experiments which followed damage over time. Three such experiments, in three different organisms, suggest essentially the same model.

The key assumption is that there is a driver of aging- a type of damage x that varies over time and drives the complex changes in agiung.
The model has the form rate of change of x= production - removal+noise
The SR model equation is made of rising houses eta t, saturating removal beta x/(k+x), so that
dx/dt=eta t -beta x/(k+x) +sqrt(2 epsilon) xi

Senescent cells in mice a key form of damage that drives aging is senescent cells. They stand between the level of molecular hallmarks inside the cell (DNA, protein and mitochondrial damage) and the level of organ systems (inflammation, stem cell exhaustion and more).

Senescent cells are the topic of intense current research- including Valery Krizhanovsky's lab here at Weizmann. They are not a single thing- they have different manifestations in each tissue and cell type. There is a spectrum of cell states between healthy, damaged and senescent. For the sake of simplicity we will for now treat all senescent cells in the body as a single number- the senescent cell load X.

To get a feeling for the dynamics of senescent cells with age, let's consider an experiment by (Burd et al. 2013) who measured senescent-cell abundance in 33 mice every 8 weeks for 80 weeks. To measure whole-body senescent cell amounts, they used genetic engineering to produce mice that emit photons in proportion to the number of senescent cells (Figure 3.1). the photons are produced by a gene from fireflies called luciferase that produces light when it acts on a certain substrate.

Burd introduced the luciferase gene into the mouse genome and placed it under the control of a DNA element, called the p16 promoter, that is primarily activated in senescent cells. Therefore, the senescent cells in these mice make luciferase. When the substrate for luciferase is injected, the mice produce light. Mice

normally don’t make photons, so the light emitted from the mice provides an estimate of senescent-cell abundance, X.

The experiment has several limitations, such as stronger absorption of light from inner regions, genetic disruption of the natural p16 system which enhanced the risk of cancer so the experiment could not probe very old ages, and experimental noise. But the experiment is a good starting point.

Looking at total light emitted from these mice as a measurement of senescent cells X, we see that X rises and falls around an increasing trajectory with age (Figure 3.1). This suggests two timescales: a fast timescale of fluctuations over weeks, and a slow timescale in which X rises over years (Figure 3.2). This fast-slow timescale separation is a key element in our understanding of aging.

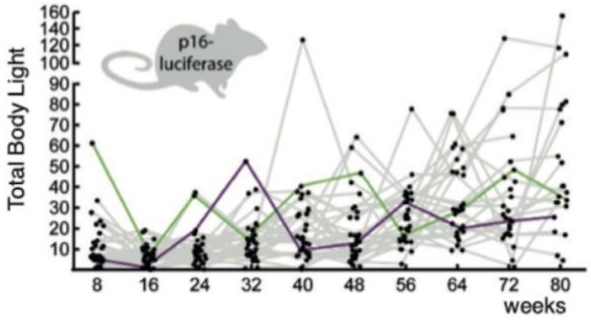


Figure 3.8 Dynamics of a senescent-cell reporter in 33 mice over 80 weeks. Light from a p16-luciferase reporter was measured every 8 weeks. Lines connect the data for each individual mouse. Green and purple lines are example trajectories. Adapted from Burd et al. (2013).

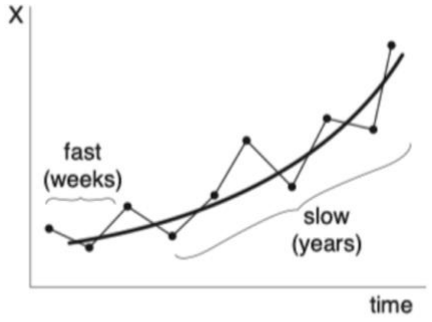


Figure 3.9 Senescent-cell data shows separation of timescales, with fast fluctuations over weeks around a slow rise over years.

Analyzing the data provides five quantitative patterns to build a theory of damage:

i. The average X grows at an accelerating rate with age (Figure 3.3 shows the average of the data in Figure 3.1) Such accelerating accumulation of senescent cells with age is also seen in human tissues.

ii. The variation of X between individuals rises with age. Old mice have a larger range of X than young mice. Some old mice even have X levels similar to young mice. (Figure 3.4 shows the standard deviation of the data in Figure 3.3.)

iii. *relative* heterogeneity drops with age: the standard deviation of X grows more slowly than the mean. Thus, individuals become more similar to each other in **relative** terms. The ratio of standard deviation to the mean, called the coefficient of variation (CV=std/mean), drops with age (Figure 3.5); its inverse 1/CV rises approximately linearly with age (Figure 3.5 inset). The declining heterogeneity means that damage X becomes more similar in relative terms between individuals with age.

