Why and how we age: the way towards an unlimited lifespan

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the way towards an unlimited lifespan

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Introduction

This book is a small collection of the works I have published in recent years, some with the authoritative contribution of Nicola Ferrara and Giuseppe Rengo, regarding the fascinating theme of aging interpreted as an adaptive phenomenon, that is a genetically programmed and regulated phenomenon.
Each chapter corresponds to an article on a scientific journal or a chapter in a peer reviewed book. The texts and figures correspond to the original ones but in some cases, especially for the first two works, they have been modified to make them uniform with the expressions and graphic representations used in the subsequent works.
Moreover, some typographical typos and some oversight have been corrected and the bibliographic references have been unified in a single section.
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Giacinto Libertini

Chapter 1

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The Role of Telomere-Telomerase System in Age-Related Fitness Decline, a Tameable Process

Giacinto Libertini

Abstract

In our body there is a continuous cell turnover. Every day innumerable cells die by programmed cell death, in particular apoptosis, and are replaced by others deriving from stem cells. With the passing of time, this turnover is limited by sophisticated mechanisms, genetically determined and regulated, which control the telomere-telomerase system and therefore cell duplication capacity (replicative senescence) and overall functionality (cell senescence).

Alterations of cell turnover mechanisms cause dramatic syndromes, such as dyskeratosis congenita and Werner syndrome, while the normal age-related slowdown and stopping of this turnover causes a fitness decline that is defined senescence in its more advanced expressions. The fitness decline documented in the wild for many species should not be confused with the mortality increment observed for animals, as *Caenorhabditis elegans* and *Drosophila melanogaster*, in artificial conditions at ages non-existent in the wild.

Many species are not subject to this fitness decline and, in the case their individuals reach very old ages in the wild, are defined as ageless animals or species with 'negligible senescence'. For some of them, the functionality of the telomere-telomerase system has been documented as unvaried at older ages.

Indeed, the fitness decline appears not an inevitable decay but a very sophisticated function, favoured for its greater inclusive fitness in particular selective conditions, and, being a function, in principle modifiable and governable. This leads to the prospect that senescence will be tamed in the not too distant future, in particular by control of, or more audaciously, by a modification of, the genetic determinants of the telomere-telomerase system. Such a prospect is radically different from the present advances in medical cures that are only increasing the proportion of disabled ultra-octogenarians.

Behind the Scenes: Introduction

When Darwin proposed the hypothesis of evolution by natural selection, two big problems undermined the reliability of the great theory.

The first was the existence of insect species with social organisation (bees, ants, etc.). If natural selection favours individuals with greater fitness and reproductive success, how can one explain the fact that worker individuals of these species nurse the progeny of queens and do not procreate themselves? What possible selective mechanisms can favour the genes determining such odd behaviour? Darwin, the father of modern biology, did not know how to answer: as an improvised patch for a wonderful new dress with a bad tear, he justified all this by maintaining that such behaviour was favoured because it was advantageous for the species [Darwin 1871]. He was wrong, and the correct answer was discovered nearly a century later, as will soon be discussed.

The second problem was even more serious. For many species, *Homo sapiens* included, an increase in chronological age is accompanied by a fitness decline in the wild. That is to say, mortality rates increase with age in the wild [Deevey 1947; Laws 1966, 1968; Laws and Parker 1968; Spinage 1970, 1972; Holmes and Austad 1995; Ricklefs 1998]. Regarding this fitness decline, referred to as "aging" in its more advanced expression (a popular and terrible name), if

natural selection favoured the fittest, how was this explicable? Darwin had two alternatives, both difficult and fraught with implications. The first (*nonadaptive hypothesis*) demanded the admission that natural selection was not able to favour genes suited to keeping fitness stable at increasing ages. However, was it possible that natural selection, which is thought to have moulded the eye, brain, hand and numberless marvels in numberless animal, plant and microbe species, failed in the task of keeping fitness stable at greater ages? Moreover, if this is the case, then why has this hypothetical incapacity of selection been greater for some species that age quickly and lesser for other species which age slowly or even not at all?

The other possibility (*adaptive hypothesis*) was that this fitness decline had some unknown evolutionary advantage. This hypothesis seemed even more arduous: how could an anticipated death be evolutionarily advantageous? Who could maintain such a thing without being considered a little muddled, or worse? Darwin could not give an answer to the second problem, thus it was aptly named Darwin's dilemma [Goldsmith 2003].

Some years later, August Weissmann, using extraordinary intuition, tried to give an answer. Unfortunately, he did not formulate a clear exposition or give solid scientific proofs, although he did hint that the anticipated death of old individuals was beneficial because this gave more space to new generations which was useful for the evolution of species [Weismann 1889; Kirkwood and Cremer 1982]. In short, Weissmann was a supporter of the adaptive hypothesis of fitness decline, although he later disavowed it [Weismann 1892; Kirkwood and Cremer 1982]. Furthermore, about the mechanism underlying this decline, he observed that the cells of the various organs and tissues were renewed continuously and that when this turnover slackened or stopped, the organs or tissues reduced or lost their functionality with negative effects on fitness [Kirkwood and Cremer 1982].

His adaptive hypothesis was original but not well inferred from a theoretical viewpoint. Moreover, as common experience testifies, all inanimate things deteriorate with the passing of time, so why not assume that living beings, too, are subject to the same inexorable law? In fact, "common sense" seems to strongly suggest that the nonadaptive hypothesis of fitness decline is correct, even if this requires the admission that natural selection is incapable of solving this specific problem.

On the contrary, the cell turnover mechanism hypothesised by Weissmann for the aging was easier to understand and accept but it had an unhappy fortune, too, at least for the next 70 years. Indeed, an illustrious Nobel prize winner, Alexis Carrel, demonstrated that cells explanted and cultivated *in vitro* multiplied an unlimited number of times [Carrel 1913], meaning that Weissmann's hypothesis was groundless and unacceptable.

Poor Weissmann with his intuitions seemed to miss every time!

In 1961 an obscure researcher, Leonard Hayflick, cultivated fibroblasts *in vitro* and discovered that they multiplied a limited number of times, a finding in clear contrast with Carrel's results. After having excluded any factor as a possible cause of such stoppage in cell duplication, he decided to publish his findings. However, the authoritative journal to which the paper was submitted rejected it, with the statement that it was *a priori* unacceptable since the results were in plain contrast with what had been definitively demonstrated and what was accepted as scientifically sound. Fortunately, Hayflick was stubborn and succeeded to publish his paper in a less authoritative journal which was more open to new ideas [Shay and Wright 2000].

Carrel's observations (likely due to errors in cell culture methodology) were overthrown and Weissmann's hypothesis recovered its value! Hayflick stated in 1977 that the limits in cell duplication (Hayflick limit) were the likely cause of the aging: "... if normal animal cells do indeed have only a limited capacity for division in cell culture, the manifestations of aging might very well have an intracellular basis." [Hayflick 1977]. However, this statement was in contrast with other ideas that by this time were imposing themselves about the aging [Kirkwood and Austad 2000], i.e.:

> "mutation accumulation" theory - "Aging" is due to the effects of harmful mutations, accumulated over evolutionary time, manifesting themselves at older ages when, in the wild, survivors are few or absent and, consequently, selective forces are too weak to

eliminate them [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987; Partridge and Barton 1993].

"antagonistic pleiotropy" theory - "Senescence" is caused by pleiotropic genes with beneficial effects at early ages and deleterious effects at later ages [Williams 1957; Rose 1991].

"disposable soma" theory - "Aging" has environmental or somatic and not genetic causes, and evolutionary responses to them are increasingly limited at older ages by physiological, biochemical or environmental constraints. Therefore, in the evolutionary search of an optimal allocation of metabolic resources between somatic maintenance and reproduction, the second is preferred [Kirkwood 1977; Kirkwood and Holliday 1979].

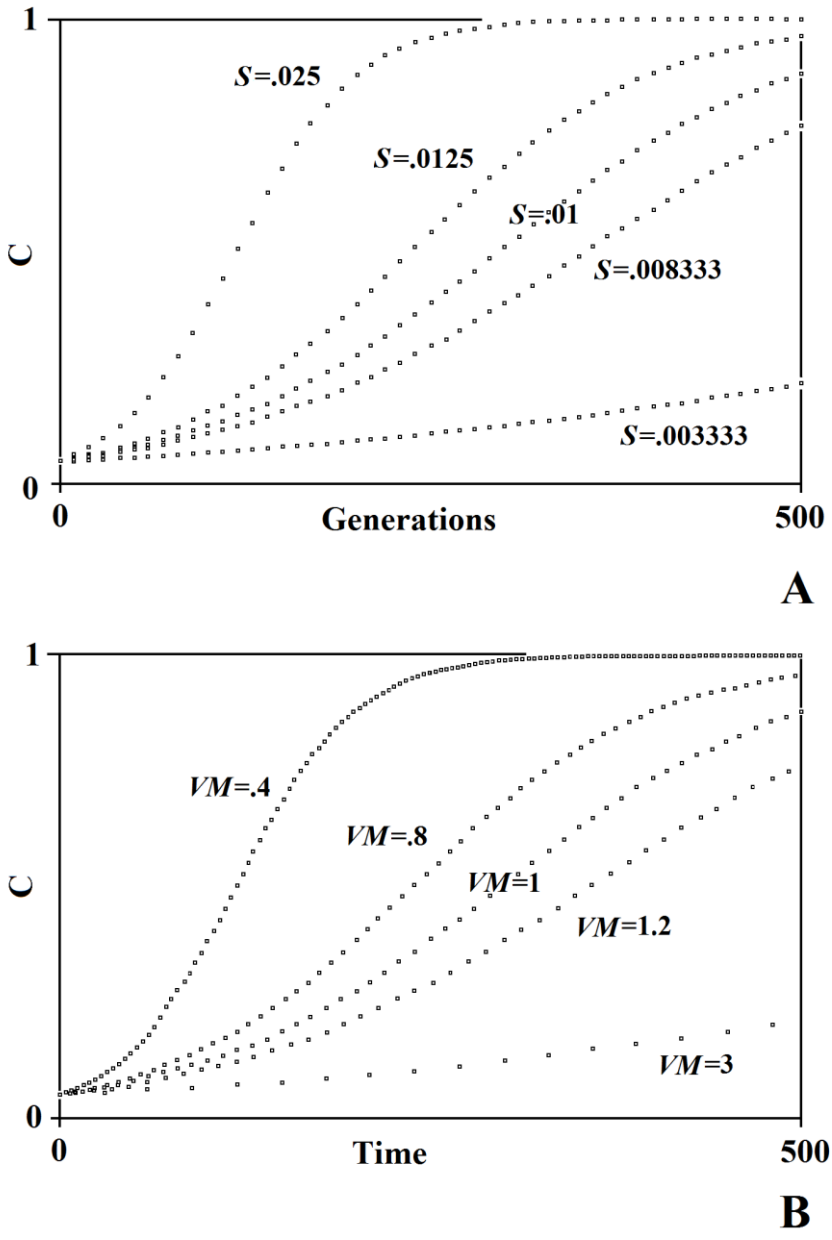


Figure 1 - (A) Spreading of a gene C according to the variation of the advantage (S) caused by the gene C, supposing a constant mean duration of life. (B) Spreading of the same gene C according to the variation of the mean duration of life (ML), supposing a constant value of S (= 0.01) [Libertini 1983, 1988, 2006].

In a relatively recent paper, Hayflick, as a prophet repudiating himself, did not mention the pivotal importance of cell duplication limits for the mechanisms of aging and instead proclaimed: "Ageing is a stochastic process that ... results from the diminishing energy available to maintain molecular fidelity. This disorder has multiple aetiologies including damage by reactive oxygen species." [Hayflick 2000]

In the years 1970-76, I was a simple student of medicine, rich in a wide ignorance and in the foolhardiness of which the ignorant persons enjoy, but —merit among the faults— was strongly struck by Hayflick's results that I considered of extreme importance for aging studies. I became an enthusiast of Hayflick's discovery and of its implications and remained firm in this belief even when Hayflick diminished or denied the importance and the implications of his results.

Stimulated by them, I tried to explain, in precise terms of natural selection, the advantage of a quicker turnover of generations that seemed to me the automatic consequence of the Hayflick limit or, in the case that this limit was unimportant, the advantage of the evident restraints in the individual duration of life for many species, man included, whichever the mechanism causing those restraints was.

Knowing what Weissmann had hinted at, and stimulated by the suggestions of two botanists [Leopold 1961; Woolhouse 1967], I formulated a mathematical model demonstrating with precision how a quicker generation turnover allowed a quicker diffusion of a favourable gene within a species. In numerical terms, this study modelled the spreading velocity of a gene within a species: the increment by x% of gene advantage or the reduction by an equal x% of the mean duration of individual life had the same effects (Figure 1).

This study was essentially the reformulation of Weissmann's intuition in mathematical terms. As examples in more comprehensible terms: populations of bacteria or insects under the action of antibiotics or insecticides become resistant to the action of these substances in the space of a certain number of generations. For a bacterium this time could be a few days, for an insect few years. For a human population, an equivalent evolution would require centuries as a consequence of the much slower turnover of generations.

However, this argument only pointed out that, all other things being equal, species with a smaller mean duration of the life (i.e. a quicker generation turnover) are favoured. This study did not prove that within a species a gene limiting the duration of life would be favoured by natural selection. Darwin's argument that this could be favoured in terms of group selection was untenable [Maynard Smith 1964, 1976] and Weissmann's intuition needed something more.

Then, I obtained a likely solution from the extraordinary results of a group of researchers [Hamilton 1964, 1970; Trivers 1971; Wilson 1975; Trivers and Hare 1976], whom in the intervening years had formulated a brilliant solution for the problem of the evolutionary mechanisms explaining the organisation of the social insects, the first of the above said great problems undermining the theory of evolution.

Until this time, accepted evolutionary arguments were only in terms of selection proportionate to the fitness of the single individual (individual fitness). In fact, for a gene causing an advantage or disadvantage (S) and acting in an individual with the capacity P of having progeny (reproductive value), the selective force (F) operating in favour or against the gene was calculated as proportional to the product of S by P:

$$F \propto S \cdot P \qquad (1)$$

In the solution maintained by those researchers, it was pointed out that for a gene existing and acting in an individual, defined as individual 1, it was necessary to also calculate the effects of the actions of the same gene on the fitness of other individuals (2, 3, ..., n) genetically related with the individual 1 (inclusive fitness). Indeed, for each gene C, it was necessary to calculate the sum of the values of the advantage or disadvantage for each individual X for which the gene C had a

consequence (S_x), multiplied by the reproductive value of each individual X (P_x), then multiplied by the coefficient of relationship between individual 1 and individual X (r_x, or the probability that gene C is present in X).

Therefore:

$$F \propto \Sigma(S_x \cdot P_x \cdot r_x) \quad \text{with X from 1 to n} \qquad (2)$$

As a simple and easily comprehensible example, a young mother with the act of nursing her child reduces her fitness (because she spends energy resources) but the same act is indispensable for the survival of her child, who has a 50% probability of having the same genes as his mother. Therefore, the small disadvantage for the mother caused by the nursing ($S_1 = -y$, with y having a small value) is clearly exceeded by the great advantage for the child ($S_2 = 1$) reduced by 50% ($r_2 = 0.5$): the inclusive fitness of a gene that determines the nursing is positive and the gene is favoured by natural selection. In numerical terms, supposing that the mother is young and her reproductive value is maximum ($P_1 = 1$) and the child reproductive value is also high ($P_2 >> 0$; supposing that only 60% of the children reach the reproductive age, $P_2 \approx 0.6$):

$$F \propto S_1 \cdot P_1 \cdot r_1 + S_2 \cdot P_2 \cdot r_2 = -y \cdot 1 \cdot 1 + 1 \cdot 0.6 \cdot 0.5 = -y + 0.3 >> 0 \qquad (3)$$

With the simpler formula (1), it is clear that the nursing gene would be eliminated by natural selection.

Other two examples are illustrated in Figure 2.

It has to be noticed that formula (2) becomes formula (1) in cases where the gene only has effects for individual 1, because the coefficient of relationship of individual 1 with himself is clearly 1 ($r_1 = 1$).

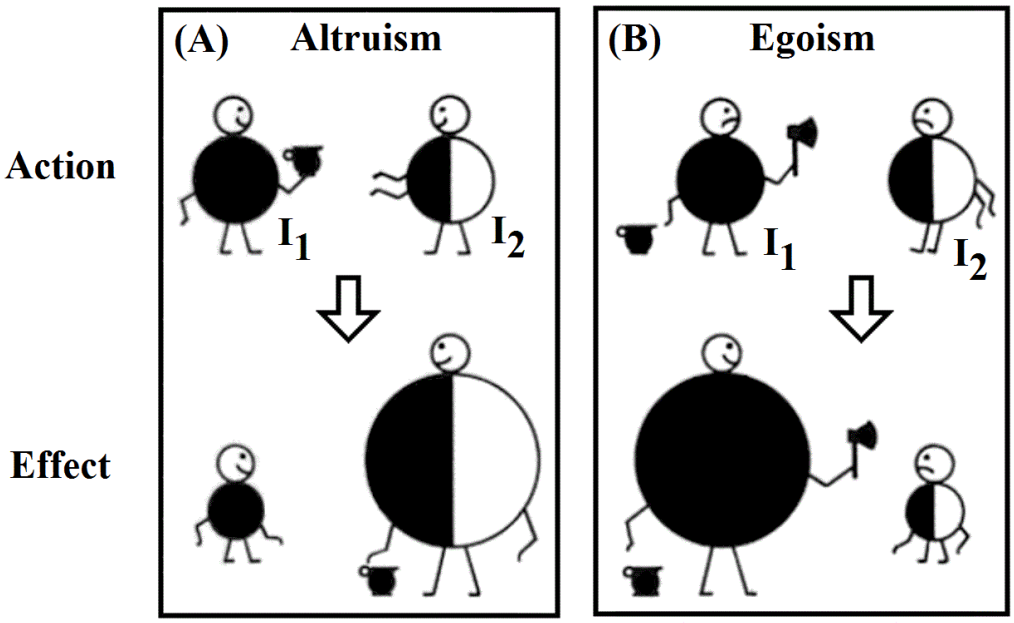


Figure 2 - Actions between two brothers (I_1 and I_2) that, being brothers, have in common half of the genes (r = 0.5). (A) "Altruism" - By effect of a gene X, I_1 gives something of his resources to I_2, increasing the fitness of I_2. His fitness is reduced but the fitness of I_2 is increased more than the double of the reduction for I_1: the gene X is favoured by selection. (B) "Egoism" – By effect of a gene X, I_1 subtracts something of the resources of I_2. His fitness is increased but the fitness of I_2 is reduced less than the half of this increase: the gene X is favoured by selection. The picture is from [Wilson 1975], partially redrawn.

Following this principle, because for the peculiar genetic mechanism with the difficult name haplodiploidy (males are haploid, while females are diploid) the coefficient of relationship between two sister bees is 0.75 while that between a mother bee and its daughter is 0.5 (see Figure 3-C), the inclusive fitness of a gene determining the nursing of a sister grub by a worker bee is favoured by natural selection more than a gene determining the nursing of a daughter grub by its mother bee. This is the same for ants.

With all the details and quibbles that such a scientific formulation involved, inclusive fitness adequately explained the social organisation of bees and ants. Darwinian theory was enriched and an unresolved problem was settled!

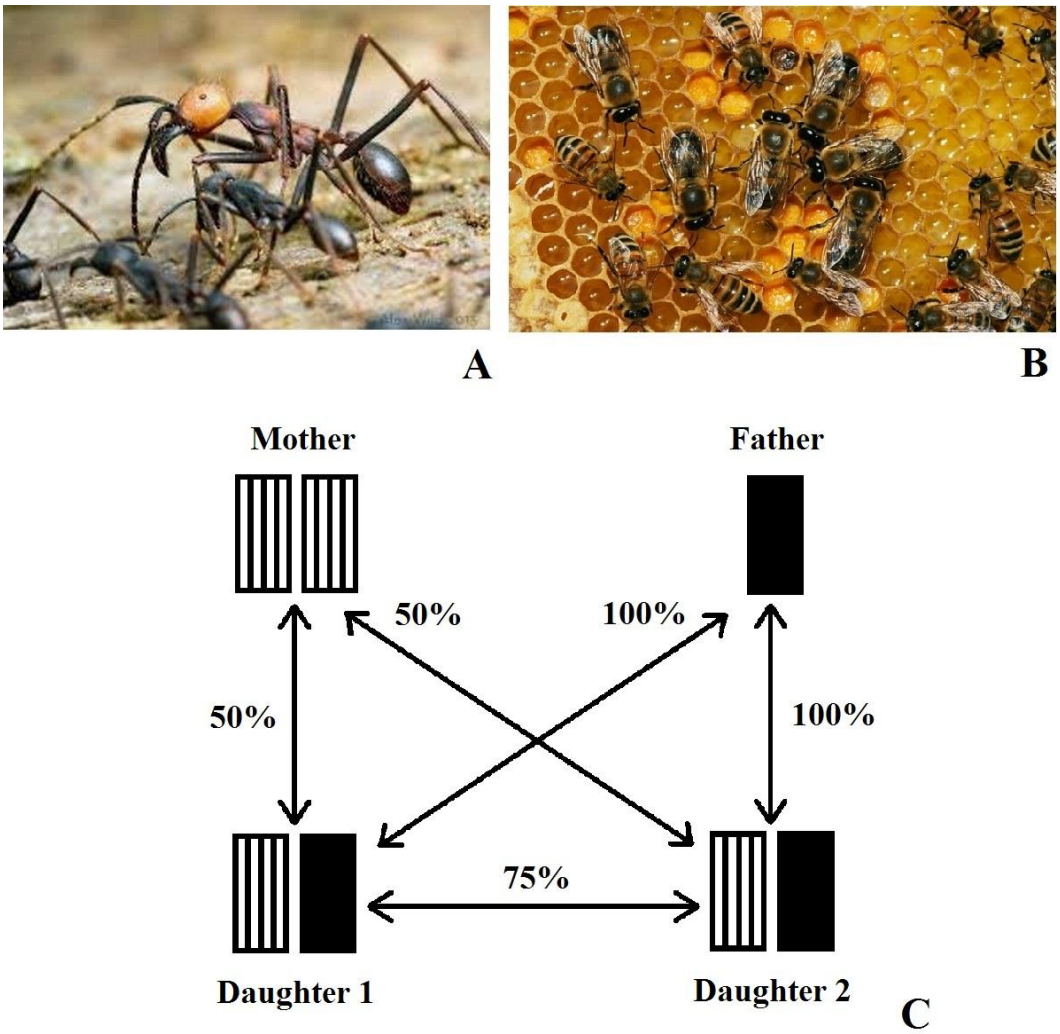


Figure 3 - (A) A queen ant with some worker ants; (B) A beehive; (C) For ants and bees, females are diploid while males are haploid. Therefore, a mother gives to a daughter 50% of its genes, while a father gives 100% of its genes. The probability that a gene is the same in the mother and the daughter, alias the coefficient of relationship (*r*) between mother and daughter, is 0.5. Otherwise, r between the two daughters is 0.75, the arithmetical mean between 50% genes in common with the mother and 100% in common with the father.

Drawing my inspiration from the same concepts, I realised that a life limiting gene was certainly harmful for individual where it acted (individual 1), but, considering that the death of individual 1 gave space for other individuals (2, 3, ... n) and allowed a quicker turnover of generation and so a quicker evolution for 2, 3, ... n, if those individuals were genetically related to individual 1, the inclusive fitness of the gene could be positive and in certain conditions (in short, a population divided in demes and numerically stable, alias K-selection [Pianka 1970]) the gene would be favoured by evolution.

Wonderful, I had a possible solution for Darwin's dilemma, the evolutionary key for the basis of aging!

I had to publish my arguments, but I feared greatly that others could steal my ideas. I thought they would be easily understood, accepted and therefore exploited by others, but how wrong I was!

Consequently, I decided that before the publication of my ideas on a scientific journal, for precaution it was necessary to print a book stating them so that any doubt of first claim would be cancelled.

Therefore, in 1983 I published in Italian a book where the above said ideas, and others, were expounded [Libertini 1983]. I sent copies of the book to many personalities of the Italian scientific world.

Well, the book was a total commercial failure and no one replied (but 21 years later Prof. Pietro Omodeo, an illustrious father of Italian biology, reminded me of the copy I'd sent to him and praised it!). However, my purpose was not economic and I diligently began trying to publish my hypothesis in the form of a scientific paper in an authoritative journal.

The task revealed itself to be very challenging. I had never published a scientific paper, my knowledge of English was rudimentary and my academic background was non-existent. Moreover, internet was not yet born and to even obtain a copy of a scientific paper was difficult for me. It was like trying to climb a Himalayan peak without any experience of mountaineering, with no training, without the help of sherpas and with inadequate and insufficient equipping. My only "force", besides the conviction of the correctness of my hypothesis, was an ignorance of this inexperience combined with a good strong dose of stubbornness. About four years were necessary but in the end, after various rejections and after various modifications and corrections of the paper, I succeeded in publishing my hypothesis in the Journal of Theoretical Biology (which rejected it two times before the acceptance!).

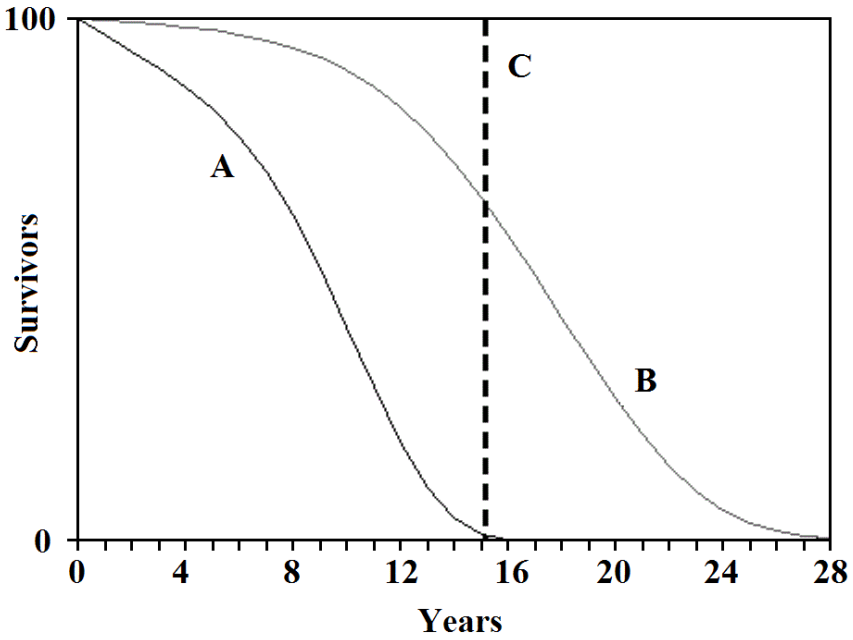


Figure 4 - Curve A: Life table in the wild of a species with a progressive fitness decline; Curve B: life table of the same species in artificial conditions of lowered mortality; Line C: arbitrarily defined line marking the beginning of the old age, or "state of senility" (at the time when the reduced fitness in the wild has become smaller than an arbitrarily established value). The fraction of individuals surpassing this line is small in the wild and the grade of their functional decay is in the arbitrarily defined range of the "state of senility". With artificially lowered mortality this fraction becomes appreciable or even preponderant [Libertini 2006].

To publish my paper, I had to specify that my topic was not on the imprecise concept of 'aging' or 'senescence', but on the "increasing mortality with increasing chronological age in the wild" (IMICAW) [Libertini 1988], a concept that has later referred to as "actuarial senescence in the wild" [Holmes and Austad 1995; Ricklefs 1998]. In effect, in the wild, the age-related fitness decline, which is a reality for many species, man included, allows only a few individuals to reach that deterioration of all the functions called old age or "state of senility", while in protected conditions (captivity, civilization, etc.) many individuals reach that age (Figure 4).

In the next weeks after publication, I received by post from each part of the world (Siberia, Bulgaria, South Africa, Alaska, Argentina, USA, France, ... even one from Italy ...) 48 requests of reprints of the paper. I satisfied all the requests and for each of them I asked for comments, criticisms and suggestions. I was in very high spirits ... but no one replied.

In the meantime, I sent a copy of the paper to an Italian journal of medical popularisation, along with a note explaining the importance of the topic and my willingness for a short informative article. At once the journal agreed to my proposal and asked me to send them the article and my photograph. Unfortunately, a few days later the journal director called me in person and, being very apologetic, informed me that the article would not be published because their trusty "experts" did not share my hypothesis. My objection that a popularisation journal should spread the news and not do a new judgement by referees, was useless: my hypothesis was too much in contrast with widely diffused and well established ideas.

Moreover, I was quite aware that I had formulated an evolutionary explanation for age-related fitness decline but had not proposed a physiological mechanism by which this decline could occur. I guessed that somehow the Hayflick limit was the key, but the topic was muddled for me and I did not dare to hazard hypotheses.

I thought that the time was not yet right and decided I should neglect any study on the topic for about fifteen years. And so I did, devoting my attention to various other things, things for which a mention here is inappropriate. Only every now and then did I turn my attention to what had once been my obsession for so long.

In 2000, reading in Nature an "authoritative" paper on aging [Kirkwood and Austad 2000], I noticed the mention, as proof of the current gerontological theories, of a paper whose title proclaimed: "Evolutionary Theories of Aging: Confirmation of a Fundamental Prediction, with Implications for the Genetic Basis and Evolution of Life Span" [Ricklefs 1998]. At once I looked for a copy of the article and then realised with astonishment that the statement of the title was in utter contrast with the results exposed in the text and with the evaluations and conclusions of the same Author! In fact, Ricklefs who was studying the life tables of many mammal and bird species in the wild, had found that the reduction of life span caused by the progressive increment of mortality ("proportion of senescent deaths" in his terminology) was inversely related to the environmental, or extrinsic, mortality (Figure 5). He declared openly that this finding was in plain contrast with the predictions of current gerontological theories! However, in my theory I had predicted that what Ricklefs termed 'the proportion of senescent deaths' should be inversely related to the extrinsic mortality - precisely as was documented by him. In my paper, I had given this paradoxical phenomenon the suggestive name "Methuselah effect" [Libertini 1988], but I had no empirical proofs of this prediction. Well, ten years after the publication of my paper, an authoritative scholar, whilst attempting to confirm predictions of the traditional theories, on the contrary found a clear proof in contrast with them and in support of my hypothesis! But, why was the contrary conclusion proclaimed in the title? I do not know, but I fear that with a title conforming to the facts and conclusions exposed in the text, the paper would have suffered many difficulties in being accepted.

However, I decided that the time was now mature for a renewed attention by myself to the topic of age-related fitness decline in the wild, and to his human terrible outcome in its amplified expression at older ages in protected conditions, namely the senile state.

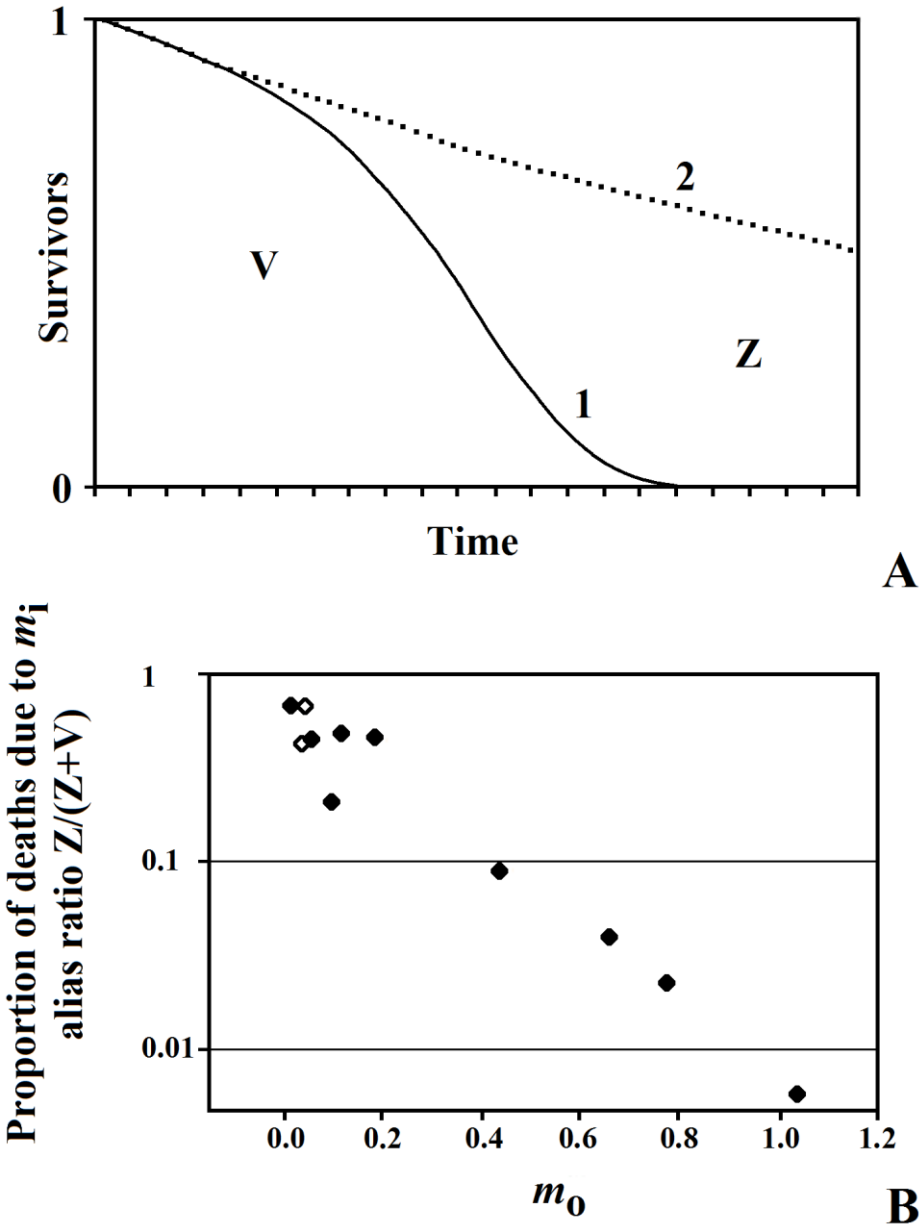


Figure 5 - Ricklefs' observation. (A) Life table in the wild of a species with an age-related increasing mortality, determined by extrinsic mortality (m_0) plus intrinsic mortality (m_i) (Curve 1, continuous line); hypothetical life tables with the action of m_0 only (Curve 2, dashed line); V is the area delimited by Curve 1; Z is the area between Curves 1 and 2, alias the "proportion of senescent deaths" (P_s). (B) Inverse significant correlation between m_0 and the proportion of deaths due to m_i (P_s), or ratio Z/(Z+V). Data are from [Ricklefs 1998], Table 2 (p. 30). Ricklefs' Figure 7 (p. 34) has been redrawn. Ordinates are in logarithmic scale. Open symbols refer to mammal species, solid symbols to bird species.

In the following months and years, I sought for and carefully consulted many papers and books regarding subjects such as telomeres, telomerase, replicative senescence, cell senescence, apoptosis, cell turnover, and diseases caused by cell turnover disorders, which I considered as deeply linked to what I investigated.

I looked for and obtained many internet contacts with scholars of various disciplines.

I wrote to Hayflick too, proclaiming my deep-rooted admiration for his essential discovery and with respectful boldness I asked for his copartnership to what I was elaborating. Hayflick replied with kindness that he did not share my opinions and that for him aging was the consequence of a

large set of cell alterations well documented by innumerable experiments. With pride and dismay I perceived that I had become the standard bearer of the implications of Hayflick's discovery - in contrast and beyond the position of the same Hayflick! An enormous weight on weak shoulders, but I was not discouraged ...

Many scholars replied to my requests, some with simple encouragements, and others with useful criticisms or suggestions. In some cases, the lack of sound objections or criticisms was an indirect confirmation of the arguments that I was proposing. However, I must express a particular gratefulness to Jerry Shay, for his useful suggestions on telomeres and telomerase, a topic in which he is an undisputed master; to Eric Le Bourg, Josh Mitteldorf and Theodore Goldsmith for their useful mentions of important papers; to Richard Ricklefs for his attention and kindness. In particular, I am profoundly grateful to Leonard Hayflick for his appreciation of my last paper (2008), which I have accepted as a sort of scientific blessing.

After some years of study and some useful rejections by qualified journals, in 2006 I published a paper [Libertini 2006] in which my hypothesis was reaffirmed and an overall interpretation of aging, both in terms of evolutionary mechanisms and in terms of underlying physiological mechanisms, was given. Moreover, I clearly expressed the differences between the current ideas about aging and the new proposed paradigm.

In the next months I wrote a further paper, published in February 2008 [Libertini 2008], where the strong empirical evidence in support of the adaptive interpretation of age-related fitness decline in the wild (in different ways formulated by various authors [Weismann 1889; Libertini 1988, 2006; Skulachev 1997; Goldsmith 2003; Longo et al. 2005; Mitteldorf 2006]) and against nonadaptive hypothesis [Medawar 1952; Williams 1957; Hamilton 1966; Edney and Gill 1968; Kirkwood 1977; Kirkwood and Holliday 1979; Mueller 1987; Rose 1991; Partridge and Barton 1993] and historical hypothesis [De Magalhães and Toussaint 2002] was given.

In the same article, I maintained that experiments about the modifications of life tables of animals such as *Caenorhabditis elegans* and *Drosophila melanogaster* were of little importance for the study of aging in species such as ours. The reason for this was because *C. elegans* and *D. melanogaster* reach ages in the laboratory that are non-existent in the wild, thus the studies are observing laboratory artefacts. Furthermore, these animals have no cell turnover, whereas our species do.

The following exposition is a description of how facts well documented by empirical evidence and supported by plausible arguments indicate the mechanisms underlying age-related fitness decline and aging, as I have described in the above said papers, but with further evidence, details and deductions.

The Protagonist: Telomere-Telomerase System

Hayflick demonstrated that cultivated human diploid fibroblast-like cells (HDF) from a variety of normal tissues have a finite growth potential *in vitro*, i.e. divide only a finite number of times ("Hayflick limit") [Hayflick and Moorhead 1961; Hayflick 1965].

Moreover, it was shown that foetal HDF display a consistently greater number of population doublings (approximately 50) than cells derived from adult tissues (20-30 doublings) [Hayflick and Moorhead 1961]. Growth potential of skin HDF from donors of different ages showed a reduction of potential doublings of 0.2 doubling per year of life [Martin et al. 1970]. A decline in growth potential was reported for epidermal keratinocyte culture [Rheinwald and Green 1975], arterial smooth-muscle cell [Bierman 1978] and lens epithelial cell [Tassin et al. 1979]. A positive relationship between growth potential of HDF cultures and the maximum life span of the species from which the cells are derived was reported [Röhme 1981].

In 1976, the Hayflick limit was documented *in vivo* too [Schneider and Mitsui 1976].

In 1975, the unknown mechanism limiting the number of duplication was shown to be in the nucleus [Wright and Hayflick 1975].

However, it was known from 1971 that DNA polymerase could not replicate a whole molecule of DNA and a little part of an end of the molecule would be unreplicated at each duplication [Olovnikov 1971; Watson 1972]. In the same years, Olovnikov hypothesised that the shortening of DNA molecule at each duplication after a certain number of times could block cell replication capacity and that this could explain the Hayflick limit [Olovnikov 1973].

In 1978, the end of the DNA molecule, defined telomere, which at each cell replication shortens [Harley et al. 1990], was shown in a protozoan species to be a simple sequence of nucleotides, TTGGGG, repeated many times [Blackburn and Gall 1978]. Later, for mammals, man included, the repeated sequence was demonstrated to be only a little different (TTAGGG) [Moyzis et al. 1988] but common to slime molds, trypanosomes, and other vertebrates and organisms [Blackburn 1991]. Its evolutionary conservation, shared even between mammals and unicellular animals, certainly indicated that the structure has great importance.

However, if the Hayflick limit originated from telomere shortening at each replication, an explanation for cells with numberless replications, such as germ line cells, was needed. The discovery of the enzyme telomerase that elongates telomeres was the solution [Greider and Blackburn 1985], and was confirmed by its presence in immortal human cell lines [Morin 1989].

This enzyme was shown to be repressed by regulatory proteins [van Steensel and de Lange 1997]. Moreover, telomerase deactivation caused telomere shortening and a reduction of growth potential [Yu et al. 1990]. Conversely, telomerase activation caused telomere lengthening and cell immortalization [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

Graduality of duplication blockage in a cell population. The simple description that cells with activated telomerase have an unlimited duplication capacity, while cells with inactivated telomerase show a limited duplication capacity strictly proportional to telomere length, was imperfectly supported by empirical data and a more sophisticated and realistic model was suggested in a review by Blackburn [Blackburn 2000]. Indeed, if somatic cell growth potential is strictly proportional to telomere length, it would be totally unimpaired up to a critical length, while under this length, namely starting from a certain number of replications, there would be a sudden slump of the growth potential. However, cell populations show a progressive reduction of growth potential starting from early ages, that is, for single cells, even with telomeres having the maximum length, the passage from "cycling state" (duplication possible) to "noncycling state" (duplication impossible) is stochastic [Pontèn et al. 1983; Jones et al. 1985]. In the aforesaid review it was suggested ("Blackburn's hypothesis") that telomere can switch stochastically between two states: capped with particular protective nucleoproteins and uncapped. Capping preserves telomere physical integrity, allowing cell division to proceed. Uncapping occurs normally in dividing cells, regardless of telomere length, but the probability of returning to the capped state is proportional to telomere length and the uncapped state, if left uncorrected too long, elicits the passage to the noncycling state (Figure 6).

Imperfect relation between telomere length and Hayflick limit. To contrast the possible objection that species, such as the mouse and hamster, with long telomeres [Slijepcevic and Hande 1999] age precociously, it is necessary to point out that Blackburn's hypothesis does not require, for different species, a fixed ratio between telomere length and the stability of the telomere-capping nucleoproteins complex. It is easily assumable that the stability of the complex and, more generally, the modulation of telomere-telomerase function is different for each species [Fossel 2004].

However, according to this model, and with the support of the aforesaid observations, a cell population with inactivated telomerase and with telomeres initially at their maximum length, shows, from the beginning, a gradual reduction of duplication capacity. This gradual reduction of duplication capacity is at first minimal, but later increases. Besides, even cells with telomerase activated and therefore telomeres always at maximum length, should show a small percentage of

cells passing to the noncycling state at each division. Stem cells, unlike germ cells, have levels of telomerase activity that are only partially able to stabilize telomere length [Holt et al. 1996] and, therefore, *in vivo* could not indefinitely replace the differentiated elements in cell populations that are in renewal [Fossel 2004].

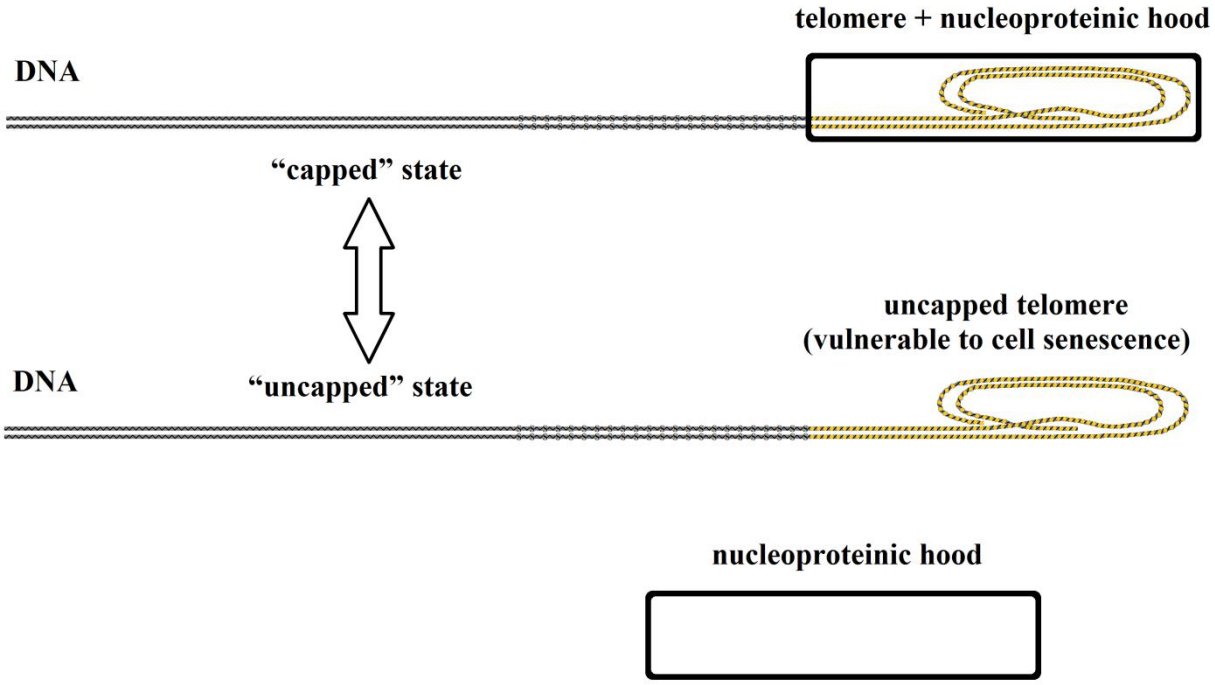


Figure 6 - Telomere switches between capped and uncapped states. The probability of being in the uncapped state increases with telomere shortening at each duplication. In the uncapped state, the telomere is seen as a broken end and this can cause an end-to-end joining that stops cell duplication capacity.

Cell senescence. In correlation with the progressive shortening of telomeric DNA, the expression of many genes, among those usually expressed in the cell, becomes impaired, jeopardizing overall cell functionality and, consequently, the functions of extracellular matrix and of other near or physiologically interdependent cells. It has been extensively and soundly documented that this decay of cell functions (cell senescence) and the progressive reduction of cell duplication capacities (replicative senescence), somehow depends on the relative shortening of telomeric DNA (Fossel's "cell senescence limited model") [Fossel 2004].

About the mechanism of this gradual alteration of gene expression: "One model of telomere-gene expression linkage is an altered chromosomal structure (Ferguson et al., 1991), such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome (Fossel, 1996; Villeponteau, 1997; Wright et al., 1999). As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) or the hood shortens (as the telomere is less capable of retaining heterochromatin). In either case, the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity (Aparicio and Gottschling, 1994; Singer et al., 1998; Stevenson and Gottschling, 1999). These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel 2004] (Figure 7).

It is likely that the proteinic "hood" and the "capping nucleoproteins" are the same thing because: 1) they act in the same part of the chromosome; 2) telomerase activation causes both

telomere lengthening with cell immortalization and the full reversal of cell senescence manifestations [Bodnar et al. 1998; Counter et al. 1998; de Lange and Jacks 1999].

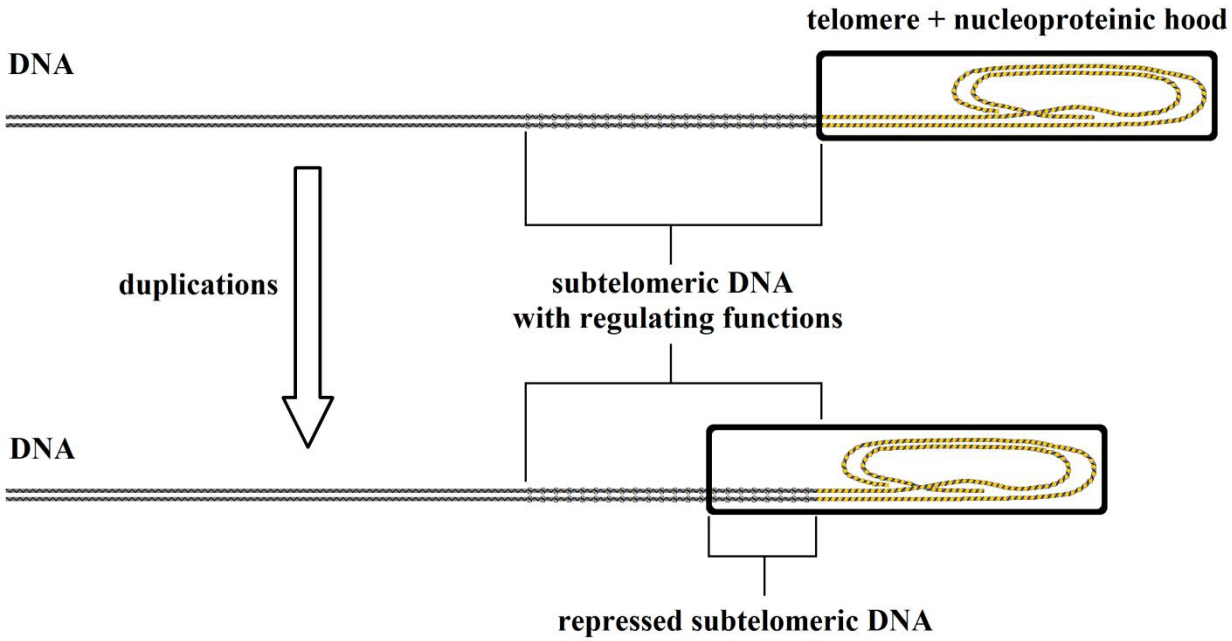


Figure 7 - With telomere progressive shortening the expression of many genes results to be impaired. It is likely that near to telomere there is a tract of DNA regulating overall cell functionality and that with telomere shortening the nucleoproteinic "hood" alters this regulation [Fossel 2004].

The arrangement and action of subtelomeric DNA, which is of great importance for cell overall functionality, is directly jeopardized by telomere shortening due to its position. This would be evolutionarily illogical and inexplicable if the hypothesis that this "illogicality" contributes to mechanisms favoured by natural selection for determining age-related fitness decay is not accepted.

The reset of telomere clock. Successful fertilization, both in reproduction and in cloned animals, requires the resetting of "telomere clock" [Fossel 2004]. In other words, cells must somehow establish the initial length of the telomeric sequence, since each subsequent shortening of this length will increase the probability of replicative senescence and cell senescence. With a particular mechanism, which is imaginable (Figure 8), a telomere regulates its future functionality without the conditioning of its initial length, whose value is "largely irrelevant" [Fossel 2004]: two *Mus* strains, one with long (20 kb) telomeres and the other with short (10 kb) telomeres have equal life spans and similar progressive alterations in gene expression patterns. The same is true for donor animals and cloned animals that are derived from cells with reduced telomeres [Fossel 2004].

The case of knockout mice. In comparison to humans, mice and other animals have a shorter life span but much longer telomeres [Slijepcevic and Hande 1999] and a baseline telomerase activity in most somatic cells [Prowse and Greider 1995]. Moreover, in mice with telomerase genetically inactivated (knockout or mTR-/- mice) four [Herrera et al. 1999] to six [Blasco et al. 1997] generations are necessary before the viability and fertility is jeopardized, although dysfunctions in organs with highly proliferative cells are shown in early generations [Lee et al. 1998; Herrera et al. 1999]. This apparently paradoxical phenomenon is easily explainable with the model illustrated in Figure 8, as expounded in Figure 9.

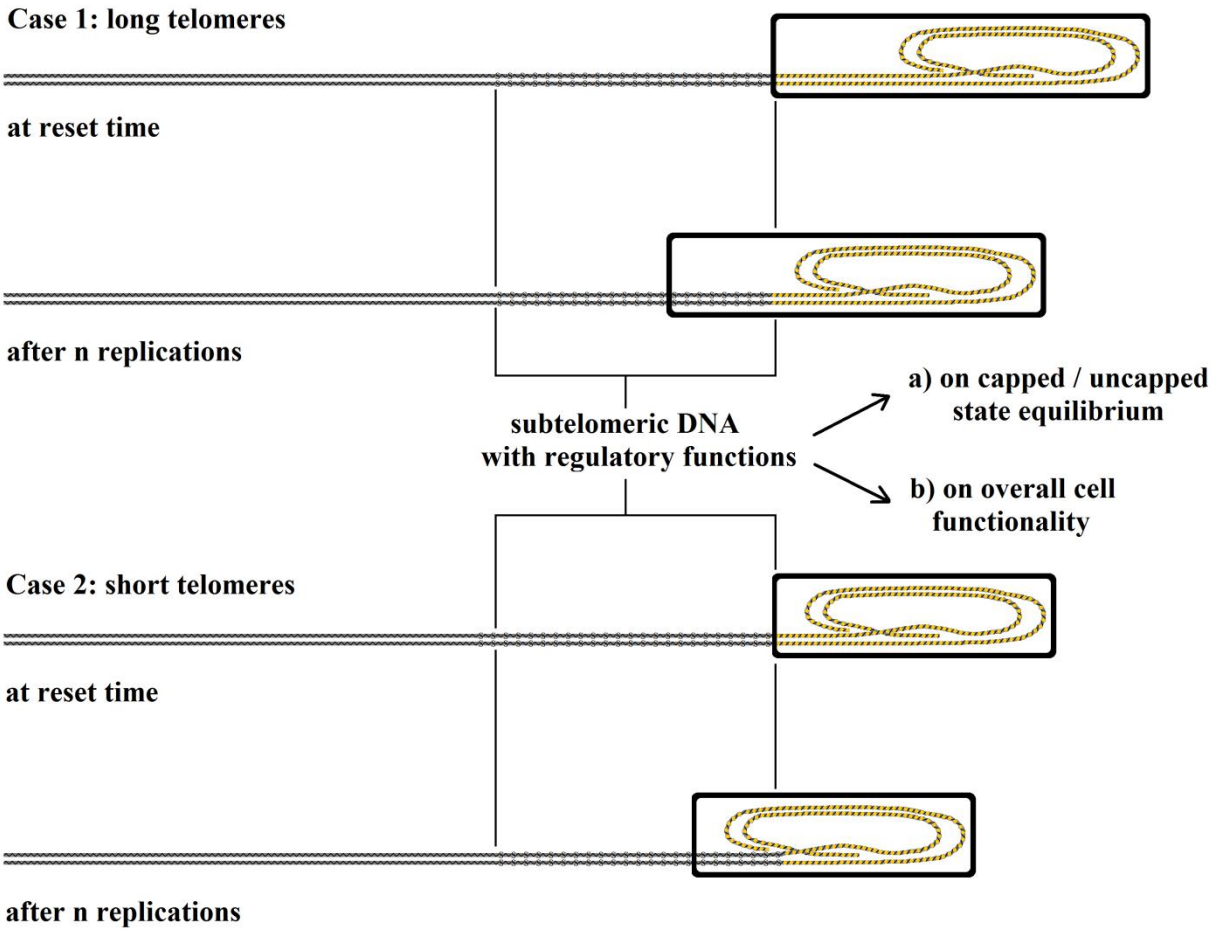


Figure 8 - In the resetting of the "telomere clock", the absolute length of the telomere is "largely irrelevant" [Fossel 2004]. During the resetting phase, the length of the nucleoproteinic hood could be shaped proportionate to telomere length and remain fixed for all the cell life. If subtelomeric DNA regulates both overall cell functionality and telomere capped/uncapped state equilibrium, this could explain the large irrelevance of the initial telomere length for the consequences of its subsequent shortenings. According to this model, the probabilities of replicative senescence and the gradualness of cell alterations after each duplication is proportional to the progressive blockage of subtelomeric DNA and in function of a prearranged pattern, typical of the species and of the cell.

With these specifications, the telomere-telomerase system appears to be a highly sophisticated mechanism, genetically determined and regulated, with pivotal importance for cell duplication capacities.

Here, we have a protagonist (telomere-telomerase system). However, we need to know the other protagonist (programmed cell death), the main action (cell turnover), and their cues for the human scene.

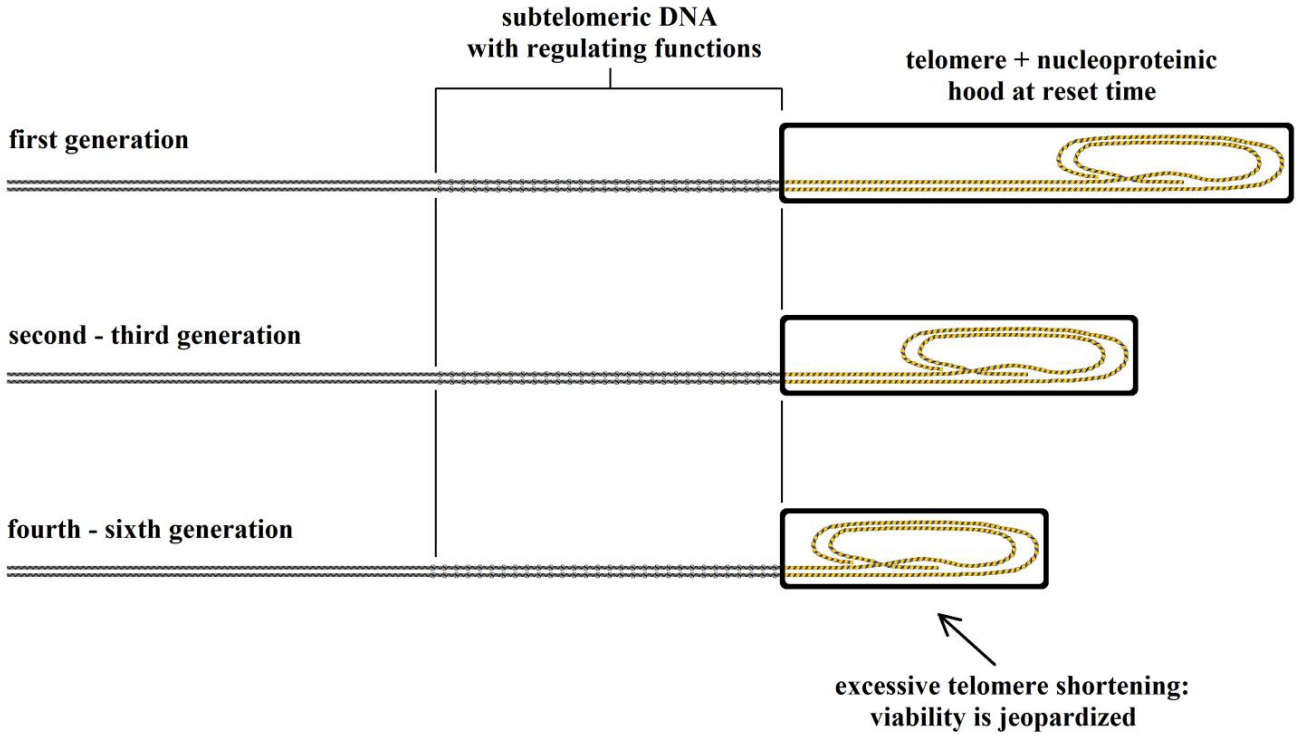


Figure 9 - According to the model of Figure 8, in knockout mice, the length of nucleoproteinic hood, shaped in the reset phase, is proportionate to telomere length so that telomere functionality is largely irrelevant of its length. The short life span of mice might be explained by a low pattern of telomere + nucleoproteinic hood complex stability.

The Other Protagonist: Programmed Cell Death

A cell may dies by necrosis because of an accidental event (injury, mechanical stress, infection, ischemia, etc.), or by a form of "programmed cell death" (PCD). The keratinization of an epidermis or hair cell is a form of PCD. Apoptosis is a peculiar form of PCD with an ordinate process of self-destruction with non-damaging disposal of cellular debris that makes it different from necrosis (see Table I). The phenomenon was for the first time described and clearly differentiated from necrosis in the observation of normal liver epatocytes [Kerr et al. 1972] (Figure 10). However, apoptosis is phylogenetically very ancient and is a characteristic of unicellular eukaryote species such as *Saccharomyces cerevisiae* [Laun et al. 2007; Kaeberlein et al. 2007].

Selective and programmed cell death by apoptosis is an integral part of multicellular organ development and an important element in lymphocyte interactions and in many pathologic mechanisms. A pivotal function of apoptosis in vertebrates is related to cell turnover in healthy adult organs [Wyllie et al. 1980; Lynch et al 1986; Medh and Thompson 2000], as documented for many tissues and organs (i.e., biliary epithelial cells [Harada et al. 2000]; gliocytes [Pontèn et al. 1983]; kidneys [Cardani and Zavanella 2000]; pancreatic β-cells [Finegood et al. 1995]; liver [Benedetti et al. 1988]; thyroid [Dremier et al. 1994]; lungs (type II alveolar epithelial cells) [Sutherland et al. 2001]; cartilage [Héraud et al. 2000]; prostate [Xia et al. 2001]; adipocytes [Prins and O'Rahilly 1997]; bone [Spelsberg et al. 1999]; skeletal muscle [Migheli et al. 1997; Pollack and Leeuwenburgh 2001]).

Table I. Some differences between necrosis and apoptosis

Necrosis	Apoptosis
Pathologic process caused by non-physiological disturbances (e.g., external injuries, inflammatory factors, lytic viruses, hypothermia, hypoxia, etc.)	Physiologic and tightly regulated process involving activation (e.g., by caspase) and enzymatic steps
Passive process with no energy requirement	Active process energy dependent
Swelling of organelles and of the whole cell; mottled chromatin condensation; random DNA fragmentation	Condensation of cell, organelles and chromatin; non-random DNA fragmentation
Loss of membrane integrity with release of cell's content	Membrane blebbing without loss of integrity
In the end, total cell lysis; the organelles are not functional	Cell falls apart into apoptotic bodies bounded by membranes; the organelles are still functional
Significant inflammatory response	No inflammatory response

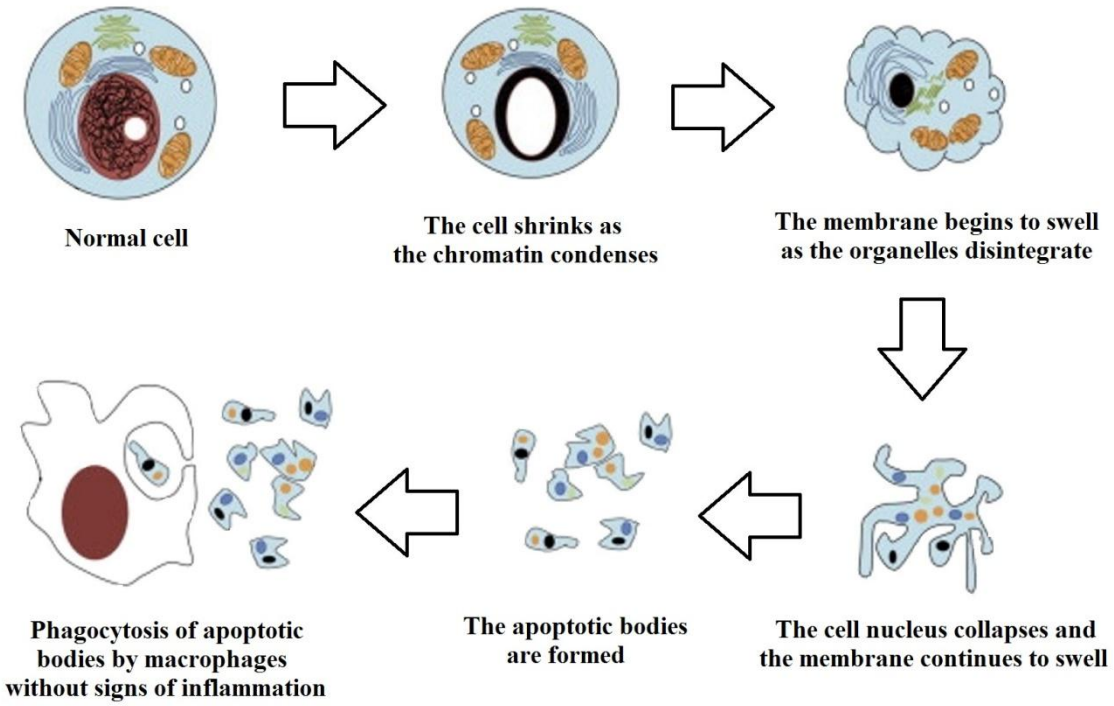


Figure 10 - A scheme of the apoptotic mechanism.

The Main Scene: Cell Turnover

"Each day, approximately 50 to 70 billion cells perish in the average adult because of programmed cell death (PCD).

Cell death in self-renewing tissues, such as the skin, gut, and bone marrow, is necessary to make room for the billions of new cells produced daily. So massive is the flux of cells through our bodies that, in a typical year, each of us will produce and, in parallel, eradicate, a mass of cells equal to almost our entire body weight" [Reed 1999] (Figure 11).

For many tissues, cell elimination is completed with the removal by macrophages (red cell) or with the detaching from the somatic surface (skin, gut), but for many other tissues and organs, often considered permanent in their cell number, there is a continuous loss of cells by apoptosis. Just in an organ, the liver, apparently stable as cell composition, in an healthy adult subject, apoptosis was described for the first time [Kerr et al. 1972].

The continuous death of cells by PCD is balanced by an equal proliferation of appropriate stem cells, which is regulated and limited by telomere-telomerase system.

Cell turnover is a general pattern in vertebrates, but not for all animals (e.g., the adult stage of the worm *Caenorhabditis elegans* has a fixed number of cells).

Figure 11 - A multitude of cells is killed every day by apoptosis. A Colleague commented that this is as The Slaughter of the Innocents (painting by Simonetta Rubinato).

In short, at least for vertebrates, three categories of cells are currently distinguished:

1. Those with high turnover: e.g., intestinal crypts cells [Andreeff et al. 2000];

2. Those with moderate turnover: e.g., cells of the deep layers of skin and endothelial cells [Marciniak and Guarente 2001], heart myocytes ("It remains a general belief that the number of myocytes in the heart is defined at birth and these cells persist throughout life ... But myocytes do not live indefinitely – they have a limited lifespan in humans and rodents. Cell loss and myocyte proliferation are part and parcel of normal homeostasis ... The old heart is characterized by a reduction in cell number and hypertrophy of the remaining myocytes, and this phenotype has been used to argue against the formation of new myocytes. But without cell regeneration the rates of cell death would imply that all myocytes would die during the first few months of a rodent's lifespan. For example, the left ventricle of a young rat contains 13×10^6 myocytes, and at any point in time 200 and 93,000 myocytes are dying by apoptosis and necrosis, respectively. Because apoptosis is completed in nearly 4 h and necrosis in roughly 24 h, 94,200 myocytes are lost in one day. Thus, 2.83×10^6 cells would die in 1 month, and the total 13×10^6 ventricular myocytes would disappear in 5 months." [Anversa and Nadal-Ginard 2002]), muscle myocytes (Stem cells from muscles of old rodents divide in culture less than cells from muscles of young rodents [Schultz and Lipton 1982]; a transplanted muscle suffers ischaemia and complete degeneration and then there is a complete regeneration by action of host myocyte stem cells that is poorer in older animals [Carlson and Faulkner 1989]; there is evidence that apoptosis is a feature in skeletal muscle fibers in several disease like chronic heart failure or Duchenne muscular dystrophy [Adams et al. 2001], that reach their deadly end when the replicative capacity of myocyte stem cells is exhausted.

3. Those with no turnover, e.g., neurones, with few possible exceptions [Horner and Gage 2000] but which always have metabolic dependence on gliocytes that are cells with turnover [Fossel 2004].

Atrophic syndrome. In correlation with a significant relative shortening of telomeric DNA, according to Fossel's "cell senescence limited model" [Fossel 2004], a tissue or an organ should show an "atrophic syndrome" with the following features:

a. reduced cell duplication capacity (replicative senescence);
b. reduced number of cells (atrophy);
c. slackened cell turnover;
d. possible substitution of missing specific cells with nonspecific cells;
e. hypertrophy of the remaining specific cells;
f. altered functions of cells with shortened telomeres or definitively in noncycling state (cell senescence);
g. vulnerability to cancer because of dysfunctional telomere-induced instability [De Pinho 2000].

A scheme for cell turnover. Cell turnover may be summarized as follows, (though obviously the modulation of this turnover will vary according to the cell type) (Figure 12):

- Stem cells with active telomerase divide themselves and originate somatic cells with replication capacity but with telomerase inactivated;
- Somatic cells with replication capacity but with telomerase inactivated originate differentiated somatic cells with no replication capacity and, after a variable number of duplications and showing a progressive overall function decay (cell senescence), pass from the cycling state to the non-cycling state;
- Somatic cell in non-cycling state (replicative senescence) with increasing cell senescence.

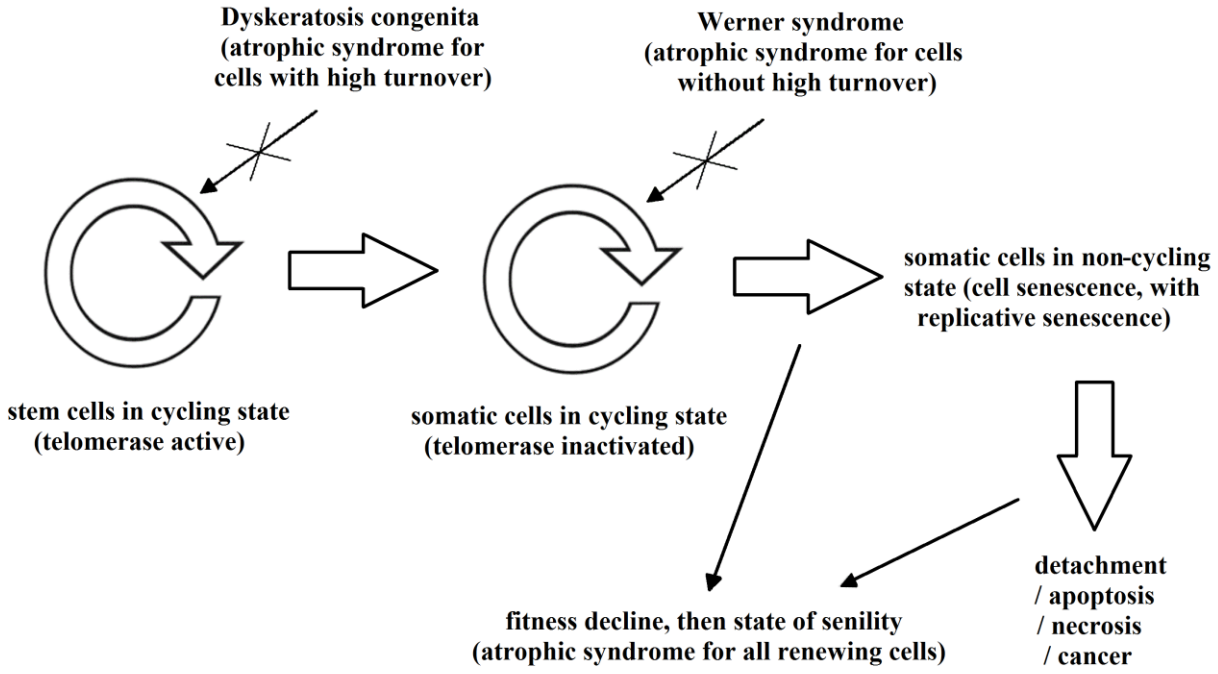


Figure 12 - Stem cells with active telomerase divide themselves and originate somatic cells with replicative capacity but with telomerase inactivated. Somatic cells in non-cycling state are originated from these. Replicative senescence and cell senescence contribute to fitness decline that in its more evident expression becomes the senile state.

Two Wrong Performances:
Dyskeratosis Congenita and Werner Syndrome

The "atrophic syndrome" with dysfunction of stem cells in the cycling state or of somatic cells in the cycling state should be observable in cells with high and moderate turnover rate, respectively, while in the old age, alias "state of senility" [Williams 1957], it should be observable in all cells and tissues.

In fact, dyskeratosis congenita, an inherited human disease [Dokal 2000], is an excellent model of the dysfunction of stem cells in the cycling state [Marciniak and Guarente 2001]. Similarly, Werner syndrome is a prototype of the dysfunction of somatic cells in the cycling state, as illustrated in a review [Martin and Oshima 2000]. The crucial differences between the two syndromes have been skilfully outlined [Marciniak and Guarente 2001].

Dyskeratosis congenita. An autosomal dominant form of *dyskeratosis congenita* (DC) is caused by mutations in the gene encoding the RNA part of telomerase [Vulliamy et al. 2001], while with the X-linked form of the disease, levels of telomerase are low and telomeres are shorter than normal [Mitchell et al. 1999]. "Problems tend to occur in tissues in which cells multiply rapidly – skin, nails, hair, gut and bone marrow – with death usually occurring as a result of bone-marrow failure." [Marciniak and Guarente 2001]

Defects in DC are present in tissues that have high rates of cell turnover, including those of the blood system and the intestinal crypts, where telomerase activity has been detected [Marciniak and Guarente 2001]:

DC patients also suffer from a higher rate of cancer that can likewise be explained by the lack of telomerase, which results in unstable chromosomes [de Lange and Jacks 1999; Artandi et al. 2000; Artandi 2002].

"By contrast, some tissues that have the capacity for cellular replacement, but do not undergo continuous cell turnover, do not express telomerase in their progenitors. It is these tissues – such as the deep layers of the skin or the lining of the blood vessels – that might be expected to suffer most from age-associated telomere depletion, as they have no ability to regenerate telomeres.

These tissues would also be greatly affected by defects in other pathways that maintain telomeres, such as DNA-recombination processes. This might explain why Werner syndrome, in which an enzyme involved in DNA processing is affected, yields a closer version of normal (if premature) ageing than does dyskeratosis congenita. In people with dyskeratosis congenita and in telomerase-deficient mice, it is tissues that normally express telomerase that one would predict to suffer most from its loss, and this proves to be the case." [Marciniak and Guarente 2001]

Table II. Alterations in dyskeratosis congenita

Organ	Cells expressing telomerase	Defect in dyskeratosis congenita
Hair	Hair follicle	Alopecia
Oral cavity	Squamous epithelium	Leukoplakia
Skin	Basal layer of epidermis	Abnormal pigmentation, nail dystrophy
Lungs	Type 2 alveolar epithelial cells	Fibrosis
Liver	?	Cirrhosis
Intestine	Intestinal crypts	Gut disorders
Testes	Spermatogonia	Hypogonadism
Bone marrow	Progenitor stem cells	Failure to produce blood cells

Werner syndrome (Figure 13). This disease is due to the dysfunction of a member of the RecQ family of helicases [Yu et al. 1996] causing a dysfunction of somatic cells in the cycling state. In this syndrome, cells show high somatic mutation rates, particularly deletions [Fukuchi et al. 1989], and a limited replication capacity [Hayflick 1965].

Figure 13 - Two cases of Werner syndrome.

Werner syndrome patients show no 'catch-up' growth and a reduced stature, premature greying and thinning of hair, atrophy of skin, regional atrophy of subcutaneous tissue, voice changes (weak, high-pitched), diminished fertility (from the third decade), premature testicular atrophy (from middle age), probably an accelerated loss of primordial ovarian follicles, cataract (from the beginning of the fourth decade), ulcerations around the Achilles' tendons and malleoli, osteoporosis, type 2 diabetes mellitus, a variety of benign and malignant neoplasms, arteriosclerosis, arteriolosclerosis and atherosclerosis, skeletal muscle atrophy, and death usually for myocardial infarction or for cancer [Martin and Oshima 2000], but "... no convincing evidence of premature senescence in the central nervous system (CNS)" [Martin and Oshima 2000]. The ratio of sarcomas to carcinomas is around 1:1, compared with 1:10 in the general ageing population [Goto et al. 1996], with origins largely from mesenchymal cells plus some other organ as thyroid, at least in Japanese subjects [Martin and Oshima 2000]. Moreover, "... the distribution of the osteoporosis is unusual; the long bones of the lower limbs can be more severely affected than those of the vertebral column. Other unusual radiological features include a characteristic osteosclerosis of the distal phalanges and subcutaneous calcification of the soft tissues. The ulcerations mentioned above are also unusual and can involve the skin around the elbows as well as the ankles." [Martin and Oshima 2000]

All these characteristics may be interpreted as an atrophic syndrome for non-high turnover cells, in consequence of the abnormality in DNA metabolism [Martin and Oshima 2000]. In particular: 1) although the crystalline lens has no cell in its core, its functionality depends on lens epithelial cells that show turnover [Tassin et al. 1979]. "Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells" [Martin and Oshima 2000], which is consistent with age-related reduction in growth potential for lens epithelial cell reported for normal human subjects [Tassin et al. 1979]; 2) "Of most interest, however, is the coupling of an abnormality in a RecQ helicase to severe atherosclerosis. In analogy with the skin ulcers seen in Werner subjects, perhaps normal Werner helicase function is required for the efficient repair of the haemodinamic shear stress of arteries. Such repair could be at the level of endothelial

cell replication" [Martin and Oshima 2000] as suggested for non-Werner subjects [Gimbrone 1999; Hill et al. 2003]; 3) Vulnerability to cancer may be explained by telomere shortness and the consequent unstable chromosomes [de Lange and Jacks 1999; Artandi et al. 2000; Artandi 2002] and this effect should be manifest for non-high turnover cells such as those of mesenchymal origins and other cells such as those of thyroid; 4) The peculiarities of osteoporosis and ulcerations, osteosclerosis of distal phalanges, subcutaneous calcification of the soft tissues, discontinuities of subcutaneous atrophy may "... reflect an unusual response to repeated, mild local trauma" [Martin and Oshima 2000], namely an altered repair capacity due to insufficient duplication capacity of the necessary repair cells; 5) CNS lesions may be secondary to vascular pathology [Martin and Oshima 2000] but could be a consequence of neuroglia atrophy [Fossel 2004]; 6) "Skeletal muscle atrophy is at least in part due to disuse, but a primary involvement of that tissue cannot yet be ruled out." [Martin and Oshima 2000]; 7) type 2 diabetes mellitus might be a consequence of β-cell atrophy, as for the same type of diabetes in non-Werner subjects an imbalance between β-cell apoptosis and regeneration rates has been suggested [Bonner-Weir 2000; Cerasi et al. 2000].

In short, dyskeratosis congenita and Werner syndrome are two model cases of segmental progeria, that is the altered functionality of only a part of cell phenotypes [Fossel 2004]. For example, in Werner syndrome there is no association with Alzheimer disease, commonly observed in the elders. It is plausible that a non-segmental progeria is utterly incompatible with life.

The Main Action: Aging in Man

A simple spontaneous hypothesis about the mechanisms underlying pathophysiological alterations in old vertebrate individuals, namely about "damage resulting from intrinsic living processes" [Masoro 1998] alias "age changes" [Hayflick 2000] alias phenomena that are "universal in the species, degenerative, progressive and intrinsic" [Davies 1998], has been inferred: the more or less precocious aging is the consequence of the less or greater genetically determined cell replication capacity and of the related cell senescence (Fossel's "cell senescence general model of aging" [Fossel 2004]).

Many experimental data support this hypothesis (e.g., for mice, the p53 tumour suppressor, activated by numerous stressors, induces apoptosis and cell cycle arrest, causing reduced longevity, osteoporosis, generalised organ atrophy and a diminished stress tolerance [Tyner et al. 2002]).

Limiting the argument to the human species because of the large quantity of available data here, if the hypothesis is true, very old individuals, that is those demonstrating "age changes" in their most extreme form - excluding "age-associated diseases" and damages by extrinsic factors (categories 2 and 3, respectively, in Masoro's 1998 classification [Masoro 1998]) -, should show widespread and pronounced signs of atrophic syndrome for all organs and tissues.

Therefore, we will disregard alterations caused by age-related diseases and, as a matter of prudence, data referred to organs for which hormonal actions are relevant and such as to confuse their analysis (endocrine glands, genital organs, etc.).

Endothelium

The correct functionality of endothelial cells is essential to avoid atherogenesis and its complications, such as cardiac infarctions, cerebral ischemia and other diseases derived from compromised blood circulation [Hill et al. 2003].

The turnover of these important cells is assured by endothelial progenitor cells, derived from bone marrow, whose number has been shown to be inversely related to age, reduced by cardiovascular risk factors (cigarette smoking, diabetes, hypertension, hypercholesteremia, etc.), and increased by drugs, such as statins, which protect organ integrity [Hill et al. 2003]. Moreover, with negative relation, the number of endothelial progenitor cells is a predictor of cardiovascular risk equal to or more significant than Framingham risk score [Hill et al. 2003; Werner et al. 2005].

In the senile state, diseases deriving from a compromised endothelial function increase exponentially in correlation with the age, even if other cardiovascular risk factors are absent [Tallis et al. 1998]. These factors anticipate and amplify the risk [Tallis et al. 1998], while drugs with organ protection qualities, as statins [Davidson 2007], ACE-inhibitors and sartans [Weir 2007a] counter their effects.

Skin

"Stratum corneal thickness is unchanged in the elderly although its moisture content and cohesiveness are reduced coupled with an increase in renewal time of damaged stratum corneum. ... Human epidermis is highly proliferative but in a steady-state condition dependent, as are other self-renewing structures, on slowly cycling, undifferentiated stem cells. These stem cells are located within the basal compartment of the epidermis – the nonserrated keratinocytes at the tips of the epidermal rete ridges. Loss of rete ridges and consequent flattening of the dermal-epidermal junction is a hallmark of intrinsically aged skin. Such flattening results in a reduction in mean surface area of the dermal-epidermal junction. One study has estimated a reduction in mean area of dermal-epidermal junction/mm^2 from 2.6 at age 21 to 40 years to 1.9 at age 61 to 80 years. These changes are accompanied by a reduction in microvilli – cytoplasmic projections from basal keratinocytes into the dermis. ... The rate of epidermal renewal is reduced in the skin of individuals aged 60 years or greater. ... Melanocytes are decreased in number in intrinsically aged epidermis, although the estimates of this decrease vary from study to study according to the methodologies used to quantitate melanocyte numbers. This said, the reduction is in the order of 8 to 20 percent per decade compared to young adult skin. ... The number of Langerhans cells is reduced in intrinsically aged epidermis, ... Gilchrest et al. demonstrated that subjects aged 62 to 86 years had a 42 percent reduction in the number of Langerhans cells in sun-protected skin as compared to young subjects aged 22 to 26 years. ... Numbers of dermal fibroblasts decrease with age ... Aged skin is relatively hypovascular, particularly due to loss of small capillaries that run perpendicular to the dermal-epidermal junction and form capillary loops. This loss is concomitant with the loss of epidermal rete ridges. Blood vessels within the reticular dermis are reduced in number and their walls are thinned. ... There is an approximate 50 percent reduction in numbers of mast cells in intrinsically aged skin. ... Eccrine glands are reduced in number and function in aged skin. ... Age probably reduces and disorganizes the nerve supply of the skin; indeed there is an approximate two-thirds reduction in numbers of Pacinian and Meissner's corpuscles with age. ... Hair, particularly scalp hair, is lost with age in both sexes. ... Nails grow more slowly in the elderly ... The study of aging skin particularly as a consequence of the ready accessibility of cutaneous tissue is one that presents a paradigm for aging of other organs." [Griffiths 1998]

Eyes

"Atrophy of the fascial planes within the eyelids may lead to herniation of the orbital fat into the lid tissue, producing the 'bags under the eyes' frequently seen in the elderly. Atrophy or disinsertion of the aponeurosis of the levator palpebrae muscle, which ordinarily supports the upper eyelid, may cause the opened lid to fail to uncover the pupil, as seen in senile ptosis, despite normal levator muscle function ... Secretory function of the lacrimal glands declines with age ..." [Brodie 1998]

For crystalline lens and photoreceptor cells, see in the next paragraph.

Orofacial Tissues and Organs

"Structural changes in human oral epithelia with aging include thinning of the epithelial cell layers (e. g., thinning of the lingual epithelial,) diminished keratinization, and simplification of epithelial structure. ... Histologic studies of aging salivary glands show a gradual loss of acinar elements, a relative increase in the proportion of ductal elements, an increase in inflammatory infiltrates, and an increase in fibrofatty tissue." [Devlin and Ferguson 1998]

"The number of taste buds decreases after age 45, resulting in a decrease in taste sensation..." [Reinus and Brandt 1998]

Gastrointestinal System

In people over 60, there is an increased prevalence of atrophic gastritis [Bird et al. 1977].

"Several histologic changes have been demonstrated in the colon, including atrophy of the muscolaris propria with an increase in the amount of fibrosis and elastin, ..." [Tepper and Katz 1998]

"Using postmortem material, Chacko *et al.* (1969) found that in an Indian population the shape of villi changed on aging. The youngest subjects had finger-shaped villi, but the frequency of broad villi and convolutions increased in specimens from older people. Webster and Leeming (1975a) described similar changes when fresh jejunal specimens from geriatric patients were compared with normal young controls. They found that in the elderly broader villi were more common, and in addition the villi were significantly shorter. ... Andrew and Andrew (1957) noticed an increase in the amount of fibrous tissue between the crypts of Lieberkuhn and a general reduction of cellularity in older mice. ... Lesher, Fry and Kohn (1961), Lesher and Sacher (1968) and Fry, Lesher and Kohn (1961), using autoradiography and tritiated thymidine, showed a prolonged generation time for duodenal crypt cells in old animals and an increased cell transit time (for cells to progress from the crypts to villous tips). In conclusion, the possible expected age changes in the small bowel of man are an increase in broad villi, with a reduction in villous height. These changes may be due to reduced cell production." [Webster 1978]

Four to six stem cells for each crypt allow the turnover of the absorptive epithelium of small intestine [Barker et al. 2007].

Liver

Liver volume declines with age [Marchesini et al. 1988], both in absolute values and in proportion to body weight [Wynne et al. 1989], and this reduction has been estimated to be about 37 percent between ages 24 and 91 [Marchesini et al. 1988]. Liver blood flow also declines with age, by about 53 percent between ages 24 and 91 [Marchesini et al. 1988]. However, while liver size declines with age, hepatocytes increase in size, unlike in the liver atrophy that accompanies starvation [James 1998].

Cirrhosis is the final stage of chronic destruction of hepatocytes caused by hepatitis, alcoholism or other factors. When hepatocyte stem cells exhaust their duplication capacities, the liver is transformed by a general atrophic process, often complicated by carcinomas caused by dysfunctional telomere-induced instability [DePinho 2000; Artandi 2002].

Diabetes

Diabetes frequency increases from youth to old age [Harris et al. 1987]. Pancreatic β-cells show turnover [Finegood et al. 1995] and it has been suggested that type 2 diabetes is caused by insufficient substitution of β-cells killed by metabolic stress [Bonner-Weir 2000; Cerasi et al. 2000]. In Werner syndrome, diabetes could be caused by impaired replicative process of β-cell stem cells with an insufficient replacement of apoptotic β-cells. In normal old individuals, the progressive exhaustion of β-cell turnover could justify the age-related progressive frequency of the disease.

Drugs effective in "organ protection", as ACE-inhibitors and sartans and statins, reduce the risk of diabetes [McCall et al. 2006; Ostergren 2007].

Heart

In the old heart there is a global loss of myocytes, with a progressive increase in myocyte cell volume per nucleus [Olivetti et al. 1991]. "With aging, there is also a progressive reduction in the number of pacemaker cells in the sinus node, with 10 percent of the number of cells present at age 20 remaining at age 75. ... Age-associated left ventricular hypertrophy is caused by an increase in

the volume but not in the number of cardiac myocytes. Fibroblasts undergo hyperplasia, and collagen is deposited in the myocardial interstitium." [Aronow 1998]

The decline of cardiac contractile capacities causes an enlargement of the heart that conceals the underlying atrophy of the contractile cells.

"... some increase in the amount of fibrous tissue and fat in the atrial myocardium with a decrease in the number of muscle fibres, and loss of fibres in the bifurcating main bundle of His and at the junction of the main bundle and its left fascicles, with lesser degrees of loss in the distal bundle branches." [Caird and Dall 1978]

Drugs effective in "organ protection", as ACE-inhibitors, sartans and statins, are effective in the prevention of atrial fibrillation [Jibrini et al. 2008; Fauchier et al. 2008].

Lungs

Lung volumes (FEV1, FVC) decline with age [Enright et al. 1993]. "The most important age-related change in the large airways is a reduction in the number of glandular epithelial cells ... the area of the alveoli falls and the alveoli and alveoli ducts enlarge. Function residual capacity, residual volume, and compliance increase. ..." [Connolly 1998] (Figure 14).

Statin use reduces decline in lung function [Alexeeff et al. 2007], justified as due to anti-inflammatory and antioxidant properties [Alexeeff et al. 2007], but that could be the consequence of effects on type II alveolar epithelial cells, analogous to those on endothelial cells [Hill et al. 2003].

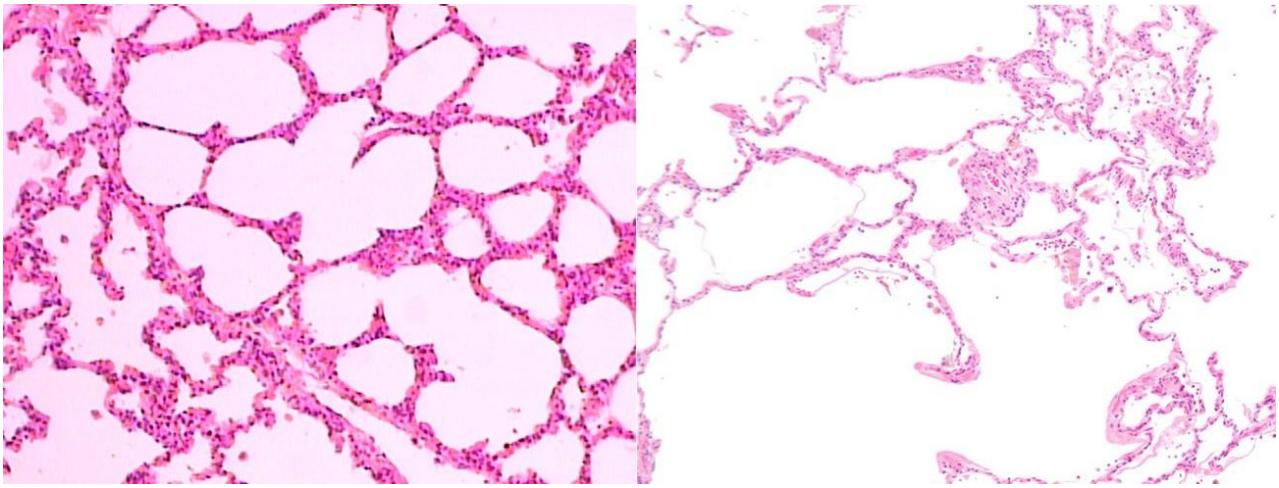


Figure 14 - Normal lung (left) in comparison with a lung affected by marked emphysema (right).

Kidneys

"Age-induced renal changes are manifested macroscopically by a reduction in weight of the kidney and a loss of parenchymal mass. According to Oliver, the average combined weight of the kidneys in different age groups is as follows: 60 years, 250 g; 70 years, 230 g; 80 years, 190 g. The decrease in weight of the kidneys corresponds to a general decrease in the size and weight of all organs. Microscopically, the most impressive changes are reductions in the number and size of nephrons. Loss of parenchymal mass leads to a widening of the interstitial spaces between the tubules. There is also an increase in the interstitial connective tissue with age. The total number of identifiable glomeruli falls with age, roughly in accord with the changes in renal weight." [Jassal et al. 1998]

Microalbuminuria, a simple marker of nephropathy, is "predictive, independently of traditional risk factors, of all-cause and cardiovascular mortality and CVD events within groups of patients with diabetes or hypertension, and in the general population ... It may ... signify systemic

endothelial dysfunction that predisposes to future cardiovascular events" [Weir 2007b], and this implicates that drugs effective in "organ protection" defend renal functionality too.

Skeletal Muscle

There is positive correlation between age and muscle atrophy, both in terms of overall muscle bulk and of the size of individual fibers [Grimby et al. 1982; Lexell et al. 1988].

"These changes are to some extent dependent on the fallout of anterior horn cells that occurs with age, but this does not completely explain the process of aging atrophy. In detailed studies it has been shown that the progressive reduction that occurs in muscle volume with aging can be detected from age 25 years and that up to 10 percent of muscle volume is lost by age 50 years. Thereafter the rate of muscle volume atrophy increases, so that by 80 years almost half the muscle has wasted. ... Both reduction in fiber number and fiber size are implicated in the loss of muscle volume." [Cumming 1998]

In Duchenne muscular dystrophy, there is a chronic destruction of myocytes that are continually replaced by the action of stem cells until these are exhausted [Adams et al. 2001].

Bone

"Once middle age is reached, the total amount of calcium in the skeleton (i.e., bone mass) starts to decline with age ... This is associated with changes in skeletal structure, resulting in it becoming weaker and more prone to sustaining fractures. For example, the bony cortex becomes thinner due to expansion of the inner medullary cavity, the trabecular network disintegrates, and there is an accumulation of microfractures. ... Bone loss in the elderly is largely a result of excess osteoclast activity, which causes both an expansion in the total number of remodelling sites and an increase in the amount of bone resorbed per individual site. .... Bone loss in the elderly is also thought to involve an age-related decline in the recruitment and synthetic capacity of osteoblasts" [Dieppe and Tobias 1998] (Figure 15).

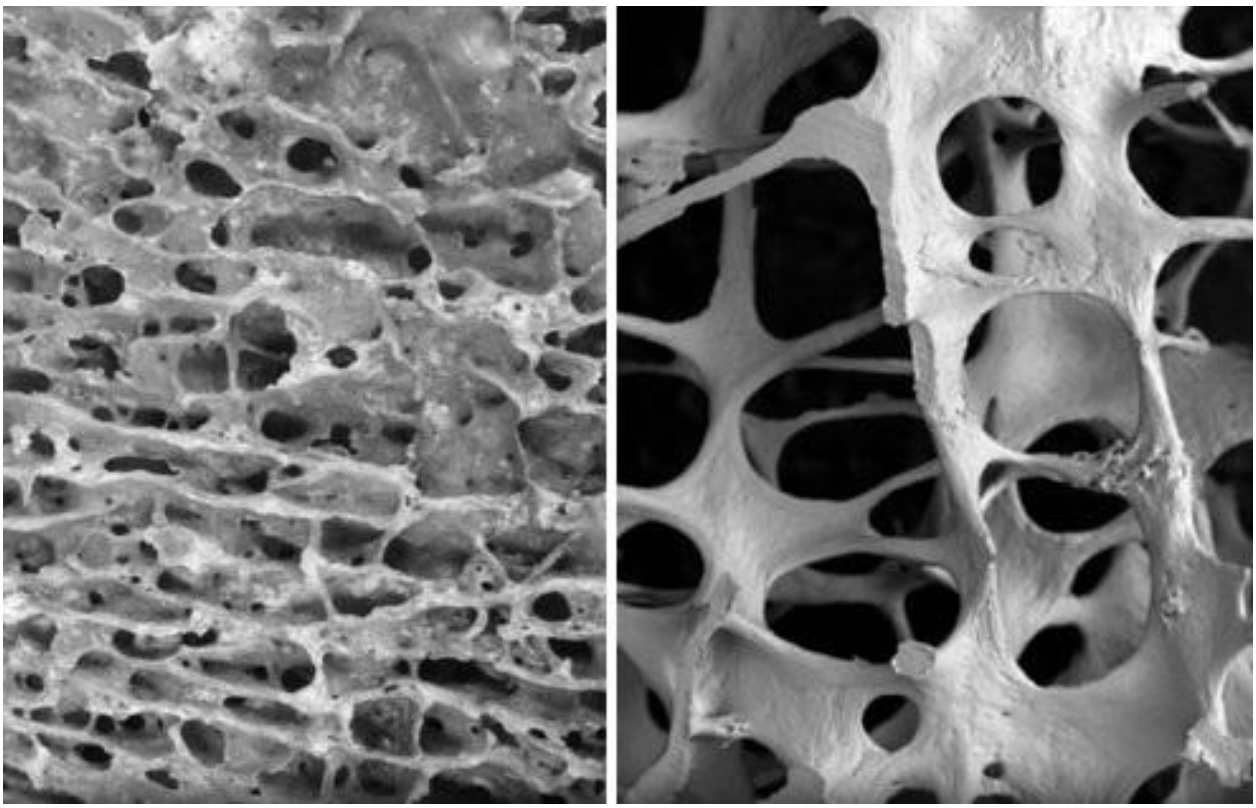


Figure 15 - A normal bone (left) in comparison with an osteoporotic bone (right). The bones of elders are sometimes described wrongly as worn-out while they are clearly atrophic.

"Involutional bone loss ... starts between the ages of 35 and 40 in both sexes, but in women there is an acceleration of bone loss in the decade after menopause. Overall, women lose 35 to 50 percent of trabecular and 25 to 30 percent of cortical bone mass with advancing age, whereas men lose 15 to 45 percent of trabecular and 5 to 15 percent of cortical bone. ... Bone loss starts between the ages of 35 and 40 years in both sexes, possibly related to impaired new bone formation, due to declining osteoblast function." [Francis 1998]

Blood

"... red cell indexes are well preserved even in centenarians. ... peripheral blood lymphocyte populations do seem to show a significant change in age, with a fall in total numbers. $CD4^+$ T-helper cells, responsible for major histocompatibility complex class II restricted recognition of foreign antigen and subsequent activation of $CD8^+$ T-suppressor, B-lymphocyte, and granulocyte effector cells of the immune response, show an overall decline with age accompanied by a reduction in capacity to produce virgin $CD4^+$ CD45RA T cells. ... Gradual involution of red marrow continues but is especially marked after the age of 70 years when iliac crest marrow cellularity is reduced to about 30 percent of that found in young adults." [Gilleece and Dexter 1998]

In vitro neutrophil functions (e. g: endothelial adherence, migration and phagocytosis capacity, granule secretory behavior, etc.) are insignificantly affected by age but *in vivo* significantly fewer neutrophils arrive at the skin abrasion sites studied in older people [MacGregor and Shalit 1990]. The proliferative capacity of T lymphocytes to nonspecific mitogens is greatly reduced with aging [Gravenstein et al. 1998].

It has been suggested that age-related functional decline in adult tissue hematopoietic stem cells limits longevity in mammals [Geiger and van Zant 2002].

Brain

"...brain weight, on average, remains fairly constant up until 60 years of age, after which a gradual decline sets in leading to an eventual loss of only some 5 percent of the original weight (60 to 70 g) by the ninth decade. ... progressive decline in nerve cell number with aging in areas such as the temporal cortex (middle and inferior temporal gyri), the pre- and post-central gyrus, the striate cortex, and the inferior and superior frontal cortex, leading to average overall losses in old age ranging from about 10 to 50 percent with the greatest changes occurring in the frontal and temporal cortex." [Mann 1998].

Neurones have no turnover but their survival depends on other cells with turnover, in particular endothelial cells of cerebral arteries and gliocytes [Fossel 2004] (see next paragraph).

Cancer

A thorough review [DePinho 2000] illustrates the well documented hypothesis of telomere dysfunction as an important cause of cancer in old age, especially for cells with higher turnover that are for the most part epithelial.

Cells/Tissues with No Turnover

Crystalline Lens

See discussion about Werner syndrome, argument 1. Besides, statin use lowers risk of nuclear cataract, the most common type of age-related cataract [Klein et al. 2006]. This has been attributed to "putative antioxidant properties" [Klein et al. 2006] but could be the consequence of effects on lens epithelial cells analogous to those on endothelial cells [Hill et al. 2003].