Such relative homogeneity might be expected for a core driver of aging- old individuals all share this driver. By contrast, downstream effects of X, like age-related diseases, depend on genetics and environment and are thus more variable

between individuals with age. Most blood tests like glucose have a rising CV with age. Thus declining CV can help us pick needles out of haystacks to find core drivers of aging- stay tuned for the next lectures.

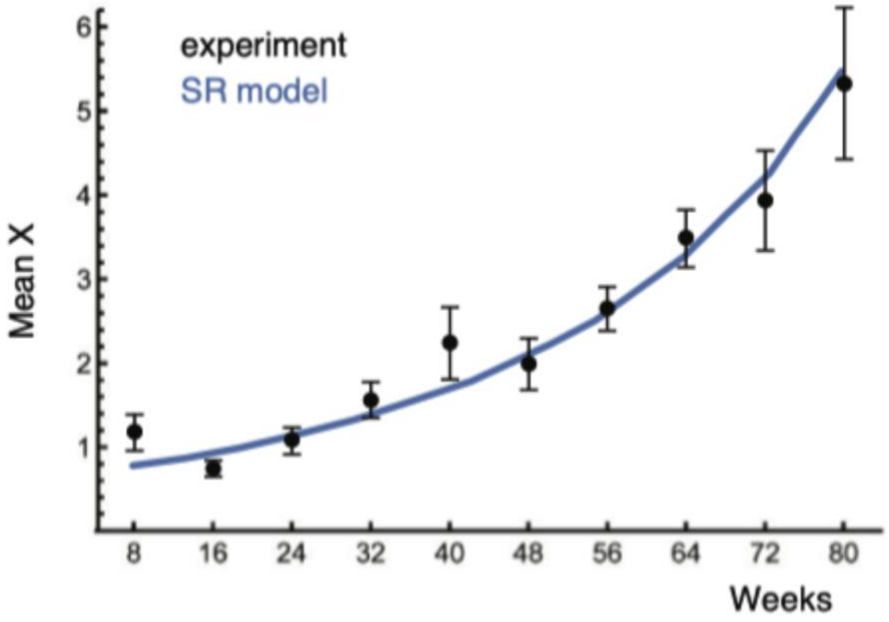


Figure 3.10

Mean level of senescent cells accelerate with age.

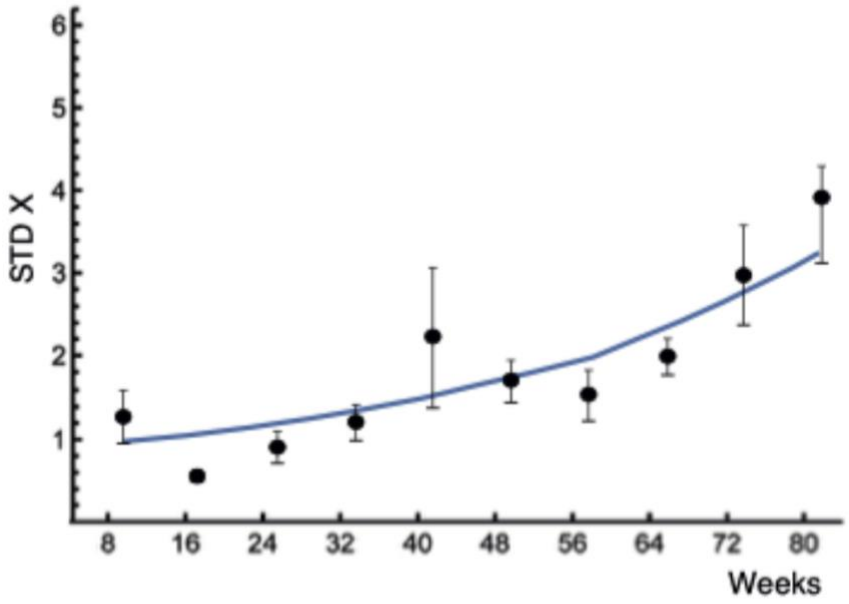


Figure 3.11
Standard deviation of senescent cells rises with age.