Photoreceptor Cells (Cones and Rods)

Retina cones and rods are highly differentiated nervous cells with no turnover. The top of these cells leans on retina pigmented cells, highly differentiated gliocytes with a turnover rate that

declines with age (Figure 16). Each day 10% of the membrane on which photopsin molecules lie are phagocytized by retina pigmented cells and substituted by an equal quantity of new membrane. Each retina pigmented cell serves 50 cones or rods and, therefore, each day a cell metabolises photopsin molecules of about 5 cones or rods, demonstrating a very high metabolic activity. Without the macrophagic activity of retina pigmented cells, photoreceptor cells cannot survive. Replicative senescence and cell senescence of retina pigmented cells limit or stop the functionality of retina cones and rods and then cause their death, i.e. age-related retina macular degeneration (AMD) [Fine et al. 2000].

With particular deficiencies of retina pigmented cells, AMD arises at lower ages and is considered a specific disease while at later ages its frequency increases exponentially and is considered a feature of the senile state.

Indeed, AMD affects 5%, 10% and 20%, respectively of subjects 60, 70 and 80 years old [Berger et al. 1999] and it is likely that a large proportion of centenarians suffer from AMD.

Smoking, diabetes, and obesity are risk factors for AMD [Klein et al. 2007].

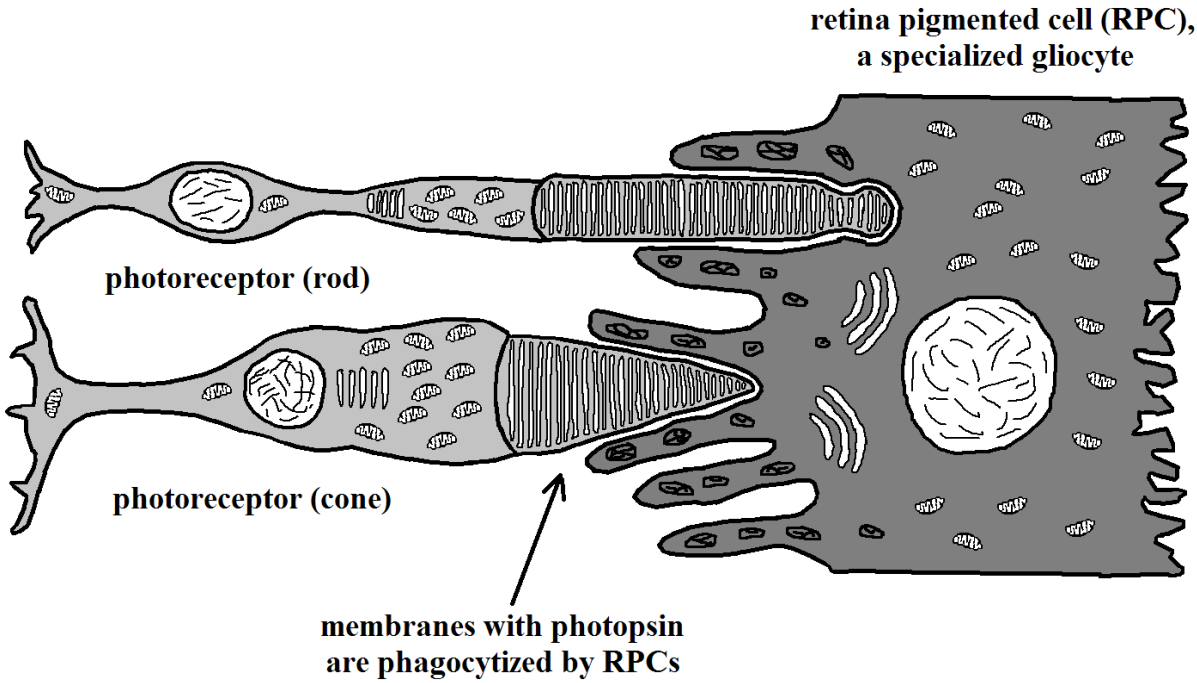


Figure 16 - A scheme of two photoreceptors and a retina pigmented cell.

Neurons

As photoreceptor cells, specialized types of neurons with no turnover, depend on other cells that are specialized types of gliocytes with turnover, perhaps other types of neurons depend from other types of gliocytes.

If this it true, replicative senescence and cell senescence of these gliocytes should cause pathologies similar to AMD. Without the key example of AMD, it has been already hypothesised that Alzheimer disease is dependent from the decline of the function of particular gliocytes (microglia cells) because of the failure of the telomere-telomerase system [Fossel 2004]: "One function of the microglia (Vekrellis et al., 2000) is degradation of β-amyloid through insulin-degrading enzyme (IDE), a function known to falter in Alzheimer disease (Bertram et al., 2000" (p. 233), "telomere lengths of circulating monocytes can serve as an independent predictor in at least

vascular dementia (von Zglinicki et al., 2000b)" (p. 235), "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." (p. 235). As for AMD, there are precocious familial cases of Alzheimers, considered as distinct diseases with distinct genetic causes [Fossel 2004], and Alzheimer frequency increases exponentially with age: 1,5% at age 65 years and 30% at 80 [Gorelick 2004], with a very high probability that a centenarians is affected by it. There is also an association between Alzheimer disease and cardiovascular factors [Vogel et al. 2006]. Drugs with organ protection qualities such as statins, ACE-inhibitors and sartans, are effective against Alzheimer disease too [Ellul et al. 2007].

Discarding the simplistic deduction that Alzheimer disease is only a consequence of vascular dysfunction, it is likely that there is a common pathogenetic mechanism: endothelial dysfunction caused by low endothelial progenitor cells in the first case, and microglia dysfunction caused by low microglia progenitor cells in the second case. In both cases the telomere-telomerase system is the primary causal factor and cardiovascular/Alzheimer risk factors accelerate telomere failure, whereas "protective" drugs counter these effects (see Figure 17).

A General Scheme

Factors that, for the turnover of each cell type, increase and reduce apoptosis rates should accelerate and slacken, respectively, the physiological age-related decline in turnover and, therefore, the onset of the related function decline (Figure 17 and Table III).

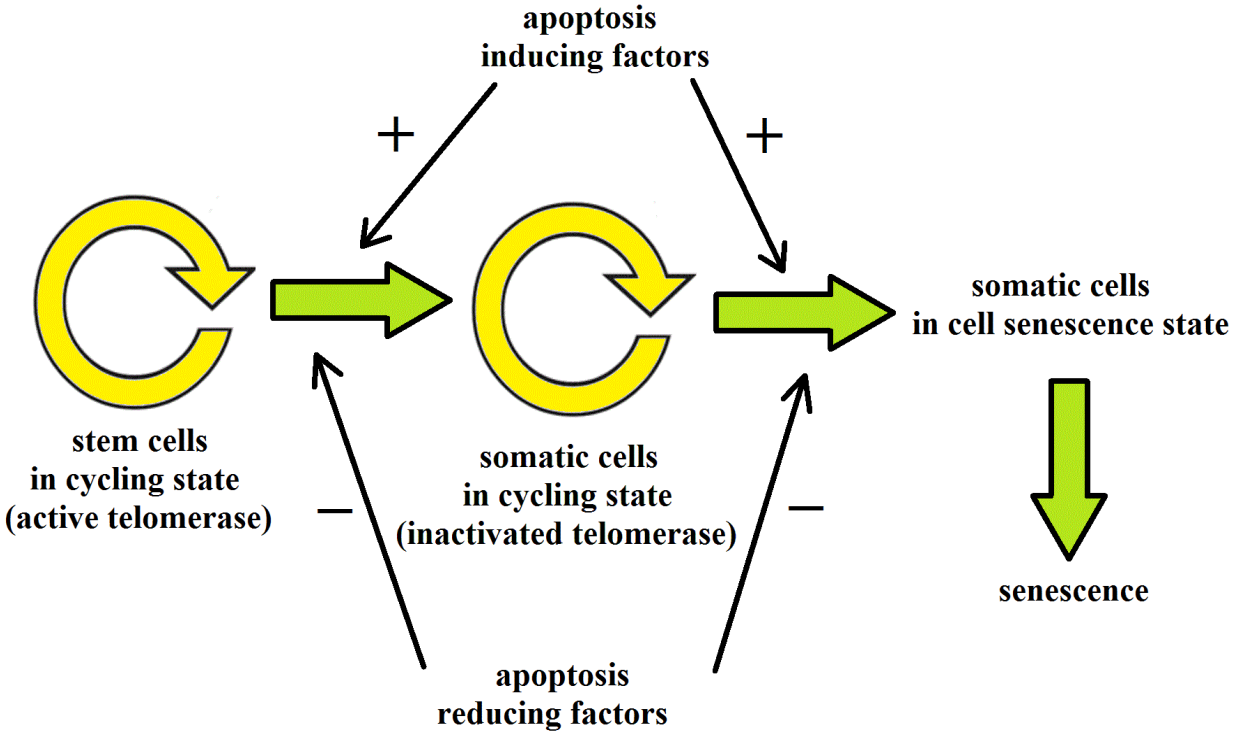


Figure 17 - A general scheme for the onset of various diseases. For example, apoptotic rate of endothelial cells is influenced by age as well as changes in the relative risk factors. It is the precise combination of these factors which determines the timing of vascular disease onset.

Table III. Abbreviations: WS = Werner syndrome; DC = dyskeratosis congenita; → X = causing X or accelerating its onset

STEM CELLS OF ...	ALTERATIONS IN THE ELDERS	APOPTOSIS INCREASING FACTORS AND THEIR EFFECTS
Alveolar type II cells	Emphysema	Smoking, chronic inhalation of noxious substances, chronic bronchitis (→ emphysema); DC (→ fibrosis)
Cardiac myocytes	Cardiac insufficiency	Myocarditis (→ dilatative cardiomyopathy)
Endothelial cells	Atherosclerosis	Smoking, hypertension, hypercholesteremia, diabetes; WS (→ atherosclerosis)
Epidermis cells	Skin atrophy	Excessive sun exposure (→ photoaging); DC (→ abnormal pigmentation, nail dystrophy); WS (→ skin atrophy)
Glomerular cells	Renal insufficiency	The same as for endothelial cells (→ renal insufficiency)
Hepatocytes	Hepatic atrophy	Chronic hepatitis, alcoholism; DC (→ cirrhosis, hepatic carcinoma)
Intestinal cells	Intestinal atrophy	DC (→ gut disorders)
Lens epithelial cells	Cataract	Exposure to radiations; WS (→ cataract)
Microglia cells	Alzheimer disease	The same as for endothelial cells (→ Alzheimer disease)
Myocytes	Muscle atrophy	Specific genetic defects (→ muscular dystrophies); WS (→ muscle atrophy)
Osteoblasts	Osteoporosis	WS (→ osteoporosis)
Pancreatic β-cells	Latent or mild diabetes	Hyperalimentation, specific viral infections; WS (→ diabetes)
Retina pigmented cells	AMD	Smoking, obesity, diabetes (→ AMD)

A Badly Interpreted Cue: Telomere-Telomerase System as Oncogenic Factor

If an age-related fitness decline is adaptive, then the existence of sophisticated mechanisms causing this decline, namely the telomere-telomerase system, is indispensable.

Conversely, if an age-related fitness decline is nonadaptive, the telomere-telomerase system needs a plausible and detailed evolutionary explanation for its existence [Libertini 2008].

A speculative justification for the effects (replicative senescence and cell senescence) of the above said system is that of a general defense against the threat of malignant tumors [Campisi 1997; Wright and Shay 2005], in a sort of evolutionary trade-off between aging and cancer restriction [Campisi 2000]. However, this hypothesis does not justify the great differences in duplication limits and overall cell functionality decay from species to species, unless the risk of malignant tumors is postulated as varying from species to species in direct correlation with the limits imposed to cell duplication capacities and to overall cell functionality by the genetic modulation of telomere-telomerase system.

Moreover, there are a number of other problems with the hypothesis that the telomere-telomerase system is a defense against cancer:

1) Lobsters and old rainbow trout, “animals with negligible senescence”, have, in the wild, the same levels of telomerase activity as young individuals [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998] and increasing problems of carcinogenesis at older ages are not plausible for them because, as their definition states, their mortality rates do not increase with age [Libertini

2008]. For these animals, telomerase action involves no evident oncogenic risk and, therefore the idea that telomerase has an oncogenic effect is implausible in these species.

2) The decline of duplication capacities and of overall cell functionality weakens immune system efficiency [Fossel 2004], which has, for a long time, been known to be inversely related to cancer incidence [Rosen 1985];

3) When telomeres are shortened, there is a great vulnerability to cancer because of dysfunctional telomere-induced instability [DePinho 2000; Artandi 2002];

4) "The role of the telomere in chromosomal stability (Blagosklonny, 2001; Campisi et al. 2001; Hackett et al., 2001) argues that telomerase protects against carcinogenesis (Chang et al., 2001; Gisselsson et al., 2001), especially early in carcinogenesis when genetic stability is critical (Elmore and Holt, 2000; Kim and Hruszkewycz, 2001; Rudolph et al., 2001), as well as protecting against aneuploidy and secondary speciation (Pathak et al., 2002). The role of telomerase depends on the stage of malignancy as well as cofactors (Oshmura et al., 2000); expression is late and permissive, not causal (Seger et al., 2002)." [Fossel 2004];

5) The telomere-telomerase system of yeast (*Saccharomyces cerevisiae*), a unicellular organism, is well studied. Individuals of this species stop their replications after 25-35 duplications [Jazwinski 1993] that is they show replicative senescence and cell senescence, although not caused by telomere shortening but by another unknown mechanism related to the number of duplications [Lesur and Campbell 2004]. A senescent yeast cell ends its life with apoptosis [Laun et al. 2001] and apoptosis is also triggered in difficult conditions [Kaeberlein et al. 2007]. In both cases, apoptosis divides the cell into metabolically active parts that are usefully and easily phagocytized by other yeast cells. This is done in an orderly way and such behavior has been plausibly interpreted as adaptive [Skulachev 2002a, 2003; Herker et al. 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf 2006]. However, in 1988 it was hypothesized that life limiting mechanisms should be favored in conditions of K-selection, namely: a) with a population numerically constant, as a consequence of a limited living-space, so that only when an individual dies there is place for a new individual; b) with dead individuals replaced prevalently by kin individuals [Libertini 1988]. Colonies of kin yeast cells in a saturated habitat are in these conditions and, therefore, empirical evidence for yeast is a confirmation of this theoretical prediction. In yeast, apoptosis, replicative senescence and cell senescence, determined by genes killing individuals where they act (= negative individual fitness) are shaped by natural selection with clear adaptive aims (= positive inclusive, or - however – supraindividual, fitness) and it is inexplicable that while these phenomena are accepted as adaptive for unicellular species, the same explanation is not considered possible for multicellular species [Kirkwood and Austad 2000]. Finally, given that yeast is a unicellular organism, the telomere-telomerase system and its actions in this species cannot have any value against cancer: the oncogenic risk is non-existent.

In short, the telomere-telomerase system is hardly justifiable as a defense against cancer risk and, lacking other explanations, only the adaptive hypothesis of age-related fitness decline appears a rational cause for its existence.

An Equivocal Cue: The Confusion between Age-Related Fitness Decline in the Wild and Mortality Increase in Laboratory Conditions

In figures 18-A1 and 18-A2, are the life tables of wild species such as the lion (*Panthera leo*) and hippopotamus (*Hippopothamus amphibius*), which demonstrate an age-related mortality increment in natural conditions. These life tables are examples among very many species, our species included, demonstrating the same phenomenon. For brevity, this "increment of mortality with increasing chronological age in the wild" has been called IMICAW [Libertini 1988].

In figures 18-B1 and 18-B2, are the life tables of animals in laboratory conditions, such as the nematode *Caenorhabditis elegans* [Finch 1990] and the fly *Drosophila melanogaster* [Finch and Hayflick 1977], which in artificial conditions demonstrate an age-related mortality increment. These life tables are examples among very many species, most of insects included, demonstrating an analogous age-related mortality increment in artificial protected conditions. For brevity, this "increment of mortality with increasing chronological age in captivity" has been called IMICAC [Libertini 1988].

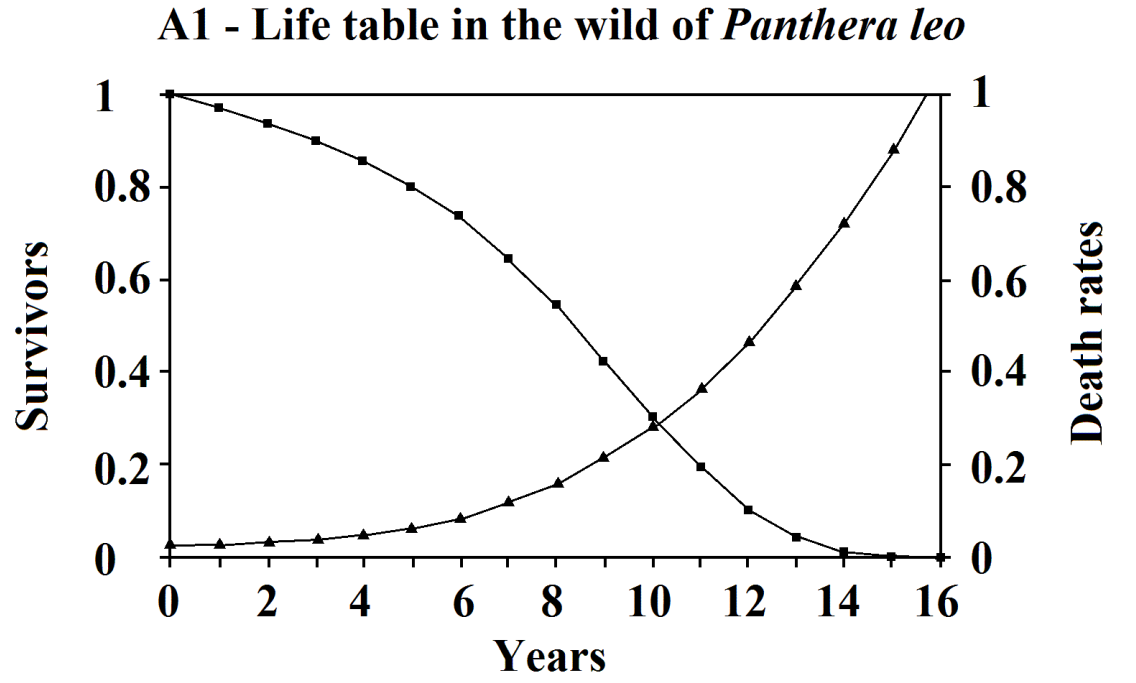
A superficial observer could suppose that IMICAW and IMICAC are the same phenomenon and therefore studies more easily done on the worm or on the fly could explain what happens in species such as the lion, hippopotamus and man.

However, a well-informed observer knows that the ages in which mortality increment starts in laboratory for the worm and for the fly, do not exist in the wild. In fact, the longevity of *Caenorhabditis elegans* "under more natural conditions is reduced up to 10 fold compared with standard laboratory culture conditions" [Van Voorhies et al. 2005] and few individuals of this species remain fertile in the wild after 10 days [Johnson 1987]. Similarly, *Drosophila melanogaster* has a reported adult life span in the wild of 10-12 days [Finch 1990].

For both these animals, the age-related increasing mortality described in figures 18-B1 and 18-B2 starts at ages non-existent in the wild, meaning that it is no more than a laboratory artefact.

Well, if IMICAW exists by definition in the wild and therefore by definition is influenced by natural selection, while on the contrary IMICAC is non-existent in the wild and therefore is not influenced by natural selection, we should have strong doubts about the conclusions of experiments about IMICAC applied to IMICAW. However, there is another essential difference.

The worm and the fly (and in general the adult insects) are composed of cells with no turnover [Finch 1990; Arking 1998], while lion, hippopotamus and man have cells and tissues with turnover. If, as it seems probable, the slowdown and later the stopping of cell turnover, and the correlated cell senescence, are pivotal elements in the fitness decline of animals such as the lion, hippopotamus and our species, it is rather dubious to use experiments on animals with no cell turnover to explain the fitness decline in animals with cell turnover. This is a basic problem, certainly of extreme weight for those interested in the explanation of aging mechanisms. However, in renowned texts on the topic, the problem is not considered [Rose 1991], and it is frequent that, in very influential journals, experiments modifying the life table of our dear worm or of our beloved fly are presented as meaningful advances in the understanding of human aging [Johnson 2007; Petrascheck et al. 2007; Kennedy 2008]!



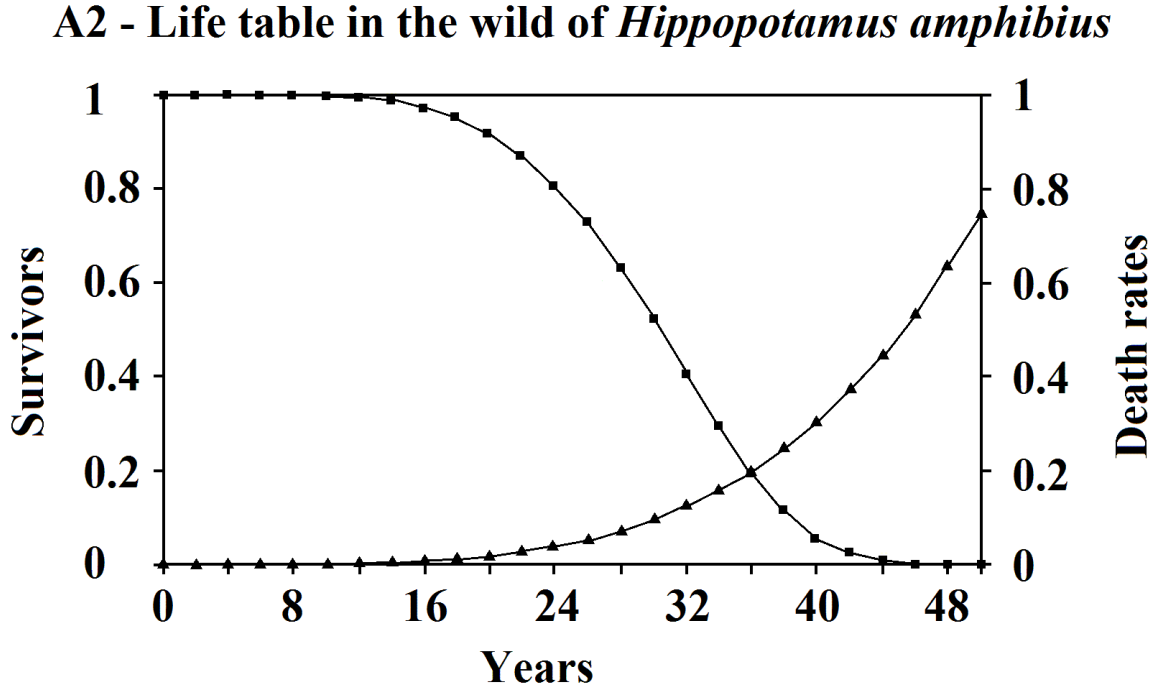
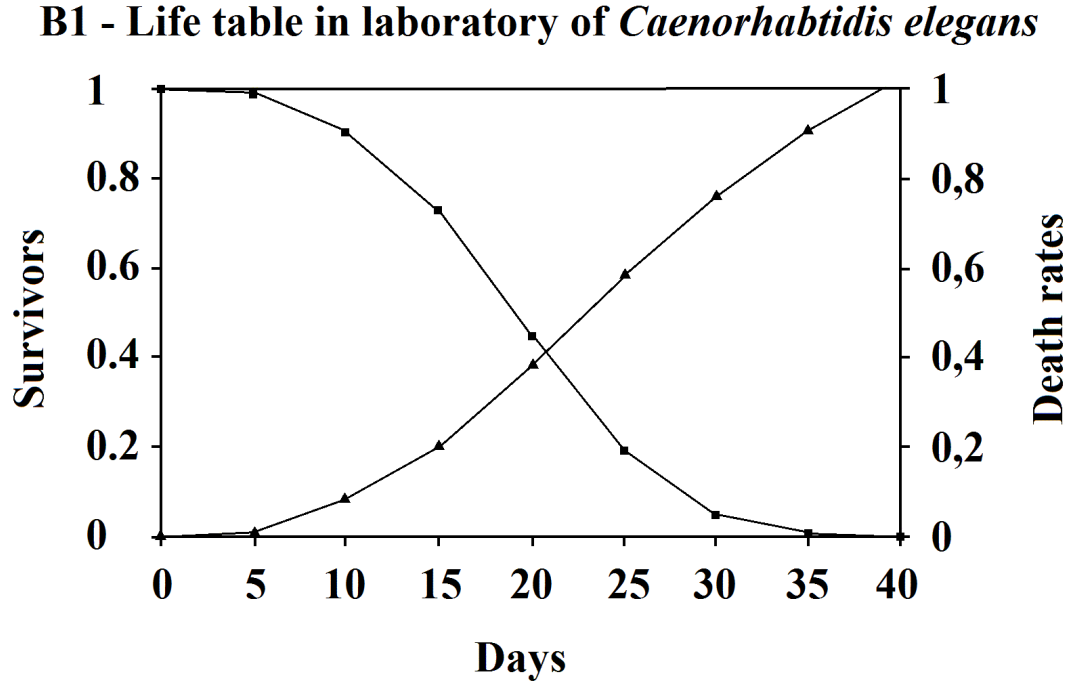


Figure 18A - Life table and death rate of: (A1) lion (*Panthera leo*) in the wild, data from [Ricklefs 1998]; (A2) hippopotamus (*Hippopotamus amphibius*) in the wild, data from [Ricklefs 1998].



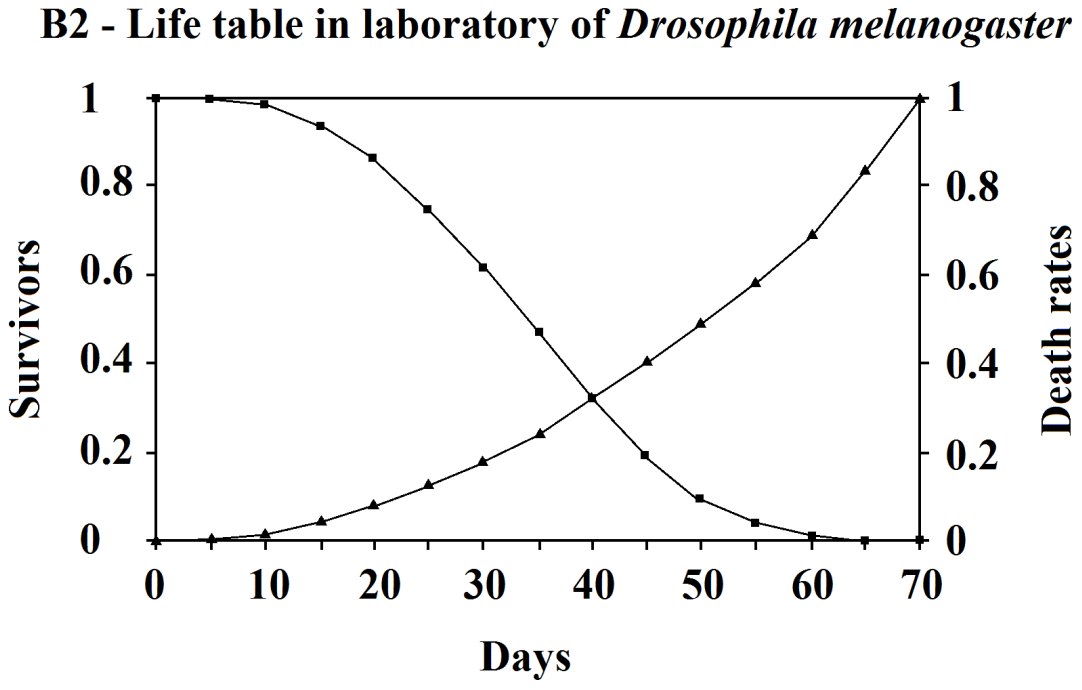


Figure 18B - Life table and death rate of: (B1) *Caenorhabditis elegans* reared in laboratory, data from [Finch 1990], Figure 6.1; (B2) *Drosophila melanogaster* reared in laboratory, data from [Finch and Hayflick 1977], Figure 10.

A Surprising Cue:
Animals with Negligible Senescence

"Negligible senescence" has been defined as the condition of species - such as rockfish, sturgeon, turtles, bivalves and possibly lobsters [Finch and Austad 2001] - which show in the wild "no observable increase in age-specific mortality rate or decrease in reproduction rate after sexual maturity; and … no observable age-related decline in physiological capacity or disease resistance" [Finch and Austad 2001]. In some cases fitness even increases with age, i.e. as a function of increasing body size [Vaupel et al. 2004].

For a theory explaining age-related fitness decline as the result of harmful factors that accumulate with the passing of time and are not sufficiently opposed by natural selection, the existence of animals reaching very old ages in the wild without any observable decline in their fitness, is a true challenge by no means solved by current theories of aging ("negligible senescence … may be in conflict with mathematical deductions from population genetics theory" [Finch and Austad 2001]). For such a theory, ageless animals have to be explained as exceptions justified by hypothetical not documented physiologic peculiarities. Particular optimization models of life-history strategies, based on the suppositions of disposable soma theory [Kirkwood 1977; Kirkwood and Holliday 1979] have been developed to justify even the cases of negative senescence [Vaupel et al. 2004].

On the contrary, for a theory explaining age-related fitness decline as caused in particular conditions by selective factors, there is a simple prediction: a species that is not in those particular conditions must be an ageless animal. This means that, for these species, survival in the wild (disregarding possible minor factors that modify fitness) is described by the simple formula:

$$Y_t = Y_0 \cdot (1 - \lambda)^t \qquad (4)$$

where Y_0 is the initial population, Y_t are the survivors at time t and λ is the mortality rate.

Survival is determined only by the parameter λ. With low values of λ, it is predicted that, in the wild, some individuals will reach very old ages. For example, if λ = 0,011306/year, the survivors after 405 years will be about 1% and the case of *Arctica islandica* specimen with an age of 405 years retrieved near Iceland in 2007 will not result surprising.

However, at an age t reached in the wild by few or no individuals, there is very little or no natural selection against a gene with harmful action only at that age ("t-gene"). Therefore, by the cumulative effects of various "t-genes", if a species demonstrating no age-related fitness decline in the wild is reared in protected conditions, it could show a progressive increase in mortality starting from ages rarely or never existing in the wild. In other words, the species could show IMICAC phenomenon (Figure 19).

As a simple corollary of formula (4) and of this phenomenon, for a group of species of the same genus, all in conditions not favouring the fitness decline, it is predicted that: (1) all species will have a stable fitness at all ages existing in the wild, with a possible little decrement at ages rarely present in the wild; (2) for each species mean life span and maximum life span in the wild will be inversely correlated with λ; (3) in protected conditions, for each species life span will be determined by variable factors, which could increase mortality starting from ages rarely or never existing in the wild and therefore variable from species to species and inversely correlated with λ. Life tables of the rockfish genus are probably a good example of these predictions [Cailliet et al. 2001].

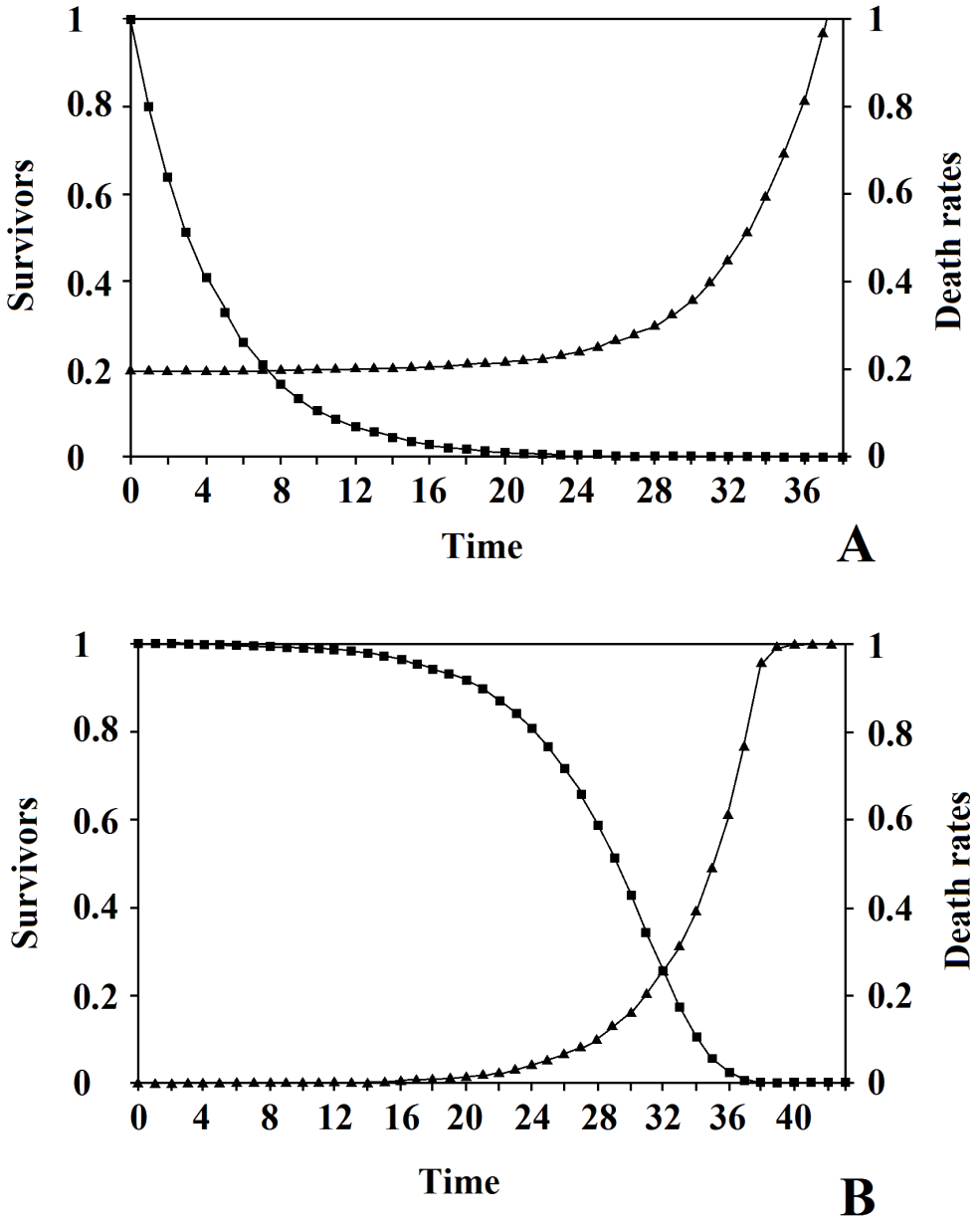


Figure 19 - Results obtained with a simulation program (IMICAC.exe [Libertini 2006], available at the Internet address www.r-site.org/ageing/simprograms.zip). (A) A species with a constant extrinsic mortality rate in the wild (=0.2/unity of time), plus the small increase of mortality rate, at ages rarely or never existing in the wild, due to the action of t-genes (20 in the simulation and with a mutation rate from inactive alleles equal to 0.00001); (B) The same species, in protected conditions with a zero extrinsic mortality rate, shows the IMICAC phenomenon due to the action of t-genes at ages not-existing in the wild.

It is interesting that for the rockfish genus, telomerase activity is constant at all ages existing in the wild [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998]. Moreover, for two species of rockfish, it has been observed that oogenesis continues at advanced ages, in contrast with long-held assumptions [De Bruin et al. 2004].

A rockfish is showed in Figure 20.

Figure 20 - A yelloweye rockfish. See also the site www.agelessanimals.org, a scientific site, directed by John C. Guerin and dedicated to: Emerging Area of Aging Research: Long-lived Animals with "Negligible Senescence".

A Poorly Understood Cue: Aging as Distinct Entity

It is very common to hear from ordinary people that someone died due to old age. However, in the official statistics no one dies due to old age! In the compilation of official death certificates, in any nation of the world, a physician, as I am, must use the international classification of diseases (ICD), in which, though there are codes for “senile dementia” or “senile cataract”, a codification for “aging” or “senescence” is non-existent. Some years ago, I compiled the death certificate for a 102-year-old great-grandmother who had no particular disease, but I could not write that she was died because she was old!

A very authoritative gerontologist wrote to me on the subject, saying: “The question you are asking me is an old one - should senescence be listed on the death certificate of someone who dies past the age of 100.

For some people for whom it is not possible to find an underlying cause, such as individuals who experience what would appear to be a collapse of their entire body all at one, I would say that senescence would be an appropriate cause of death to place as the underlying cause.

I expect the frequency of this diagnosis will increase in the coming decades. However, should such a cause of death be added to the ICD, my guess is that it would overused by attending physicians too lazy to determine underlying cause.”

Aging as a distinct cause of death is disregarded or considered as non-existent by classic gerontological theories or by official epidemiology. For such theories, aging is not a specific process but only the sum of many different diseases (Figure 21). With this paradigm, we should cure each of these diseases, while the possibility of acting on aging is unthinkable because aging does not exist as a distinct entity!

Well, if you want to understand the next paragraphs, you should accept a new idea, a radical change of the old paradigm that “gradual decline in performance with age happens by default” [De Grey 2007]: i.e. aging as a distinct phenomenon exists!

Figure 21 - According to the classic paradigm, these elders will not be killed by senescence but only by a myriad of diseases whose frequency increases with age.

Ancient and Current Scenery: Toward a Socio-Medical Nightmare

Until the beginning of the XIX century the child mortality rate was very high, the adult mortality rate was higher than present and the mortality rate for elders not very different from present. The following is an example of this historical child mortality rate. In the years 1812-1815, in the statistics from a part of the reign of Naples under king Murat [Martuscelli 1979] the number of those who died before 7 years of age was about 42-46% of the total of deaths, e.g.:

Table IV. Mortality in Naples and province at the beginning of XIX century

		< 7 years old	> 7 years old	Total
1812	(Province of Naples)	3.821 (42.62%)	5.144	8.965
1813	"	4.420 (45.44%)	5.308	9.728
1814	"	4.367 (45.24%)	5.287	9.654
1815	(City of Naples)	5.600 (42.22%)	7.664	13.264

In the next 200 years with the large improvement in economic and hygienic conditions and with the advances in medical cure, there has been a drastic lowering of children's mortality, a strong reduction of adult mortality and a relatively modest increase in life expectancy for the elders. The triplication of mean life span in these two centuries (from about 25 years in 1800 to about 75 years today) is due largely to a drastic reduction in children's mortality and contrasts strongly with an

apparently stable maximum life span. This is shown for England in Figure 22, which indicates that the survival table is becoming similar to that of a straight line followed by a sharp drop (rectangular curve). With the increasing control of tumoral, cardiovascular and other diseases (and an exponential increase in related costs), a greater rectangularization of the survival curve is a realistic forecast for the next decades.

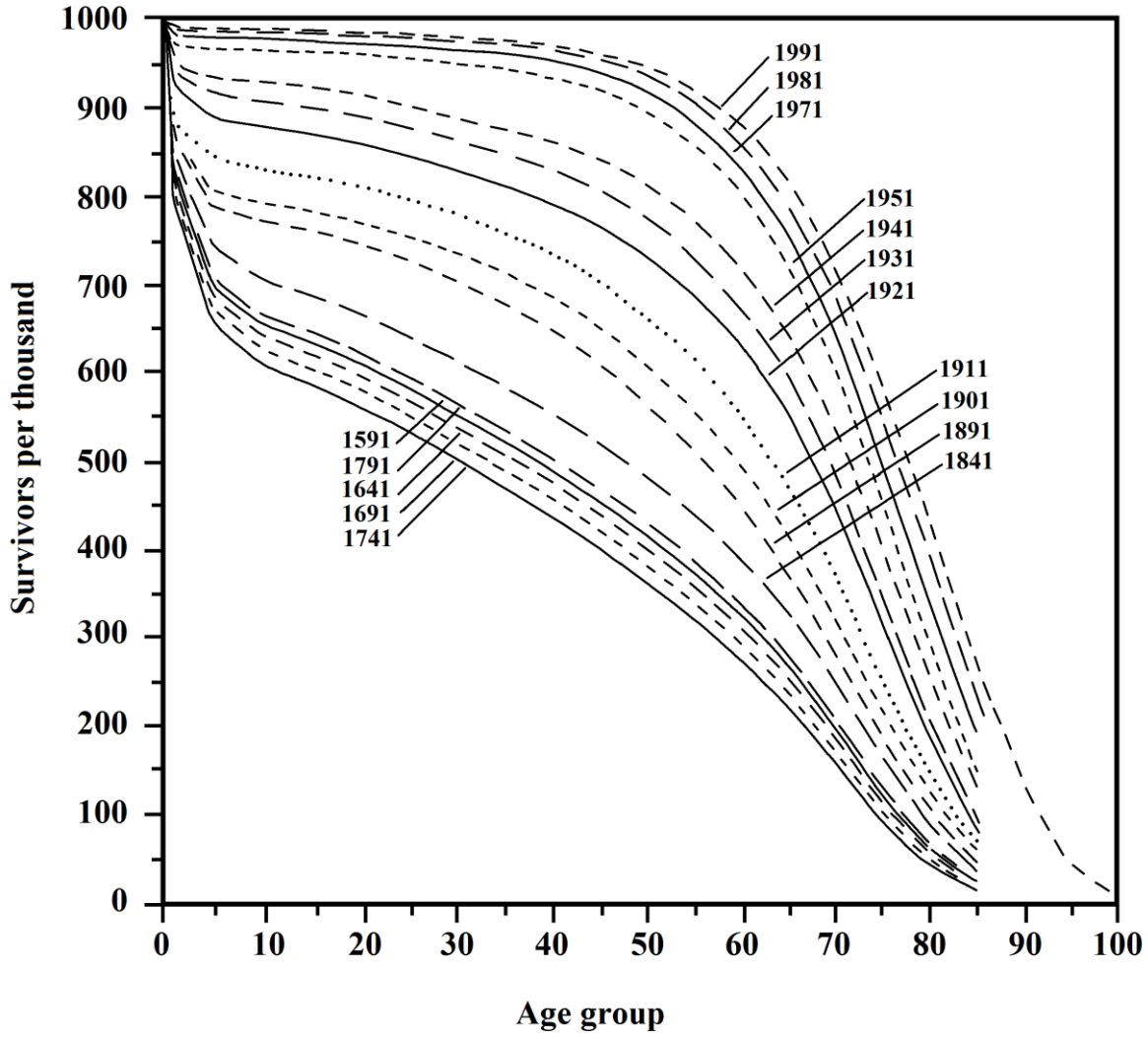


Figure 22 - Survival curves for cohorts of one thousand newborns, by age group: England, 1541-1991. Data from Cambridge Group back projection files and English Life tables up to no. 15. Work of James Oeppen [Laslett 1995].

This means that the population will be comprised of an increasing number of elderly individuals with severe troubles and pains deriving from marked osteoporosis, harsh cardiovascular and respiratory insufficiency, senile dementia, visual and auditive deficits, incontinence, etc. In short, an increasing part of the population will be seriously suffering from many diseases, in particular for the decay of cognitive and sensory capacities, and they will therefore be dependent on others. This will have heavy economic results. The progress of medicine will become the cause of a sociological and economic nightmare.

In a sense it will be the realisation of the legend of Aurora and Tithonus. Aurora, a goddess, obtained the gift of immortality from Jupiter for her beloved Tithonus, a mortal man, but neglected to ask for perpetual youth. Tithonus became older and older and never died. Lastly, out of pity, Tithonus was transformed in an animal. In 1979, Comfort said: "We are producing Tithonuses" [Comfort 1979]!

Today, we could say that a mass production of Tithonuses has been started.

A Possible Alternative Scenery: Toward the Taming of Senescence

We have two opposite general hypotheses, or paradigms, about the fitness decline and its extreme expression, i.e. 'old age' or the 'senile state'.

For the first paradigm, the phenomenon is something inescapable, inevitably inherent to the nature of life and weakly opposed by repair mechanisms in the tight limits of other prevailing demands dictated by natural selection. To counter senescence is as to oppose the force of gravity in the construction of a skyscraper: the higher one goes, the exponentially greater are the necessary efforts. In other words, the undertaking of this task becomes impossible beyond certain heights.

For the second paradigm, fitness decline is a function: suitable mechanisms, which are genetically determined and favoured by evolution because of their positive inclusive fitness, limit life span. Opposing senescence is like removing the obstacles or limiting the friction beneath a ball that rushes on a flat surface. Clearly the natural condition of such a moving body - in absence of friction and other obstacles - is an unlimited movement.

According to the first paradigm, the interpretation of senescence as a program is an absurdity because it would be of no evolutionary meaning. Therefore, the growing evidence in support of such a program is disregarded and attention is fixed only on the stochastic accumulation of damages of various types.

Conversely, according to the second paradigm a program is not at all excluded. In fact, without a program the second paradigm would be false, and attention is fixed on the events that actively determine and regulate the progressive fitness reduction. Moreover, as aging is the consequence of genetically determined mechanisms and not the sum of stochastic events, such mechanisms are the rational object of useful analysis and of possible control and modification. What for the first paradigm is an insuperable obstacle and a closed horizon, for the second paradigm is a modifiable and controllable trait, one with a limitless horizon.

To master the senile state, apart from foreseeable crucial objections of bioethical or philosophical or religious nature that will be outlined immediately after, three categories of action are required:

1) *Alterations due to cell turnover limitations* - A thorough knowledge of the mechanisms underlying cell turnover and its limitations are needed. Currently, drugs with "organ protection" properties seem to act efficaciously on some cell turnover alterations [Hill et al. 2003]. It seems more rational to propose as a future treatment the modification of regulating genes before the onset of aging manifestations, in a sort of gene "therapy". Incidentally, the term "therapy" is open to criticism since age-related fitness decline not properly a disease. Ten years ago extraordinary experiments demonstrated that the insertion of an active telomerase gene or, in general, telomerase activation, eliminates replicative senescence and the effects of cell senescence [Bodnar et al. 1998; Counter et al. 1998; de Lange and Jacks 1999]. This indicates that the effects of many factors on aging, oxidative substances included, are reversible consequences of cell senescence and not the cause of aging [Fossel 2004].

Presently, gene therapy is possible, or in trial, for only a few diseases and with the insertion of the appropriate gene in a random DNA position [Fischer 2001; Meyer and Finer 2001; Flotte 2007], therefore with the possible noxious modifications of other genes, e.g., oncogene suppressors (Figure 23). This is a strong contraindication for an indiscriminate use of this therapeutic technique.

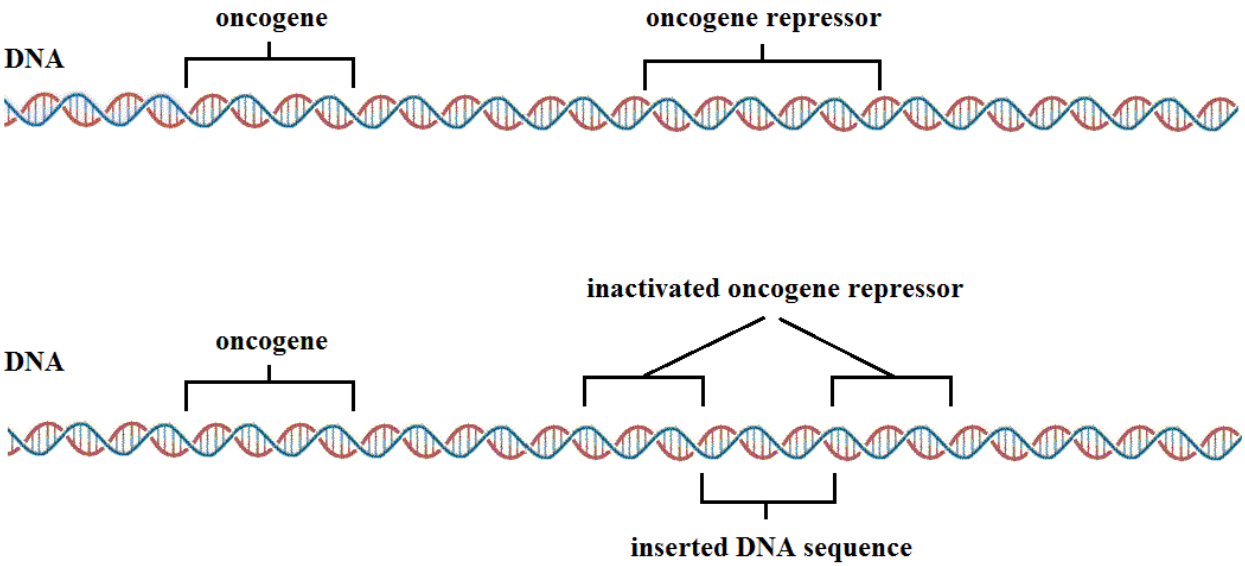


Figure 23 - The insertion of a DNA sequence in a random point may cause damage that is fatal for the whole organism, e.g. inactivating an oncogene repressor.

Furthermore, to limit this danger, the gene is inserted in only a fraction of cells that, if somatic, are substituted by turnover, which gradually cancels the therapeutic effects. Ideally, gene therapy should recognise a known sequence with no function and present as unique copy in the whole DNA and then, with a safe vector, insert the gene, breaking the known sequence in a precise point so that a second insertion would be impossible because the known sequence is modified (Figure 24). Moreover, the gene should be inserted in the majority, or prefereably all, cells (stem cells included) so that its elimination by cell turnover would be avoided. With these specifications, apart from any possible bioethical objections, gene therapy in non-germ line cells could be proposed to modify telomere-telomerase system so that age-related fitness decline will be postponed or even nullified.

Later, the possible application of the same techniques to germ line cells, namely the possibility to obtain a status of "negligible senescence", probably will become only a bioethical/philosophical/ religious problem.

2) *Age-associated diseases* – At ages rarely or never present in the wild, natural selection against genes causing a disease is weak or non-existent. Such diseases can be defined as "age associated disease" because they are an evolutionary consequence of age-related increasing mortality in the wild. A thorough knowledge of each of the associated diseases is a plain preliminary condition. The next step is the achievement of treatments to completely control each of them, namely avoiding that with growing age their harm, even if reduced, accumulates. However, as age-associated diseases are very common in the elderly [Horan 1998] and the coexistence of diverse age-associated diseases in the same individual is common [Horan 1998]. For this reason, the modification of the altered genes before the beginning of the symptoms seems the ideal treatment. About gene therapy, see the preceding paragraph.

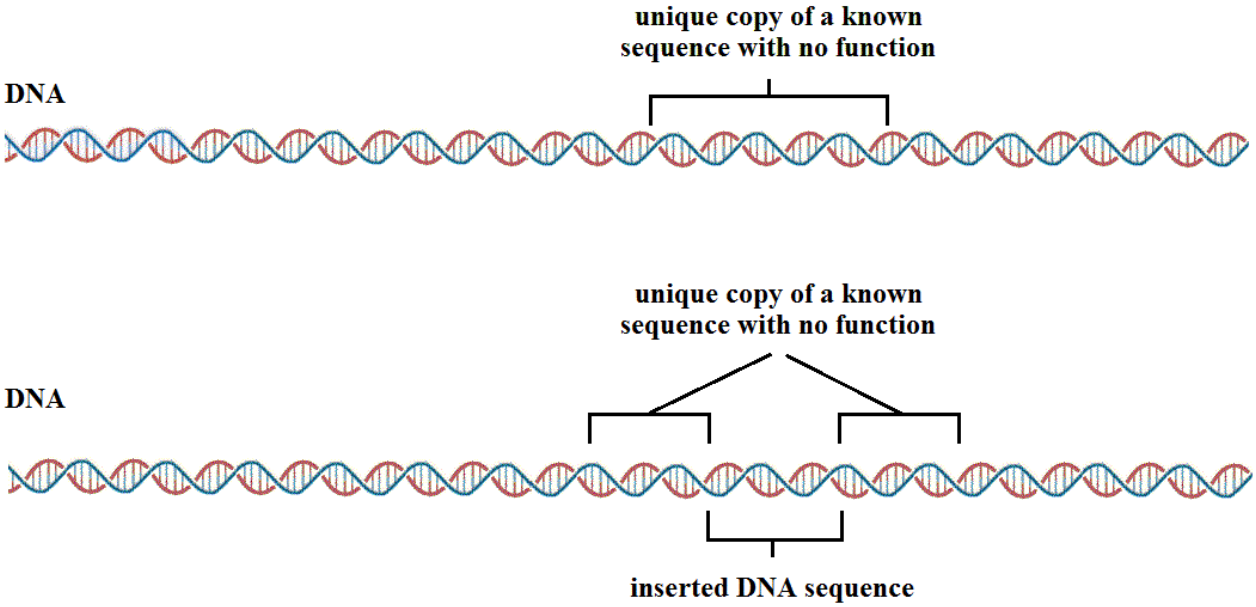


Figure 24 - The sequence is inserted breaking a known sequence existing as a unique copy in the whole genome and with no function. The breaking of this unique sequence makes impossible another gene insertion in the same cell.

3) *Alterations due to wear and tear factors* - The main example of this is tooth wear. Besides their replacement with prosthesis, multiple dentitions, namely a periodic tooth renewal as is found in other species, is imaginable. "The senescence of human teeth consists not of their wearing out but of their lack of replacement when worn out." [Williams 1957]. Thus period tooth renewal would be possible by means of germ line cell modifications, although the aforementioned ethical objections would still remain.

Bioethical/philosophical/religious objections. The possible treatments mentioned above pose great technical obstacles, none of them in principle insuperable, but there are two much greater problems, the solutions to which are not at all scientific or technical (Figure 25):

I - The first is that to modify natural aging to a slower or even a zero rate (negligible senescence) constitutes an enormous change of human nature, and is not merely the correction of a disease. For this, and even more for any hypothesis of germ cell modification, it is easy to anticipate strong bioethical, philosophical or religious objections, or even accusations of blasphemy[Note 1] or of ὕβρις[Note 2].

II - The second, still greater, difficulty, is that changes in civilisation resulting from senescence slowing or even from a non-senescent condition, would certainly be extreme and full of uncertainties. The roots of our civilisation, organisational structure and cultural traditions are based upon the philosophical idea and religious creed that life span limitation is ineluctable. The drastic change of such a reality would be a revolution greater than any other revolution ever experienced by our species.

Figure 25 - To act, or not to act on telomere-telomerase system: that is the question.

The Need of a New Dramaturgy: Conclusion

The change of an ancient paradigm seems indispensable. Age-related increasing mortality is not an unavoidable fate, as the old paradigm claims. To the contrary, it is a trait, the underlying mechanisms of which can now be described in precise detail, which has been actively moulded by selection. Although such a trait seems paradoxical because it does not improve individual fitness, the hypothesis that it has been moulded by selection means that it is modifiable and tameable in principle. The old paradigm considers fitness decline to be a maladaptive character that can only be partially opposed [De Grey 2005]. By considering aging as "a specific biological function" [Skulachev 1997], then, the new paradigm offers a more optimistic approach to treating aging.

However, the change of a paradigm is always a scientific revolution and this usually requires a new generation [Kuhn 1962].

Note 1) "Then God said, 'Let Us make man in Our image, after Our likeness ..." Genesis 1:26; "So God created man in His own image, in the image of God He created Him ..." Genesis 1:27.

Note 2) In the Greek classic culture a mortal that presumed to measure himself against the gods, regarding himself or searching for being like to them, became guilty of ὕβρις, namely of unforgivable impious pride and arrogance toward the deity.

Chapter 2

Libertini G (2009b) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle. In: Bentely JV, Keller MA, eds, *Handbook on Longevity Genetics, Diet and Disease*. Nova Science Publ., New York, pp. 35-95.

Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle

Giacinto Libertini

Abstract

Life span is limited by the effects of diseases and ageing. For the aim of a longer life span, it is indispensable that a rational analysis of the primary causes of these phenomena is not limited to the description of their physio-pathological mechanisms. If Dobzhansky's statement that "nothing in biology makes sense, except in the light of evolution" is true, it appears logical to maintain that evolution theory must be the main tool for such analysis.

From an evolutionary point of view, diseases are the predictable consequence of: 1) defects in the maintenance and transmission of genetic information; 2) alterations of the ecological niche to which the species is adapted (in particular, for our species, due to civilisation); 3) interactions with other species (bacteria, viruses, fungi, protozoa, parasitic worms, etc.); and 4) conditions for which the species is not adapted.

Moreover, evolution theory allows the paradoxical prediction that, in particular ecological conditions, kin selection favours a progressive fitness decline, usually, in its more evident manifestations, referred to as ageing and that must not be classified as a disease. This decline is genetically determined and regulated and is obtained with a progressive limitation of cell turnover through a sophisticated modulation of the telomere-telomerase system.

A lifestyle compatible with the ecological niche to which our species is adapted and good medical treatment permit the attainment of the highest life span defined by the genetic program of our species (within the limits of individual genetic peculiarities) but do not allow the overcoming of the maximal values of longevity defined by the same program.

To increase these values, it is indispensable to modify the genetic planning of ageing, with a different modulation of the telomere-telomerase system.

In principle, granted that this will be considered ethically acceptable, it is possible to propose a modification of that part of the genetic program that modulates ageing so that an unlimited survival is obtained, similar to the so-called "negligible senescence" observed for many animal and plant species.

A possible schedule to achieve this aim and the effects on human civilisation are outlined.

Premise

Disease is usually defined as an alteration of physiological conditions. If it is true that evolutionary mechanisms are indispensable for the full understanding of any biologic phenomenon [Dobzhansky 1973], it is necessary to investigate if and how diseases and other phenomena causing suffering, disability and/or death are explainable and classifiable in evolutionary terms and whether from this approach useful indications may be deduced.

This question is the object of so-called Darwinian or evolutionary medicine [Williams and Nesse 1991; Nesse and Williams 1994; Trevathan et al. 1999, 2008a; Stearns 1999; Stearns and Koella 2008], proposed in 1991 [Williams and Nesse 1991] but with some known forerunners [Trevathan et al. 2008b].

In fact, the main concept of evolutionary medicine, the "discordance" between the conditions to which our species is adapted and actual conditions of life as a very important cause of disease, was already clearly expressed and well documented before the term "Darwinian medicine" was formulated [Eaton, Shostak and Konner 1988].

Another forerunner [Libertini 1983] stated many of the concepts expressed by Williams and Nesse [Williams and Nesse 1991; Nesse and Williams 1994] with a substantial difference. For current evolutionary medicine, in accordance with prevalent gerontological ideas [Kirkwood and Austad 2000], ageing is the result of insufficient selection for a greater longevity and, in particular, of a trade-off between better somatic maintenance and reproduction capacity versus greater longevity [Austad and Finch 2008]. Alternatively, it was proposed that mechanisms underlying ageing are favoured by kin selection, in particular ecological conditions [Libertini 1983, 1988], and therefore age-related fitness decline should be considered a physiological function and not a set of unrelated pathological conditions insufficiently countered by natural selection. This paradoxical and heretical different interpretation of ageing, which is in accordance with the general hypothesis of ageing as adaptive phenomenon [Weismann 1884; Skulachev 1997; Goldsmith 2003, 2004, 2006, 2008; Longo et al. 2005], has been reaffirmed recently with the support of empirical evidence that disproves the classic interpretation [Libertini 2006, 2008]. Moreover, regarding the possibility of drastic modifications of human longevity, ageing considered as "a specific biological function rather than the result of a disorder in complex living systems" [Skulachev 1997] allows totally new perspectives, based both on theoretical arguments and on the extraordinary advances in the understanding of the telomere-telomerase system, apoptosis, cell turnover and related arguments.

The Basic Question

Anatomy, physiology and behaviour characterising each species, ours included, are modelled and influenced by natural selection which has acted for innumerable generations. As natural selection improves fitness and reproduction capacity, a logical prediction is that individuals of a species should have the best fitness and reproduction capacity, with the exception of rare particular cases.

Yet, individuals of our species suffer from many diseases or other disabling conditions, and death is a common end to many of these conditions (Figure 1).

The incredible complexity and the amazing capacities of the eyes, the brain, the metabolic pathways and innumerable other characteristics of living beings are a marvellous fruit of natural selection, but diseases and other disabling conditions are a clear challenge for our confidence in the power of natural selection [Nesse and Williams 1994].

In short, is the evolutionary design of our species, although complex and admirable, a partial failure because we are afflicted by many imperfections and severe defects? Alternatively, are these imperfections and severe defects intrinsic to the evolutionary process?

This question is particularly important for the understanding of the causes of diseases that torment our species.

The problem is not a useless theoretical disquisition [Nesse 2008]: a rational answer to this question is the basis for the comprehension of the primary causes of diseases and similar conditions, for elaborating correct strategies to limit morbidity and mortality and for enhancing life span (mean duration of life) and longevity (the greatest duration of life).

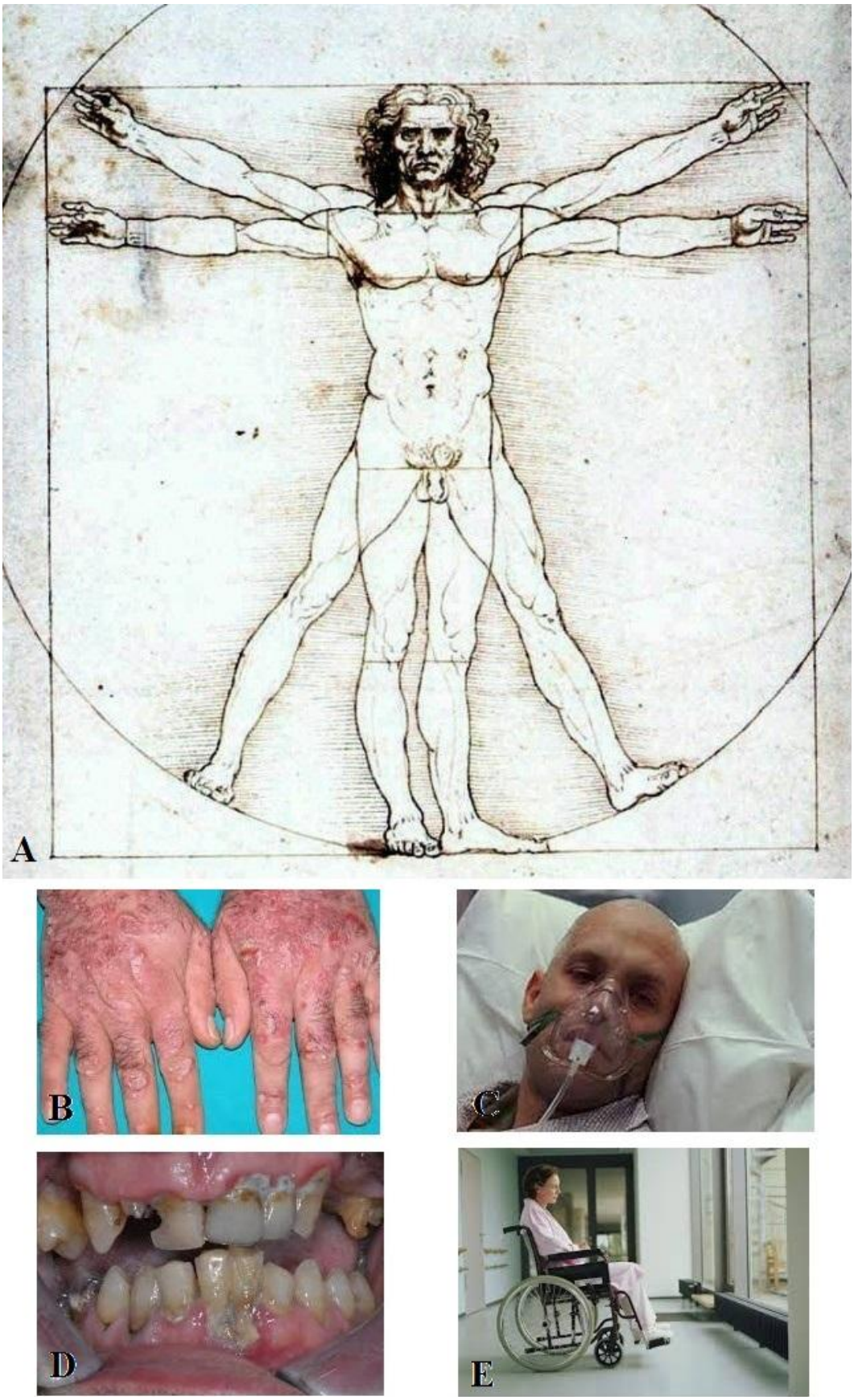


Figure 1 - A) Vitruvian man (Leonardo da Vinci). This image could be the symbol of natural selection that shapes an organism without inappropriate imperfections. B) to E) Images illustrating some of the many conditions afflicting our species and that seem to indicate the failure of natural selection.

Evolutionary Classification of Diseases and Other Phenomena Causing Suffering, Disability and Death

1. Diseases Caused by Alterations of the Genotype

The preservation of genetic information and its transfer from a generation to the next is imperfect, a fact that is fundamental for the whole evolutionary theory as without genetic diversity selection would be impossible. Genetic information rules a sophisticated program that determines organism development and functions, both very complicated phenomena. A random modification in a very complex structure is, as more probable event if not neutral, an alteration that is a cause of dysfunction (Figure 2). Natural selection acts against the spreading of harmful alterations. Therefore, it is predictable that in a species there will be many alterations of genetic information, each with a low frequency by effect of natural selection.

Figure 2 - A random modification in a complex structure is a probable cause of breakdown.

The equilibrium frequency between the onset of new cases of genetic alterations and their elimination by natural selection is easily calculable. If the harmful gene C is recessive, its equilibrium frequency (C_e) will be:

$$C_e = \sqrt{-v/s} = \sqrt{v/[s]} \quad (1)$$

where v = mutation rate from an inactive allele (C'); -s = damage caused by C (the value is negative as C is harmful); [s] = absolute value of s (for the calculation of the formulas 1-7, see Appendix).

Using Hardy-Weinberg formula (CC + 2 CC' + C'C' = 1), the equilibrium frequency of the phenotype expressing the disadvantageous condition (P_e) will be:

$$P_e = C_e^2 = v/[s] \quad (2)$$

If C is dominant, its equilibrium frequency will be:

$$C_e = \frac{2\,s + 2\,\sqrt{s^2 + 3\,s\,v}}{6\,s} = \frac{1 - \sqrt{1 - 3\,v/[s]}}{3} \approx 0.5\ v/[s] \qquad (3)$$

and the equilibrium frequency of the phenotype expressing the disadvantageous condition (P_e) will be:

$$P_e = C_e^2 + 2\,C_e\,(1-C_e) = 2\,C_e - C_e^2 = \frac{(1 - \sqrt{1 + 3\,v/s})\,(5 + \sqrt{1 + 3\,v/s})}{9} \approx v/[s] \qquad (4)$$

These equilibrium frequencies are illustrated in Figure 3.

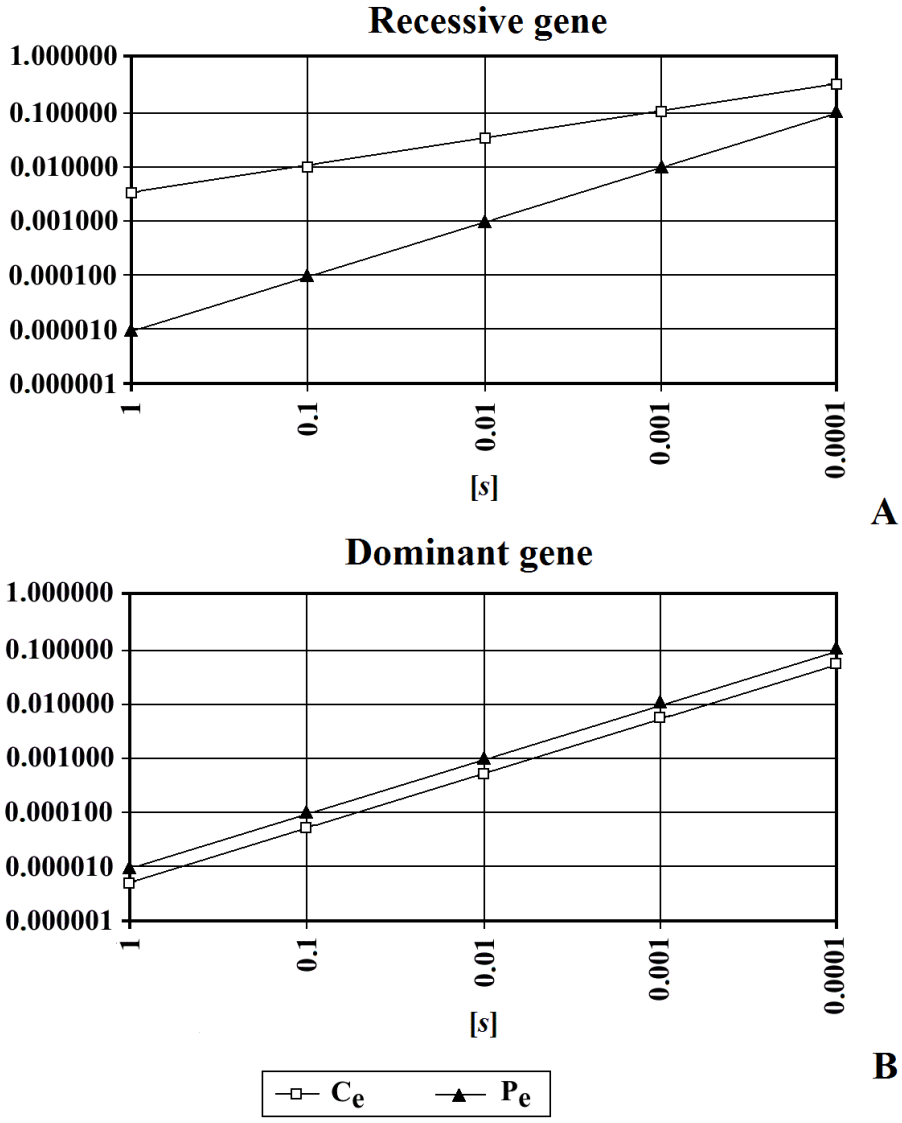


Figure 3 - A) Equilibrium gene frequency (C_e) and phenotypic frequency (P_e) for a recessive harmful gene. B) Equilibrium gene frequency (C_e) and phenotypic frequency (P_e) for a dominant harmful gene.

For chromosome alterations, v can be interpreted as the frequency of the onset of a chromosome alteration and its equilibrium frequency, which coincides with its phenotypic frequency, is:

$$P_e = C_e = v/[s] \quad (5)$$

as for a noxious gene in a haploid organism.

If n types of mutations, with a mean mutation rate v, can transform neutral alleles into C, the equilibrium phenotypic frequency of C will be:

$$P_e = n\,(v/[s]) \quad (6)$$

These formulas mean that with small values of v (e.g., $v < 0.00001$), if [s] is not very small, the predicted frequency of a disease (P_e) caused by particular alterations of the genotype is very small, that is harmful alleles are constantly and efficaciously removed by natural selection.

This is not true in two cases.

1) If the value of [s] is very small, e.g., in the case that the harmful expression of the gene is at ages when only a few individuals survive and their remaining expectation of life and therefore their reproductive value is minimal, P_e will be not small. This is an argument of the "mutation accumulation" theory of ageing (see later).

2) If C is disadvantageous in the homozygous state ($s < 0$) and advantageous in the heterozygote state ($s' > 0$), its equilibrium frequency will be:

$$C_e = -\frac{2s'}{s - 4s'} = \frac{2s'}{[s] + 4s'} \quad (7)$$

and it is easy to calculate the equilibrium frequencies of homozygous and heterozygote conditions (if $s > 0$ and $s' < 0$, $C_e=1$). The high frequency of some types of genetically determined anaemias (sickle cell anaemia, thalassaemia, G6PD deficiency, etc.), which are mild in the heterozygote state and deadly in the homozygous state, is explained by their advantage against malaria in the heterozygote state [Trevathan et al. 2008b].

However, disregarding these particular cases, the theoretical prediction is that in a species there will be many diseases caused by alterations of the genotype, each with a very low frequency (greater when many different mutations alter the same gene or the same metabolic pathway) but with an overall frequency not small.

2. Diseases Caused by Alterations of the Ecological Niche

A modification of the ecological conditions to which a species is adapted, is a change in a very complex and ordinate system. Therefore, a modification of the ecological niche will be, as more probable event if not neutral, a cause of physiological dysfunctions (Figure 4).

Evolution is a slow process. If a species has been for a long time (thousands of generations) in a particular ecological niche (climatic conditions, behavioural and nutritional habits, relations with other species, etc.), the species should be considered as well adapted.

If the ecological niche changes, the adaptation to the new conditions may require times very long for the human standards, e.g., thousands of generations, which means 20,000-30,000 years for each thousand of generations.

From the origins to about 10,000 years ago, our species lived in Palaeolithic conditions (Stone Age) and, presumably, was well adapted to this "ancestral condition". With the Neolithic revolution, agriculture and breeding modified strongly our ecological niche. Afterwards, the massive urbanisation, the huge increase in demographic density, technological innovations, the industrial revolution, etc., have caused even greater changes.


Figure 4 - A random modification in a very complex ordered sequence is a probable cause of disharmony.

Only a partial adaptation to the new conditions is documented or plausible. For example, adult Stone Age men were unable to digest fresh milk after being weaned and the greater part of modern men have the same inability except some populations in Europe, western India and sub-Saharan Africa that, having reared cattle from thousands of years, have acquired the capacity to digest fresh milk in adulthood [Stearns et al. 2008].

The radical modification of our conditions of life has strikingly worsened the mean health of modern men in comparison with populations living in Stone Age-like conditions (hunter-gatherers or foragers). Some years ago, there were a few remaining populations with these lifestyle (e.g., some Australian aborigines, Hadza in Tanzania, !Kung of Botswana, Ache of Paraguay, Efè of the Democratic Republic of the Congo, and Agta of the Philippines [Trevathan et al. 2008b]) and they showed almost no dental caries [Price 1939], hypertension, diabetes, obesity, cardiovascular affections, cancer, psychological and emotional ailment [Eaton, Konner and Shostak 1988], although more than 8% of the individuals exceeded 60 years of age [Blurton Jones et al. 2002].

Some examples of particular alterations of our ecological niche and the consequent diseases are listed in Table 1. A complete list with the discussion of the particular pathological mechanism for each disease would require a textbook.

Table 1

Alterations of the ecological niche -> Diseases
Excessive ingestion of salt -> hypertension [Eaton, Shostak and Konner 1988; Bragulat and de la Sierra 2002; Rodriguez-Iturbe et al. 2007] (-> heart hypertrophy, congestive heart failure, arrhythmia and sudden death [Morse et al. 2005]) (Figure 5)
Excessive time spent focusing close up or in improper conditions of vision -> myopia [Fredrick 2002] (up to 70–90% of a population affected [Chow et al. 1990; Wong et al. 2000]), refractive defects (myopia, astigmatism, hyperopia) [Kee and Deng 2008]
Excessive ingestion of unsaturated fats, caloric foods, meat with high fat content -> obesity (->renal cell carcinoma [Lipworth et al. 2006], heart hypertrophy, congestive heart failure, arrhythmia and sudden death [Morse et al. 2005]) (Figure 6), type 2-diabetes (Figure 7) and increased vascular risk (-> myocardial infarct, cerebral ischemia, infarcts in all the vascular districts, heart hypertrophy and failure, etc.) [Eaton, Shostak and Konner 1988]
Occupational noise, smoking, high Body Mass Index -> hearing loss [Fransen et al. 2008]
Excessive exposure to noise -> hearing loss [Eaton, Shostak and Konner 1988; Daniel 2007]
Smoking and/or air pollution -> chronic bronchitis [Viegi et al. 2006], emphysema [Taraseviciene-Stewart and Voelkel 2008]
Smoking -> coronary heart and other cardiovascular diseases, chronic respiratory diseases, pregnancy complications, and respiratory diseases in children [Giovino 2007], lung [Giovino 2007; Clavel 2007]/larynx [Clavel 2007; La Vecchia et al. 2008]/bladder [Clavel 2007; Janković and Radosavljević 2007]/kidney [Lipworth et al. 2006]/pancreas [Hart et al. 2008] carcinoma, peptic ulcer [Halter and Brignoli 1998; Parasher and Eastwood 2000]
Excessive ingestion of simple and refined carbohydrates (in particular sugar) and other dietary modifications -> dental caries, pyorrhoea, crowded teeth [Price 1939; Eaton, Shostak and Konner 1988] (Figure 8)
Scarce ingestion of fibre -> constipation, colon diverticulosis, colon carcinoma, stomach carcinoma, type 2-diabetes, metabolic syndrome and cardiovascular diseases [Trepel 2004], appendicitis [Arnbjörnsson 1983; Adamidis et al. 2000]
Scarce ingestion of calcium and reduced physical activity -> osteoporosis [Eaton, Shostak and Konner 1988; National Institutes of Health 2000], back pain [Eaton, Shostak and Konner 1988]
Reduced exposure to natural allergens in the childhood -> allergies [Janeway et al. 2001]
Exposure to chemical substances artificially synthesised -> allergic diseases [Kirchner 2002]
Altered conditions of sociality, stress of civilised condition -> mental and psychiatric disorders [Eaton, Shostak and Konner 1988; Nesse and Williams 1994]
Various factors -> increased incidence of various types of cancer [Eaton, Shostak and Konner 1988; Greaves 2000]
Alcoholism -> hepatic steatosis, steatohepatitis, cirrhosis [Adachi and Brenner 2005], larynx carcinoma [La Vecchia et al. 2008]

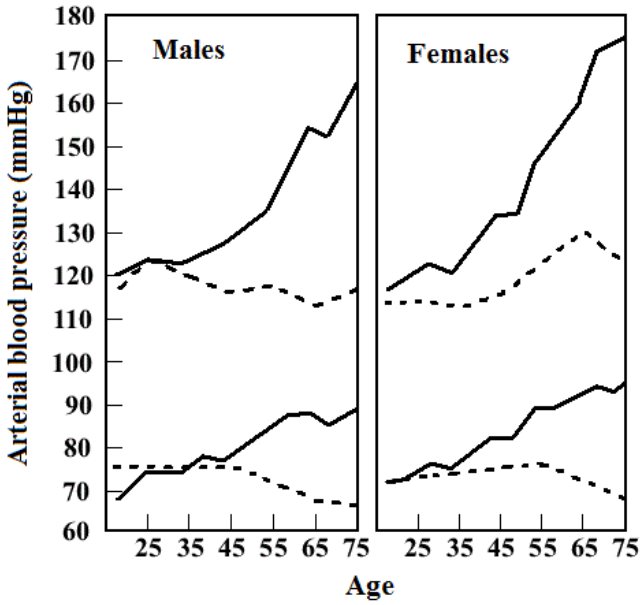


Figure 5 - Arterial blood pressures in !Kung individuals (dashed lines) and in London citizens (continuous lines). Figure from [Truswell et al. 1972], partially redrawn.

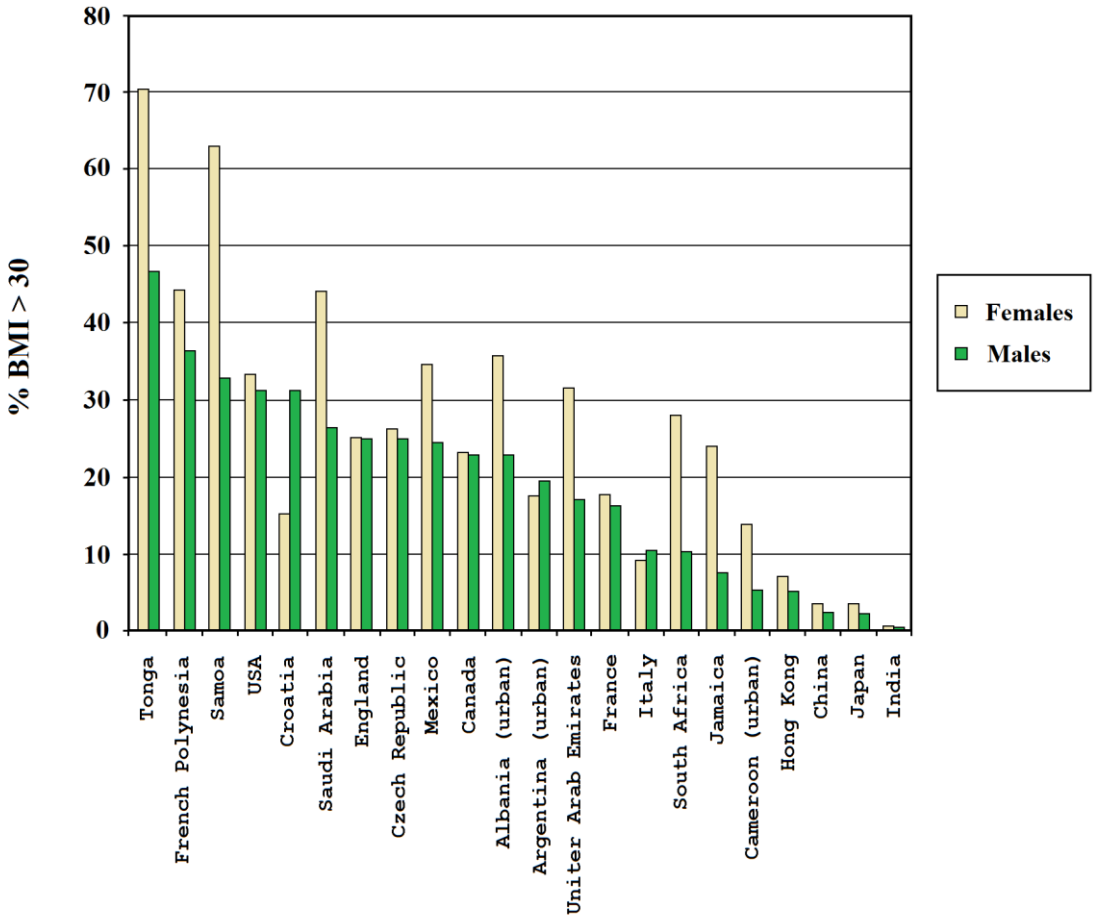


Figure 6 - Frequencies of Body Mass Index > 30 in some countries. Data from International Obesity Task Force (from late 1990s to 2002; http://www.iotf.org/database/documents/GlobalPrevalenceofAdultObesity July08pdfv2.pdf). Some years ago, for the few remaining hunter-gatherer populations, obesity was a rarity [Eaton, Konner and Shostak 1988].

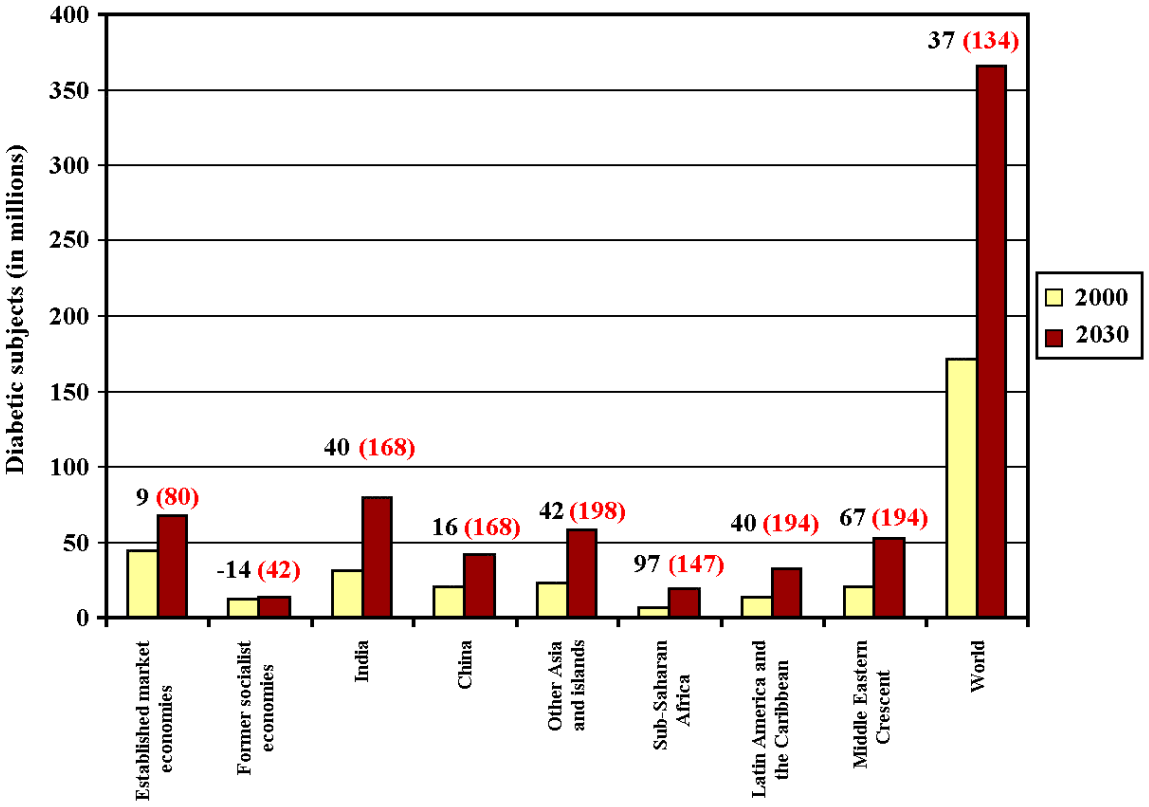


Figure 7 - Estimated numbers (in millions) of people with diabetes by region for 2000 and 2030. Percentage of change in total population and (between brackets) percentage of change in population >65 years of age are also indicated [Wild et al. 2004]. Diabetes was a rarity in the few remaining hunter-gatherer populations [Eaton, Konner and Shostak 1988].

Figure 8 - In the upper side: photos of indigenous and of skulls from people following ancestral dietary habits (“teeth ... excellent and free from dental caries”); in the lower side: photos of indigenous following modern diets (multiple dental caries, “crowding of the teeth”, “changes in facial form”, pyorrhoea) [Price 1939]. After about 70 years from publication, the evidence and the teaching of the extraordinary book of Price (called the “Charles Darwin of nutrition”) is still perfectly topical and could be a symbol of the damages caused by thoughtless changes of the ecological niche.

It is essential a distinction between “proximate” and “evolutionary” causes of a disease [Nesse and Williams 1994]. Evolutionary (or ultimate or primary) causes explain “why” diseases happen. Proximate (or near) causes explain “how” diseases manifest themselves.

Genes making an individual vulnerable to a disease in particular conditions are the "proximate" causes, while the "evolutionary" cause is that an individual is exposed to ecological conditions to which the species is not adapted. For example, a "normal" modern diet includes an intake of salt more than ten times that estimated to be ingested in prehistoric times or in modern hunter-gatherer societies. Our body is not adapted to this "normal" intake of salt and, after years of excessive use of salt, "hypertension-predisposing" genes cause hypertension. The true cause of hypertension is the abnormal excessive intake of salt to which the organism is not adapted (evolutionary cause) and not the existence of "hypertension-predisposing" genes (proximate cause). The "normality" of modern diet is correct in its statistical meaning (average intake of salt in a modern population) as it is correct to say that blindness is statistically "normal" in a community of blind men. It should be stressed that "normal" modern diet is largely abnormal in evolutionary terms and that "hypertension-predisposing" genes are normal genes that in the modern harmful conditions of life cause hypertension. The pathological condition is the modern "normal" diet and not "hypertension-predisposing" genes and the attention should be focused on the real causes and their possible correction and not on proximate causes (Figure 9).

A B

Figure 9 - A) The victims of the Holocaust numbered in the millions (Hebrews, Roms, gays, political dissidents, etc.). Misleading interpretation (proximate cause): this was caused by their race, religious creed, etc. Correct interpretation (primary cause): this was caused by an insane and murderous ideology. B) The victims of diabetes, hypertension, atherosclerosis and their complications number in the tens of millions. Misleading interpretation (proximate cause): this is caused by their diabetes-, hypertension-, atherosclerosis-predisposing genes. Correct interpretation (primary cause): this is caused by alterations of the ecological niche (too many calories and unsaturated fats, too much salt, etc.) to which the organism is not adapted.

The mismatch between the ecological niche to which our genes are well adapted and the actual habits is the true ("primary") cause of large part of our ills ("Discordance Hypothesis") [Eaton, Shostak and Konner 1988] (Figure 10).

3. Diseases Caused by Interactions with Other Species

As there is continuous competition among the species with conflictual evolutionary exigencies and, in particular, between an organism and its parasites (bacteria, viruses, fungi, protozoa, parasitic worms, etc.), a third general cause of disease is predictable.

The number of bacteria living in the gut of each man has been estimated to be ten times the number of his cells [Mullard 2008] and there are trillions of other bacteria living on our skin, mucosae and elsewhere on or in our body. Large part of these bacteria live with us without being harmful and often are useful, e.g., hindering settlement and attack of bacterial pathogenic species.

“The total set of over 1400 pathogen species breaks down into over 200 viruses, over 500 bacteria and rickettsia, over 300 fungi, over 50 protozoa, almost 300 helminths, and at least 2 kinds of prion” [Woolhouse and Antia 2008].

A

B

Figure 10 - A) Bushmen tribes (!Kung San, Botswana), some years ago one of the few remaining hunter-gatherer populations, had a lifestyle analogous to Stone Age societies. They were quite well adapted to their ancient ecological niche and diseases such as hypertension, diabetes, obesity, cardiovascular affections, cancer, psychological and emotional ailments, dental caries, myopia, astigmatism, etc., were rare events for them; B) Modern men. Their genes are practically the same of hunter-gatherer men but there is a noxious “discordance” between their habits and the ecological niche to which their genes are adapted: the above-mentioned affections, and many others, are the terrible consequence [Eaton, Shostak and Konner 1988].

In this category of diseases, natural selection acts both on the side of the host and on the side of its parasites. The result must be a compromise between the competing exigencies to survive and propagate of both host and parasites.

The relationship between an organism and its parasites is analogous to that between a prey and its predators, and in it we have a troublesome position analogous to that of a prey. However, it is predictable that, in a similar way to what happens in the prey-predators case [Wilson 1975], to minimise disadvantages and maximise advantages both for host and parasite, parasites will damage more very young, sick and old individuals, with low reproductive potentiality, and less intermediate ages and healthy individuals with greater reproductive potentiality.

4. Diseases Caused by Conditions beyond Adaptation Range

Conditions beyond the adaptation range of the species (e.g., the fall of a man from an excessive height, the trauma of a violent car accident, etc.) are a sure cause of physiological dysfunctions or death.

5. Disease-Like Phenomena Caused by Natural Selection

Natural selection may determine disease-like phenomena, which may be apparently harmful or which are surely harmful in terms of individual fitness.

For this category of phenomena, which cannot be defined as diseases, a subdivision is necessary on the grounds of the selective mechanisms involved.

5.1. Phenomena That Are Defences against Harmful Agents

Natural selection favours physiological mechanisms that are defensive against infections and other harmful agents or conditions (e.g., fever, cough, sneezes, itch, inflammatory phenomena, nociceptive pain, diarrhoea, iron deficiency, morning sickness, emotions such as anxiety, fear, etc.) [Williams and Nesse 1991].

In particular: 1) iron deficiency, especially in pregnancy, appears an effective defence against infectious diseases [Nesse and Williams 1994; Denic and Agarwal 2007] because: "Acquiring iron is a fundamental step in the development of a pathogen, and the complexity and redundancy of both host and pathogen mechanisms to acquire iron and control flux and availability illustrate the longstanding and ongoing battle for iron." [Doherty 2007]; 2) morning sickness of the pregnant woman protects the embryo from foods containing teratogen chemicals (e.g., natural toxic chemicals in vegetables) or potentially infected (e.g., meats, fish, poultry, and eggs) [Flaxman and Sherman 2000, 2008].

In certain cases, which must be considered as pathological noxious conditions of alteration of physiological and beneficial functions, the defensive mechanism is excessive or inappropriate or harmfully altered by a parasite to increase its propagation (e.g., diarrhoea in infections by *Vibrio cholerae* [Vanden Broeck et al. 2007]).

5.2. Phenomena Damaging Other Individuals Genetically Related but Improving Overall Fitness of Progeny

Vertebrate immune system must discriminate between antigens of each host individual and those of the parasites, which try to overcome immunologic defences by using for their coverings proteins with the same antigenicity of the host (antigen mimicry). The defence of the host against antigen mimicry is to have the greatest inter-individual variability of antigen formulas so that a mimicry adapt to infect all the potential hosts is impossible [Libertini 1983]. The major histocompatibility complex (MHC) is the main tool by which the host organism obtains an extraordinary antigen variability. Differences between antigenic formulas of host and parasite give greater resistance to the infection while similarities cause susceptibility. Correlations between resistance or susceptibility to several infectious or infection-related diseases and specific human MHC alleles are well documented [Lechler and Warrens 2000; Shiina et al. 2004].

The best progeny is that with the greater antigen variability. This may be obtained through MHC-mediated mate choice and with post-copulatory selection. The first phenomenon has been observed in several vertebrate taxa and is widespread in nature [Slev et al. 2006]. MHC genes influence human mating preferences. Women college students rated the odours of MHC-dissimilar men as being 'more pleasant' than those of MHC-similar men [Wedekind et al. 1995; Wedekind and Füri 1997]. In an isolate, ethnically homogenous community, significantly fewer couples was observed to match at a 16-locus MHC haplotype [Ober et al. 1997; Ober et al. 1999].

With the second phenomenon, also referred to as 'cryptic female choice' [Loisiel et al. 2008], miscarriage eliminates the production of offspring with lesser antigen variability having a future decreased fitness due to diminished disease potential resistance [Apanius et al. 1997]. For animals, post-copulatory selection is well documented [Tregenza and Wedell 2000]. For humans, in a study, an excess of MHC-heterozygotes was found in newborn males [Dorak et al. 2002]. A series of studies on an isolate and ethnically homogenous community have documented that couples with shared HLA-DR alleles in comparison with couples not sharing the same alleles have significantly less children [Ober and van der Ken 1997], a greater interval between pregnancies [Ober 1992] and a greater pregnancy loss rate [Ober et al. 1998].

In this sub-category, natural selection determines the death of healthy embryo individuals to optimise the survival potentiality of progeny. Infanticide or the abandonment of healthy new-born babies when the resources are insufficient are ancient and widespread behaviours [Scrimshaw 1984], apparently determined by analogous evolutionary necessities of not giving place to progeny with reduced survival possibilities and that could subtract precious resources to kin individuals [Eaton, Shostak and Konner 1988]. For animals, analogous behaviours are well known [Wilson 1975]. This sub-category indicates that natural selection may cause physiological events that could be interpreted as pathological or behaviours considered ethically unacceptable in our culture. This implicates that from an ethical point of view not all the effects of natural selection can be accepted uncritically or regarded in principle as not to be modified.

5.3. Phenomena Damaging the Individual but Favoured by Kin Selection

In the classic definition of natural selection, the variation of the frequency of a gene X between two generations (Δ_x) is depending on the advantage or disadvantage s caused by X, alias the variation of fitness, and of the reproductive value P of the individual in which X acts:

$$\Delta_x \propto s \cdot P \tag{8}$$

The definitions of inclusive fitness and kin selection have strongly modified this concept [Hamilton 1964, 1970; Trivers 1971; Wilson 1975; Trivers and Hare 1976]. If a gene X, present in the individual I_1, determines effects on I_1 and on other individuals I_2, I_3, ... I_n genetically related (kins) to I_1, with coefficients of relationship (probability of genes in common) equal to r_2, r_3, ... r_n, respectively, and with reproductive values equals to P_1, P_2, P_3, ... P_n, respectively, to evaluate the spreading or decay of X within the species, the effects on the fitnesses of all individuals involved must be considered:

$$\Delta_x \propto \Sigma (s_z \cdot P_z \cdot r_z) \tag{9}$$

with z varying from 1 to n.

Only if no other individual than I_1 is involved in the action of X, formula (9) is transformed in the classic formula (8), as $r_1 = 1$.

This conceptual revolution allowed a convincing explanation of the social organisation of ants and bees and of many other otherwise inexplicable phenomena [Wilson 1975].

Inclusive fitness and kin selection are indispensable to understand the phenomena illustrated in this sub-category.

5.3.1. Altruistic Actions

For animals, behaviours or actions that damage or kill the individuals expressing them but increase the survival probabilities of kin individuals are well documented [Wilson 1975], e.g., the defence from predators of a drove of yellow baboons (*Papio cynocephalus*) [Altman and Altman 1970] or of chacma baboons (*Papio ursinus*) [Hall 1960] by the predominant males with great individual risk.

Actions damaging an individual and favouring kin individuals do not necessitate a wilful choice or even the existence of a nervous system. For example, apoptosis is a form of cell death genetically determined and highly regulated, for the first time described as phenomenon other than necrosis in normal liver epatocytes [Kerr et al. 1972] and typical of eukaryotic organisms, even if monocellular [Fröhlich et al. 2007]. In the yeast (*Saccharomyces cerevisiae*), the scarcity of nutrients triggers the apoptosis of older individuals enhancing "the chances of the rest of the population to survive and to sporulate, thus increasing the probability that the clone will survive" [Büttner et al. 2006] and this is explained as "altruistic cell death" [Büttner et al. 2006], alias as an "altruistic behaviour" [Fröhlich and Madeo 2000] caused by kin selection.

For humans, behaviours and actions that reduce fitness or jeopardise health, and even life of individuals committing them, are well known and can be interpreted as altruistic behaviours determined by kin selection [Silk 1980; Rachlin and Jones 2008].

5.3.2. Ageing

For many species, ours included, an age-related fitness decline is well documented both in wild and in protected conditions [Deevey 1947; Laws 1966, 1968; Laws and Parker 1968; Spinage 1970, 1972; Finch 1990; Holmes and Austad 1995; Ricklefs 1998]. This fitness decline is illustrated by the age-related continuous decline of athletic performances (Figure 11), which is mirrored in the age-related mortality increase: "No one would consider a man in his thirties senile, yet, according to athletic records and life tables, senescence is rampant during this decade" [Williams 1957].