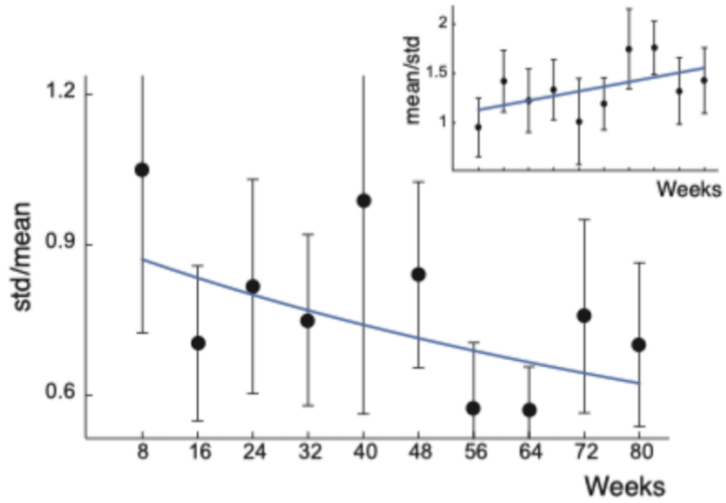


Figure 3.12 Coefficient of variation, defined as CV=std/mean, of senescent cells drops with age. Inset: 1/CV rises with age.

iv. Distributions of X between individuals at a given age are skewed to the right. There are more individuals above average X than below it (Figure 3.6). The

skewness of these distributions gradually drops with age, as they become more symmetric.

v. The autocorrelation time of X increases with age (Figure 3.7 right). A mouse that is higher or lower than average stays so for longer periods the older it is (Figure 3.7 left panels). Thus, with age, the stochastic variation in X becomes more persistent.

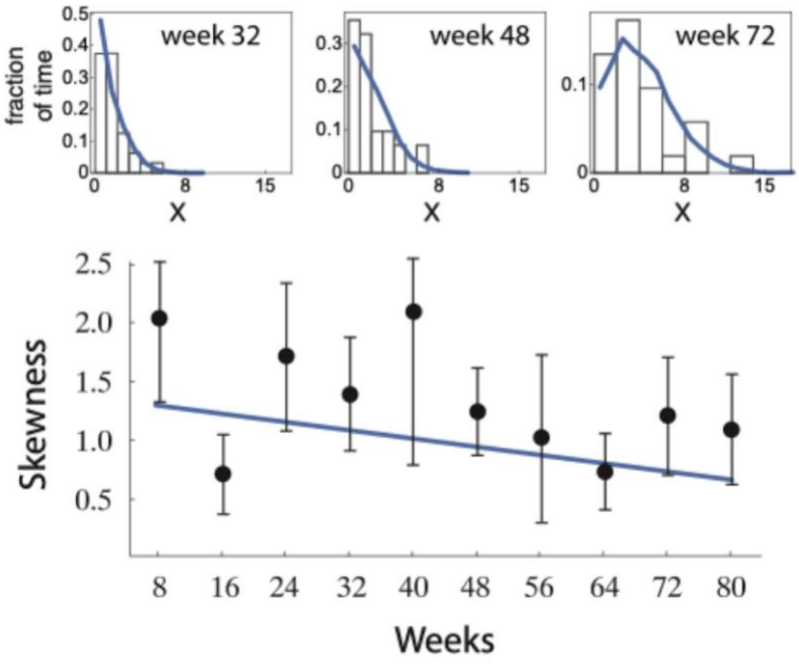


Figure 3.13 Damage distributions are skewed to the right and skewness drops with age.

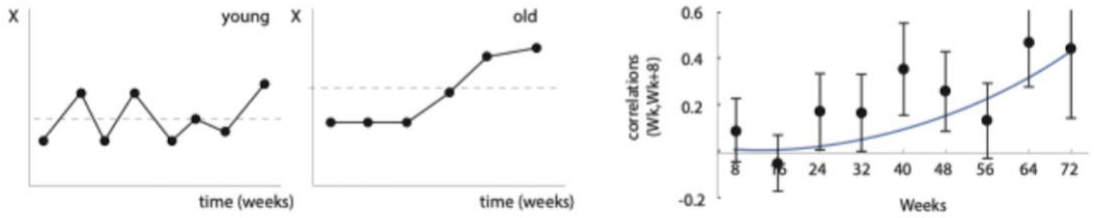


Figure 3.14 Damage fluctuates rapidly in young and becomes more persistent with age as seen in rising autocorrelation time.

Single celled organism shows similar damage trajectories

The same features are found in a completely different organism and different damage. This is the bacterium E. coli - a single celled organism. When provided with food E. coli can divide indefinitely. But when starved, e coli cells all die, like us. And like us, they die at different times - They show the Gompertz law with a typical lifespan of about 100 hours.

Yifan Yang measured damage in E. coli by using a molecule - the die called PI- that only enters the cell when the cell membrane is damaged. The die then binds the DNA inside the cell and becomes Fluorescent. Measuring fluorescence over time In individual starved E. coli cells, Yifan analyzed 600 damage trajectories. The damage trajectories show the same features as the mice senescent cell data- accelerating mean and std, decreasing CV and so on. There appears to be Universality in damage dynamics between bacteria and mice.

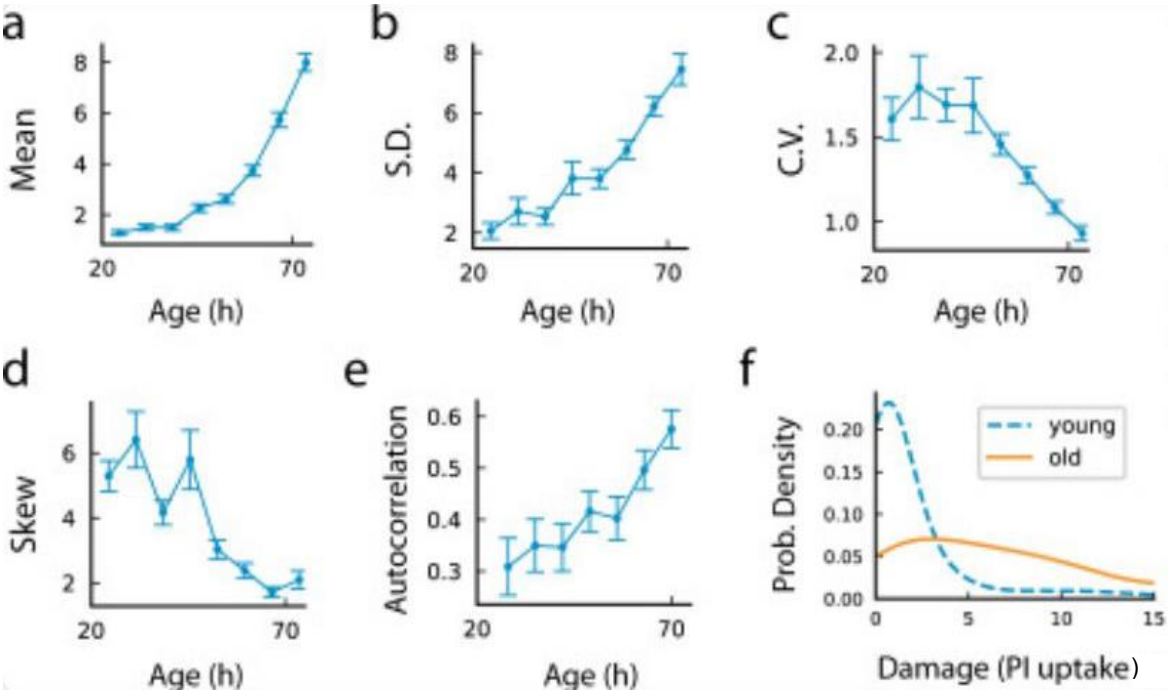


Figure 3.15

Human frailty index shows similar statistics Interestingly, these dynamical features are also shared with the **human frailty index**, one of the best predictors of mortality in humans introduced by Rockwood et al (Mitnitski et al. 2002). The frailty index is simple to define - it is the fraction of deficits a person has out of a list of deficits, ranging from back pain and hearing loss to diabetes and cancer. Thus the frailty index can range between zero – no deficits, and one – all deficits on the list. Since damage drives different deficits in each person depending on genetics and environment, the frailty index is a good proxy for damage because it averages out over many types of deficits.

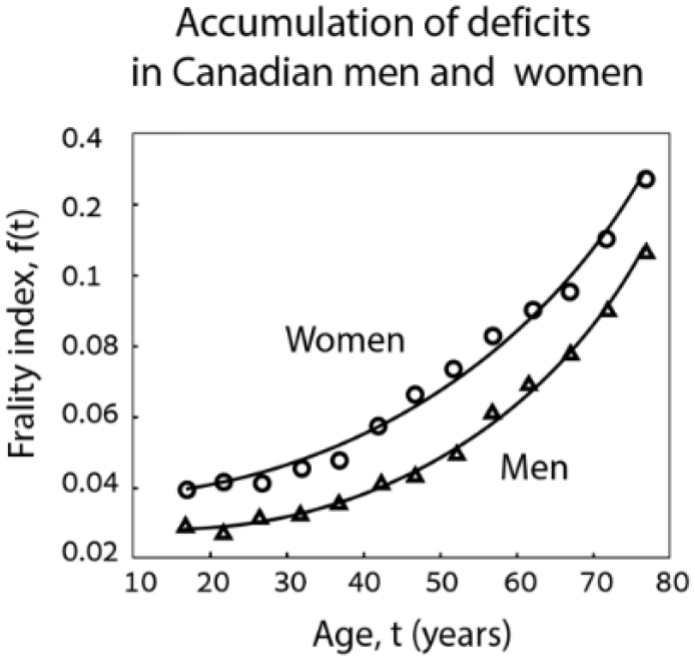


Figure 3.16 *Frailty index – the fraction of health deficits an individual has from a list of deficits – accelerates with age. Adapted from (Mitnitski et al. 2002a).*

The average frailty index increases in an accelerating way with age (Fig 6.8). The distribution of frailty becomes wider and skewed to high values with age (Fig 6.9A).