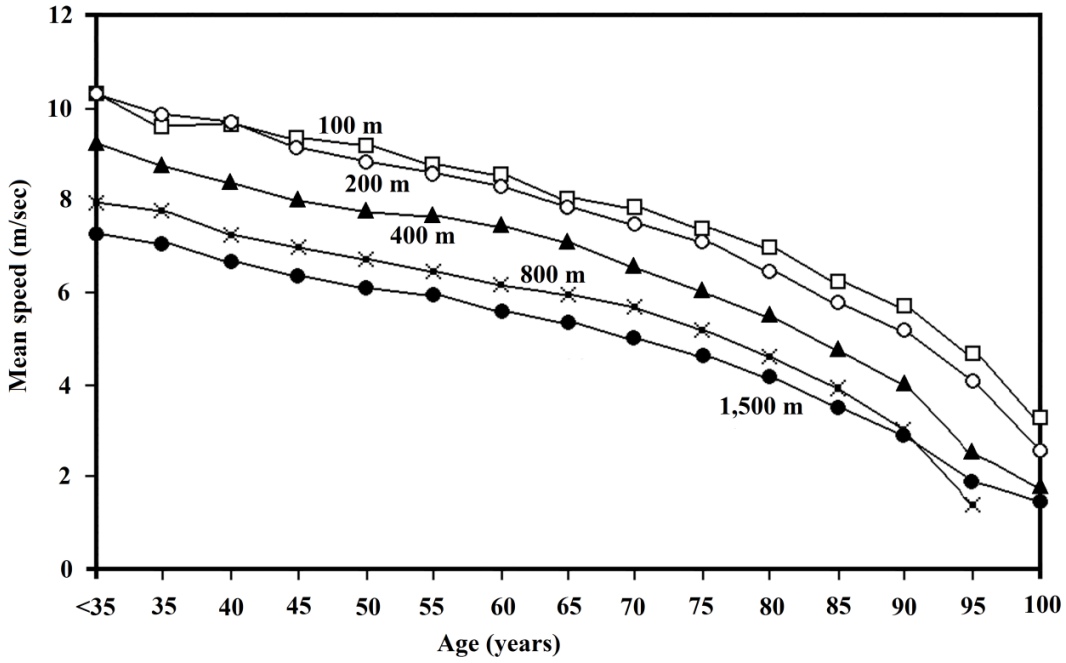


Figure 11 - Age-related fitness decline. Source of data: for age group < 35 (world records): [World records in athletics 2017]; for other age groups:[World records in athletics for age groups 2017].

In its more advanced manifestations, this phenomenon is universally known as ageing/senescence, but the use of these terms is scientifically tricky being often referred only to the more evident expressions of the fitness decline, rarely observable in the wild ("...there is scant evidence that senescence contributes significantly to mortality in the wild. ...As a rule wild animals simply do not live long enough to grow old" [Kirkwood and Austad 2000]). To avoid misunderstandings, other terms precisely describing the phenomenon as "increasing mortality with increasing chronological age in the wild" (IMICAW) [Libertini 1988] or "actuarial senescence in the wild" [Holmes and Austad 1995; Ricklefs 1998] or "age-related fitness decline in the wild", which do not refer to lower limits for the grade of fitness decline, are preferable.

A widespread opinion is that the age-related fitness decline in the wild is the result of insufficient selection at older ages against harmful mutations accumulated over evolutionary time (mutation accumulation theory) [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987; Partridge and Barton 1993].

Against this hypothesis, it has been demonstrated that even with a great number of noxious gene expressing their harmful action at ages with a few survivors, natural selection reduces greatly frequencies and effects of the noxious genes so that life table is scarcely modified by their action (Figure 12). The conclusion, till now not falsified, is that mutation accumulation theory is untenable as explanation of age-related fitness decline in the wild [Libertini 1983, 1988, 2006].

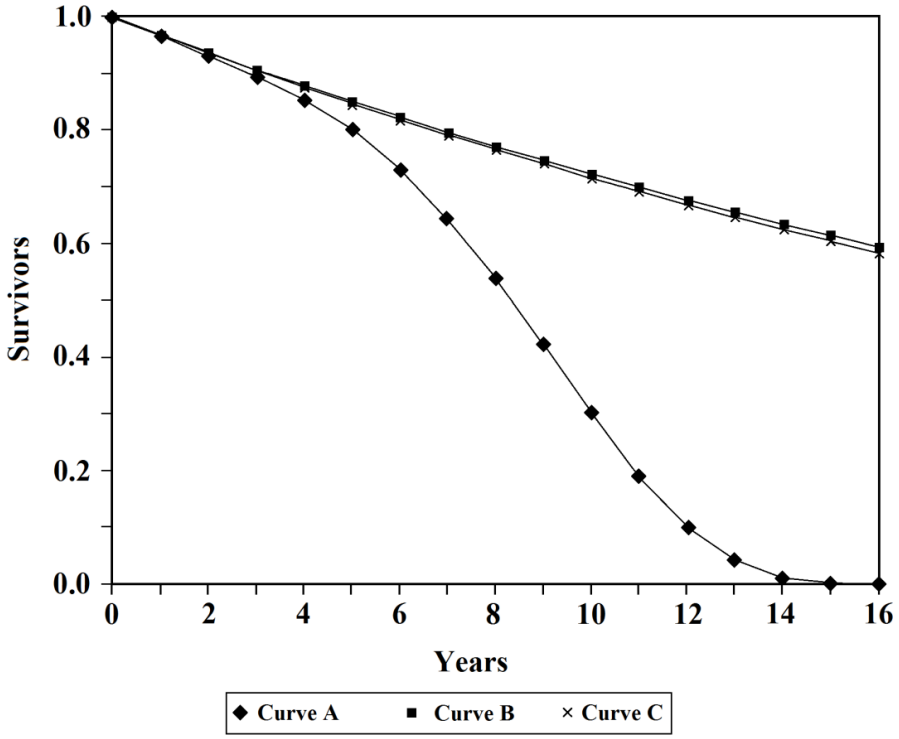


Figure 12 - Curve A is the life table in the wild of a real species showing an age-related fitness decline. Curve B is a hypothetical life table of the same species with only the extrinsic mortality at its lowest value and without the age-related fitness decline. Curve C is a hypothetical life table with the same mortality of curve B plus the effects of a great number (n = 500/year) of noxious genes acting at years t_1, t_2, ... Curve A is quite different from curve C and, therefore, it is unjustifiable as an effect of noxious genes insufficiently eliminated by natural selection [Libertini 2006].

To overcome the weakness of mutation accumulation theory, two new hypotheses were proposed. The first (antagonistic pleiotropy theory) suggested that fitness decline was determined by pleiotropic genes, beneficial at early ages and harmful at later ages [Williams 1957; Rose 1991].

The second (disposable soma theory) proposed that the causes of fitness decline were environmental or somatic and that at older ages natural selection was limited by physiological or environmental constraints, so that, in the subdivision of metabolic resources between reproduction and maintenance, reproduction was preferred [Kirkwood 1977; Kirkwood and Holliday 1979].

However, "Few plausible candidates for antagonistically pleiotropic genes have been recognized, and the physiological mechanisms connecting opposing early and late effects on fitness are not well characterized ..." [Ricklefs 1998] and there is no proof of trade-off between greater reproduction and lesser longevity for primate and humans [Le Bourg 2001]. In an authoritative paper [Kirkwood and Austad 2000], no example of trade-off is reported for animals that show age-related fitness decline in the wild (documentation of trade-offs for animals that show age-related fitness decline in artificial conditions is reported, but this is a different phenomenon not subjected to natural selection [Libertini 2006, 2008]).

The common view of these three theories is that they consider age-related fitness decline as a nonadaptive phenomenon. Current gerontological theories reply to "Darwin's dilemma" [Goldsmith 2003, 2006] (Is ageing nonadaptive and therefore a great example of failure of natural selection or adaptive by being somehow evolutionarily advantageous?) maintaining that natural selection fails to make individuals very long-lived or not showing age-related fitness decline.

In clear contrast with this idea, age-related fitness decline has been explained as evolutionary advantageous in terms of kin selection in particular ecological conditions [Libertini 1983, 1988, 2006]. Afterwards, in accordance with the theoretical arguments but independently of them, empirical data in support of an adaptive meaning of fitness decline and against hypotheses interpreting age-related fitness decline as nonadaptive have been presented [Libertini 2008].

Indeed, only the heretical idea that age-related fitness decline is adaptive, hinted by various authors [Weismann 1884; Libertini 1988, 2006; Skulachev 1997; Longo et al. 2005], allows to justify:

1) the existence of species with no age-related fitness decline in the wild. The individuals of many species survive in the wild till remarkable ages showing no detectable fitness decline (e.g., sturgeon, rockfish, turtles, bivalve mollusks, certain perennial trees, etc.; "animals with negligible senescence" [Finch 1990]). For this phenomenon, truly strange for nonadaptive theories [Finch and Austad 2001], particular variants of disposable soma theory have been developed [Kirkwood 1977; Kirkwood and Holliday 1979], even to justify the case in which mortality rate decreases at greater ages [Vaupel et al. 2004]. However, these variants have the taste of adaptations *ad hoc* to justify data contrasting with theoretical predictions.

2) the inverse relation of extrinsic and intrinsic mortality documented for some bird and mammal species in the wild [Ricklefs 1998]. Current nonadaptive theories predict explicitly a direct relation and Ricklefs states clearly in his discussion that this prediction is confuted by empirical data [Ricklefs 1998]. On the contrary, adaptive theory predicts the inverse relation observed [Libertini 1983, 1988, 2006, 2008].

3) the existence of sophisticated mechanisms, genetically determined and regulated, progressively limiting cell turnover and cell functionality. The telomere-telomerase system limits cell duplication capacities (replicative senescence) and, consequently, cell turnover and, moreover, causes a progressive decay of cell functionality (cell senescence) [Fossel 2004]. These mechanisms, genetically modulated and determined, which are a plausible cause of the progressive fitness decline [Fossel 2004; Libertini 2006], are not explained by nonadaptive hypothesis while are necessary for the validity of the adaptive hypothesis [Libertini 2008]. In particular, nonadaptive hypothesis tries to explain replicative senescence and cell senescence as a defence against malignant neoplasia [Campisi 1997; Wright and Shay 2005], that is a terrible evolutionary trade-off between ageing and defence against cancer [Campisi 2000], but: a) old individuals of "animals with negligible

senescence" such as rainbow trout and lobster show in the wild the same telomerase activity of young individuals [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998] and no increase in cancer vulnerability, as their stable mortality rates prove; b) replicative senescence and cell senescence weaken the efficiency of immune system [Fossel 2004], a factor inversely related to cancer vulnerability and incidence [Rosen 1985]; c) shortened telomeres increases cancer probabilities because of dysfunctional telomere-induced instability [DePinho 2000; Artandi 2002]. Moreover, replicative senescence and cell senescence, although not caused by telomere shortening but by another unknown mechanism related to the number of duplications, are well documented in eukaryotic species such as yeast [Jazwinski 1993; Fabrizio and Longo 2007; Laun et al. 2007], which being unicellular species cannot be affected by cancer. However, these phenomena and others strictly associated [Laun et al. 2001; Kaeberlein et al. 2007] observed in yeast have been interpreted as adaptive [Skulachev 2002a, 2003; Herker et al. 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf 2006] and they are consistent with the explanation that they determine a greater evolution rate and are favoured in conditions of K-selection [Libertini 1988].

Telomere-Telomerase System

It is known for many years that, in general, normal cells have a limited capacity of duplication (Hayflick limit) *in vitro* [Hayflick and Moorhead 1961; Hayflick 1965] and *in vivo* [Schneider and Mitsui 1976], documented for many types of cells [Rheinwald and Green 1975; Bierman 1978; Tassin et al. 1979], related to the life span of the species from which cells are derived [Röhme 1981], inversely related to the ages of donors of origin [Martin et al. 1970] and caused by something acting in the nucleus [Wright and Hayflick 1975].

The cause of the Hayflick limit was hypothesised to be the progressive shortening of the DNA molecule at each duplication [Olovnikov 1973], as DNA polymerase cannot replicate a whole molecule of DNA and a little terminal portion of DNA would be ignored in replication [Olovnikov 1971; Watson 1972].

One of the ends of DNA molecule (telomere) is constituted by a repetitive sequence, shown to be TTGGGG in a protozoan [Blackburn and Gall 1978], and later for mammals, man included, to be only a little different (TTAGGG) [Moyzis et al. 1988] but common to many other species [Blackburn 1991]. As hypothesised, telomere was proved to shorten at each duplication [Harley et al. 1990].

An enzyme, telomerase, capable to elongate telomere at each duplication, annulling DNA polymerase insufficiency, explained the existence of cells with unlimited duplication capacities, such as germ line cells [Greider and Blackburn 1985]. It was shown that telomerase is present in immortal human cell lines [Morin 1989] and repressed by regulatory proteins [van Steensel and de Lange 1997]. Its deactivation causes telomere shortening at each replication and the reduction of duplication capacity [Yu et al. 1990], while with its activation telomeres resulted elongated and cells acquired unlimited duplication capacities [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

The final blockage of cell duplications (replicative senescence) is not an abrupt phenomenon but a progressive increase in the probability of blockage depending on telomere residual length [Pontèn et al. 1983; Jones et al. 1985]. Telomere is capped by particular protective nucleoproteins and oscillates between capped and uncapped states, with the duration of the capped state in direct relation to telomere length and with vulnerability for the passage to "noncycling state" (replicative senescence) in the uncapped state [Blackburn 2000].

As stem cells, unlike germ cells, have levels of telomerase activity capable to restore only partially telomere length [Holt et al. 1996], *in vivo* stem cells even with partially shortened telomere, that is with a slight probability to pass to replicative senescence, could not duplicate unlimitedly [Fossel 2004].

The modulation of the telomere-telomerase function is likely different for each species [Fossel 2004] and this could explain why species with long telomeres [Slijepcevic and Hande 1999] age precociously.

In correlation with telomere shortening, the overall cell functionality declines (cell senescence). This decay, as replicative senescence, is surely in correlation with the relative shortening of telomere (Fossel's "cell senescence limited model") [Fossel 2004]. In particular, experiments provoking telomerase activation reverse both replicative senescence and cell senescence [Bodnar et al. 1998; Counter et al. 1998; de Lange and Jacks 1999]. The mechanism of cell senescence is likely a progressive repression of a subtelomeric DNA portion (transcriptional silencing), which regulates the overall cell functionality, caused by the progressive sliding of the protective nucleoproteins ("heterochromatin 'hood' ") of probable fixed length capping telomere and adjacent DNA in correlation with telomere shortening [Fossel 2004] (Figure 13).

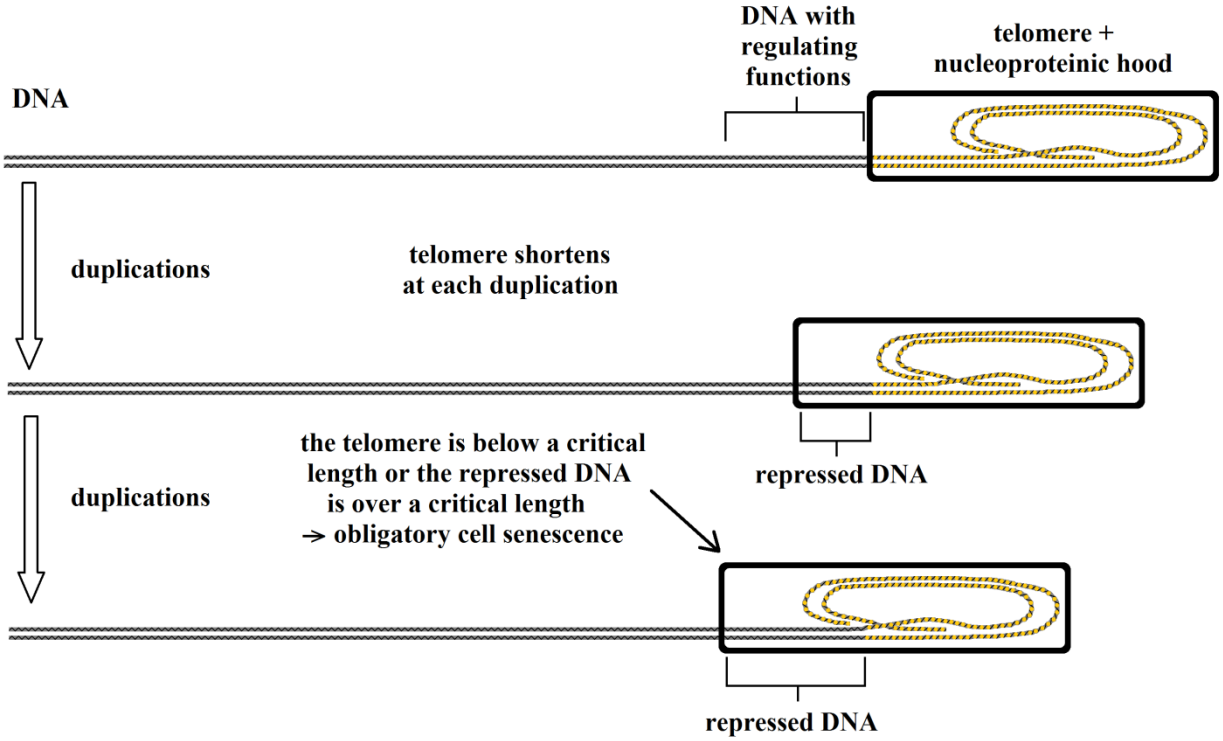


Figure 13 - Telomere progressive shortening impairs the expression of many genes. It is likely the existence near to the telomere of a tract of DNA regulating overall cell functionality: with telomere shortening the nucleoproteinic "hood" capping the telomere slides down and alters this regulation.

The placing of a portion of DNA with essential regulatory activities in a position progressively impaired by telomere shortening is a strong element in support of the hypothesis that telomere-telomerase limits are adaptive.

In short, the limitation of cell duplication capacities and its modulation appear clearly to be not caused by insuperable physiological constraints but determined and regulated by genes specifically favoured by natural selection as adaptive.

Cell Turnover

Our body is composed of cells in continuous turnover with rates different for each cell type. It has been estimated that each year a mass of cell equal to our entire body weight is lost and substituted [Reed 1999]. Even for some types of cells considered perennial, there is now evidence that they are subject to turnover (heart myocytes [Anversa and Nadal-Ginard 2002], muscle

myocytes [Schultz and Lipton 1982; Carlson and Faulkner 1989; Adams et al. 2001]). For other types of cells surely perennial, there is dependence on other cells with turnover, such as for the neurons on the gliocytes [Fossel 2004].

In normal conditions, cell elimination is the result of various forms of "programmed cell death" (PCD), such as removal by macrophages (red cells), keratinization and detaching from the somatic surface (skin) and apoptosis. In particular, apoptosis, an ordinate process of self-destruction with non-damaging disposal of cellular debris, was described for the first time as a phenomenon different from necrosis in a normal liver [Kerr et al. 1972], is related to cell turnover in healthy adult organs [Wyllie et al. 1980; Lynch et al. 1986; Medh and Thompson 2000] and is documented for many healthy tissues and organs [Pontèn et al. 1983; Benedetti et al. 1988; Dremier et al. 1994; Finegood et al. 1995; Migheli et al. 1997; Prins and O'Rahilly 1997; Spelsberg et al. 1999; Cardani and Zavanella 2000; Harada et al. 2000; Hèraud et al. 2000; Pollack and Leeuwenburgh 2001; Sutherland et al. 2001; Xia et al. 2001].

The continuous elimination of cells by PCD must be balanced with the replication of appropriate stem cells and this cell turnover is limited by the genetic regulation of the telomere-telomerase system.

In short, for vertebrates but not for all animals (e.g., the adult stage of *Caenhorabditis elegans* has a fixed number of cells), three categories of cells are currently distinguished:

1) Those with high turnover: e.g., intestinal crypts cells [Andreeff et al. 2000];

2) Those with moderate turnover: e.g., cells of the deep layers of skin and endothelial cells [Marciniak and Guarente 2001], heart myocytes [Anversa and Nadal-Ginard 2002], muscle myocytes [Schultz and Lipton 1982; Carlson and Faulkner 1989; Adams et al. 2001].

3) Those with no turnover, e.g., neurons, with a few possible exceptions [Horner and Gage 2000] but always metabolically depending on gliocytes that are cells with turnover [Fossel 2004].

Atrophic Syndrome

The progressive shortening of telomeres, if we accept Fossel's cell senescence limited model [Fossel 2004], causes an "atrophic syndrome" characterised by:

a) increasing number of cells in replicative senescence (overall reduction of cell duplication capacities);

b) slowdown of the cell turnover;

c) reduction of the overall number of cells (atrophy);

d) hypertrophy of the remaining specific cells;

e) possible substitution of the missing cells with nonspecific cells;

f) increasing number of cell with altered functions (cell senescence);

g) dysfunctional telomere-induced instability with consequent vulnerability to cancer [DePinho 2000].

A General Scheme for Ageing

It is easy to infer that cell turnover limitations caused by the telomere-telomerase system and linked or derived phenomena (cell senescence, atrophic syndrome, etc.) cause all the morphological and functional alterations that determine the fitness decline and, in their more advanced expression, the senile state (Fossel's cell senescence general model of ageing [Fossel 2004; Libertini 2006]) (Figure 14).

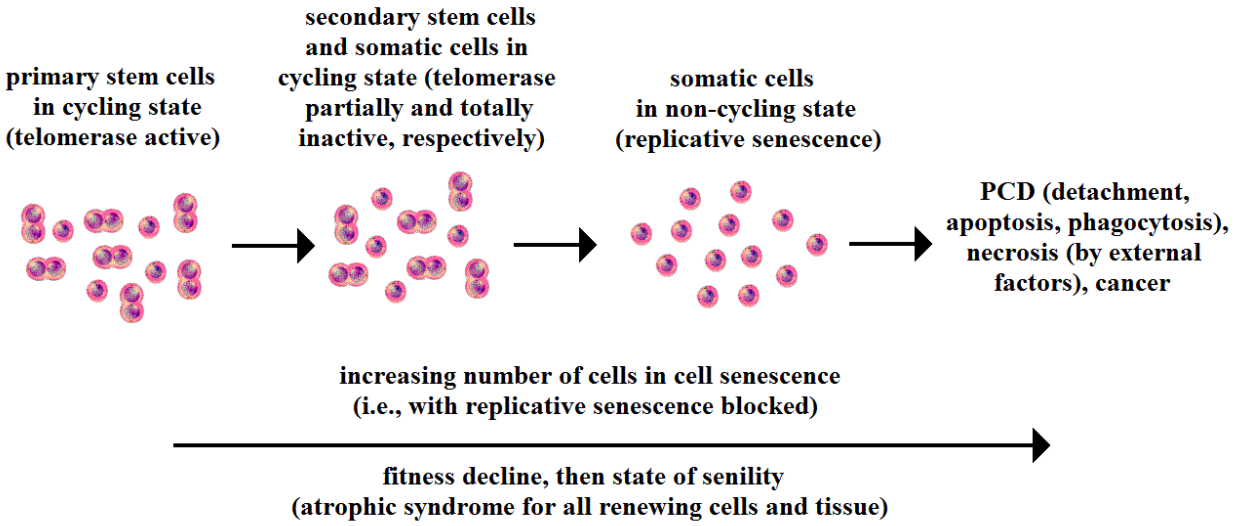


Figure 14 - From primary stem cells with active telomerase, secondary stem cells and somatic cells with replicative capacity originate, but with telomerase partially and totally inactivated, respectively. From both types of cells, somatic cells in replicative senescence originate. Replicative senescence and cell senescence contribute to fitness decline that gradually becomes the senile state.

In support of this hypothesis:

1) for mice, a factor inducing apoptosis and cell cycle arrest, provokes osteoporosis, a diminished stress tolerance, atrophy of all organs and a reduced longevity [Tyner et al. 2002];

2) in a rare human genetic disease (dyskeratosis congenita [Dokal 2000]), in which telomerase activity is low and telomeres are shorter than normal [Mitchell et al. 1999], tissues in which cells multiply rapidly (skin, nails, hair, gut and bone marrow, etc.) manifest precociously severe dysfunctions (alopecia, nail dystrophy, gut disorders, failure to produce blood cells, etc.) [Marciniak and Guarente 2001]. In this syndrome there is also a high cancer rate due to telomerase deficiency that cause unstable chromosomes [de Lange and Jacks 1999; Artandi et al. 2000].

3) in another genetic disease, Werner syndrome, in which cell replication is impaired [Fukuchi et al. 1989; Yu et al. 1996] and there is a limited replication capacity [Martin et al. 1970], tissues composed of cell with moderate turnover suffer from severe alterations (e.g., alterations in lens epithelial cells, endothelial cells, Langherans β-cells, various types of derma cells provoke cataracts, atherosclerosis, type 2-diabetes, regional atrophy of subcutaneous tissue and skin atrophy, respectively) [Martin and Oshima 2000].

Examples of Normally Ageing Tissues

Intestinal Villi

In each intestinal crypt, there are four to six stem cells that with their intensive duplication activity renew continuously the epithelium of the small intestine [Barker et al. 2007]. In healthy old individuals, in comparison with young individuals the transit time for cells from crypts to villous tips increases and villi become broader, shorter and with less cellularity [Webster 1978] (Figure 15). These changes, surely due to a declining mitotic activity of crypt stem cells, as hypothesised from a long time [Webster 1978], reduce intestinal functionality and, likely, overall fitness.

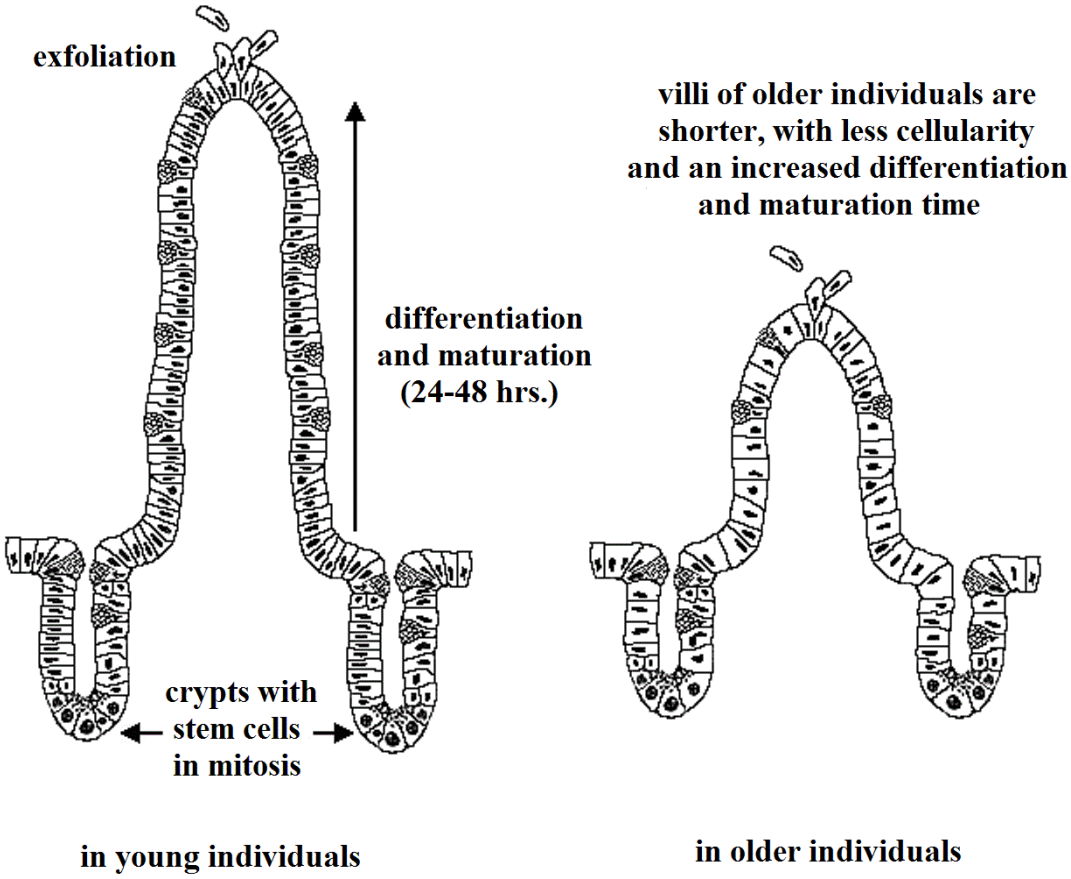


Figure 15 - Intestinal villi in young and older individuals.

Endothelium

Endothelial cells manifest a continuous turnover assured by endothelial progenitor cells, derived by primary stem cells of bone marrow, and numerically in inverse relation with age [Hill et al. 2003]. A slackened turnover of endothelial cells increases the probability of endothelial dysfunction and, therefore, of diseases derived from altered blood circulation such as myocardial infarction and cerebral ischemia: indeed, the number of endothelial progenitor cells is a predictor of cardiovascular risk equal to or more significant than Framinghan risk score [Hill et al. 2003; Werner et al. 2005]. Diseases derived from compromised blood circulation are a common end to the life of healthy old individuals with no particular risk factor [Tallis et al. 1998].

Epidermis

Human epidermis turnover is determined by stem cells located in the dermal-epidermal junction, a corrugated surface. In old subjects, dermal-epidermal junction is flattened, an indirect sign of the reduction of epidermis stem cells, and the rate of epidermal renewal is reduced [Griffiths 1998]. In derma, as a likely consequence of the exhaustion of specific stem cells, a general reduction of all its components (melanocytes, Langerhans cells, dermal fibroblasts, capillaries, blood vessels within the reticular dermis, mast cells, eccrine glands, hair. etc.) is reported and nails grow more slowly [Griffiths 1998].

Photoreceptor Cells

Photoreceptor cells (cones and rods) are highly differentiated nervous cells with no turnover, but metabolically depending from other cells with turnover, retina pigmented cells, which are highly differentiated gliocytes. Each day, with an extraordinary metabolic activity, every retina pigmented

cell phagocytizes about 10% of the membranes with photopsin molecules of about 50 photoreceptor cells. With the age-related decline of retina pigmented cell turnover, the deficiency of their function kills the photoreceptors not served. This is above all manifested in the functionality of the more sensitive part of the retina, the macula, from which the name "age-related retina macular degeneration" (AMD) [Fine et al. 2000]. AMD affects 5%, 10% and 20% of subjects 60, 70 and 80 years old, respectively [Berger et al. 1999], and it is likely that a large proportion of older individuals suffer from AMD.

Neurons

Neurons are perennial cells but their vitality depends on other cells (e.g., microglia, a type of gliocytes) that show turnover. The hypothesis that Alzheimer Disease (AD) is caused by replicative senescence and cell senescence of microglia cells has been proposed [Fossel 1996, 2004].

Microglia cells degrade β-amyloid protein [Qiu et al. 1998; Vekrellis et al. 2000] and this function is known to be altered in AD [Bertram et al. 2000] with the consequent noxious accumulation of the protein.

Telomeres have been shown to be significantly shorter in patients with probable AD than in apparently healthy control subjects [von Zglinicki et al. 2000]. AD could have, at least partially, a vascular aetiology due to age-related endothelial dysfunction [Fossel 2004] but "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." [Fossel 2004]

An interesting comparison between AD and AMD is possible: both are probably determined by the death of cells with no turnover as a likely consequence of the age-related failure of cells with turnover (Figure 16). Moreover, AD frequency, as AMD, affects 1,5% of USA and Europe population at age 65 years and 30% at 80 [Gorelick 2004] and a centenarian has a high probability of suffering from it.

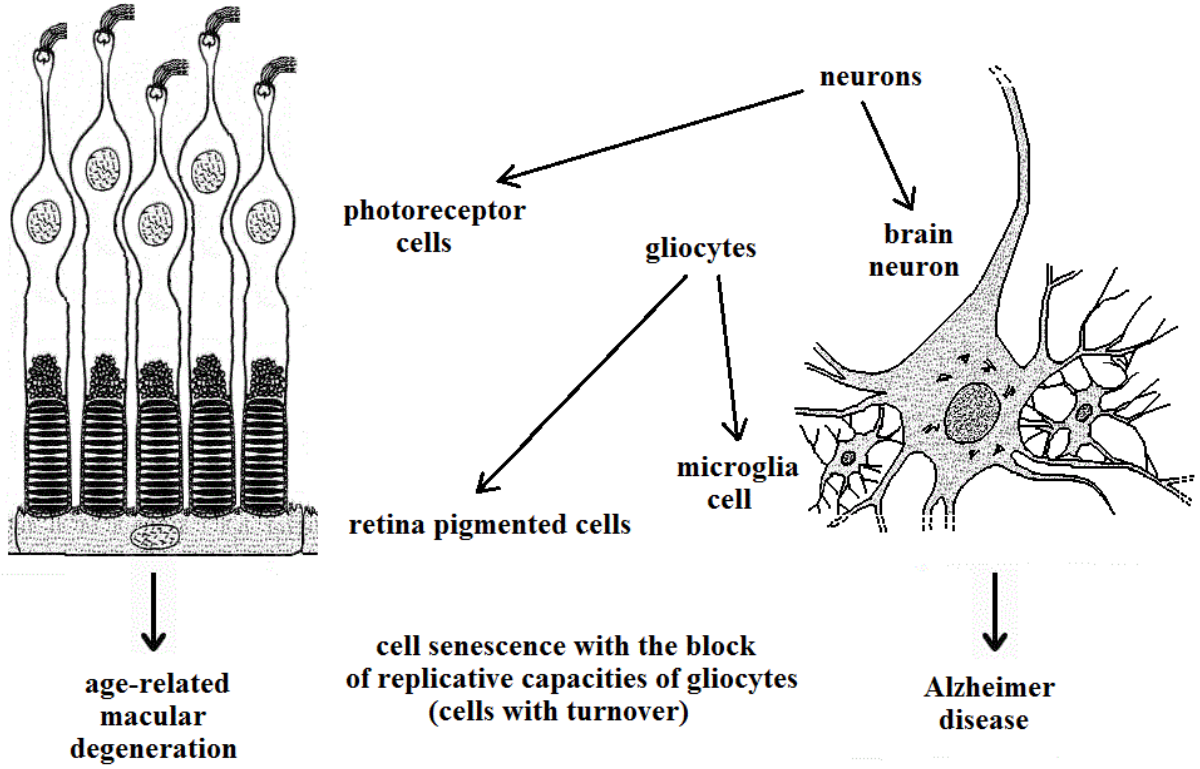


Figure 16 - Schemes of retina photoreceptors and of a brain neuron (both neurons) served by two types of differentiated gliocytes, retina pigmented cells and microglia cells, respectively. Gradual cell senescence and cell senescence of retina pigmented cells and of microglia cells cause age-related macular degeneration and Alzheimer disease, respectively.

Other Organs/Tissues

In relation to the progressive failure of cell turnover, senescence in healthy subjects is also characterised by age-related: bone loss (-> osteoporosis), muscle atrophy (-> sarcopenia), reduction in the number and in the size of nephrons with consequent decline of renal function (-> renal insufficiency), atrophy of pulmonary alveoli (-> latent/manifest emphysema), decline of liver volume with increased size of the remaining epatocytes (-> latent/manifest hepatic insufficiency), loss of myocytes with hypertrophy of the remaining myocytes and enlargement of cardiac cavities (-> cardiac failure), reduction in the number of pacemaker cells in the sinus node (-> atrioventricular block and other arrhythmias), decline in the number and activity of pancreatic β-cells (-> latent/manifest diabetes mellitus), atrophy of gastric mucosa (-> atrophic gastritis), declining activity of lens epithelial cells (-> nuclear cataracts), atrophy of large intestine, atrophy of salivary glands, decrease of taste buds, thinning of the lingual epithelial, involution of red marrow with increasing decline of the number and activity of cells with hematological and immunological functions, etc. [Tallis et al. 1998].

Moreover, telomere dysfunction in cells in replicative senescence, in particular those, mostly epithelial, with higher turnover, is a significant cause of cancer in older individuals [DePinho 2000].

Finally, we must consider the numberless complications for many organs deriving by the progressive impairment of endothelial, neuronal and immunological functions and, in general, by the interlacement of the decline of several functions [Tallis et al. 1998].

Ageing in short

The empirical evidence shows that an ageing individual suffers from a generalised atrophic syndrome and that death will be caused by the critical failure of one or several impaired functions. The atrophy of each tissue or organ is explained by the decline in cell turnover (Fossel's cell senescence general model of ageing [Fossel 2004; Libertini 2006]), which is caused by the limits of the telomere-telomerase system (Fossel's "cell senescence limited model" [Fossel 2004]).

The ageing phenomenon is therefore caused by limits genetically determined and regulated in a complex system [Libertini 2006] and these limits can be evolutionarily justified only accepting an adaptive meaning for fitness decline [Goldsmith 2003, 2006; Libertini 2006, 2008].

This conception is radically different from that currently accepted, which maintains:

1) *Ageing does not describe a distinct entity and is only an useful term to describe the numberless afflictions of the old age*. In fact, in the International Classification of Diseases-9-CM [ICD-9-CM 2016], which has code numbers for each disease or physiological event needing medical advice (e.g., pregnancy, delivery, etc.), there is not a code for ageing/senescence but only a code for senility/old age (797) among "Symptoms, signs and ill-defined conditions". Likewise, in the ICD-10 [ICD-10 2016] there is only a code for senility/old age (R54) in the chapter XVIII, titled "Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (R00-R99)", paragraph "General symptoms and signs (R50-R69)", and therefore not including distinct diseases or physiological events. In fact, for current medicine and in official classifications, ageing as a distinct entity is nonexistent and old individuals never die by ageing but, in general, by one or more diseases typical of old age.

2) *Old individuals have tissues and organs that have been progressively impaired by a lot of different damaging factors, in particular oxidizing agents*. This thesis appears to ignore cell turnover and the experiments where cells in replicative senescence and with all the manifestations of cell senescence are transformed by telomerase activation into cells with unlimited replication capacities and without signs of cell senescence [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999]. "... there is something fundamental

controlling the occurrence or accumulation of cellular free radical damage, something controlling the balance between damage and homeostasis. Free radical damage accumulates in somatic cells, but homeostasis is a sufficient match in germ cell lines. Alteration in gene expression, modulated by telomere length, is a likely candidate for such control." [Fossel 2004] Oxidative damage becomes a problem when cell turnover slackens and cell senescence increases: limits in the telomere-telomerase system are the primary cause and oxidative damage only a consequence [Fossel 2004].

3) *Ageing, being the consequence of numberless and random factors, is in principle not likely controllable and strong efforts could obtain only a slowing down of an inevitable process.* This is contrast with the evidence of the telomere-telomerase system, cell turnover, atrophic syndrome caused by cell turnover decline, and experiments documenting that at cell level senescence is totally reversible and avoidable [Fossel 2004].

4) *Ageing is something intrinsic to the condition of the living being, in particular of multicellular organisms, and therefore it is inevitable.* This is in plain contrast with the existence of many species that in the wild show no increase in mortality (e.g., rockfish, sturgeon, turtles, bivalves, etc. [Finch and Austad 2001]) and defined, if with considerable life spans, "animals with negligible senescence" [Finch 1990] or "ageless animals" (http://www.agelessanimals.org/). There are even species with mortality decreasing with age, e.g., depending on an increasing body size [Vaupel et al. 2004]. On the contrary, individuals of some unicellular eukaryotic species age, that is the telomere-telomerase system allows only a limited number of duplications (replicative senescence) and, in relation to the previous number of duplications, causes a decline in the overall functionality of the cell (cell senescence) with an increasing sensibility to apoptotic stimuli [Jazwinski 1993; Fabrizio and Longo 2007; Laun et al. 2007].

5) *Age-related increasing mortality, alias fitness decline, cannot be adaptive because natural selection favours individuals with greater fitness.* Natural selection favours genes with positive inclusive fitness, even with negative individual fitness [Hamilton 1964, 1970; Trivers 1971; Wilson 1975; Trivers and Hare 1976], and, therefore, in principle a gene causing age-related fitness decline, that is negative individual fitness, could be favoured by natural selection in particular ecological conditions [Libertini 2006]. The existence, in the wild, both of species with fitness decline and of species without fitness decline are a demonstration that unavoidable universal senescence-causing factors are unlikely and that both cases are somehow adaptive. It is remarkable that replicative senescence and cell senescence in yeast are weighed as adaptive [Skulachev 2002a, 2003; Herker et al. 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf 2006] and the logical consequence would be to accept as possible that the effects of the telomere-telomerase system in multicellular organisms are adaptive too.

Weight of Ageing for Life Span and Longevity

The effects of fitness decline on life span (mean duration of life, ML) and longevity are huge and often underestimated. For a species with a fixed mortality rate (λ), survivors at time t (Y_t) are given by the formula:

$$Y_t = Y_0 (1 - \lambda)^t \qquad (10)$$

With $Y_0 = 1$, life span (ML) is:

$$ML = {}_{t=0}[(1 - \lambda)^t]^{t=h} = - \frac{1}{\mathrm{Log}_e(1 - \lambda)} \qquad (11)$$

and the time t when Y_t individuals survive is:

$$t = \frac{\mathrm{Log}(Y_t)}{\mathrm{Log}(1 - \lambda)} \quad (12)$$

In the USA, 2005 statistics say that for the total population the probability of dying between ages 20 to 25 and between ages 25 to 30 were 0.004869 and 0.004865, respectively, which is about 0.00097/year [National vital statistics reports 2008]. With $\lambda = 0.00097$, without the age-related mortality increase the life expectancy of 20-30 years old individuals ($ML_{20\text{-}30}$) would be about 1,030 years and 1% would be alive after 4,745 years.

Excluding accidents (unintentional injuries) and homicides, which caused in 2005 about half of the deaths for 20-30 years old individuals [National vital statistics reports 2008], that is roughly halving the value of λ to 0.0005, $ML_{20\text{-}30}$ would be about 1,999 years and 1% would be alive after 9,208 years!

The same statistics say that the probability of dying between ages 55 to 60 was 0.036299, which is about 0.00726/year [National vital statistics reports 2008], 7.45 times the mortality of 20-25 and 25-30 years cohorts. With this value $ML_{55\text{-}60}$ would be about 137 years and 1% would be alive after 632 years.

It must be underlined that an individual without an age-related increasing mortality rate would have an "unlimited longevity" but this should not be confused with the concept of "immortality" (infinite longevity and life span). In fact, an individual of a species with negligible senescence (ageless animal) or even of a species with age-related decreasing mortality (negative senescence [Vaupel et al. 2004]) dies by events that are mortal at any age (severe infections, accidents, predation, killing by other individuals of the same species, etc.; in short, extrinsic mortality) and, moreover, at ages when practically no individual survives in the wild for the extrinsic mortality and so there is no natural selection, if the individual survives because reared in protected conditions, it is possible the onset of unforeseeable and deadly internal imbalances. Therefore, a hypothetical man with no age-related increasing mortality should be in the condition of unlimited longevity but limited life span.

Interactions between Diseases of Different Categories and between Ageing and Diseases

Evolutionary interactions between diseases of different categories and between diseases and disease-like phenomena, in particular senescence, are important. For the sake of brevity, only some interactions will be outlined.

A. Interactions between Diseases Caused by Alterations of the Genotype (Category 1) and Ageing (Category 5.3.2)

Equilibrium frequency of a harmful gene C (C_e) and of its phenotypic expression (P_e) are both in inverse function of [s] (absolute value of the disadvantage s; see formulas 1-7), which is depending on the reproductive value of the individuals damaged by C. In species with age-related fitness decline, as older individual have a smaller expectation of life and, therefore, a smaller reproductive value, if C damages older individuals, C_e and P_e will be greater than for another harmful gene damaging younger individuals, that is a disease caused by an alteration of genotype that manifests itself at older ages (e.g., Huntington's disease) is expected to have higher frequency.

In numerical terms, if a dominant harmful gene C kills (s = -1) at an age when in the wild (or in the ancestral condition) only 1% of the population survives and the frequency of mutation into C from neutral alleles is 0.00001, the frequency of the diseases (P_e) is expected to be:

$$P_e \approx 0.00001/[0.01 \cdot -1] = 0.001 \quad (13)$$

The opposite concept, the hypothesis that age-related fitness decline is caused by the combined effect of many harmful genes acting at older ages and insufficiently eliminated by natural selection (mutation accumulation theory) is untenable because contradicted by theoretical arguments [Libertini 1983, 1988, 2006] and unsupported by empirical evidence.

B. Interactions between Diseases Caused by Interactions with Other Species (Category 3) and Ageing (Category 5.3.2)

A parasite damaging an older individual of a host species with age-related fitness decline, causes a disadvantage lower than a parasite damaging a younger individual, as older individuals have less reproductive value. Therefore, it is expected that older individuals will suffer from the effects of parasite actions more severely that younger individuals as natural selection is less effective when the reproductive value is lower. For humans, the greater gravity of infections in older individuals is well known and documented [Tallis et al. 1998].

C. Interactions between Diseases Caused by Alterations of the Ecological Niche (Category 2) and Diseases Caused by Interactions with Other Species (Category 3)

The huge and continuous modifications of human ecological niche caused by technological innovations, urbanisation, demographic growth, changes of lifestyle, foods, hygienic habits, etc., have greatly altered the conditions to which the species is adapted. Fragile and intricate evolutionary balances between the man and his numerous parasites, attained after thousands of human generations (and millions of parasite generations) have been crushed with catastrophic results, worse than for any other human disaster or calamitous event, war included. Some examples:

1) The extraordinary growth of human population and of its demographic density, its aggregation in urban crowds with water polluted and habitations infested by infected animals, the cohabitation or proximity with bred animals, dangerous hygienic habits, etc., have provoked from the ancient times dreadful epidemics (black death, bubonic plague, smallpox, typhus, cholera, influenza, hepatitis A, tuberculosis, HIV, etc.) with the deaths of hundreds of millions of men. From 1347 to 1640, Black Death, a disease probably different from bubonic plague [Cohn 2002] was the scourge of Europe and other parts of the world, with more than 100 millions of victims (Figure 17). In 1918-1920 a single epidemic of influenza (Spanish flu) killed perhaps 40-50 million people worldwide [Patterson and Pyle 1991] or, according to current estimates, 50-100 millions [Knobler et al. 2005], that is from 2 to 5 times the deaths caused by World War I in five years. When Europeans reached the America, they were selected, much more than American indigenous people, by centuries of terrible epidemics. The germs that they spread unintentionally in America were dangerous for them but very frequently lethal for indigenous populations, which were devastated by smallpox, measles, influenza and other diseases for which they had no evolutionary experience [McMichael 2004].

2) Statistical data show that alterations of the ecological niche and, on the other hand, corrections of these alterations are far more important of non-preventive medical treatment, antibiotics included. In fact, in the USA infectious disease mortality rate has strongly declined before the introduction of sulphonamides and penicillin, and the usage of these drugs and of many new antibiotics has not changed sensibly the decline of mortality by infections [Armstrong et al. 1999]. In recent years, mortality by infections is increasing because of HIV diffusion and, perhaps, of increasing antibiotic resistance (Figure 18).

Indeed, use and abuse of antibiotics and chemotherapeutic substances have selected antibiotic resistant bacteria with weighty consequences (about 90,000 U.S. residents die each year by nosocomial infections [Bergstrom and Feldgarden 2008]). Even vaccines, a medical triumph, if not properly planned or used, can “provoke and even be overcome by pathogen evolution” [Read and Mackinnon 2008].

Figure 17 – *The plague in Mercatello open space*, Naples 1656, a painting of Domenico Gargiulo, known as Micco Spadaro.

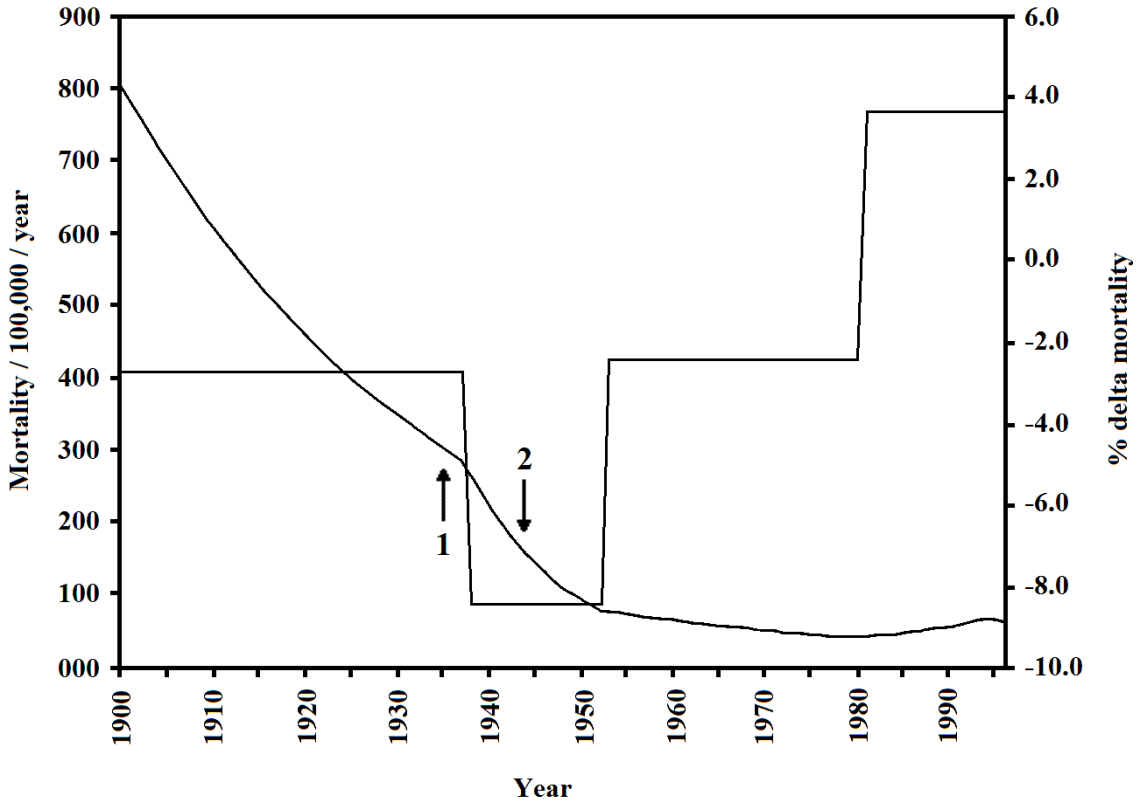


Figure 18 - Overall trends in infectious disease mortality rate and per cent variation of mortality rate in the USA from 1900 to 1996 [Armstrong et al. 1999]. The episodic strong increase in mortality due to 1918 influenza pandemic has been disregarded. Sulfonamids were released in 1935 (arrow 1) and the beginning of clinical use of penicillin was in 1943 (arrow 2) but there is no clear effect of their use on mortality rates.