The standard deviation of frailty also grows with age. However, it grows more slowly than the mean. Therefore, the relative heterogeneity, the CV **coefficient of variation** goes down with age (Fig 6.9b). Again, the variation between individuals in frailty rises in absolute terms, but drops in relative terms. There are differences in what deficits each person has, but in relative terms frailty becomes more similar between individuals with age.

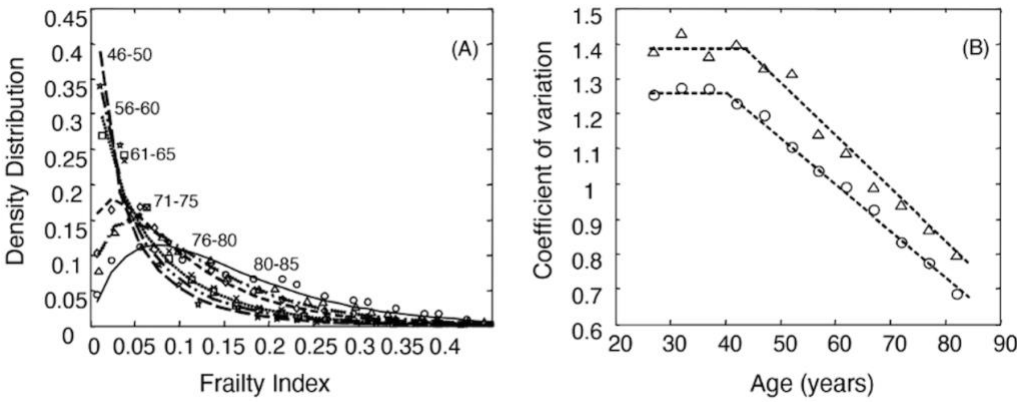


Figure 3.17 *Distribution of frailty index is skewed to the right, and shows decreasing coefficient of variation with age. Adapted from (Rockwood, Mogilner, and Mitnitski 2004).*

This may be a good moment for a nice deep sigh of relief.

The Saturating Removal Model is the simplest equation that fits these datasets

Remarkably, the same model is the best minimal model to fit these three types of datasets. This was the strategy of Omer Karin in his PhD with me (Karin et al. 2019). Omer scanned a wide class of models (Figure 3.8).
The models are based on two timescales, one fast and one slow: X is produced and removed on a timescale that is much faster than the timescale of the lifespan. This separation of timescales allows us to write an equation for the rate of change

of X in which the production and removal rates vary slowly and can depend on age. The model also includes stochastic noise. Thus,

dX/dt = production - removal + noise

Omer tried general forms for production(X,t) and removal(X,t).

He thus allowed production and removal to depend on time t and damage X in biologically reasonable ways like Michaelis Menten terms for X and linear terms for t. There are 16 possible model choices- time can affect production or not is 2 possibilities, x can affect production or not is another 2 possibilities, and the same for removal, totaling 2^4=16 models architectures.

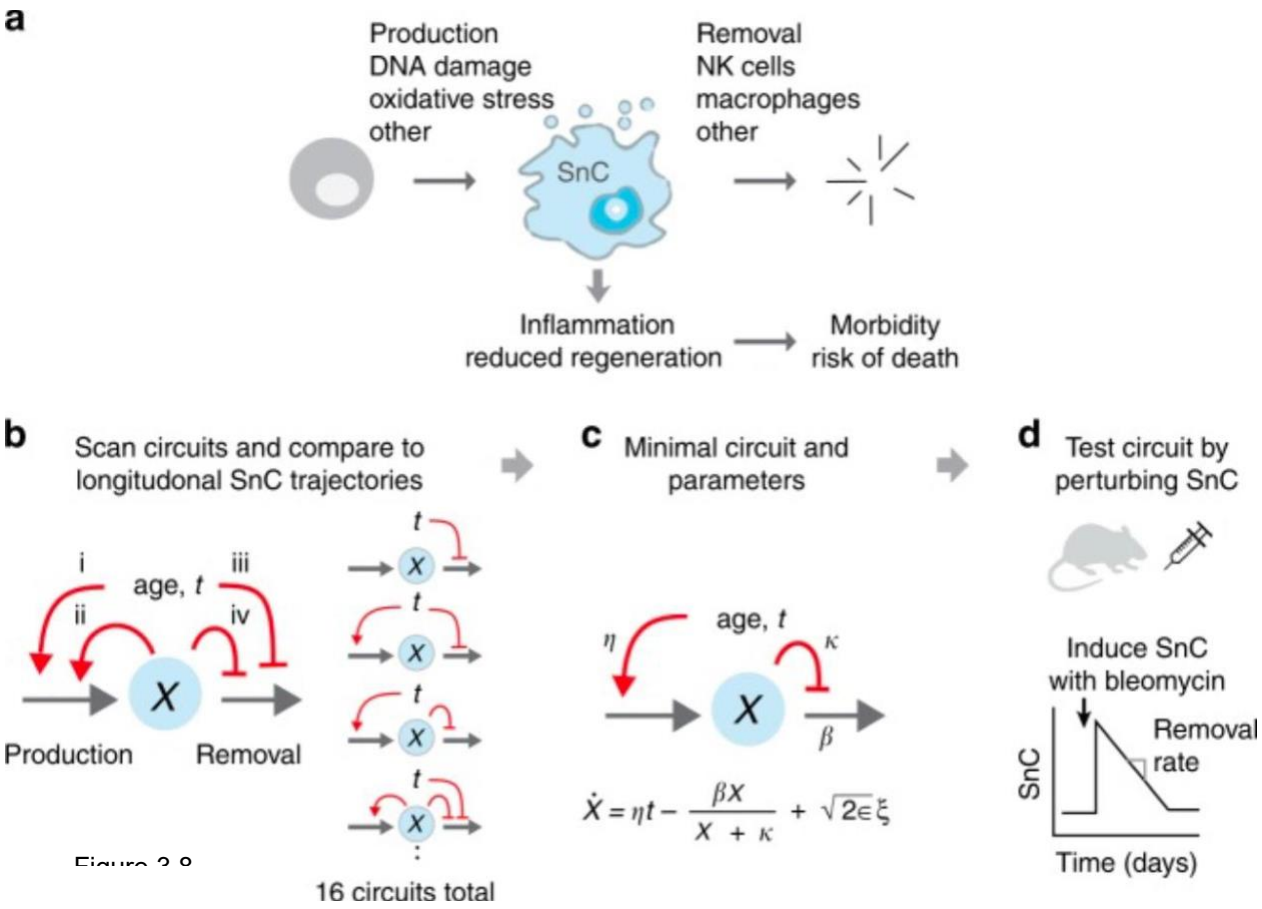


Figure 3.18

For each of these 16 possibilities Omer determined the best fit parameters to the data and compared their goodness of fit.
(Technically, he used the full dynamic trajectory dataset and computed maximum likelihood using stochastic simulations- how likely the observed trajectories are given each model and parameter choices).
Happily, there was one minimal model that outperformed the rest. A few years later Yifan Yang found that the same minimal model is also the best at explaining damage measurements in the bacterium E. coli. The model is therefore agnostic as to the type of damage represented by X- and can apply to different organisms.

So here is the winning model, which teaches us a lot about the biology of aging.

First, the production rate of X rises linearly with age in the model: production = ηt

Again, let's make sure that we understand that there are two timescales: a fast scale (days-weeks in mice) in which damage reaches steady state, and a slow timescale (years in mice) over which production rate ηt changes.

The idea behind this production process is based on an additional form of damage, y, that is not removed. As we saw above, since y is not removed it rises linearly with age y=p t. The damage y generates damage x. Thus y may be called 'damage producing units'.

What can this y be, what entity is not removed, accumulates with time and produces senescent cells?
It can not be just any cell, like a skin cell removed in about days, a gut cell removed in 5 days, a blood cell removed in months or a liver cell removed in one year- because these cells do not last decades.
There are two classes of cells that last in our body a lifetime. One class are permanent cells that do not divide- like neurons and heart muscle. These cells go dysfunctional one at a time at a low rate, providing y=p t. We will consider these in the lecture on neurodegeneration.

Of more immediate interest now is another form of cells- stem cells. As mentioned before, stem cells divide very rarely to self- renew and also produce differentiated cells D that make up our organs like skin lung gut and blood (each of which has its own specialized stem
cells). These D cells divide a number of times (transit amplifying cells) to generate the massive amounts cells of the organ that need to be replaced each day.