3) Hygienic or iper-hygienic habits restrict and delay the first exposure to microbes and parasitic worms or make impossible infections or infestations. These modifications of the ecological niche, in principle always beneficial for traditional medicine, on the contrary for evolutionary medicine are potentially harmful and dangerous.

Delayed exposure to poliovirus is a likely cause of modern epidemics of poliomyelitis, a scourge till worldwide application of polio vaccines [McMichael 2004]. Poliovirus has been for thousands of years an endemic pathogen, which rarely caused poliomyelitis or infantile paralysis, until the 1880s, when major epidemics of poliomyelitis began to occur in Europe and, soon after, in the United States [Trevelyan et al. 2005].

Reduced exposure to allergens, especially in early life, caused by modern extreme hygiene, is a significant risk factor for allergy and is the most likely explanation, at present, for the extraordinary worldwide increase in atopic allergy [Janeway et al. 2001].

"Hygiene hypothesis" maintains that exposure to bacteria, viruses and parasitic worms during childhood protects against the development of allergies [Cooper 2004] and atopic diseases [von Mutius 2002]. Allergies may be caused by a delayed establishment of gut flora in infants [Emanuelsson and Spangfort 2007].

For diabetes mellitus type 1, an autoimmune disorder, it is hypothesised that "... increased hygiene may contribute to an imbalance of the immune system, facilitating autoimmune reactions [against β-cells] when virus infections, or proteins like cow's milk or gluten, provoke." [Ludvigsson 2006]

Some intestinal worms secrete chemicals that suppress the immune system to prevent the host from attacking the parasite [Carvalho et al. 2006] and without these substances the immune system becomes unbalanced and oversensitive [Yazdanbakhsh et al. 2002]. Clinical trials have been initiated to test the effectiveness of certain worms in treating some allergies [Falcone and Pritchard 2005].

The deliberate infestation with a parasitic worm (helminthic therapy) is a promising treatment for several autoimmune diseases such as Crohn's disease [Hunter and McKay 2004; Summers et al. 2005; Croese et al. 2006], ulcerative colitis [Summers et al. 2005], multiple sclerosis [Correale and Farez 2007], allergic asthma [Falcone and Pritchard 2005; Leonardi-Bee et al. 2006], etc., whose incidence is greatly increased in recent years and, moreover, is greater in industrialised countries in comparison with developing countries with less strict hygienic habits [Pugliatti et al. 2002; Weinstock et al. 2004; Leonardi-Bee et al. 2006; Zaccone et al. 2006]. These autoimmune and allergic disorders, and others, are explained by the Hygiene hypothesis, which is in short a thesis of evolutionary medicine.

4) The abuse of soaps, deodorants, detergents, disinfectants, etc., modifies normal microbial flora of epidermis and external mucosae (especially of armpits, genitals and hands) and causes the spreading of pathogens, in particular fungi (personal observation).

D. Interactions between Diseases Caused by Alterations of the Ecological Niche (Category 2) and Ageing (Category 5.3.2)

Various alterations of the ecological niche and the diseases caused by them (cigarette smoking, diabetes mellitus, hypertension, hypercholesteremia, obesity, alcoholism, etc.; "risk factors") increase physiological cell turnover, likely provoking a greater apoptosis rate, and therefore accelerate the onset of manifestations that without them should be present only in older individuals [Hill et al. 2003]. The effects of risk factors are countered by drugs with organ protection qualities ("protective drugs") such as statins [Hill et al. 2003; Davidson 2007], ACE-inhibitors and sartans [Weir 2007a], probably by normalisation of apoptosis rate.

Risk factors increase the frequency of cardiovascular diseases and accelerate their onset [Tallis et al. 1998]. Smoking, diabetes, and obesity are risk factors for AMD [Klein et al. 2007]. There is association between Alzheimer disease and risk factors [Vogel et al. 2006].

Protective drugs reduce the risk of cardiovascular diseases [Davidson 2007; Weir 2007a] and of diabetes [McCall et al. 2006; Ostergren 2007], are effective in the prevention of atrial fibrillation [Jibrini et al. 2008; Fauchier et al. 2008] and against Alzheimer disease [Ellul et al. 2007].

Statins reduce decline in lung function [Alexeeff et al. 2007] and lower the risk of nuclear cataract [Klein et al. 2006].

Some diseases caused by alterations of the genotype, such as Werner syndrome and dyskeratosis congenita have analogous effects of risk factors on cells with moderate and high turnover, respectively [Marciniak and Guarente 2001] (Figure 19).

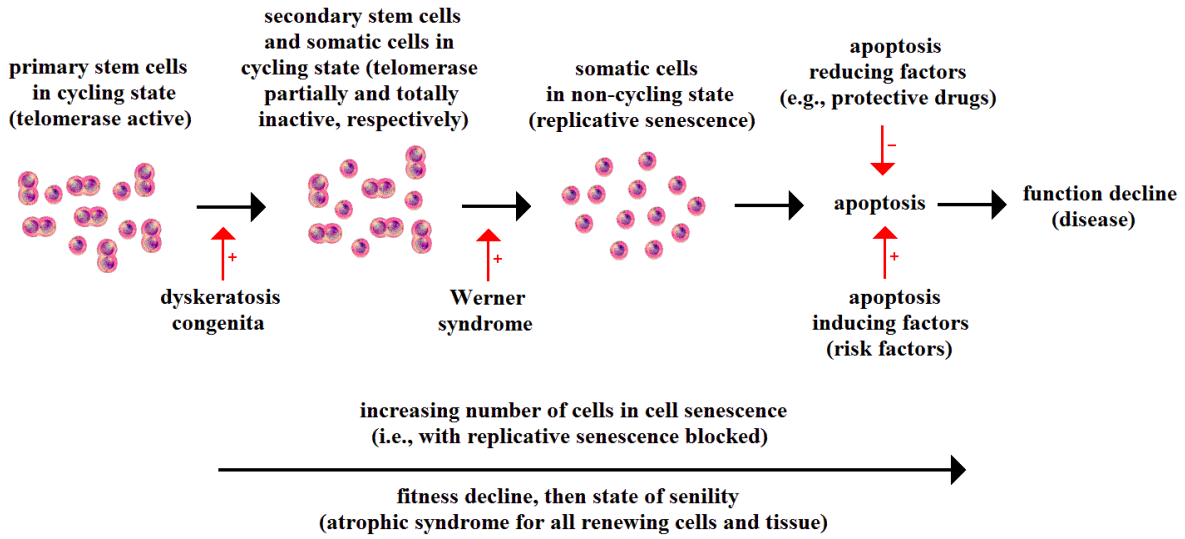


Figure 19 - Risk factors (and some genetic diseases) increase apoptosis rate and cell turnover. Protective drugs counter the effect of risk factors but it is not documented the capacity of reducing physiological turnover rate.

Comparison between Evolutionary Disease Categories and the Breakdown Types of a Machine

It is possible a comparison between the various disease categories classified in evolutionary terms and the various types of breakdowns of a machine (e.g., a car) (Figure 20).

The industrialist is certainly very careful in its manufacture, but it is inevitable that some cars will be sold with one or more faults in construction. As for more frequent defects the manufacturer will adopt opportune measures, it is predictable that each particular defect will have a limited frequency. The breakdowns caused by these defects are analogous to the diseases caused by alterations of the genotype.

A car has a range of operative conditions, e.g., specific types of oil, lubricants and fuel to be used, particular maintenance operations to be observed, etc. The owners not observing manufacturer indications expose their cars to conditions for which the car is not designed and therefore they will risk failures. The breakdowns with these causes are analogous to the diseases caused by alterations of the ecological niche.

In their use, cars can collide with other motor-vehicles or be damaged by vandals. These harms are analogous to the diseases caused by interactions with other species.

If the owner uses the car on roads with gravely uneven surface or to cross a stream or for other conditions for which the car is by no means designed, it is probable a damage, which is analogous to the diseases caused by conditions beyond adaptation range.

As years go, the car wears out, breakdowns becomes more and more frequent, repairs more and more expensive and difficult, spare parts are of not easy finding, until the car is unusable and must be scraped. In part, this is due to mechanical wear and to some components that cannot be substituted (or with replacements too expensive). In part, this is because cars have a built-in obsolescence, that is they are designed not for the greatest duration but to last for a certain period after which the machine must begin to break down with increasing frequency and with increasing costs for the owner until he is induced to buy a new machine. Built-in obsolescence and its consequences are analogous to ageing manifestations.

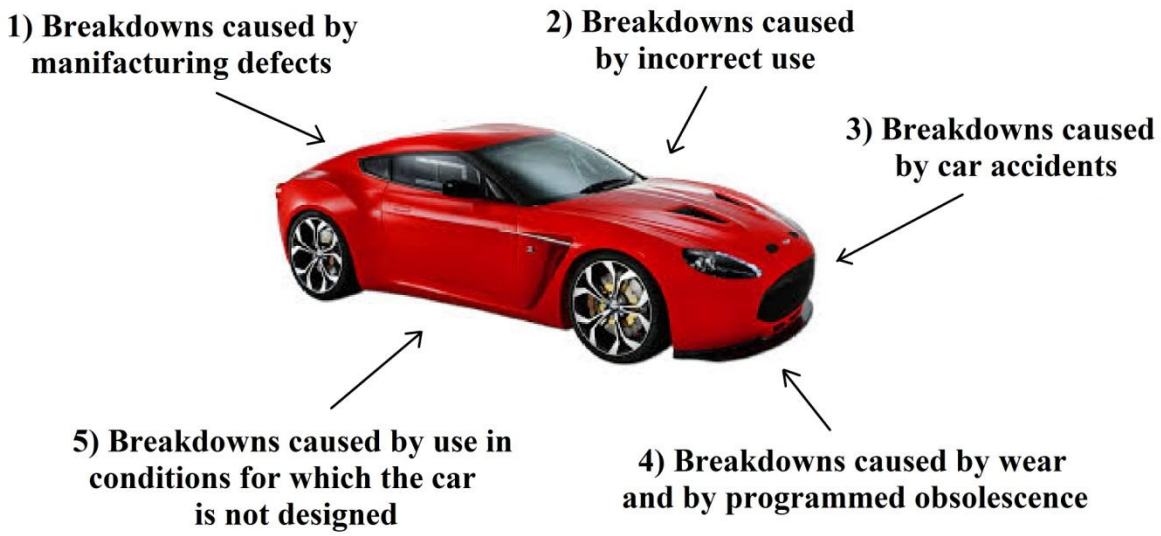


Figure 20 - Categories of car breakdowns, in analogy with the evolutionary classification of diseases and the peculiar phenomenon of ageing.

The concept of planned or built-in obsolescence is less known and obvious than that of mechanical wear.

In 1983, I wrote:

"Built-in obsolescence is that characteristic of an industrial product, specifically planned and pursued, for which the product deteriorates and becomes more and more hardly repairable after a definite time, even though being reliable and fully usable before that time.

Built-in obsolescence causes a waste of materials and a considerable economic overload for the consumer, but has at least three important advantages.

The first is to avoid that the annual part of renewal of a product in a stable market be minimal. For example, a nation in which there are 10 millions of motor-vehicles, with a mean duration of ten years, demands for replacement an annual production of 1 million of motor-vehicles. If the mean duration of a car would increase to 20 years, the annual production should fall to 0.5 millions, with catastrophic consequences for proceeds and employment. The second advantage is the introduction of new technologies with speed inversely proportional to the mean duration of the product. A product with unlimited duration would delay or even make economically disadvantageous the use of new and more effective technologies. The third advantage is that a productive system organised for a quick and continuous renewal is easily adaptable to: a) an unexpected market growth; b) the opening of new markets; c) the conversion to the production of other items; d) the transformation in military industry, etc. On the contrary, the production of goods with very long duration, as there is a minimal annual production, is not much fit to the aforesaid events.

In this regard, I have the following beliefs.

Built-in obsolescence is a hidden pillar of the modern ‘consumer culture’. Neither manufacturers nor trade unions, nor politicians have interest to publicise such pillar.

The consumer believes that it is not possible to make products with greater duration or that the necessary modifications would render the product too expensive. These opinions are wrong and on the contrary considerable efforts in the design of an industrial consumer product are directed to make the product both accurate and reliable until a certain time and afterwards not much reliable and more and more expensively repairable.

Built-in obsolescence of an industrial product and the programmed senescence of a living being are two very different phenomena, yet the analogies are considerable and not superficial. With opportune modifications of the terms, the main common aim is that to allow to the industrial product or to the living being the greatest evolution, the greatest adaptability to new conditions, the greatest competitiveness in the struggle.

If the considerations stated regarding senescence are true:

It is tragic to observe that the man and his machines share a last fate similar in its essence.

It is ironic to consider that the modern technology even in this has been preceded and exceeded by the Nature.

It is incredible that in a civilisation in which built-in obsolescence is fundamental, it is not known that the living world obeys to a parallel logic.” (translated from Italian [Libertini 1983]).

From Wikipedia (14/08/2008)
Article: Planned Obsolescence

“Planned obsolescence (also built-in obsolescence in the United Kingdom) is the process of a product becoming obsolete and/or non-functional after a certain period or amount of use in a way that is planned or designed by the manufacturer. Planned obsolescence has potential benefits for a producer because the product fails and the consumer is under pressure to purchase again, whether from the same manufacturer (a replacement part or a newer model), or from a competitor, which might also rely on planned obsolescence. For an industry, planned obsolescence stimulates demand by encouraging purchasers to buy again sooner if they still want a functioning product. Built-in obsolescence is in many different products, from vehicles to light bulbs, from buildings to software. There is, however, the potential backlash of consumers who learn that the manufacturer invested money to make the product obsolete faster; such consumers might turn to a producer, if any, which offers a more durable alternative.

Planned obsolescence was first developed in the 1920s and 1930s when mass production had opened every minute aspect of the production process to exacting analysis.

Estimates of planned obsolescence can influence a company's decisions regarding product engineering. Therefore, the company can use the least expensive components that satisfy product lifetime projections. Such decisions are part of a broader discipline known as value engineering.

The use of planned obsolescence is not always easy to pinpoint, and it is complicated by related problems, such as competing technologies or creeping featurism, which expands functionality in newer product versions.

- Rationale behind the strategy

A new product development strategy that seeks to make existing products obsolete may appear counter intuitive, particularly if coming from a leading marketer of the existing products. Why would a firm deliberately endeavour to reduce the value of its existing product portfolio?

The rationale behind the strategy is to generate long-term sales volume by reducing the time between repeat purchases (referred to as shortening the replacement cycle). Firms that pursue this strategy believe that the additional sales revenue it creates more than offsets the additional costs of research and development and opportunity costs of existing product line cannibalization. However, the rewards are by no means certain: in a competitive industry, this can be a risky strategy because

consumers may decide to buy from competitors. Because of this, gaining by this strategy requires fooling the consumers on the actual cost per use of the item in comparison to the competition.

Shortening the replacement cycle has many critics as well as supporters. Critics such as Vance Packard claim the process wastes resources and exploits customers. Resources are used up making changes, often cosmetic changes, that are not of great value to the customer. Supporters claim it drives technological advances and contributes to material well-being. They claim that a market structure of planned obsolescence and rapid innovation may be preferred to long-lasting products and slow innovation. In a fast paced competitive industry, market success requires that products are made obsolete by actively developing replacements. Waiting for a competitor to make products obsolete is a sure guarantee of future demise ..."

An Example of Difference between the Current Classification of Diseases and the Evolutionary Classification

Some subjects suffer in juvenile age from myocardial infarction or cerebral ischemia as outcome of serious hereditary hypercholesteremia or other genetic diseases. Others, smokers and/or obese and/or diabetic and/or hypertensive middle-aged subjects, are hit by the same affections. Others, non-smokers non-obese non-diabetic old subjects, suffer from the same diseases.

In the traditional classification, the three groups of patients are classified together, while in the evolutionary classification their affections are divided in three distinct groups of diseases.

It being understood that the aforesaid diseases in their manifestations are treated in the same way, in the evolutionary classification there is a clear-cut distinction.

In the first case, we have diseases deriving from alterations of the genotype: practically they are not preventable (with the exception of therapeutic abortion that is not exactly a prevention), it is possible a precocious identification, pharmacological treatment reduces the risk, ideal therapy is genetic, ideal prevention is eugenics before conception.

In the second case, we have diseases deriving from alterations of the ecological niche. Effective prevention is possible and should be the best choice. Pharmacological treatment reduces the risk of new events but the correction of ecological niche alterations should be the main measure.

In the third case, we have diseases deriving from a physiological function as ageing appears to be. Prevention is not possible. Pharmacological treatment could reduce but not cancel the risk, which increases with the age. The only effective measure could be a genetic modification of ageing regulating and determining mechanisms.

Strategies to Reduce Morbidity Rates and to Increase Life Span and Longevity

1. Actions for the First Evolutionary Category of Diseases (Caused by Alterations of the Genotype)

Today: If possible, precocious identification of subjects with genetic diseases and their pharmacological treatment. Therapeutic abortion in some cases.

In the future:

Option A - Overcoming the limits of current gene therapy [Dropulic and Carter 2008], in particular the transitory success of the therapy, caused by cell turnover if the corrected gene is not inserted in stem cells, and the possibility that an insertion may inactivate a suppressor oncogene and arouse a cancer (insertional oncogenesis) [Porteus et al. 2006] (Figure 21), development of methods with which it will possible to insert in a sure way in the genome of the patient the corrected gene in a position not causing possible dangerous interference with other genes. Treatment of genetic diseases with gene therapy, in a way that the therapeutic method is unvarying and only the genetic

inserted sequence changes. Limitation of the reproduction of subjects with genetic alterations, subordinating the reproduction to controls, if possible, of the genetic condition of the foetus.

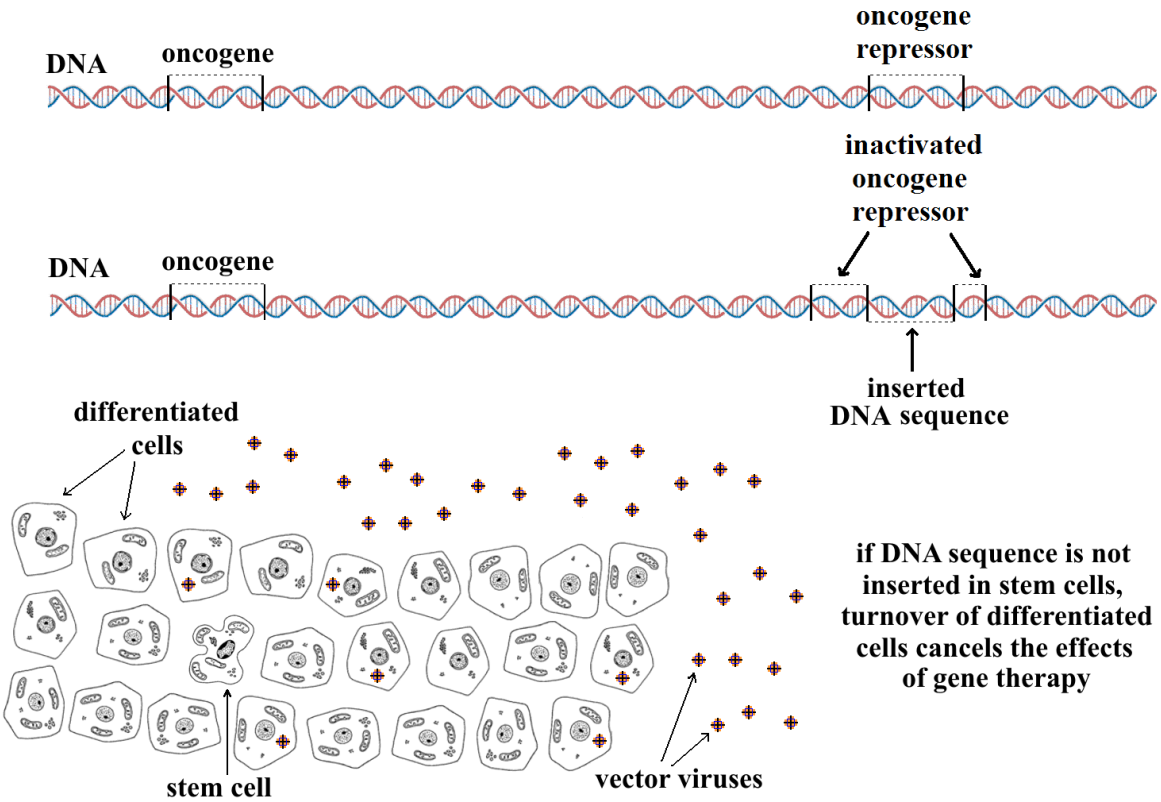


Figure 21 - Current gene therapy. DNA sequence is inserted in a random position by a vector virus. If an insertion inactivates a suppressor oncogene, this may cause a cancer. The type of vector virus and/or limits in the dose of viruses inoculated may cause the transformation only of differentiated cells and not of the rare stem cells and, consequently, the transitory success of the therapy, because cell turnover gradually substitutes differentiated cells with new cells originated from non-transformed stem cells.

Option B - Development of methods with which it will possible to substitute in a sure way the altered gene with the corrected gene in the genome of the patient and in all his cells, germinal cells included (Figures 22 and 23). Reproduction without restrictions as long as it is verified that the substitution has been made correctly.

Effects: Limitation of morbidity and mortality deriving from genetic diseases. Limitations of the progressive increase in their incidence in the future generations. Limited increase in the mean duration of life. No increase in longevity.

2. Actions for the Second Category of Diseases (Caused by Alterations of the Ecological Niche)

Today: Generally, after the manifestation of the disease, it is strongly advised to avoid risk factors. The people and many physicians have not a precise knowledge of the risk factors. The affection is defined disease and there is not a full awareness that the pathological condition is the alteration of the ecological niche.

In the future: Optimal knowledge of the ecological conditions to which our species is adapted. It is necessary to define the risk for each alteration of the ecological niche and to spread in the population and the medical categories the knowledge of the risks. Prevalent utilisation of the resources for prevention. Adoption of measures discouraging risk behaviours. The cures for subjects not observing preventive measures and advice must be the last bastion.

Effects: Drastic reduction of all the pathologies classifiable in this category with parallel reduction of morbidity and mortality. Reduction of the related sanitary costs. Increase in life span. No increase in longevity.

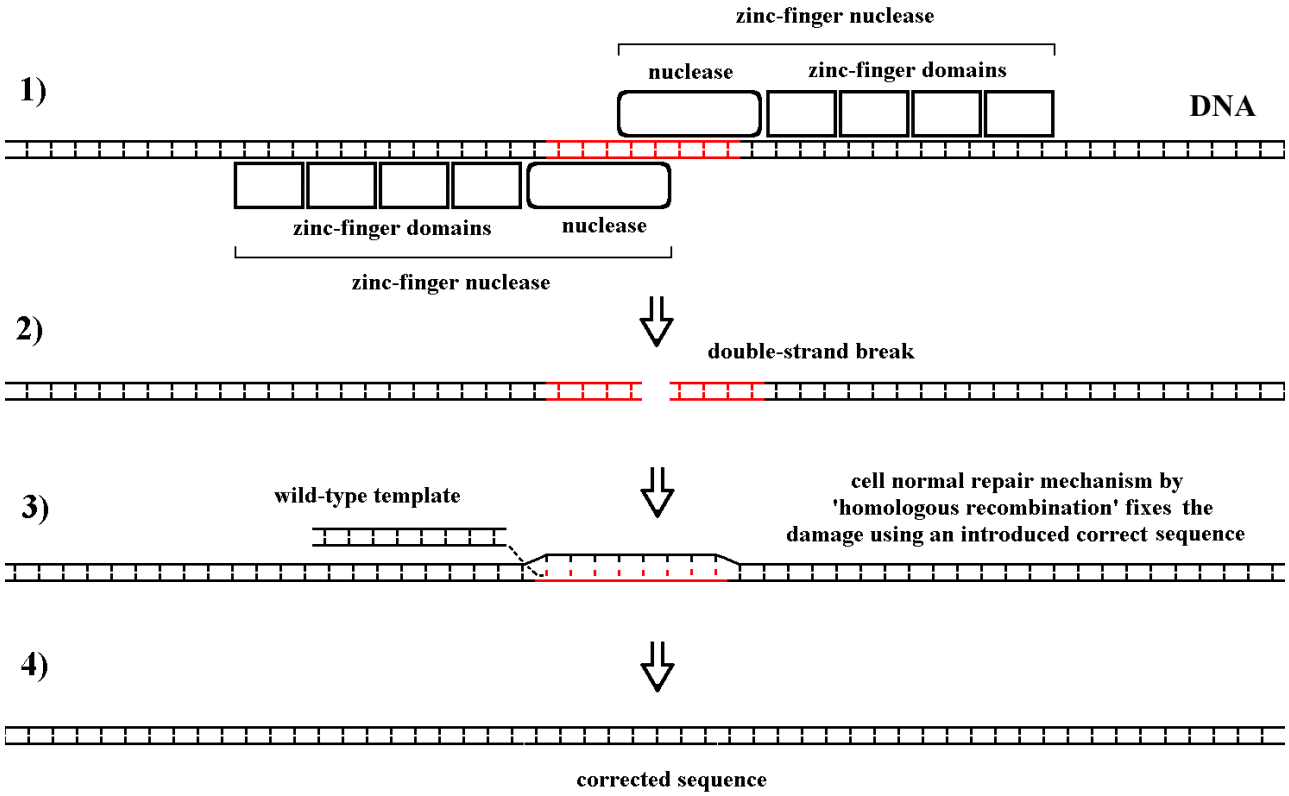


Figure 22 - Option B. The corrected gene is inserted in substitution of the altered gene. With the use of two zinc-finger nucleases, composed of zinc-finger domains (each specific for a particular three-base DNA sequence) and a nuclease (a Type IIS restriction enzyme), it is possible to break DNA double-strand in a precise point with the successive correction by normal cell DNA-repair system by using an introduced DNA corrected sequence [Urnov et al. 2005]. This method appears very promising [High 2005].

3. Actions for the Third Category of Diseases (Caused by Interactions with Other Species)

Today: Indiscriminate actions against any type of infection or parasitosis. Excessive or inappropriate use of antibiotics. Scarce attention to the ancestral ecological relationship between man and microbial species and parasites.

In the future: Widening of the study of the relations between man and his parasites. Reduction or elimination of the circumstances that increase the danger of parasitic infection and epidemics. Limits in the excessive or inappropriate use of antibiotics. A greater and prevalent use of preventive measures and vaccines.

Effects: Reduction of morbidity and mortality. Reduction of antibiotic-resistance cases. Increase in life span. No increase in longevity.

4. Actions for the Fourth Category of Diseases (Caused by Conditions beyond Adaptation Range)

Today: Actions that reduce risk conditions.

In the future: More careful actions to reduce risk conditions (e.g., safer cars and roads, greater severity and observance of safety measures at work, etc.)

Effects: Reduction of morbidity and mortality. Increase in life span. No increase in longevity.

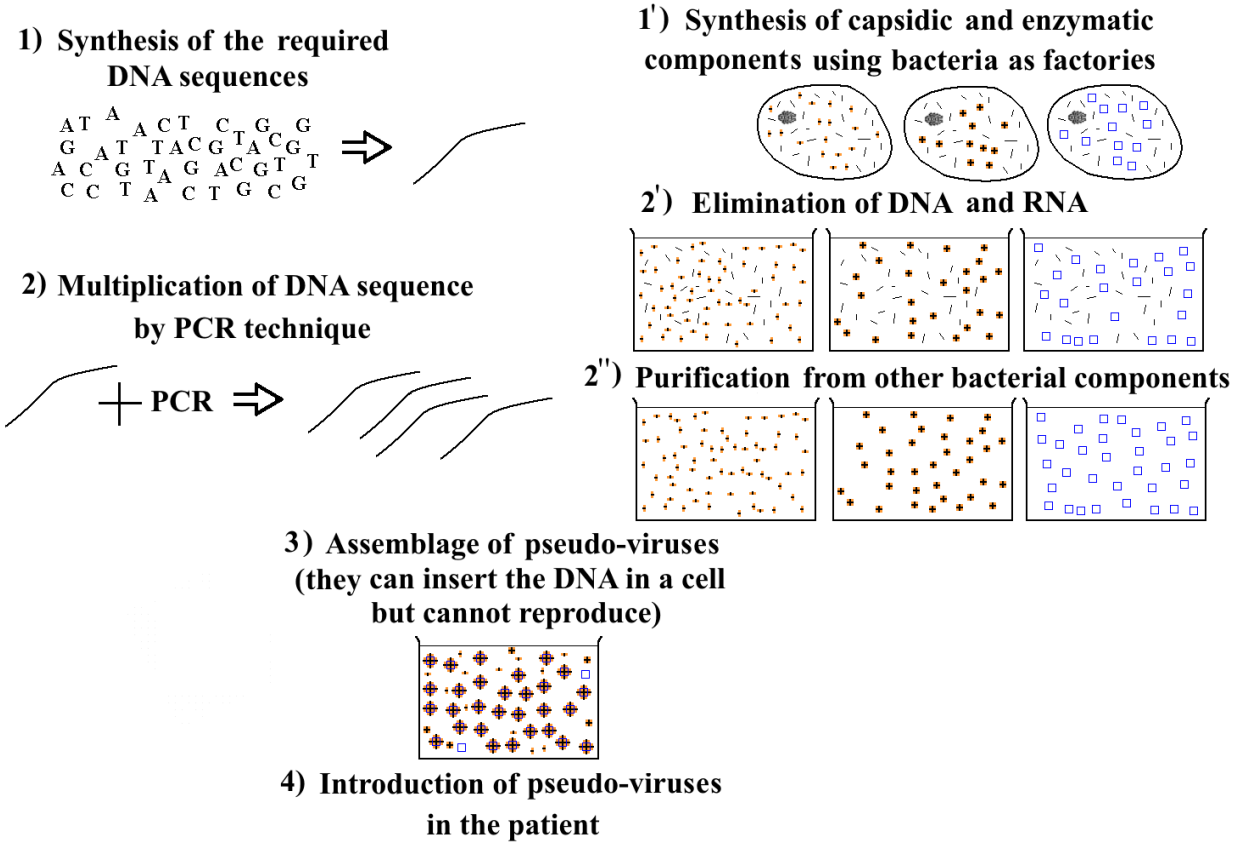


Figure 23 - Creation of gene vectors (hypothetical scheme). The required DNA sequences (for the specific zinc-finger nucleases, for the gene to be modified, etc.) are created starting from defective viral sequences and from single nucleotides and multiplied by using PCR technique. Capsidic components and enzymes essential for the assemblage and activation of pseudo-virus are synthesised by using transformed bacteria and later eliminating DNA, RNA and other bacterial components. DNA sequence and capsidic and enzymatic components are assembled creating pseudo-viruses able to insert or substitute a DNA sequence in a cell but not to reproduce.

5. Actions for Ageing

Today: Ageing is considered not a physiological event but a mixed set of diseases with age-related increasing frequency and severity. Ageing manifestations are empirically treated for their dysfunctions and in analogy with diseases showing the same dysfunctions. The cures allow often an increase in survival time in conditions of low quality of life.

In future: It is indispensable to acquire the awareness that ageing is something other than a disease phenomenon and that needs specific measures. It is possible to conceive an ambitious project for the solution of the problem in four steps:

Step 1

Parallel pursuit of various targets (duration: at least a decade)

a) Widening of the studies on the telomere-telomerase system;

b) The same for apoptosis phenomenon;

c) The same for cell turnover of all tissues and its effect on the functions of the organs;

d) The same for the morphogenesis of the organs, in particular for the dentition;

e) Development of genetic techniques for the effective and precise insertion of a genetic sequence in a point of the genome not causing dangerous alterations;

f) Development of genetic techniques for the effective and precise substitution of a genetic sequence with another sequence;

g) Research of possible safe drugs to modify the telomere-telomerase system and/or cell turnover (or other) so that longevity is increased.

Step 2
Parallel pursuit of various targets (duration: at least a decade)

a) Experiments on animals of insertion of genetic sequences to modify the modulation of the telomere-telomerase system for increasing longevity;
b) The same with techniques of genetic substitution;
c) First applications of the above-mentioned techniques for the treatment of severe genetic diseases;
d) First applications of the above-mentioned techniques for the treatment of age-related severe diseases such as age-related macular degeneration and Alzheimer's disease;
e) As with (a) and (b) to obtain multiple dentitions;
f) Experiments on animals of possible drugs with increasing longevity qualities.

Step 3
Duration: at least two decades

a) First experiments on man of gene therapy (but not on germinal cells) and of possible drugs with increasing longevity qualities;
b) Verification of the results and progressive widening of the experiments.

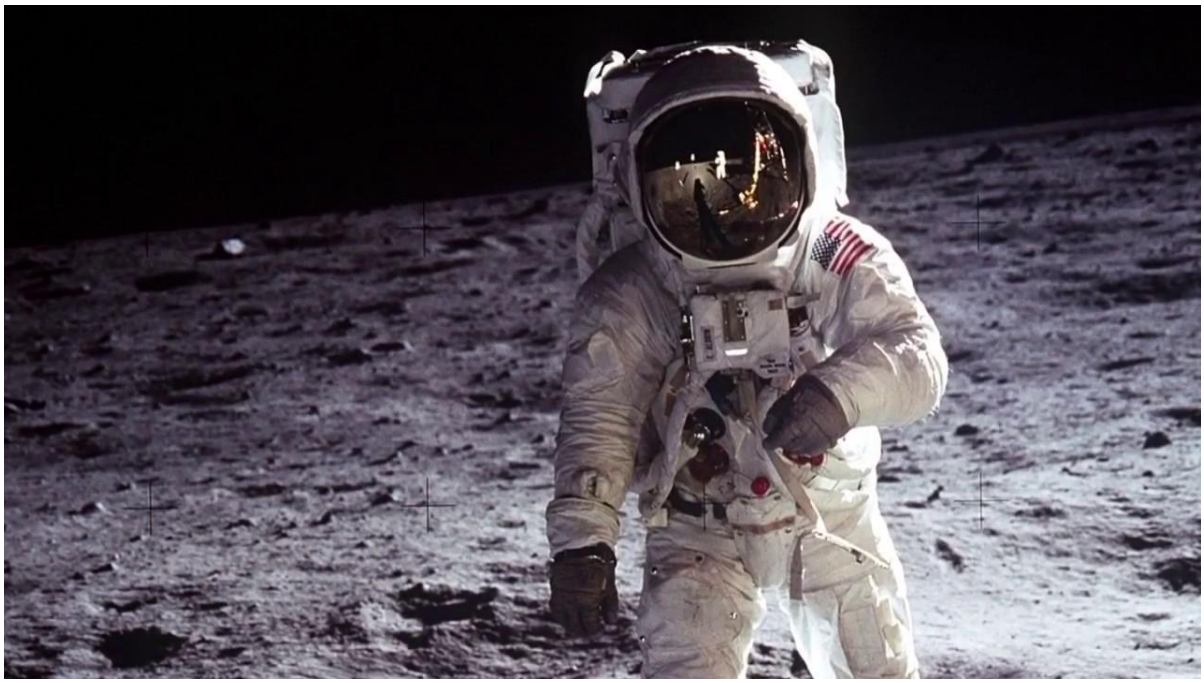


Figure 24 - President John F. Kennedy focused NASA, founded in 1958 by Eisenhower, on sending astronauts to the moon by the end of the 1960s. This aim was achieved on July 20, 1969 (from NASA official site http://www.nasa.gov/).

Step 4
Duration: indeterminate

a) Possible experimentation and application of gene therapy on human germinal cells;
b) Applications on a large scale of safe and tested techniques and drugs

Effects: Increase in the mean duration of life deriving from longevity increase.

For the extreme weight of the argument, the creation of an apposite international agency, adequately funded, could be useful, with the specific aim of controlling ageing and, as a very important corollary, genetic diseases, following the example and the wonderful outcomes of NASA (Figure 24).

Characteristics of a Future Age with Unlimited Longevity

When Moses, by then very old and just before his death, saw from the mountain of Nebo the Promised Land [Deuteronomy, 34], certainly the Hebrews wept for joy and imagined the ending of all their torments. However, the Promised Land, though ending the many pains they had suffered in Sinai, was the beginning of many other sufferings, struggles and disillusions.

A world with unlimited longevity would be as the Promised Land: many pains of today would end, but many others would begin. Our descendants would commiserate with us for our limited life span and longevity but would envy us for so many other things.

A society composed of individuals with very long longevity cannot be in a simple way the same society of today. All or most would have to change.

A very small risk to life considered today acceptable because the expectation of life is a few decades becomes unacceptable if there is a very long expectation of life. Rigorous measures to prevent incidents - with a severity currently unimaginable - would be the rule.

Today, procreation is free and one of the rights considered inviolable. The limitation of only one child per family imposed in China seems to most people an unacceptable limitation. In a society with a very long life span, births would be regulated and limited in proportion. Children would be a rare exception, cuddled and protected by whole communities. Motherhood would become a rare privilege.

Today, marriage is a life-long oath and its break is a trauma strongly discouraged. Marriage would be transformed into a temporary engagement with specific rules and limitations.

Powerful and/or rich persons would be able to obtain peaks of power and/or wealth today inconceivable. Many rules would be arranged to limit the excesses and to assure turnover in power management.

Today, a man studies for a certain period of his life, then works for another period and then retires on a pension, enjoying the fruits of his work. This way of life would not be possible any longer. Perhaps there would be an alternation between periods of work and others of rest or study.

The mean level of education would increase enormously, and cases of persons with various degrees and specialisations would be frequent as well, because after a certain period there would be a psychological demand to change the object of study and work.

There would be extreme attention to beautiful, artistic and poetical things, and there would be supreme examples of lovers of artistic disciplines, but also monstrous examples of egoism and wickedness.

But there would be also the spread of what the Romans called *tedium vitae*, and perhaps suicide would become the main cause of death.

The wars - in memory - would become a symbol of extreme madness, but the world would be static and uniform. Our descendants would commiserate with us for our innumerable wars and yet in historical action representations would pursue those emotions that they would lack entirely in everyday life - a little like when we deplore the troubles of the past centuries but are fascinated by representations of warriors fighting with swords, bows and other ancient weapons.

And what are we to say of philosophy, religion, politics, poetry, sociology, psychology, etc.? All changes if the expectation of life is immensely great.

Cynics and unbelievers would state that God, religion and philosophy are reformulated and adapted to the new society, showing once again to be only creations of the human mind.

Mystics and believers would state instead that God, religion and philosophy are unchanged in their essence and that a life unlimited in its duration allows a better level of comprehension, because we would be less limited by physical ties.

Economics and politics would have radically different aims. Today, we plan for the contemporary generation and a little - if one is farsighted - for the next. In the future, men would think first to the future, as the contemporary generation would have to live in that time.

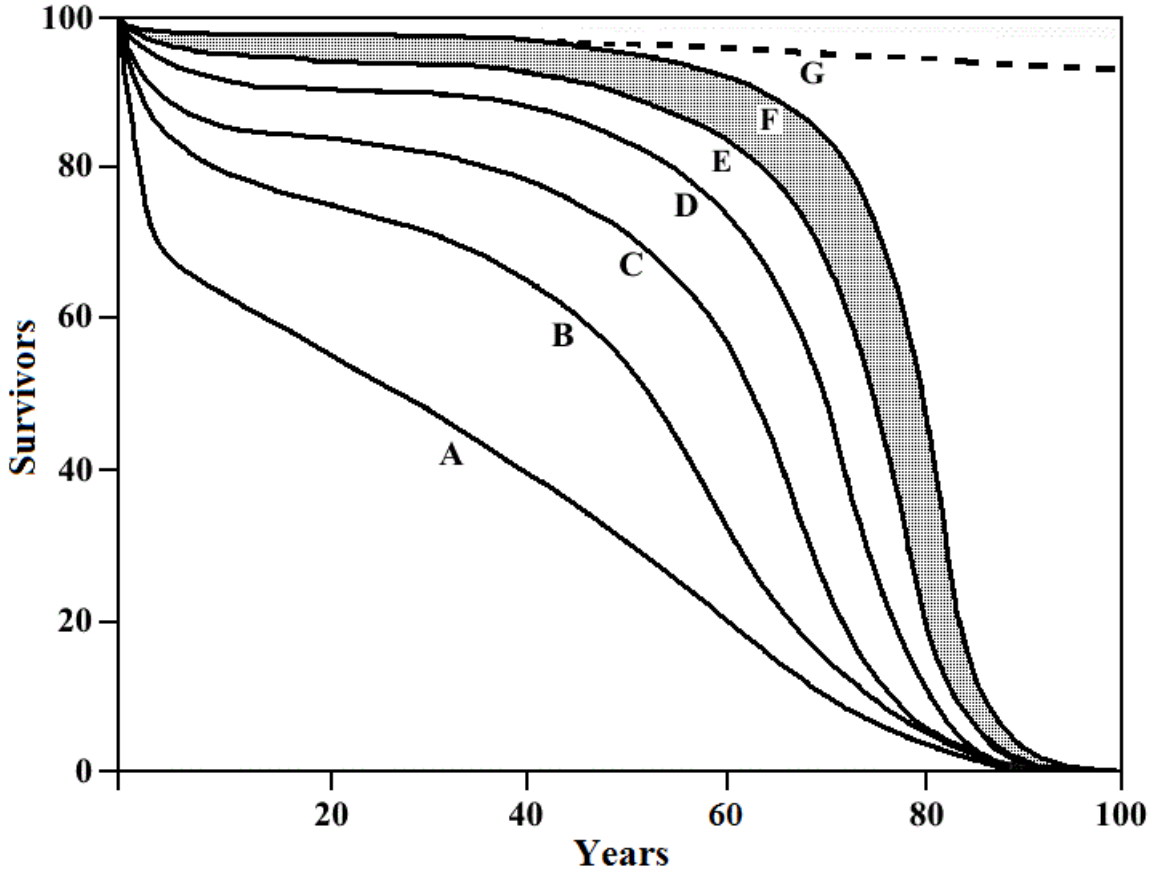


Figure 25 - Life tables of human species (inspired by Figure 0.1 in [Comfort 1979]) illustrating a historical progressive increase in life span while longevity appears unchanged (curves A-E). Actual condition in developed countries is roughly indicated by curve E. With good preventive measures and better health treatment curve F is a likely outcome, with a little further increase in life span (dashed area) but not in longevity. Only with a modification of the progressive increase in mortality caused by intrinsic factors (ageing) will a drastic increase in life span and longevity be possible (curve G).

Conclusion

The development and the efficacious application on a large scale of safe techniques of gene therapy would reduce the consequences of diseases of category 1 (diseases caused by alterations of the genotype).

Respect for the ecological conditions to which the human species is adapted would largely reduce morbidity and mortality of diseases of category 2 (diseases caused by alterations of the ecological niche).

A better comprehension of the interactions between our species and its parasites and of the ecological conditions that minimise their damage, a greater use of vaccines and a more intelligent use of antibiotics would relieve the impact of diseases of category 3 (diseases caused by interactions with other species).

Strong precautionary measures would reduce the impact of diseases of category 4 (diseases caused by conditions beyond adaptation range).

Improvements in health cures and greater social assistance would improve the survival and the quality of life of elderly persons.

The entirety of the aforesaid measures would increase the mean duration of life, but longevity would be unvaried (Figure 25), except for a greater survival in very bad conditions of older persons.

A modification of our genetic program in the part that limits longevity would increase life span and longevity without a theoretical limit. It will be essential to make decisions regarding the ethical nature and advisability of this possibility, but here there is a boundary between science and politics, religion and human free will.

Appendix: Calculation of Equilibrium Frequencies

C is a gene having an advantage or disadvantage s in the homozygous condition and s' in the heterozygote condition. C' is its inactive allele. The notations C_n and C'_n indicate the frequency at the n-th generation of C and C', respectively. The frequency of mutation of C' in C is indicated with v and that of C in C' with u.

The frequencies of C_{n+1} and C'_{n+1} are given by:

$$C_{n+1} = \frac{C_n + C_n^2\, s + 2\, C_n\, C'_n\, s' + C'_n\, v - C_n}{T} \qquad \text{(A1)}$$

$$C'_{n+1} = \frac{C'_n + 2\, C_n\, C'_n\, s' - C'_n\, v + C_n\, u}{T} \qquad \text{(A2)}$$

where T is the sum of numerators.

Formula (A1) can be written as:

$$C_{n+1} == \frac{C_n\,[1 + C_n\, s + 2\,(1 - C_n)\, s' - v - u] + v}{1 + 4\, C_n\, s' + C_n^2\,(s - 4\, s')} \qquad \text{(A3)}$$

There is equilibrium condition when the frequency of C does not change passing from a generation to the next, that is, when:

$$C_{n+1} = C_n = C_e \qquad \text{(A4)}$$

Substituting in (A3), we have:

$$C_e\,[1 + 4\, C_e\, s' + C_e^2\,(s - 4\, s')] = C_e\,[1 + C_e\, s + 2\,(1 - C_e)\, s' - v - u] + v \qquad \text{(A5)}$$

The solutions of this third grade equation are long and complex.

With a recessive harmful gene (s' = 0), supposing the simplification: $s < 0$; $u = 0$, formula (A5) becomes:

$$C_e\,(1 + C_e^2\, s) = C_e\,(1 + C_e\, s - v) + v \qquad \text{(A6)}$$

and the solutions are:

$$1,\ -\sqrt{-v/s},\ \sqrt{-v/s} \qquad \text{(A7)}$$

Discarding solutions 1 and 2 and recalling that $s < 0$, we can write:

$$C_e = \sqrt{v/[s]} \qquad \text{(A8)}$$

where [s] means the absolute value of s.

For Hardy-Weinberg equilibrium ($CC + 2\ CC' + C'C' = 1$), the equilibrium frequency of the phenotype expressing the disadvantageous condition will be:

$$P_e = C_e^2 = v/[s] \qquad \text{(A9)}$$

In the case of a dominant harmful gene, with the simplification: $s = s' < 0$; $u = 0$, formula (A5) becomes:

$$C_e (1 - 3\ C_e^2\ s + 4\ C_e\ s) = C_e (1 - C_e\ s + 2\ s - v) + v \qquad \text{(A10)}$$

and the solutions are:

$$1, \quad \frac{2\ s - 2\ \sqrt{s^2 + 3\ s\ v}}{6\ s}, \quad \frac{2\ s + 2\ \sqrt{s^2 + 3\ s\ v}}{6\ s} \qquad \text{(A11)}$$

Discarding solutions 1 and 2, and by considering that $s = -[s]$:

$$C_e = \frac{2\ s + 2\ \sqrt{s^2 + 3\ s\ v}}{6\ s} = \frac{1 - \sqrt{1 - 3\ v/[s]}}{3} \approx 0{,}5\ v/[s] \qquad \text{(A12)}$$

For Hardy-Weinberg equilibrium, the equilibrium frequency of the phenotype expressing the disadvantageous condition (P_e) will be:

$$P_e = C_e C_e + 2\ C_e\ C'_e = 2\ C_e - C_e^2 \approx 2\ (0.5\ v/[s]) - (0.5\ v/[s])^2 \approx v/[s] \qquad \text{(A13)}$$

that is, for a dominant harmful gene the frequency of the phenotype is almost identical to that for a recessive gene.

In the case of a gene harmful in the recessive condition ($s < 0$) and advantageous in the heterozygote condition ($s' > 0$), with the simplifications $u = 0$; $v = 0$, formula (A5) becomes:

$$C_e [1 + 4\ C_e\ s' + C_e^2\ (s - 4\ s')] = C_e [1 + C_e\ s + 2\ (1 - C_e)\ s'] \qquad \text{(A14)}$$

and the solutions are:

$$0, \quad 1, \quad -\frac{2\ s'}{s - 4\ s'} \qquad \text{(A15)}$$

The first solution is valid if $s < 0$ and $s' < 0$. The second solution is valid if $s > 0$ and $s' <= 0$. The third solution is valid if $s' > 0$. Therefore, discarding solutions 1 and 2:

$$C_e = -\frac{2\ s'}{s - 4\ s'} = \frac{2\ s'}{[s] + 4\ s'} \qquad \text{(A16)}$$

For Hardy-Weinberg equilibrium, equilibrium frequencies of phenotypes in homozygous and heterozygote conditions are given by C_e^2 and $2\ C_e\ (1- C_e)$, respectively.

For chromosome alterations, it is useful to consider a chromosome alteration as an altered gene in a haploid organism, underlining that equilibrium frequency of a chromosome alteration (C_e) and equilibrium phenotypic frequency (P_e) coincide.

Therefore, supposing $s < 0$ and $u = 0$:

$$C_{n+1} = \frac{C_n - C_n\, s + (1 - C_n)\, v}{C_n - C_n\, s + (1- C_n)\, v - (1 - C_n)\, v} = \frac{C_n\,(1 - s - v) + v}{1 - C_n\, s} \qquad \text{(A17)}$$

$$C_e\,(1 - C_e\, s) = C_e\,(1 - s - v) + v \qquad \text{(A18)}$$

The solutions are:

$$1, \quad -v/s \qquad \text{(A19)}$$

that is: $C_e = P_e = v/[s]$ (A20)

as for P_e of recessive or dominant genes in a diploid organism.

Chapter 3

Libertini G (2012a) Classification of Phenoptotic Phenomena. *Biochem (Mosc)* 77, 707-15.

Classification of phenoptotic phenomena

Giacinto Libertini

Abstract

Phenoptosis is defined as the programmed death of an organism. In a more detailed formulation of the concept, it is the death of an individual caused by its own actions or by actions of close relatives (and not by accidents or age-independent diseases) which is determined by genes that are favoured by natural selection and in certain cases increase the evolvability of organisms.