At a slow but substantial rate, stems cells S become epigenetically altered. The epigenetic alteration most relevant for aging, according to work in multiple organisms, is changes in histone acetylation that cause opening of chromatin in the wrong (normally closed) places including telomeres. These changes occur due to random biochemical processes, and also as an aftermath of DNA repair that rearranges the epigenome locally. The important role of epigenetic alterations with age was called the information theory of aging by David Sinclair.

If the alteration harms the stem cell, that altered cell loses to the other stem cells and is eventually removed.
But some alterations do not harm the stem cells, and these altered stem cells are not removed. This means more and more altered stem cells accumulate, y=p t. They pass on their alteration to their daughter cells D - in general stem cells copy epigenetic information when they divide [.https://doi.org/10.1038/s41588-023-01476-x]
They also dump their protein and mitochondrial damage to their differentiated daughter cells so the stem cells can remain fresh and last a lifetime.

The daughter cells D divide rapidly and the epigenetic alterations they inherited from their stem cell mother causes them to have DNA breaks and other problems. This causes them to become senescent. Thus each altered stem cell will generate a column of damaged and senescent cells.

What about removal of senescent cells? The removal of X is carried out by the immune system, such as NK cells that kill senescent cells and macrophages that

clear the debris. These are immune cell garbage trucks. Note for future use that these trucks have other roles as well- fighting virus infected cells and cancer cells.

If this removal worked at a constant rate β per senescent cell, the removal term would be − β X. However, this does not match the data. As we saw above, The equation is dX/dt = ηt- β X, whose quasi-steady-state solution is a linear rise of X with age, X st = ηt /β . This is ruled out by the data which shows an accelerating rise with age (Figure 3.3).

Instead, the winning model explains the accelerating rise of X by a removal rate that drops with the number of senescent cells. In other words, removal saturates; senescent cells inhibit their own removal. Such a drop could be due to several processes: immune cells that remove senescent cells are downregulated if they kill too often, and they can be inhibited by factors that the senescent cells produce. The drop of removal rate can also be simply due to a saturation effect, in which the removing cells become increasingly outnumbered by senescent cells.

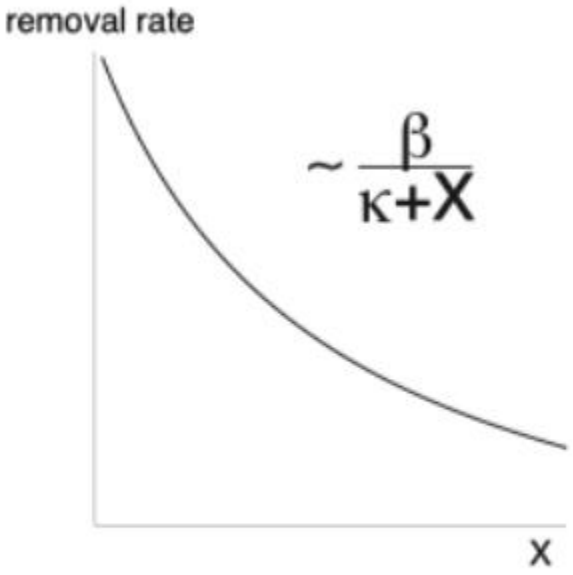
To model the saturation, we use a Michaelis–Menten form which is good both for inhibition due to secreted factors and for saturation

Removal = $\beta \frac{X}{X+\kappa}$

where β is the maximal senescent-cell removal capacity, in units of senescent cells/time, and κ is the concentration of X at which they inhibit half of their own removal rate. The removal β rate *per senescent cell* thus drops with senescent cells abundance, $\frac{\beta}{X+\kappa}$ (Figure 3.9), and the number of senescent cells lost per unit time is that rate times X, namely $\beta \frac{X}{X+\kappa}$. Combining production and removal, we obtain a model for the rate of change of X:

$$\frac{dX}{dt} = \eta t - \beta \frac{X}{X+\kappa}$$

Note that this model assumes that maximal removal capacity β does not decline with age. Adding such a decline, namely β (t) , generally leaves the conclusions the same. For simplicity, we ignore this possibility and recall that NK cell and macrophage numbers do not drop strongly with age in humans.



Removal rate per senescent cell drops with senescent-cell abundance X.

Figure 3.19

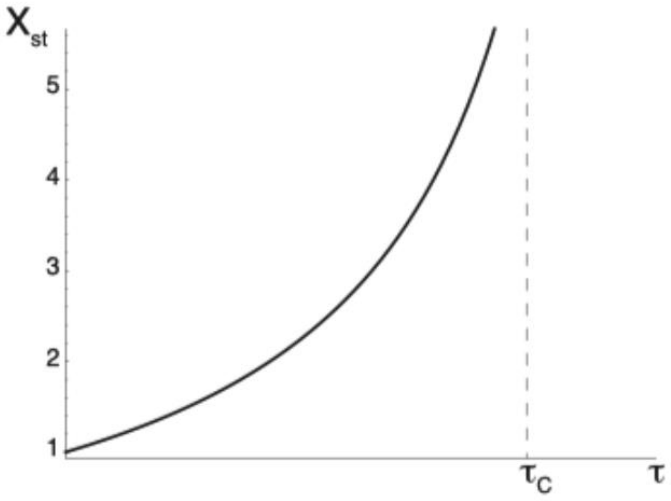
Let's compute the steady state of X. On the fast timescale of weeks, the production rate ηt can be considered as constant. Setting dX/ dt = 0 in Eq. 3.1, we find that the (quasi-) steady-state X of is

$$X_{st} \approx \frac{\kappa\eta t}{\beta - \eta t}$$

Thus, X st rises linearly with age at first. Then, the term on the bottom becomes closer and closer to zero. The rise of X accelerates and diverges at a critical age t_c

= β /η (Figure 3.10). Figure 3.3 shows a good agreement between data in dots and the model in black.

When X levels rise high enough, they reach levels not compatible with life. Thus, the critical age t_ c = β /η where X diverges is a rough approximation for the mean lifespan. This equation indicates that lifespan can be extended by increasing the removal capacity β , or by reducing the senescent-cell production rate η. More trucks or slower construction of damage producing units extend longevity.



Mean senescent cells *X* accelerates and diverges at a critical age.

Figure 3.20

The saturating removal model is thus a combination of two of our toy models from the beginning of the lecture-
Damage that is not removed,y produces x. Since y rises linearly with age, production of x also rises linearly with age as eta t. Removal of x saturates. Together this generates an accelerating rise of damage - as you can see in a rate plot in which the production line rises as age progresses, and the crossing point

with the removal curve accelerates to higher and higher values of x. When production exceeds maximal removal
There is no longer a (quasi) steady state since the lines no longer cross. Damage rises steeply- this situation requires simulations to solve

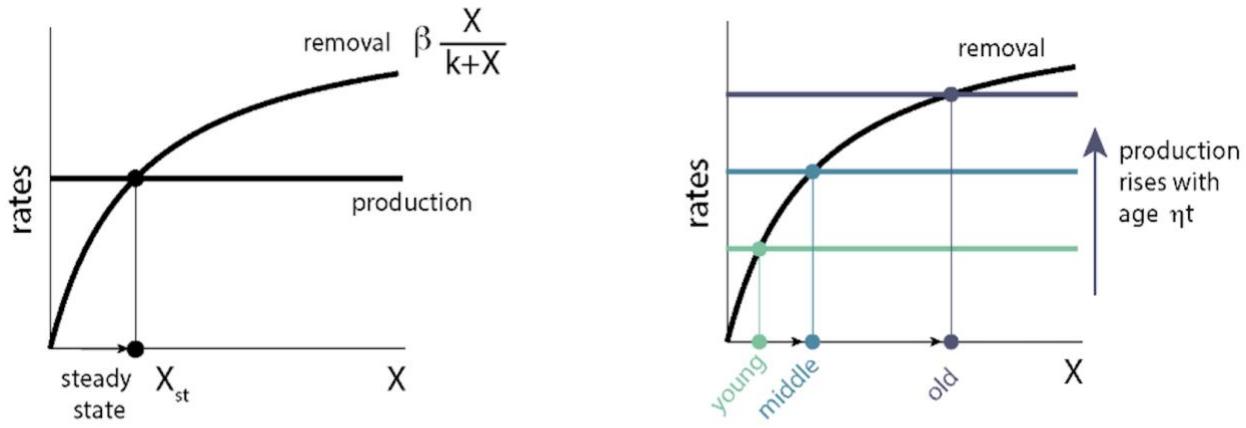


Figure 3.21 A rate plot shows that steady-state damage, the point at which production and removal curves intersect, accelerates with age.

Let's honour our busy immune trucks with a song; in the lecture you did great job as backup
Singers! . Trucks work at night- immune clearance is higher when we sleep- and as we will discuss later, sleep is one of the four pillars of health, all night long!

Truck song/ music the Doors

Keep your eyes on the road and your hands upon the wheel x2
Picking up the Garbage and I just can't get my fill

I said roll trucky roll x 3
All night long!

Me and my crew used to keep our village clean x2
Now it's a city and the garbage keeps piling in

I said roll…

Lord have mercy it’s my third shift in a row
My hands are shaking and my eyelids dropping low
Can’t stop now- there’s tons of garbage left to go

I said roll…