This category of phenomena cannot be justified in terms of individual selection and needs always a justification in terms of supra-individual selection.

Four types of phenoptosis are proposed (**A**: Obligatory and rapid ph.; **B**: Obligatory and slow ph.; **C**: Optional ph.; **D**: Indirect ph.). Examples of each type and subtype are given.

The classification is discussed in its meaning and implications, and compared with another classification of end life types largely based on the classical concept of senescence.

Introduction

In the first Darwinian concept of natural selection, a character is favoured when it determines a greater fitness for the survival or for the reproduction capacity of the individual having it [Darwin 1859].

In this view, after the discovery that characters are determined by genes, a general formula illustrating the frequency variation from a generation to the next, determined by natural selection, of a gene C (Δ_c) acting in an individual I, could be:

$$\Delta_c \propto S \cdot P \qquad (1)$$

where:

S = advantage/disadvantage for I caused by the gene C; P = residual capacity of I of having a progeny at the age when the gene manifests its action (reproductive value).

But, this formula does not explain the unselfish actions of parental care, or the cases in which reproduction entails obligatorily the death of a parent or when the adult individual decays more or less rapidly just after the reproduction. In these cases, with a somehow strained interpretation, it is necessary that the meaning of the term S is not limited to the strict individual advantage, but includes the advantages/disadvantages determined by the effects of gene C on other individuals genetically related.

A solution for these difficulties is the concept of "inclusive fitness" [Hamilton 1964, 1970; Trivers 1970; Wilson 1975], where the calculation of the selective forces considers all the individuals for which the actions of a character (that is, of the gene/genes determining it) have some effect:

$$\Delta_c \propto \sum_{x=1}^{n} (S_x \cdot P_x \cdot r_x) \qquad (2)$$

where:

n = number of individuals for which the character has some effect; S_x = advantage/disadvantage for the individual x; P_x = reproductive value of individual x; r_x = coefficient of relationship between individual x and individual I.

This formula explains very well parental care and many other unselfish (and selfish) behaviours, included the cases of parental deaths connected to reproduction. The concept of inclusive fitness (kin selection) is not alternative to the classic view, but is an extension of it: individual fitness is a particular case of inclusive fitness where only individual I is involved in the effects of the character. In mathematical terms, with n=1 and considering that r_1=1, the formula (2) coincides with formula (1).

Kin selection concepts have been largely used for some decades to explain also eusociality, in particular by using the "haplodiploid hypothesis" as justification of eusociality in many *Hymenoptera* species of ants, wasps and bees. But, formulas based on inclusive fitness concept become unworkable when multiple synergistic effects in the interactions between individuals must be considered. Moreover, many not haplodiploid species show eusociality (e.g., termites) and "The association between haplodiploidy and eusociality fell below statistical significance." [Nowak et al. 2010]. It has been argued that "standard natural selection theory in the context of precise models of population structure represents a simpler and superior approach" [Nowak et al. 2010].

On the other side, kin selection applied to populations composed by a single or few clones is a form of group selection that does not imply unacceptable postulates. And other cases are proposable where this or that type of mathematical/logical approach could be admissible.

This small preamble does not want to discuss the validity and the limits of the various methods and concepts of population genetics used to describe and study actions and behaviours implying unselfish actions, but only to underline two common features:

1) It is important to consider the possible effects of the actions of a character, or a gene, on other individuals, namely it is necessary to consider mechanisms of supra-individual selection, which can be variously defined and calculated (kin selection, precise models of population structure, group selection, mechanisms that favour the rate and the possibilities of evolution, or evolvability [Kirschner and Gerhart 1998], conditions that favour semelparity or parent sacrifice, etc.), although this does not exclude that in most cases a character, or gene, has no effect on other individuals;

2) The selection may favour in particular cases a character, or a gene, that is damaging for the survival of the individual where the character acts. This does not exclude that in most cases a character favoured by natural selection is not damaging for individual fitness.

These general ideas are not new in the Darwinian concept of evolution, as the same Darwin said: "A tribe including many members who … were always ready to aid one another, and to sacrifice themselves for the common good, would be victorious over most other tribes; and this would be natural selection." [Darwin 1871]

Moreover, the idea of individual sacrifice for the common good is old as the human civilization and it was even expressed by a philosophical mind in terms that somehow anticipate Darwinism, as already underlined in a recent meeting [Skulachev 2010]:

"Schopenhauer wrote: *The individual is … not only exposed to destruction in a thousand ways from the most insignificant accidents, but is even destined for this and is led towards it by nature herself, from the moment that individual has served the maintenance of the species*. Today, this statement needs only one specification, i.e. the term 'species' should be replaced by 'species-inherent genetic program'As a rule, interests of individual coincide with those of the genetic program which requires individual to exist, multiply and evolve. However, in certain cases, these two kinds of interests are opposite, so the genetic program forces individual to operate in a way that is counter-productive for individual. In extreme cases, ... it favours elimination, rather than survival, of an individual." For Schopenhauer's quotation, see [Schopenhauer 1819].

Figure 1 – Prof. Vladimir Skulachev.

Definition of “phenoptosis”

It is incredible that until few years ago, namely up to the suggestion of the neologism “phenoptosis” by Skulachev (Fig. 1) in 1997 [Skulachev 1997, 1999a], in the immense scientific vocabulary there was no term indicating the death of an individual, when not determined by accidents or age-independent diseases, as an event provoked by particular mechanisms genetically regulated, namely programmed, and therefore somehow favoured by natural selection.

This is even more strange if we consider that, for many years prior to 1997, innumerable cases of individuals dying by action of mechanisms clearly programmed were known in the animal and vegetable worlds. For example, in the monumental and very well documented 1990 Finch's textbook [Finch 1990], a whole long chapter is devoted to the “Rapid senescence and sudden death”, but the Author did not consider necessary to coin a specific name for this frequent type of events.

It is true that, relatively only a few years before, there was the description of apoptosis [Kerr et al. 1972], namely the death of a cell not caused by accidents but as a phenomenon genetically programmed and having a set of functions, that is adaptive (Later, other forms of “programmed cell death” have been defined and studied, but this is a topic beyond the scope of this work). Moreover, the description of programmed cell death in prokaryotes (“proapoptosis”, [Hochman 1997]) and the definition of the programmed death of a mitochondrion (“mitoptosis”, [Skulachev 1999b]) was even more recent.

Really, it must be considered the scientific and cultural resistance to accept fully the notion that natural selection for a character can lead to phenomena such as promoting the death of the individual having the character: seemingly, this is the exact opposite of the pivotal concept of Darwinism, namely that natural selection favours everything useful for survival and reproduction of an individual, whereas what is harmful to survival and reproduction is opposed.

However, beyond these considerations, in the original definition of Skulachev, “Phenoptosis” is “the programmed death of an individual” [Skulachev 1999a]. The term was coined in analogy with apoptosis, mitoptosis and proapoptosis. The following term “organoptosis” [Skulachev 2003] was coined in analogy with them.

A more detailed definition of the concept is as follows:

“Phenoptosis is the death of an individual caused by its own actions or by actions of close relatives (siblicide; in particular, the parent-caused death of an offspring or filial infanticide) and not caused primarily by accidents or diseases or external factors, which is determined, regulated or influenced by genes favoured by natural selection.”

Phenoptosis cannot be justified in terms of individual selection and needs always a justification in terms of supra-individual selection. On the contrary, a death with no explanation in terms of supra-individual selection must have specific non-selective determinants.

As parallel considerations:

a) Apoptosis in monocellular organisms and proapoptosis in eubacteria, when these phenomena have an adaptive meaning, are synonyms of phenoptosis for the individuals killed by these phenomena;

b) In all its functions in multicellular eukaryotes, apoptosis and other forms of programmed cell death can be considered as analogous phenomena or, better, an evolution of the death of a monocellular individual in a clone where the reproduction is reserved to specialized cells. Moreover, an analogy is possible with the sacrifice of an individual in a eusocial species where the reproduction is reserved to few individuals.

c) Organoptosis is the organized death of many cells by apoptosis, and should be considered not a different phenomenon but only as a coordinated manifestation of apoptosis in many cells;

d) On the contrary, mitoptosis is a similar but different phenomenon and should be analyzed in the awkward evolutionary context of the interactions between a complex host (the multicellular eukaryote) and clones of symbionts (the mitochondria in a cell).

Classification of phenoptotic phenomena

Here, a possible classification of phenoptotic phenomena is proposed.

Its aim is not the definition of arbitrary boundaries between types and subtypes of the phenomenon, but to underline different types of phenoptosis that generally need different explanations in terms of natural selection.

At the end of the classification of phenoptotic phenomena, the absence of them is opposed as last different category.

A) Obligatory and rapid phenoptosis

A-1) *Related to the reproductive cycle*;

A-2) *Deriving in general from characteristics of the life cycle*;

B) Obligatory and slow phenoptosis

B-1) *Duplications-related increasing probability of apoptosis in monocellular eukaryotes*;

B-2) *Age-related increasing mortality in multicellular eukaryotes*;

C) Optional phenoptosis

C-1) *Determined by biochemical mechanisms*;

C-2) *Determined by behavioural mechanisms*;

D) Indirect phenoptosis

D-1) *Determined by biochemical mechanisms*;

D-2) *Determined by behavioural mechanisms*;

and, on the contrary:

E) Absence of phenoptosis

E-1) *With high constant mortality rate*;

E-2) *With small or moderate constant mortality rate*;

E-2) *With age-related decreasing mortality rate.*

A) Obligatory and rapid phenoptosis

Definition

Phenoptosis is defined as obligatory and rapid when, as a rule, it happens in all the individuals of a species in a relatively short time.

This type of phenoptosis is in the range of the phenomena defined by Finch as “Rapid senescence and sudden death” [Finch 1990]. In some cases of semelparity and rapid senescence, the phenomenon is triggered by particular environmental or physiological signals [Finch 1990], but this is only a temporal modulation of a fixed life cycle and not a form of optional phenoptosis (see later). The concept of sudden phenoptosis does not imply a short lifespan, e.g.: “Various species of the thick-stemmed bamboos (*Phyllostachys*) have prolonged phases of vegetative growth that last

for many years or decades (7, 30, 60, or 120 years) according to the species, before suddenly flowering and dying ...” [Finch 1990]

Subtypes

A-1) *Related to the reproductive cycle*

Examples:

- Semelparity and sudden death after reproduction in many species of Salmoniformes and Anguilliformes, in some species of dasyurid marsupials and of rodents, in many species of plants, in particular monocarpic angiosperms [Finch 1990]. In particular, Finch says: “Many botanists emphasize that plant senescence is an orderly and active process (Leopold, 1961; Noodén, 1988a, 1988b, 1988c)” [Finch 1990];

- Endotokic matricide, that is maternal death as the obligatory result of birth, shown by some invertebrates, in which “the young kill their mother by boring through her body wall” or cannibalizing her body [Finch 1990].

A-2) *Deriving in general from characteristics of the life cycle*

Examples:

- Aphagy in adult insects: “Aphagy from defective mouthparts or digestive organs is very common during the adult phases of insects (Weismann, 1889b; Metchnikoff, 1915; Norris, 1934; Brues, 1946; Wigglesworth, 1972; Dunlap-Pianka et al., 1977) and is *the* limiting factor in the adult lifespan of many short-lived species. This phenomenon is, inarguably, programmed senescence. ...” [Finch 1990];

- Lack of anatomic parts in male rotifers [Finch 1990].

B) Obligatory and slow phenoptosis (or, shortly, slow phenoptosis)

Definition

Phenoptosis is defined as obligatory and slow when it is characterized by an age-related progressively increasing probability of death, that is a progressively decreasing fitness. The expression “slow phenoptosis”, in relation to the age-related increasing mortality shown by many species, was proposed by Skulachev [Skulachev 2002b, 2010].

Subtypes

B-1) *Duplications-related increasing probability of apoptosis in monocellular eukaryotes*

Example:

- In the mother cell lineage of yeast, the death by apoptosis follows an exponential dynamics [Laun et al. 2007] that mimics the increment of mortality rate in multicellular eukaryotes. In Finch's classification of senescence phenomena [Finch 1990] this subtype of phenoptosis is classified in the chapter “Rapid senescence & sudden death”, section “Reproduction-related rapid senescence and sudden death”, as the deaths are related to the reproduction-related duplications in the mother cell lineage.

This phenomenon has been suggested as adaptive [Büttner et al. 2006]: “apoptosis coupled to chronological and replicative aging limits longevity that would maintain ancient genetic variants within the population and, therefore, favor genetic conservatism.”

Lewis argues against this interpretation [Lewis 2000] with the argument that a yeast cell of the mother lineage dies by apoptosis after n duplication (n = 25-35 in laboratory conditions [Jazwinski 1993]) and that the death of a single individual among $2^n = 10^7$-10^{10} descendants is irrelevant for any hypothesis considering the phenomenon as somehow favoured by natural selection. Against this argument: it is important not the death of a single individual among innumerable descendants, but the exponentially progressive increasing probability of apoptosis, which causes a quicker generation turnover and contrasts the “genetic conservatism” mentioned by Büttner et al.

B-2) *Age-related increasing mortality in multicellular eukaryotes*

Examples:

- Many species of multicellular eukaryotes show an “increasing mortality with increasing chronological age in the wild” [Libertini 2006, 2008], defined with its acronym (“IMICAW”,

[Libertini 1988]) or as “actuarial senescence” [Holmes and Austad 1995] or as “age-related fitness decline in the wild” [Libertini 2009a], or described as “Gradual senescence with definite lifespan” [Finch 1990].

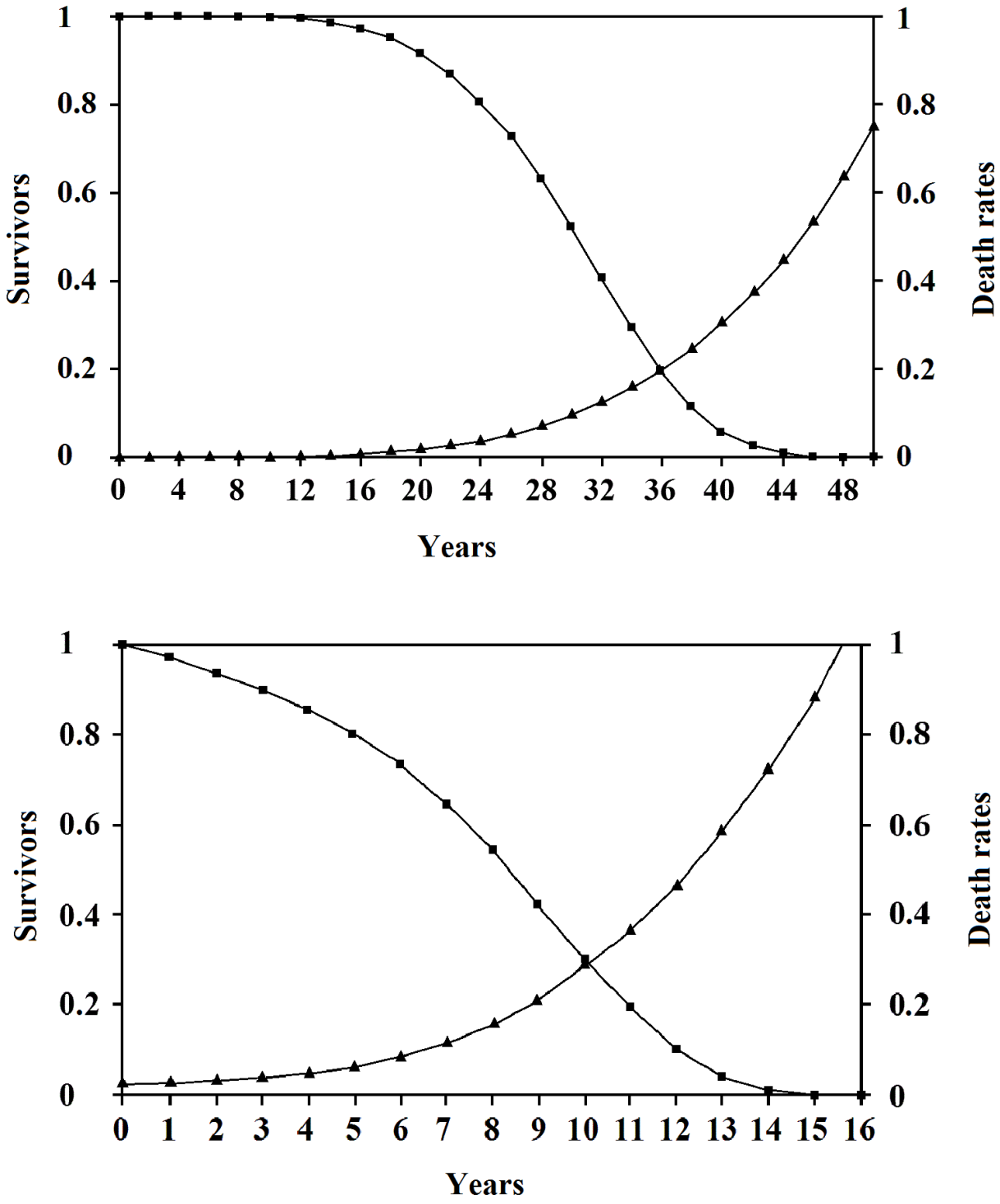


Figure 2 - Life tables and death rates in wild conditions of hippopotamus, *Hippopotamus amphibius* (top), and lion, *Panthera leo* (bottom); data from [Ricklefs 1998].

The first three definitions are descriptive and imply no explanation for the phenomenon. On the contrary, according to the common meaning attributed to the term senescence/ageing, namely the

unavoidable age-related deterioration of everything, both animate or inanimate, Finch's definition seems to mean, intentionally or not, a gradual unavoidable deterioration caused by factors not determined by natural selection. On the other side, the definition "slow phenoptosis" used in this classification assumes a selective advantage for the phenomenon, as underlined in the section Definition of Phenoptosis.

There are some facts:

a) The age-related progressive fitness decline is well documented in natural conditions. On the basis of Ricklefs' data [Ricklefs 1998] it is possible to define the life tables for many animals (Fig. 2). As this phenomenon exists in natural conditions, it is subject to natural selection and, therefore, needs an explanation based on the selective pressures - positive and negative, individual and supra-individual - to which it is subject;

b) In the wild, the older individuals, which show the advanced signs of what is commonly said senescence, are rare or, in their more advanced expressions, not-existent; but this fact does not contradict the existence in the wild of the age-related mortality increase;

c) About the phenomenon, it has been observed that it cannot be explained by selection as it is certainly harmful to survival and reproductive capacity of the individual showing it [Kirkwood and Austad 2000]. This consideration is unacceptable as it disregards possible explanations based on supra-individual types of selection.

C) Optional phenoptosis

Definition

Phenoptosis is defined as optional when it is triggered only in particular conditions on the basis of genetically determined mechanisms that favour or oblige to the phenomenon.

Subtypes

C-1) *Determined by biochemical mechanisms*

Phenoptosis is triggered only in particular conditions in which it entails an advantage for individuals of the same group/deme/tribe (likely related) or of the same clone (by definition related).

Examples:

- Proapoptosis in eubacteria as

a) bacterial phytoplankton mass suicide as defence against viruses [Lane 2008]. In particular, "As most plankton in a bloom are near identical genetically, from the perspective of their genes, a die-off that creates enough scorched earth to stop the viral advance can make sense" [Lane 2008];

b) bacterial suicide triggered by phage infection, "thereby curtailing viral multiplication and protecting nearby *E. coli* from infection" [Raff 1998];

c) the "built-in suicide module" activated by antibiotics in *E. coli* [Engelberg-Kulka et al. 2004] and other bacteria [Lewis 2000].

The programmed cell death in prokaryotes has been defined as "proapoptosis" and hypothesised as phylogenetic precursor of eukaryotic apoptosis [Hochman 1997], with which it shares various features [Koonin and Aravind 2002]. The fact that this type of phenomena is genetically determined or programmed has been underlined [Lewis 2000; Skulachev 2003].

- Apoptosis in monocellular eukaryotes

In yeast, an individual, composed by a single cell, divides in a mother cell and a daughter cell. For the mother cell lineage, in relation to the number of duplications there is an increasing vulnerability to apoptosis [Laun et al. 2001; Herker et al. 2004; Büttner et al. 2006; Fabrizio and Longo 2008] and the death rate increment follows an exponential dynamics [Laun et al. 2007]. In combination with this increasing propensity to apoptosis, it is triggered by: a) unsuccessful mating [Büttner et al. 2006]; b) dwindling nutrients [Granot et al. 2003]; c) chemical alterations [Madeo et al. 1999].

Cellular fragments of individuals died by apoptosis do not damage near cells and are usefully phagocytised by them, which, therefore, "are able to survive longer with substances released by dying cells" [Herker et al. 2004].

Yeast apoptosis is interpreted as adaptive being useful to the survival of the clone, likely made up of kin individuals [Skulachev 1999b, 2002a, 2003; Herker et al. 2004; Fabrizio e al. 2004; Longo et al. 2005; Mitteldorf 2006; Skulachev and Longo 2005]. On the contrary, the apoptosis triggered by toxin secreted by competing yeast tribes [Büttner et al. 2006], is not adaptive for the attacked tribe and clearly means an exploitation of apoptotic mechanisms by the assailants.

C-2) *Determined by behavioural mechanisms*

In particular conditions, selection favours behaviours that are risky or deadly for the individual showing it, but that increase survival probability of related individuals.

The adjective "behavioural" means that the mechanisms require the action of a nervous system, with or without the action of instinct and/or intelligence and/or awareness, however defined or conceived. The adjective does not imply that the functions of a nervous system are not based on or influenced by biochemical mechanisms, hormones included.

Examples:

- Unselfish and deadly behaviours in invertebrates

Behaviours of individual sacrifices in eusocial insect species (ants, bees, termites, etc.) are well-known [Wilson 1975].

- Unselfish and deadly behaviours in vertebrates

Unselfish behaviours that jeopardize one's survival for the benefit of others are common in social vertebrate species. For example, with great individual risk, the predominant males of yellow baboons (*Papio cynocephalus*) [Altmann and Altmann 1970] and chacma baboons (*Papio ursinus*) [Hall 1960] place themselves in the most exposed positions to defend their drove from predators.

For various species of birds, there are the distraction behaviours shown by parents with great individual risk to save offspring threatened by predators [Armstrong 1947; Brown 1962; Gramza 1967].

- Unselfish and potentially deadly behaviours in man

There are countless cases in which one or more individuals sacrifice their own life, or at least endanger seriously it, to save the lives of others. When a man does such acts of sacrifice, this is usually attributed to the choice of the individual, as an expression of free will, and not to mechanisms determined by genes because this second interpretation would be in fact a denial of the free will. The question becomes religious and philosophical and any scientific value is lost. However, a plausible scientific thesis is that, both for eusocial vertebrates and for man, there are not genes that determine rigidly particular behaviors under specific conditions, but that particular combinations of genes determine a neuroendocrine development (or whatever it is correct to describe it) that under certain conditions tends to favor certain types of reaction or strategies.

For example, in a state of imminent danger, some will die trying to save those around them, others will flee in the attempt to save their lives, even if this affects the survival chances of others. The choice between these two opposing strategies is also strongly influenced by the degree of relationship between the individual who must choose the strategy and the people whose survival is threatened: it is very likely that the first strategy is chosen if their offspring are threatened and less likely to do so if the contrary is true.

In animals, analogous behaviors are interpreted as determined by instincts, namely it is recognized that in some way the behavior has been shaped by natural selection. In humans, while acknowledging that in large part they are determined by acts of will, it would be objective to admit that there are instinctive components determined by natural selection as well as for the animals. Moreover, wanting to be stringent, for the part that is determined by the will, perhaps it is not wrong to argue that intelligence, consciousness, will, and all those characters that somehow make us different from other evolutionarily near species, are a result of natural selection and therefore, although more indirectly, were shaped by similar selection pressures.

D) Indirect phenoptosis

Definition

Phenoptosis is indirect when, in particular conditions, there is the death of an individual caused by its close relative, in particular the case of the parent-caused death of an offspring.

Subtypes

D-1) *Determined by biochemical mechanisms*

Examples:

- In the mouse, a new partner of a female kills the new-born offspring. So, when a new male takes over, a female aborts its own young. This is interpreted as adaptive because saves for the mother time and energy, deriving from the predictable killing of the young after birth [Bruce 1959].

- In vertebrates, it is indispensable that immune system discriminates between antigens of each host individual and those of the parasites, which try to overcome immunologic defences by using for their coverings proteins with the same antigenicity of the host (antigen mimicry). The defence of the host against antigen mimicry is to have the greatest inter-individual variability of antigen formulas so that a mimicry adapted to infect all the potential hosts is impossible. The major histocompatibility complex (MHC) is the main tool by which the host organism obtains an extraordinary antigen variability. Differences between antigenic formulas of host and parasite give greater resistance to the infection while similarities cause susceptibility. Correlations between resistance or susceptibility to several infectious or infection-related diseases and specific human MHC alleles are well documented [Lechler and Warrens 2000; Shiina et al. 2004].

As the best progeny is that with the greater antigen variability, MHC-mediated mate choice and post-copulatory selection try to achieve this result. The first case is widespread in nature and is documented for several vertebrate taxa [Slev et al. 2006], and also for our species: a) Women college students rated the odours of MHC-dissimilar men as being 'more pleasant' than those of MHC-similar men [Wedekind et al. 1995; Wedekind and Füri 1997]; b) In an isolate, ethnically homogenous community, significantly fewer couples were observed to match at a 16-locus MHC haplotype [Ober et al. 1997; Ober et al. 1999].

The second case, also defined as 'cryptic female choice' [Loisiel et al. 2008], spontaneous and non-pathologic miscarriages eliminate the offspring with lesser antigen variability with a decreased fitness due to reduced potential resistance to infective diseases [Apanius et al. 1997]. Post-copulatory selection is well documented in animals [Tregenza and Wedell 2000]. A study on human subjects documented an excess of MHC-heterozygotes in newborn males [Dorak et al. 2002]. Several studies on an ethnically homogenous and isolate community documented that couples with shared HLA-DR alleles in comparison with couples not sharing the same alleles have: 1) significantly fewer children [Ober and van der Ven 1997]; 2) a greater interval between pregnancies [Ober 1992]; 3) a greater pregnancy loss rate [Ober et al. 1998].

- "... sonograms of women in the first trimester of pregnancy reveal that twins are conceived two to four times more often than they are born; in the majority of cases, the smaller of the two foetuses disappears by the third trimester and is apparently reabsorbed by the mother (Robinson and Caines, 1977; Varma, 1979)." [Hausfater and Hrdy 1984]. The phenomenon (the "vanishing twin", reviewed in 1998 [Landy and Keith 1998]) should be evaluated considering that one of the common determinants of filial infanticide (see later) is the twin birth as scant resources do not allow the successful breeding of two children at the same time.

D-2) *Determined by behavioural mechanisms*

For the meaning attributed to the adjective "behavioural", see subtype C-2.

Filial infanticide is the killing of an offspring by its own parents.

Examples:

- For our species, the abandonment of healthy new-born babies or the direct filial infanticide when the resources are insufficient are widespread and ancient behaviours [Scrimshaw 1984]. The plausible evolutionary interpretation of these acts, which are present even in modern societies, is

that progeny with reduced survival possibility subtracts precious resources to parents and kin individuals [Eaton, Shostak and Konner 1988].

- In the animals, filial infanticide is widespread and often involves cannibalism [Hausfater and Hrdy 1984].

E) Absence of phenoptosis

Definition

Slow phenoptosis is absent when in the wild the mortality rate does not increase in relation with age[1].

Subtypes

E-1) *With high constant mortality rate*

In this case, which is very frequent among the insects and many other invertebrates as the famous *C. elegans*, in the wild the mortality is so high that it is rare or virtually not-existent the possibility that an individual reaches the ages at which, in protected laboratory conditions, it is possible to observe a progressive increase of mortality [Finch 1990]. This "increasing mortality with increasing chronological age in captivity" (IMICAC [Libertini 1988]) is clearly different from IMICAW phenomenon because, being absent in natural conditions, cannot be influenced by natural selection, while the contrary is true for IMICAW. However, as for these species generally there is almost neither cell turnover nor capacity of repairing parts of their soma that are worn out or damaged, it could be discussed if the characteristics of their life cycle should be classified within the category "A) Obligatory and rapid phenoptosis", subtype "A-2) *Deriving in general from characteristics of the life cycle*". In its textbook [Finch 1990], Finch chose to classify these phenomena within the category "Rapid senescence and sudden death" but it is necessary to underline that many of the observations reported by him are referred to ages existing only in laboratory conditions. Wanting an exact observance of the classification criteria of this work, which refers to phenoptosis phenomenon and not to senescence in its broad meaning as in the case of Finch's textbook, if for a species the death deriving from an absent cell turnover or from unrepaired mechanical wear or damage, happens in natural conditions (at least in a non-minimal percentage) we should choose the classification in the subtype A-2, because in this case it is a programmed part of the life cycle, as it is directly influenced by natural selection. On the contrary, if the death for the above-said causes is practically a laboratory phenomenon, the classification should be in the present subtype E-1, because the death is due to the absence of a selection useful for a life extended to ages non-existing in the wild. However, the difference is unimportant in its essence. In both interpretations, the natural selection cannot or does not act for a greater duration of the life, e. g. favouring mechanisms of cell turnover or of repair of parts worn out or damaged, because the life cycle is such that a greater duration of life does not involve any advantage.

E-2) *With small or moderate constant mortality rate*

Finch provides a broad overview of animal and plant species for which in natural conditions an age-related fitness decline, alias increased mortality, is not observed and this condition is defined as

[1] "In the lens proteins (crystallins) of big whales spontaneous isomerization of L-amino acids to B-amino acids occurs at any age. Crystallins are synthesized during formation of lens and originally contain, as any other proteins, only L-amino acids. Crystallins are practically not replaced during entire life of the whale. L-D isomerization is not encoded by genomes and it is a chemical property of amino acids. Fortunately, this process is very slow (2% per 10 years). However, after 200 years, 40% of L-amino acids are already isomerized to D-amino acids in crystallins, an event strongly affecting the spatial structure of these proteins and apparently their unique ability to be absolutely transparent for the visible light. If such a process results in formation of cataract, it may lead to blindness which should make impossible the life of old whale in the ocean. He should die, such a death being age-dependent. And nevertheless it cannot be regarded as slow phenoptosis." [Observation of the Vladimir Skulachev]. However, such non-adaptive phenomena compromise survival at ages rarely or never existing in wild conditions and so are not included in the definition of slow phenoptosis.

"negligible senescence" [Finch 1990], that is an absence of signs of aging to an extent to be detectable or statistically significant. The absence of an age-related increasing mortality for a species does not mean that the individuals of the species are immortal. After a certain time T, in function of the mortality rate, the probability that an individual is still alive is minimal. Besides, from that age T, by definition natural selection cannot have an impact and it is impossible that a harmful event acting after age T can favour the development of a character contrasting the damage.

For the supporters of the idea that ageing is something inevitable, this is interpreted as a confirmation of the idea. For the supporters of the contrary idea that ageing is something genetically programmed, the phenomenon of functions decay for species not showing such a decline at ages existing in the wild and then showing the decline at later ages existing only in captivity conditions, is indicated with a specific name (IMICAC) and is not confused with the phenomenon IMICAW [Libertini 1988].

E-3) *With age-related decreasing mortality rate*

The phenomenon for which the mortality rate decreases in relation with the age, also defined "negative senescence" [Vaupel et al. 2004], is shown by some species for which there is no function decline and, on the contrary, other factors, as a greater soma, causes a reduced predation and, so, an age-related decreasing mortality.

The definition "negative senescence" is misleading, as one could understand it as a form of reverse aging. In actual fact, it is a case of small or moderate constant mortality rate (subtype E-2) with the addition of a reduction of mortality due to other factors, as a reduced predation. Therefore, subtype E-3 should be considered only a variant of the previous subtype.

Coexistence of several types of phenoptosis in the same species

A single individual can die only once, but the many individuals of a species can die according to more than one type of phenoptosis as there is no reason for which several types of phenoptosis cannot coexist in a single species.

In particular, limiting as example the discussion to our species, we are subject to: optional phenoptosis (subtype C-2: *Determined by behavioural mechanisms*); slow phenoptosis (subtype B-2: *Age-related increasing mortality in multicellular eukaryotes*); indirect phenoptosis (subtype D-1: *Determined by biochemical mechanisms*, and D-2: *Determined by behavioural mechanisms*).

Moreover, within our body, there are numberless cases of phenomena that are similar to phenoptosis: a) every day, countless apoptotic events for cells in turnover; b) in particular cases, mitoptosis; c) in morphogenetic phases, organoptosis; and d) very likely, within the billions of eubacteria living on our teguments and inside our cavities, cases of proapoptosis.

General schema for the study of a phenoptotic phenomenon

First of all, it is necessary to establish that a phenomenon X is within the limits of phenoptosis definition.

Then, it is important to distinguish between:

I) Primary causes, that is the evolutionary determinants and the specific genetic mechanisms favoured by natural selection;

II) Secondary causes, that is the physiological mechanisms which determine and modulate the phenomenon;

III) Tertiary causes, that is the final causes of death.

E.g., in aphagous insects, which in the adult stage lack mouthparts or digestive organs and can survive only a limited time, we have: I) evolutionary determinants that favor such a strange (for us vertebrates) condition; II) molecular mechanisms, genetically determined and regulated, which cause the above said defects; III) the consequent deadly starvation.

Finally, it is necessary to compare similar species where phenoptosis is present only in some of them (or, for a single species, only in particular conditions), and study the evolutionary (primary causes) and physiological (secondary causes) determinants of the presence/absence of the phenomenon.

E.g., in Salmoniformes, ten out of ninety genera show semelparity followed by death while the other genera show iteroparity [Finch 1990]; *Alosa sapidissima*, a herring, show semelparity at lower latitudes "while iteroparity increases linearly with the degree latitude" [Finch 1990].

Conclusion

The schematic classification of the phenoptotic phenomena exposed in this paper is not at all in competition for completeness and documentation with Finch's textbook that covers broadly the endless variety of ways according to which individuals of different species end their lives [Finch 1990] or with analogous manuals.

The approach of the two types of classification is significantly different and must be somehow discussed.

Finch describes the modes of the end of life, defining them as different types of senescence. It is implicit in this approach the concept that all bodies undergo senescence: the fact that many species of animals and plants do not show detectable signs of aging is exposed by Finch, but no plausible and/or general explanation is given.

On the contrary, the classification exposed in this paper is based on the concept that each phenoptotic phenomenon shown by a species presumes a definite evolutionary advantage, because without it the phenomenon could not exist and, so, the general principle, according to which natural selection favours those who best survive, should be applied.

In this alternative view, the default condition favoured by natural selection is the absence of age-related fitness decline, obviously in ages present in wild conditions, namely what Finch calls "negligible senescence" but that should be called "absence of senescence": on the contrary, the presence of phenomena for which the fitness is reduced, suddenly or slowly, always or in particular conditions, is necessarily due to special evolutionary necessities that somehow justify their existence.

It should be noted that, in most cases of the phenoptotic phenomena, the prevailing view in the scientific world is that they are determined by specific evolutionary necessities. The only big exception to this common vision is the slow phenoptosis for multicellular eukaryotes, namely what is commonly called aging. For this particular category of events, the prevailing opinion is that they are the result of insufficient selection for a longer lifespan.

A few isolated heretics, a tiny minority, argue with various arguments and in various ways that slow phenoptosis is the active result of natural selection, as it is for other types of phenoptosis, and not the outcome of insufficient selection for a longer lifespan.

It is ironic to note that these isolated heretics in the wider context of the scientific opinions about phenoptotic phenomena are part of a large majority and, on the contrary, the non-heretics about the slow phenoptosis are heretics in the broader context of phenoptosis.

However, the phenoptosis concept has also a great practical meaning. It is a turning point from a paradigm in which senescence is considered a non-physiological phenomenon, a sum of many different forms of decay and wear, in short a simple name over a mass of various out of control processes, to a new paradigm where ageing is determined and regulated by genes, has an evolutionary meaning, physiological mechanisms and phylogenetic correlations and is a particular expression of a broader category of phenomena, phenoptosis, which has a central importance in biology and deserves the best studies.

Chapter 4

Libertini G (2012b) Phenoptosis, Another Specialized Neologism, or the Mark of a Widespread Revolution? *Biochem (Mosc)* 77, 795-8.

Phenoptosis, another specialized neologism, or the mark of a widespread revolution?

Giacinto Libertini

Abstract

The classical approach of evolutionism is based on the concept of the survival of the fittest individuals. More and more data indicate that natural selection often acts with supra-individual mechanisms favoring genes and actions harmful for the individual. The most striking type of cases is when an individual kills himself or his offspring by actions genetically determined or favored.

The neologism "phenoptosis" describes these events and implicates that they are not evolutionary anomalies but physiological phenomena determined by natural selection.

The most important and familiar kind of phenoptosis, the "slow phenoptosis" or aging, which is currently considered an inevitable and scarcely changeable event, is transformed by this different interpretation into a function, in principle modifiable and manageable.

Perhaps, the neologism "phenoptosis" will represent, together with the term supra-individual selection, the mark of a vital enrichment of evolutionism, conceived in broader terms of which the individual selection is just a particular case, and will be referred to as the brand and the standard for the start of a new era.

* * *

In the classic formulation of Darwinism, evolution follows from the survival of the fittest to live and reproduce. It is clearly a concept based primarily on the selection at the individual level.

But, from its initial conception, the idea of selection only at the individual level was impossible. In fact, reproduction necessarily requires actions involving two parents - or at least one in the cases of parthenogenesis - and one or more young, and even with only two individuals the selection is no longer about a single individual. As trivial example, a mother breastfeeding a child subtracts resources to herself to allow the survival of the child, and she should limit this loss of resources to increase her possibilities of survival and of future reproduction, while the child has opposite needs. A simplistic remedy is to consider the offspring as a genetic extension of the individual, which remains the object of selection, and the problem seems solved, albeit with some bias.

However, the situation quickly becomes much more complex, with quite disconcerting consequences, even for apparently very simple cases. Let us consider the black eagle (*Aquila verreauxii*) (Fig. 1-A) that can successfully breed only one chick at a time because the difficulties of finding food for two or more young would cause the death of all offspring. In a hasty assessment, the most logical action may seem - in evolutionary terms - to lay a single egg and provide the best of nutrition and survival for that single offspring only. On the contrary, the bird lays two eggs and one of them hatches before the other. The young that is born first kills its brother with blows to the face and body until it lies inert or dead, all without the mother trying to stop him in any way [Gargett 1990]. This behavior seems strange and unnecessarily cruel but has its own logic. The second egg has a backup function; it is used in cases where the first egg does not hatch or in cases where the first born is unable to offend or defend itself. The elimination of the other young of the brood is essential for the survival of the first. The apparent waste of resources and the cruelty of the action can be justified in evolutionary terms only by appealing to super-individual causes.

This case is not limited to black eagles or even to birds as a category: "Pandas [*Ailuropoda melanoleuca*] (Fig. 1-B) routinely give birth to twins but nurse only one. The second is dropped to the ground and left to die. … In a variety of predatory birds – pelicans, eagles, boobies, cranes – siblings play the role of executioner. Two eggs are laid, and they hatch at unusually long intervals for birds, several days apart. The first chick to hatch gains an immediate size and strength advantage over its younger sibling. When the second chick hatches, it faces an unrelenting assault from its brother or sister that ends only when one, almost always the younger, dies" [Forbes 2005]; in sand tiger sharks (*Carcharias taurus*) (Fig. 1-C), the first to hatch in the maternal uterus searches and kills all its own brothers and sisters [Gilmore et al. 1983].

Our species (Fig. 1-D) is also strongly involved in this type of phenomena.

At menarche, it is estimated that a woman has 300,000 egg cells, or oocytes. Each month, a group of oocytes is stimulated by a hormone (FSH), but the egg cell that first reaches a certain dimension eliminates all other oocytes by biochemically inducing their suicide by apoptosis. Furthermore, in the early stages of embryo formation, only those fertilized eggs without genetic defects are allowed to implant in the womb. In other words, there are many early abortions which are generally not recognized as such. In the next weeks, many fetuses are eliminated because they are somehow defective, or even simply because they are one of a couple of twins: "Multiple births are rare in humans, but multiple conceptions are not. As many as 1 in every 8 pregnancies begins as twin conceptions, though few survive intact to birth; twins constitute only 1 in every 80 to 100 live births …" [Forbes 2005].

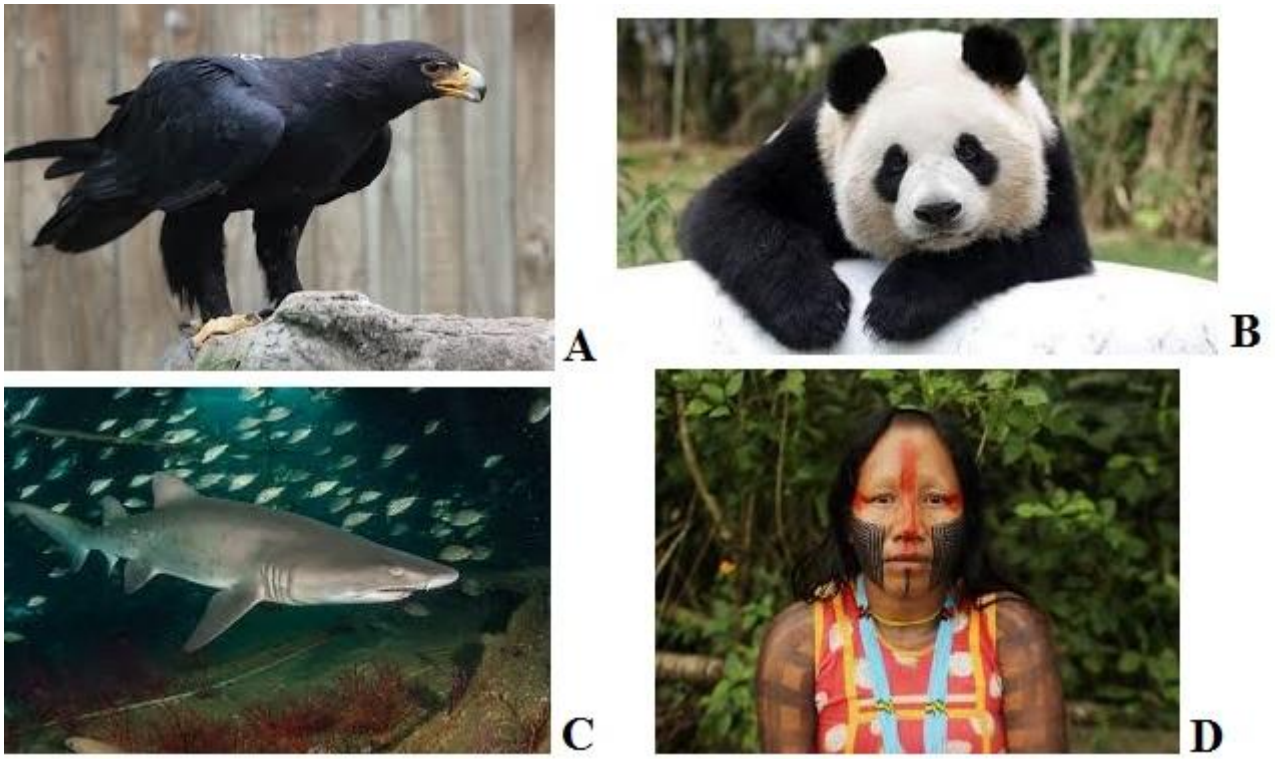


Figure 1 – A: black eagle (*Aquila verreauxii*); B: giant panda (*Ailuropoda melanoleuca*); C: sand tiger shark (*Carcharias taurus*); D = female of *Homo sapiens*. These four vertebrate species show all phenoptotic phenomena characterized by the elimination of some offspring.

Other fetuses are eliminated because they have scarce antigen variability and this reduces the resistance to infective diseases [Apanius et al. 1997]. Evidence of this phenomenon ('cryptic female choice' [Loisel et al. 2008]) was well documented in an isolated community [Ober 1992; Ober and van der Ven 1997; Ober et al. 1998].

After birth, the killing of the offspring continues. In the study of 60 primitive societies, 112 circumstances (not single cases!) in which newborns were habitually killed was reported. There were various motivations for these killings, including inadequate parental resources (40), deformed or ill newborns (21), or the birth of twins (14) [Hausfater and Hrdy 1984].

This series of phenomena is just one of many categories of events for which the selection conceived in individual terms is clearly inadequate. In fact, the countless forms of grouping and social behavior at all levels and types, and in particular the so-called eusociality of many species of ants, bees and termites, are utterly inexplicable without using mechanisms of supra-individual selection.

In many cases, to explain these phenomena one has to appeal to group-level- or even species-level-benefits. These arguments were challenged in general [Maynard Smith 1964, 1976], but a new model was formulated that solved many of these difficulties and seemed to give a conclusive answer.

This new theory, "kin selection" [Hamilton 1964, 1970; Trivers 1971], no longer regarded the individual as the central point of the selection but the gene. From this perspective, the researcher must consider the fact that a gene is present both in the individual where it acts and in related individuals upon which its actions have an impact. The advantages and disadvantages for all these individuals must be taken into account when assessing whether or not a gene is favored by natural selection.

Kin selection is a powerful explanatory tool, and it can shed light on many behaviors with relative ease. For this reason, it is considered to be the theory that founded sociobiology, and for a time it was considered the key to explaining the eusociality observed in many species of insects (bees and ants, in particular) [Wilson 1975; Trivers and Hare 1976].

However, justifying insect eusociality by appealing to haplodiploidy - as kin selection does - has since been disputed. This is because many non-haplodiploid species (e.g., termites) are eusocial and the association between eusociality and haplodiploidy does not seem statistically significant. Moreover, models of population structure appear to be a better approach to justify and study eusociality [Nowak et al. 2010].

Besides, kin selection for demes in competition composed by one or few clones in actual fact is group selection. Thus kin selection cannot always be considered an alternative to the taboo of group selection.

There are other hypotheses, models or types of mathematical approach that have also been proposed to account for supra-individual selection (e.g., selective mechanisms that increase the rate and the possibilities of evolution, or evolvability [Earl and Deem 2004; Colegrave and Collins 2008]).

All these considerations are not at all a careful and accurate description of the theoretical debate that is about the analysis of evolutionary mechanisms regarding supra-individual selection. Their aim is simply to highlight that evolutionism has been gradually transformed from an evaluation of selective mechanisms formulated exclusively, or primarily, in terms of individual selection, into a more extensive supra-individual evaluation, of which the strictly individual level of selection is only a special case.

* * *

As part of this transformation, a very important fact was missing and, vice versa, the consequent inclusion of this fact in a much broader context was lacking.

It is well known that many species of animals and plants have a lifespan that is strictly and clearly planned. In his authoritative textbook, Finch dedicated a whole long chapter to these species [Finch 1990]. The fact that for many species the death of individuals is genetically programmed and favored by natural selection is therefore nothing new.

But, it is also well known that for many species, including our own, there is from a certain age (30 years for our species), a time-related gradual decline of fitness, that is an age-related increase in mortality rate. The current opinion is that this phenomenon is the result of weak selection at ages in which few individuals survive, or alternatively of selection of characters that are advantageous at young ages but harmful at older ages. Overall, the current view is therefore that this age-related increasing mortality is a phenomenon caused by unavoidable factors, which are either not directly favored by selection or insufficiently opposed by it. However, there are a number of lines of evidence against these mainstream views – evidence which suggests that the phenomenon is planned and directly favored by selection. This evidence includes:

1) The existence of many species in which the phenomenon does not exist and there is equal fitness at any age [Comfort 1979; Congdon et al. 2003], species defined as having "negligible senescence" [Finch 1990], or even an age-related increase of fitness, called "negative senescence" [Vaupel et al. 2004]. This is not explained by current theories;

2) According to the current view, there should be a positive correlation between high environmental (or extrinsic) mortality and intrinsic mortality, but empirical data show that the correlation is inverse [Ricklefs 1998], as it should be if the phenomenon was programmed and favored by selection [Libertini 1988, 2008]. Not one of the supporters of current theories has ever attempted to explain this contradiction, or even admitted that a problem exists;

3) The interpretation of the phenomenon as something programmed necessarily requires the existence of specific mechanisms, genetically determined and regulated, which leads to the fitness decline. Conversely, if the phenomenon is not programmed, the existence of aging-causing mechanisms is not expected and indeed they would be in total conflict with this interpretation [Libertini 2008]. In regard to these mechanisms, there is increasing documentation and awareness that the decline of physical functions is determined by limitations in cell turnover, which in turn are controlled by the telomere-telomerase system and by the progressive activation of a specific on/off program defined as cell senescence [Fossel 2004]. In particular, cell senescence, which has been considered a "fundamental cellular program" [Ben-Porath and Weinberg 2005], is reversed to the off state, with the return to youthful conditions and the reactivation of duplication capacities, by telomerase introduction in somatic cells [Bodnar et al. 1998; Counter et al. 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999]. Moreover, telomerase reactivation in aged mice with artificially blocked telomerase shows a marked reversal of all degenerative manifestations, even for the nervous system [Jaskelioff et al. 2011]. These results are hardly justifiable in keeping with the non-programmed hypotheses of senescence: the justification of telomere-telomerase system as a general defense against cancer, the only proposed explanation, is weak and contradicted by empirical data and theoretical arguments [Libertini 2008; Milewski 2010].

4) Moreover, the current evolutionary interpretations of aging (specifically, antagonistic pleiotropy and disposable soma hypotheses) are based on the assumption that the beneficial effect of a longer life span cannot be obtained without incurring a cost [Goldsmith 2008b]. But, this should be proven. Currently the evidence in support of this assumption is not only lacking, but there is clear evidence against it in particular important cases. For example, A) there is a clash between the predictions of disposable soma theory and the association of caloric restriction with a greater longevity [Mitteldorf 2001]; B) there is the possibility of evolving "both a long life and alternative anti-cancer defences" [Milewski 2010].

To mark (and emphasise) the idea that individuals are sacrificed by supra-individual selective mechanisms, the neologism "phenoptosis" was coined [Skulachev 1999a].

Strangely, no one before Skulachev – who is not an evolutionary biologist - had thought to unify under a single term phenomena very different from each other in the mechanisms and expressions but firmly united by a common and well-known evolutionary logic: the individual is completely expendable if supra-individual selective mechanisms require this.

With the same logic, the age-related growth of mortality in species such as ours has been defined as "slow phenoptosis", a beautiful expression coined by the same Scholar [Skulachev 2002b, 2010],

marking the evolutionary analogy with many other forms of phenoptosis that are entirely different in their specific causes and mechanisms.

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The analogy between the terms phenoptosis and apoptosis (the word which inspired the neologism) is not just semantics.

By considering a multicellular organism as an immense clone highly organized and differentiated (and it should be noted that the first multicellular organisms derived from clones that have gradually acquired an increasing cellular specialization and organization), the apoptosis of a cell in a multicellular organism is phylogenetically similar to the phenoptosis of an individual within a clone.

Moreover, if we consider that bacterial proapoptosis [Hochman 1997], sometimes expressed in the form of mass suicide [Lane 2008] and modulated by mechanisms clearly phylogenetically related to apoptosis in unicellular eukaryotes [Koonin and Aravind 2002], which also show forms of single [Büttner et al. 2006] and mass suicide [Granot et al. 2003], the underground ties and the analogies among bacterial proapoptosis (alias phenoptosis in these unicellular organisms), apoptosis of single-cell eukaryotes (alias phenoptosis in this case too), apoptosis in multicellular organisms, and phenoptosis of multicellular organisms, become even more evident.

***

How is it possible to mark with symbolic words the distinction between an idea of evolution focused on the individual and a different conception based mainly on mechanisms of supra-individual selection (and of which the individual selection is only a special case)?

The term “kin selection” is too restrictive and only indicates a method, although very important, of analysis of supra-individual selection. The expression “selfish gene” has too much of the flavor of a selection based on the single gene, rather than the single individual, and does not reflect well the assumed central importance of the supra-individual selection.

Perhaps, there are two words that best interpret the new concept in the distinction from the old idea.

The first is the expression “supra-individual selection” that describes a category of tools of analysis and evaluation (here not precisely defined) in contrast to the oversimplified view of the first conception.

The second is the very expression “phenoptosis”, both because it pinpoints the sacrifice of the individuals as a pivotal characteristic of evolution, and because it places in the centre a particular category of phenoptosis, the slow phenoptosis, indicating too that, being a genetically programmed and regulated function, it is also open to modifications and control.

In a more general framework, in particular outside the scientific world, the transition from methodologies focused on individual selection to others focused on supra-individual selection does not appear capable of arousing noteworthy attention. But, if we consider that this step involves the transformation from the concept that aging is an inevitable and scarcely changeable event to a new outlook for which aging is a function, in principle modifiable and manageable, this will certainly be object of the greatest interest.

For these reasons, the neologism “phenoptosis”, to which the name of this journal has been dedicated, is not just another technical term to use in a small circle of specialist scholars, but potentially a term to be referred to as the brand and the standard for the start of a new era.

Chapter 5

Libertini G (2013) Evidence for Aging Theories from the Study of a Hunter-Gatherer People (Ache of Paraguay). *Biochem. (Mosc.)* 78, 1023-32.

Evidence for aging theories from the study of a hunter-gatherer people (Ache of Paraguay)

Giacinto Libertini

Abstract

In late seventies, a small tribal population of Paraguay, the Ache, living under natural conditions, was studied. Data from this population turn out useful for considerations about evolutionary hypotheses on aging phenomenon:

1) Ache show an age-related increasing mortality, which limits strongly the mean duration of life, as observed in other studies on mammal and bird species.

2) According to current theories on aging, in the wild very few or no individual reach old age and, so, aging cannot be directly influenced by natural selection. However, data from our population show that a significant proportion of the population reaches in the wild 60 and 70 years of age.

3) Data from Ache are also in agreement with the observation about an inverse correlation between extrinsic mortality and the deaths due to the age-related increasing mortality.

4) For many gerontologists, the age-related decline of vital functions is a consequence of the gradual decline of cell turnover, genetically determined and regulated by the declining duplication capacities of stem cells. The current interpretation is that these restrictions are a general defense against the proliferation of any tumoral mass. However, among wild Ache cancer is virtually unknown in non-elderly subjects, and only among older individuals there are deaths attributable to oncological diseases. Moreover, fitness decline begins long before oncological diseases have fatal effects in significant numbers. This completely disproves current hypothesis, because a supposed defense against a deadly disease cannot exterminate a population before the disease begins to kill.

These data are consistent with similar data from other species studied under natural conditions, and bring new arguments against the non-adaptive interpretation of aging and in support of the adaptive interpretation.

Introduction

For the study of a species, it is fundamental to observe it under natural conditions. For the human species, the closest condition to the natural one is that of the few residual hunter-gatherer populations, which is equivalent to the human condition in the Paleolithic period.

These populations live in areas of difficult access, as such it is very difficult to contact and study them. Moreover, when they come in contact with “civilized” subjects, most of them fall victim of infectious diseases they are not adapted to. It is also worth noting that these people are illiterate, do not use numbers or have specific memories of past events.

The precise study of a hunter-gatherer population is therefore a formidable scientific challenge.

One of the most comprehensive studies on the field was conducted in the late seventies on a small tribal population of Paraguay, the Ache, best known with the depreciative name Guayaki used by their “civilized” neighbors. The report of this study was published in 1996 [Hill and Hurtado 1996].

Many data are unique or with few matches in other similar studies [Howell 1979; Melancon 1982; Early and Peters 1990].

In this paper, I want to summarize some of the data from Hill and Hurtado's study concerning the life table of this hunter-gatherer people in wild conditions and the causes of their mortality. These data will be used for considerations about the evolutionary hypotheses on aging phenomenon.

Part 1 - Data from life table of Ache people and their implications for the evolutionary hypotheses about ageing

Hill and Hurtado's very demanding study ("fourteen years of data collection" among the Ache and "nearly five years of writing" [Hill and Hurtado 1996]) is a very valuable work and an almost unique source of information. Such a study would be unlikely repeatable because hunter-gatherer populations are disappearing. Figures 1-3 summarize the life table of Ache under natural conditions prior to the close and friendly contact with the modern populations (Not friendly contacts with "civilized" people were in existence for many years as indiscriminate killings of Ache as soon as they were spotted by Paraguayans or, in other cases, their capture and enslavement). Figure 3 compares the data obtained from the Ache with those obtained from the Yanomamo, one of the few other populations studied under natural conditions.

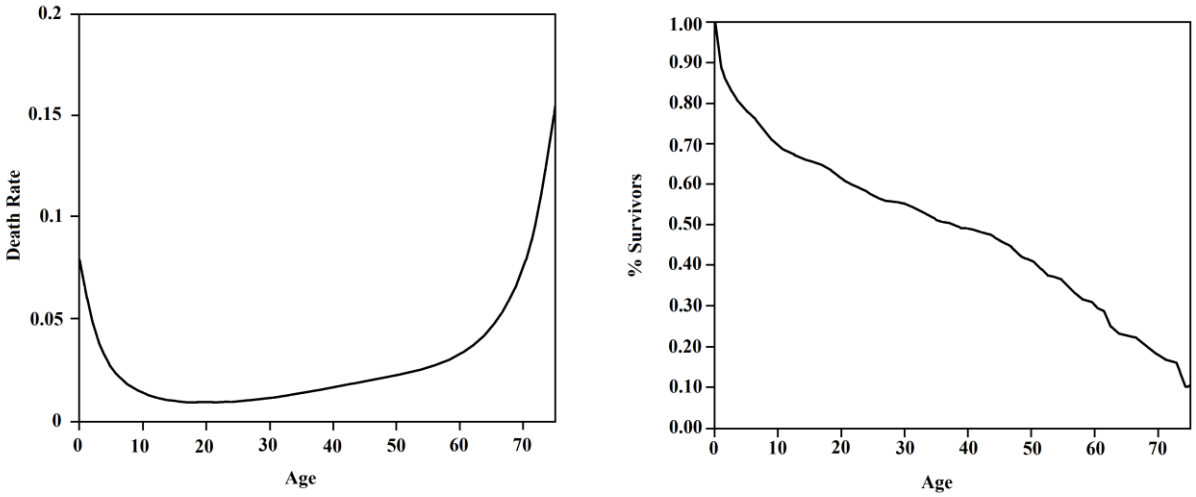


Figure 1 - Probability of death in function of age (left) and life table (right) of Ache people in wild conditions (forest period). Data from [Hill and Hurtado 1996].

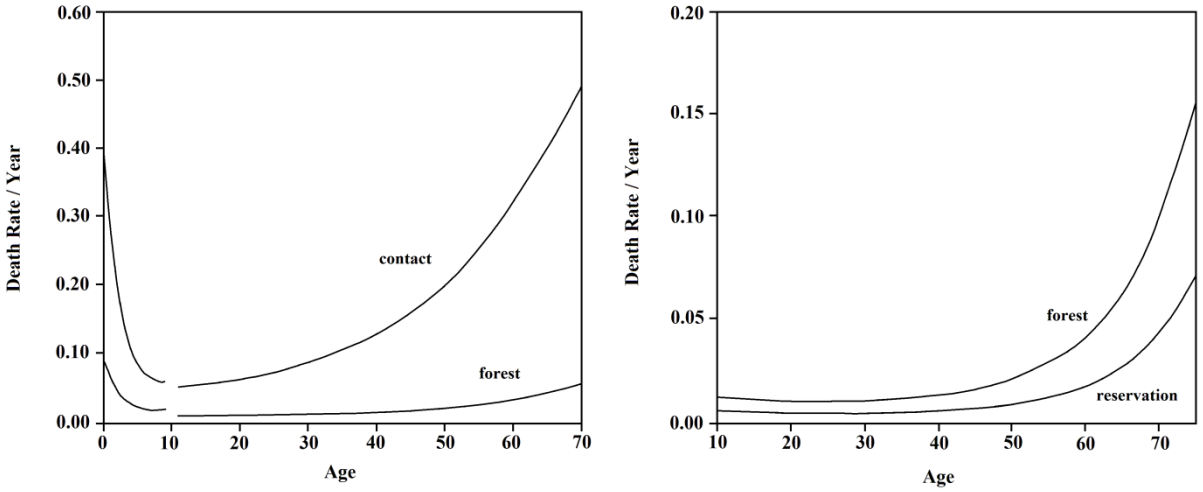


Figure 2 - Comparison of age-specific mortality between the period lived in the forest and during the first contact (left) or during the life in the reservation (right). Data from [Hill and Hurtado 1996].

The data document that, in wild conditions, the Ache - despite the ruthless killings and captures by Paraguayans, which caused about one third of the overall mortality (missing persons included) - had a life table similar to that of modern populations, with a high and decreasing mortality in the first part of the life, then a phase in which the mortality was low and stable, with a minimum at around 15-20 years (about 0.9%/year), and finally a third phase in which the mortality grew slowly at first and then in a strong way.

It is noteworthy the fact that, at ages 60 and 70 years, approximately 30% and 20%, respectively, of Ache survived.

Another important fact is that the increased mortality reported for the Ache in wild conditions in the third phase is not at all indifferent for the overall mean duration of life (ML).

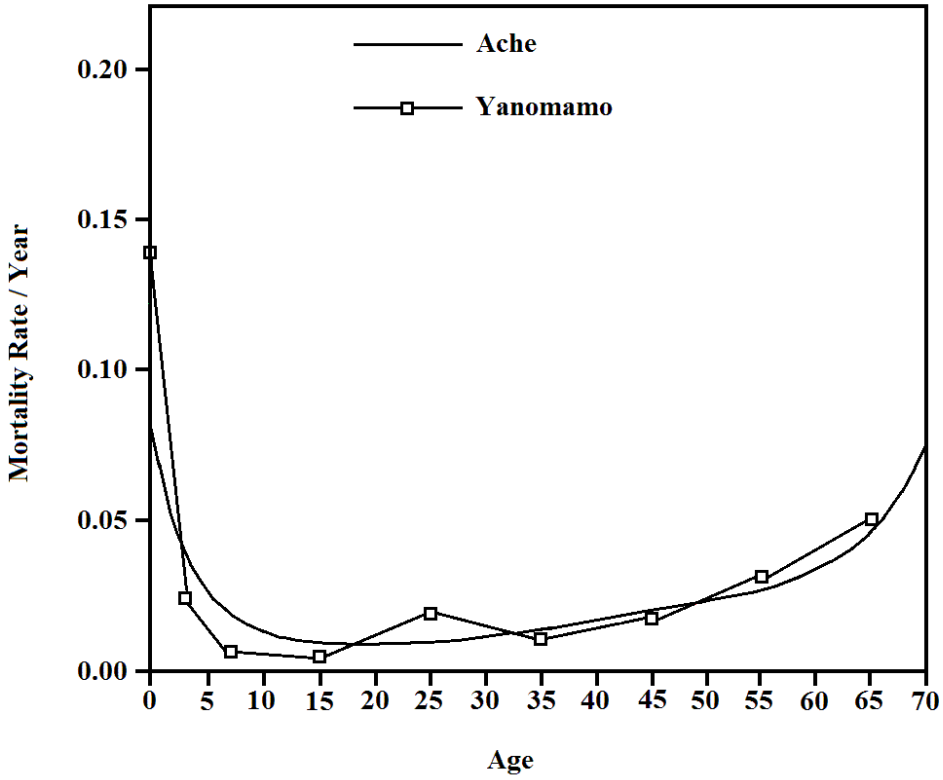


Figure 3 - Comparison between the life tables of Ache and Yanomamo, another hunter-gatherer population. The curves are essentially overlapping. Data from [Hill and Hurtado 1996] and [Early and Peters 1990].

Figure 4 shows the Ache life table in natural conditions and the hypothetical life table that would occur in the case that there was no age-related increase of mortality, i.e. in the case in which individuals would not grow old. In the real life table, the ML is equal to 38.8 years, while in the hypothetical life table ML is equal to 87.75 years, with a ratio (Ratio 1) between the two values equal to 2.260. It is worth noting that the length of the abscissas is extended up to 580 years, since - with a mortality rate of 0.9%/year, which is about the estimated minimum mortality in natural conditions – at that age even the 0.5% of the population would survive!

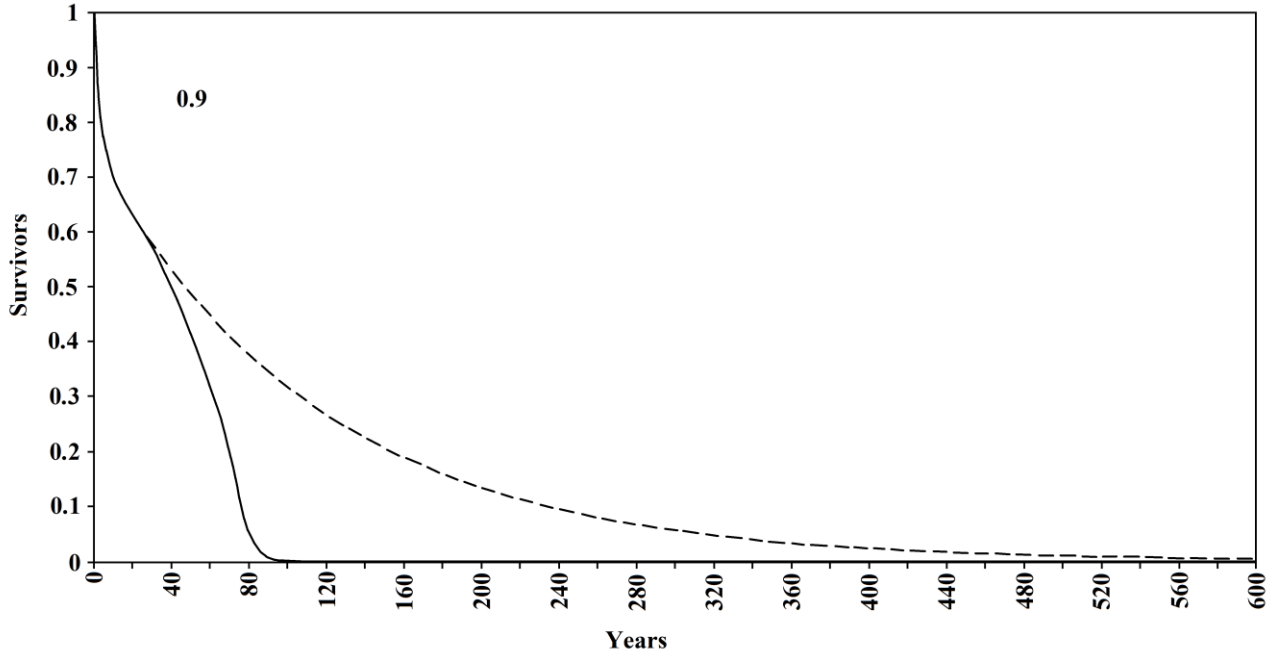


Figure 4 - Continuous line: life table of Ache in natural conditions (forest period), data from [Hill and Hurtado 1996]; dashed line: hypothetical life table without age-related increasing mortality.

If we consider only the individuals surviving at the age of 20 years (figure 5), the ML of Ache in the wild was 20 + 38.1 = 58.11 years, while for the hypothetical curve the ML is 20 + 116.04 = 136.04 years!, with a ratio (Ratio 2) between the two values equal to 3.044. In figure 5, abscissas extend up to 640 years, since at the age of 634 years about 0.5% of the population would survive!

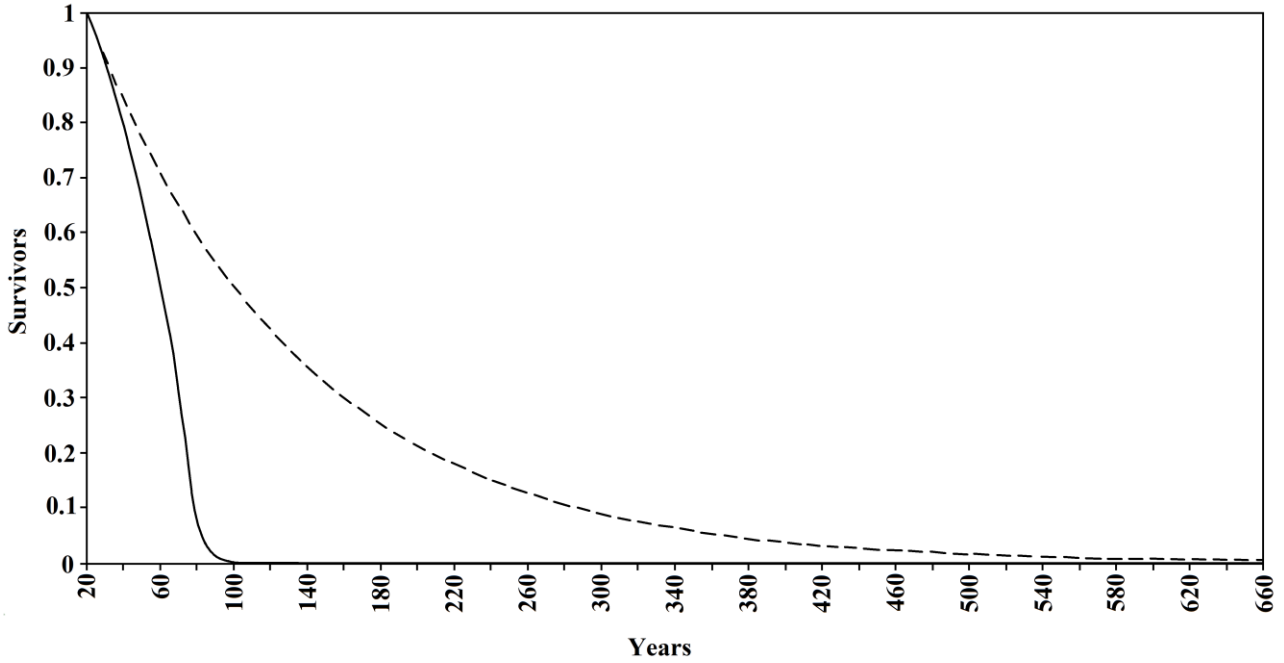


Figure 5 - The same of figure 4, but only individuals surviving at the age of 20 years are considered.

The hypothetical curves of figures 4-5, in the sections where the mortality is constant, is calculated by using the simple formula:

$$Y_t = Y_0 (1 - m_0)^t \quad (1)$$

where Y_0 = survivors at time 0; Y_t = survivors at time t; m_0 = minimum mortality; t = time.

Ratio 1 and Ratio 2 may be visualized, in each figure, as the ratio between the area defined by the hypothetical curve and the area subtended by the real curve.

These results, for a human population in wild conditions, are consistent with those reported in a previous work for various species of mammals [Libertini 1988], where the range of Ratio 1 and Ratio 2 were 1.55-3.21 and 2.42-5.09, respectively (Table 1).

Table 1 – Ratio 1: ratio between ML in natural conditions and ML in the hypothetical condition that the mortality rate remains stable on its lowest value, for various mammal species; Ratio 2: the same of Ratio 1 but excluding the first periods of life, i.e. before mortality reaches its lowest value. Data from [Libertini 1988], sources: (a) [Spinage 1972]; (b) [Laws 1966]; (c) [Laws 1968]; (d) [Spinage 1970]; (e) [Deevey 1947], and from [Hill and Hurtado 1996] (in italics). For all: sex combined; time unit = year.

Species	Source of data	Ratio 1	Ratio 2
Zebra	a	2.03	3.20
Hippopotamus	c	2.81	4.45
Elephant	b	1.67	2.42
Waterbuck	d	2.57	4.02
Warthog	a	1.55	2.85
Impala	a	2.64	3.85
Buffalo	a	2.21	3.46
Dall mountain sheep	e	3.21	5.09
H. sapiens (Ache people)	*[Hill and Hurtado 1996]*	*2.26*	*3.04*

These data show that, for the Ache people in wild conditions:

1) the increase of mortality is evident at ages existing in natural conditions;

2) such mortality increase strongly reduces the ML;

3) a substantial part of the population reaches ages commonly considered as senile.

Since the age-related increase of mortality, i.e. the age-related reduction of fitness alias aging, is clearly present in natural conditions, it is therefore certainly subjected to natural selection. This contradicts a basic assumption of the non-adaptive theories of aging (mutation accumulation theory [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987; Partridge and Barton 1993], antagonistic pleiotropic theory [Williams 1957; Rose 1991], disposable soma theory [Kirkwood 1977; Kirkwood and Holliday 1979]), according to which aging is not subject to natural selection for the rarity of elderly individuals under natural conditions: "… there is scant evidence that senescence contributes significantly to mortality in the wild … As a rule, wild animals simply do not live long enough to grow old. Therefore, natural selection has limited opportunity to exert a direct influence over the process of senescence" [Kirkwood and Austad 2000]

It is good to point out the following.

Although the percentages of Ache who reached the ages of 60 and 70 years are certainly remarkable and perhaps surprising (see fig. 1), the key issue is not so much the percentages of individuals who reach those ages but the percentage of individuals who, by age-related fitness reduction, die before. It is important to avoid the confusion between the gradual decline of fitness, or aging, and the end result of this process, namely the elderly individuals (see the previous quotation).

The age-related fitness decline is clearly a phenomenon subject to natural selection and in need of an evolutionary explanation, regardless of the percentage – in wild conditions - of individuals called "elders" because they have reached a certain threshold of decline, arbitrarily defined.

Another important fact is that these results are also in agreement with Ricklefs' observation [Ricklefs 1998] about an inverse correlation – documented in some mammal and bird species - between extrinsic mortality (m_0) and the deaths due to the age-related increasing mortality (P_s), a relation that disproves non-adaptive aging theories and supports adaptive aging hypotheses [Libertini 2008], which consider vertebrate aging a type of phenoptosis, or programmed death of an individual [Skulachev 1997, 1999a].

With a minimum mortality (m_0, approximately 1%/year) and a share of deaths due to senescence (P_s, on the ordinates) equal to about 67%, the position of the human species (Ache people) in the graph (fig. 7 from [Ricklefs 1998]) is highlighted with an open square.

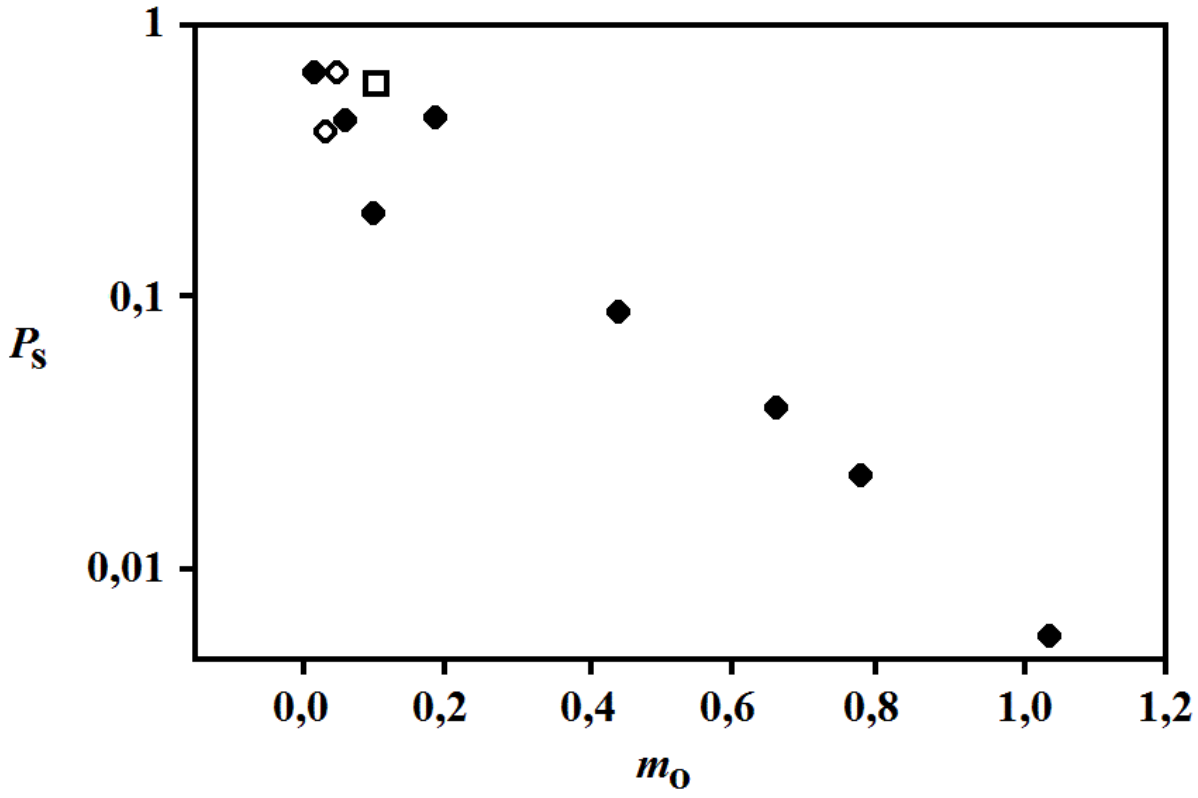


Figure 6 - Inverse relation between extrinsic mortality (m_0) and the proportion of deaths (P_s) due to intrinsic mortality (m_i), i.e. the deaths due to the age-related increasing mortality. Open rhombs = mammal species; solid rhombs = bird species; open square = Ache people in wild conditions. Data are from [Ricklefs 1998], Table 2 (p. 30). Ricklefs' fig. 7 (p. 34) has been redrawn and the datum from Ache people [Hill and Hurtado 1996] has been added. Ordinates are in logarithmic scale.

Part 2 - Data about death causes in Ache people and their implications for the hypothesis of age-related fitness decline as a defence against cancer

Death causes for Ache people under natural conditions (forest period) are shown in Table 2 [Hill and Hurtado 1996].

Table 2 – Causes of death for Ache people in natural conditions (forest period). Data from [Hill and Hurtado 1996]

In children aged 0-3 years

Violence and accidents		
Homicide/neglect	52	
Captured/shot by Paraguayan	21	
Accidents	3	
Total:	76	58.02%
Infections/ Intoxications		
Various causes	36	
Total:	36	27.48%
Congenital causes		
Unspecified newborn death/defective	17	
Childbirth/Mother had no milk	2	
Total:	19	14.50%
Total:	131	100%

In children aged 4-14 years

Violence and accidents		
Homicide/neglect	17	
Captured/shot by Paraguayan	56	
Accidents	11	
Total:	84	84.85%
Infections/Intoxications		
Various causes	11	
Total:	11	11.11%
Other causes		
Sick (unspecified)/sick in lungs	4	
Total:	4	4.04%
Total:	99	100%

In adult aged 15-59 years

Violence and accidents		
Buried alive	1	
Left behind	1	
Club fight	6	
Homicide, killed by Ache	3	
Shot by Paraguayan	46	
Captured by Paraguayan	1	
Snakebite	15	
Eaten by jaguar	8	
Hit by lightning	3	
Fell from tree/hit by falling tree	2	
Lost	1	
Total:	87	69.05%
Infections/Intoxications		
Fever after eating pichu larvae	8	
Fever after eating kracho larvae	5	
Fever after eating honey	6	
Fever after eating palm starch/corn	2	
Malaria	2	
Fever after touching blood	3	
Skin infection/sores on neck	2	
Swollen body/systemic infection	3	
Total:	31	24.60%
Other causes		
Childbirth	3	
Stomach problems	1	
Liver problems	1	
Sick in lungs	1	
Sick (unspecified)	1	
Old age	1	
Total:	8	6.35%
Total:	126	100%

In adult aged 60+ years

Violence and accidents		
Buried	1	
Left behind	2	
Club fight	2	
Shot by Paraguayan	4	
Eaten by jaguar	1	
Snakebite	3	
Lost	3	
Total:	16	59.26%
Other causes		
Diarrhea	3	
Sick (unspecified)	2	
Old age	6	
Total:	11	40.74%
Total:	27	100%

Overall number of deaths

Age 0-3 years	131	34.20%
Age 4-14 years	99	25.85%
Age 15-59 years	126	32.90%
Age 60+ years	27	7.05%
Total:	383	100%

It is worth noting that, in Ache people in wild conditions, the main causes of death for modern western populations (heart attacks, diabetes, hypertension, etc.) are absent. Moreover, cases of death by cancer are not reported, although, in the group “adult aged 60+ years”, some deaths attributed

generically to unspecified causes or to "old age" could be the result of neoplastic diseases. In any case, the data indicate that neoplastic diseases were rare events in Ache people in the forest period. This rarity of cancer in primitive conditions is not a new thing and is confirmed elsewhere.

For example, some anecdotal, but authoritative, information about the immunity from cancer of primitive populations are reported by Price [Price 1939]:

Dr. J. Romig, "a surgeon [of Anchorage] of great skill and with an experience among the Eskimos and the Indians, both the primitives and the modernized … stated that in his thirty-six years of contact with these people he had never seen a case of malignant disease among the truly primitive Eskimos and Indians, although it frequently occurs when they become modernized." (p. 83)

Dr. J. R. Nimmo, the government physician in charge for Torres Strait Islands people told dr. Price that: "in his thirteen years with them he had not seen a single case of malignancy, and seen only one that he had suspected might be malignancy among the entire four thousand native populations. He stated that during this same period he had operated on several dozen malignancies for the white populations, which numbers about three hundred." (p. 179)

These data can be used to settle the dispute between non-adaptive and adaptive interpretations of aging. Non-adaptive theories do not predict at all the existence of genetically determined and regulated mechanisms that progressively reduce the fitness. The existence of such mechanisms would indeed falsify non-adaptive theories, forcing their total abandonment.

On the contrary, for the plausibility of aging adaptive theories (e.g., [Libertini 1988]), it is indispensable the existence of genetically determined and regulated mechanisms for the age-related mortality increase [Libertini 2006, 2009a].

The age-related decline of vital functions is well explained as a consequence of the gradual decline of cell turnover, determined by the declining duplication capacities of stem cells by effect of telomere-telomerase system [Fossel 2004; Libertini 2009a]. This cell turnover decline is in accordance with the adaptive interpretation of aging, while, for the non-adaptive interpretation, it cannot be accepted as a mechanism causing the senescence and, therefore, a different rationale is absolutely necessary.

The absence of a valid or at least plausible explanation for these mechanisms, would indeed disprove all non-adaptive aging theories, transforming them, once and for all, from potentially valid theories in hypotheses of historical value.

This would change the validity of a huge number of works and experiences based on the assumption of aging as non-adaptive phenomenon. Some of the best researchers have therefore sought a plausible justification for the above-mentioned mechanisms different from that which explains them purely and simply as determinants of aging.

The current authoritative justification, in fact the only hypothesis widespread and supported in the scientific world, is that these restrictions are a general defense against cancer, because they would limit the pathological proliferation of any tumoral mass [Campisi 1997, 2000; Wright and Shay 2005].

If we compare the age-related increasing mortality in natural conditions (Ache population, fig. 7 – A) with the incidence of cancer and the deaths caused by it in a modern population (Great Britain, fig. 7 – B), at first sight this explanation could seem plausible.

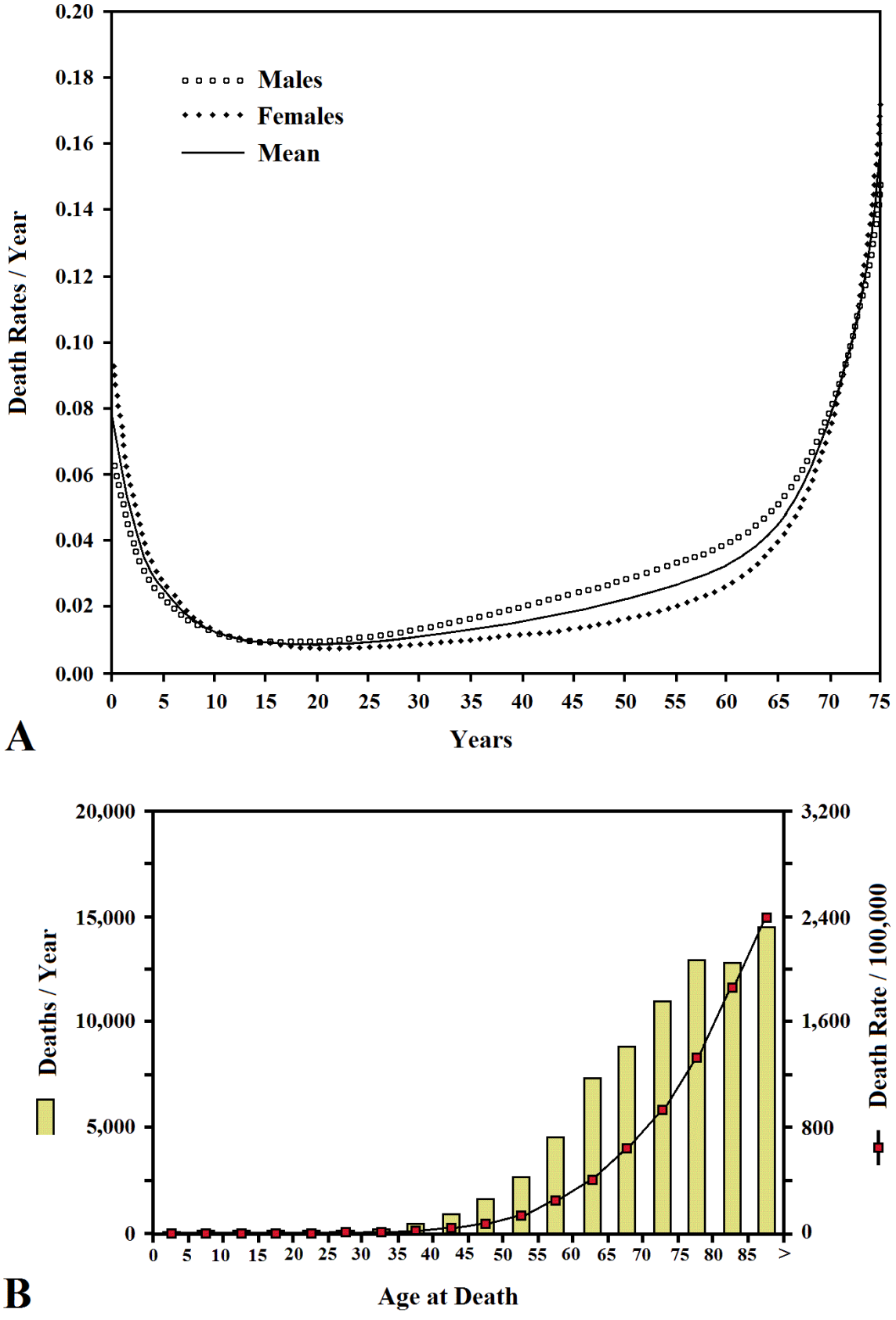


Figure 7 - A) Life table of Ache people in natural conditions (forest period); data from [Hill and Hurtado 1996]; B) Incidence of cancer and cancer deaths in a modern western population (UK) [General Register Office for Scotland 2010, etc.].

The appearance is misleading and data presented in such a way lead to a false confirmation of current opinion. It is necessary to draw, on a single graph and using a single scale, the rates of: A) total mortality for Ache under natural conditions; B) plausible cancer death rates in wild Aches; and, by comparison: C) cancer death rates in the modern population (figure 8). It is evident that, in the wild, the increase in overall mortality (over the minimum value of about 1%) anticipates and is much higher than the mortality from cancer both in the wild and in the modern population.

Moreover, under natural conditions, when there are the first possible cases of deaths by cancer, fitness decline has already determined the death of most individuals.

This completely disproves the hypothesis that the reduction of cell duplication capacities would be a defense against cancer: it would be like arguing that a defense against a deadly disease has the effect of mass-killing before the disease begins to kill!

On the contrary, according to the adaptive hypothesis of aging, the decline of defence against cancer results also from the decline of cell replication capacities and the cases of cancer in old age are part of aging characteristics [Libertini 2009a].

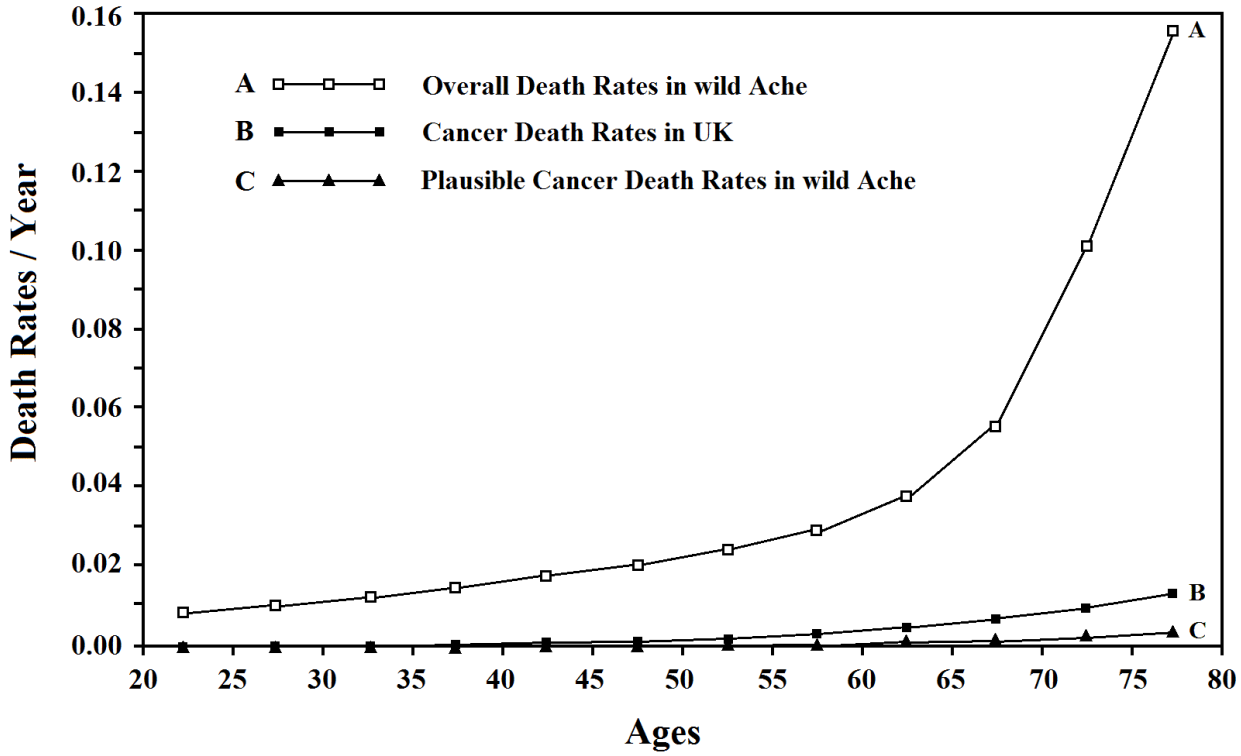


Figure 8 - Total mortality and plausible cancer death rates for Ache under natural conditions and cancer death rates in a modern population (Great Britain).

Conclusion

For aging research, the observation of a species under natural conditions is essential because it allows the evaluation of the phenomenon in the real conditions in which natural selection acts. This enables the avoidance of possible misconceptions on which any theoretical construction would be fallacious and the verification of whether, in natural conditions, some theoretical predictions are confirmed or falsified.

The study of the human species under natural conditions should give, and gives, results similar to those obtained from studies of other vertebrate species:

1) According to current non-adaptive interpretation of aging, very few or no individual reach old age and, so, aging cannot be directly influenced by natural selection [Kirkwood and Austad 2000].

However, data from a human population in the wild show that a significant proportion of the population reaches 60 and 70 years of age (about 30% and 20%, respectively).

Moreover, if we consider not the ill-defined concepts of “aging” and “old age” but the age-related mortality increase, a perfectly definable parameter, this phenomenon greatly reduces the mean duration of life (67% in Ache people in wild conditions!) and, therefore, it is absurd to consider this increase in mortality as something that is not influenced by natural selection or of no importance for selective process.

These data are consistent with similar data from other vertebrate species studied under natural conditions [Ricklefs 1998] and invalidate the main theoretical tenet of current evolutionary hypotheses about aging.

2) Non-adaptive aging theories predict a direct relation between the deaths due to the age-related increasing mortality and extrinsic mortality [Kirkwood and Austad 2000] while adaptive aging theories predict an inverse relation [Libertini 1988, 2008]. Ricklefs’ data on some mammal and bird species [Ricklefs 1998] confirm the prediction of adaptive aging hypothesis and falsify the opposite thesis. Ache life table is perfectly consistent with Ricklefs’ data.

3) Non-adaptive aging theories offer no valid explanation about the known limitations in cell replication and cell turnover that are the most plausible interpretation of age-related fitness decline.

The supporters of non-adaptive theories of aging, in the attempt to formulate a plausible explanation, hypothesize that these limits are a general defense against cancer. However, data from a population in natural conditions, and even from modern populations where cancer incidence is greatly increased, show that deaths from cancer are chronologically subsequent to the age-related increase in mortality and have a frequency much lower than the deaths caused by this increase in mortality. This shows that from a logical point of view the hypothesis that the aforesaid limits are a general defense against cancer is untenable.

In short, the study of a human population under natural conditions brings new arguments against the non-adaptive interpretation of aging. This hypothesis (actually, a group of ill defined theories) is commonly presented as the true and only scientific explanation of aging, but, when a hypothesis is widely and repeatedly disproved by empirical data, the scientific method requires that it must be considered as no longer scientifically acceptable and confined within the hypotheses of historical interest.

The blowgun of the humble Ache has a poison that turns out to be fatal to old and widespread beliefs.

Figure 9 - An Ache hunter.

Chapter 6

Libertini G (2014a) Programmed aging paradigm: how we get old. *Biochem. (Mosc.)* 79(10): 1004-16.

Programmed aging paradigm: how we get old

Giacinto Libertini

Abstract

According to the traditional explanations ("old paradigm"), aging is due to the progressive accumulation of heterogeneous damages that are insufficiently contrasted by natural selection. An opposite interpretation ("new paradigm") sees aging as selectively advantageous in terms of supra-individual natural selection, and this implies the indispensable existence of genetically controlled specific mechanisms that determine it. The aim of this work is to expound synthetically the progressive alterations that mark the aging by showing how these changes are clearly defined and regulated by genes. The possibility of such a description, based on sound evidence, is an essential element for the plausibility of the new paradigm, and a fundamental argument against the tenability of the old paradigm.

Introduction

For "aging", here precisely defined as "age-related progressive fitness decline/mortality increase", there are two antithetical general explanations [Goldsmith 2013] that have very important opposed implications and, so, deserve the definition of paradigms.

The first, here defined as "old paradigm", explains aging as the effect of various damaging factors insufficiently opposed by natural selection [Kirkwood and Austad 2000]. This paradigm implies that natural selection is successful in the achievement of numberless extraordinary functions and organs, while for contrasting aging it is capable only of limited effectiveness!

The second explanation, here defined as "new paradigm", justifies aging as a physiologic phenomenon determined and favored, in particular conditions, by supra-individual selection [Libertini 1988]. This paradigm implies that natural selection is successful in the achievement of aging as in other numberless extraordinary functions and organs!

The two paradigms, by definition, are alternative and incompatible with each other.

For the new paradigm, aging is a particular type of "phenoptosis", an important neologism proposed by Skulachev [Skulachev 1997; Libertini 2012a], which includes a large category of well-known phenomena [Finch 1990] characterized by the self-sacrifice of an individual, genetically caused/induced and regulated, and favored by natural selection, clearly in terms of supra-individual selection. Examples of phenoptosis types, well described by Finch [Finch 1990], are:

- Aphagy;
- Autogeny;
- Hormonally triggered senescence in plants;
- Death after spawning;
- Death of the male associated with mating/reproduction;
- Endotokic matricide;
- ...

and, according to the new paradigm:

- Aging ("slow phenoptosis" [Skulachev 2002b])

Here, I do not want to discuss arguments and evidence for or against the two paradigms, but only focus on a key topic: how we age, i.e. a general description of aging process in our species (as for mammals in general) on the basis of mechanisms genetically determined and regulated.

In fact, the new paradigm predicts and requires the existence of specific mechanisms, genetically determined and regulated, which cause aging [Libertini 2008]. On the contrary, the old paradigm excludes the possibility that such mechanisms exist: their existence would therefore demonstrate that the old paradigm is false [Kirkwood and Austad 2000].

Only manifest and accepted evidence will be used in the following exposition.

Evidence

1) Programmed Cell Death

Cells may die by necrosis, as a result of accidental events (infection, mechanical stress, trauma, ischemia, etc.), or by one of various types of programmed cell death (PCD), e.g.:

- keratinization of epidermis or hair cells;
- detachment of cells from the lining of intestines or other body cavities;
- osteocytes phagocytized by osteoclasts;
- transformation of erythroblasts in erythrocytes and their subsequent removal by macrophages;
- apoptosis, an ordinate process of self-destruction with non-damaging disposal of cellular debris that makes it different from necrosis. The phenomenon was for the first time described and clearly differentiated from necrosis in the observation of normal liver epatocytes [Kerr et al. 1972]. A pivotal function of apoptosis in vertebrates is related to the cell turnover in healthy adult organs, as well documented for many tissues and organs [Libertini 2009a].

It must be underlined that the term PCD is often used as synonymous of apoptosis, but this is a wrong simplification!

2) Cell Turnover

The endless death of cells by PCD is balanced by an equal proliferation of appropriate stem cells: "Each day, approximately 50 to 70 billion cells perish in the average adult because of programmed cell death (PCD). Cell death in self-renewing tissues, such as the skin, gut, and bone marrow, is necessary to make room for the billions of new cells produced daily. So massive is the flux of cells through our bodies that, in a typical year, each of us will produce and, in parallel, eradicate, a mass of cells equal to almost our entire body weight" [Reed 1999].

This "massive" turnover (about 690,000 cells per second!) is restricted by duplication limits caused by telomere-telomerase system (see below).

Cell turnover is a general pattern in vertebrates, but not for all animals (e.g., the adult stage of the worm *Caenorhabditis elegans* has a fixed number of cells) [Finch 1990].

The rhythm of cell turnover varies greatly depending on cell type and organ, e.g. in the intestinal epithelium "cells are replaced every three to six days" [Alberts et al. 2013], while "bone has a turnover time of about ten years in humans" [Alberts et al. 2013] and "the heart is replaced roughly every 4.5 years" [Anversa et al. 2006]. Other data about cell turnover rhythms are reported elsewhere [Richardson et al. 2014].

3) Limits in cell duplication

Cell replication, which is essential for cell turnover, is restricted by known mechanisms. Limits in the number of cell duplication were demonstrated by Hayflick in 1961 [Hayflick and Moorhead 1961]. Olovnikov hypothesised that, as DNA molecule shortens at each duplication, this could explain the finite number of duplications [Olovnikov 1971, 1973] (As a matter of fact, it was later documented, for many cell types, that telomere length shows an age-related progressively shortening [Takubo et al. 2010]). The end of DNA molecule (telomere) was demonstrated, first in a protozoan species, to be a simple repeated sequence of nucleotides [Blackburn and Gall 1978]. The discovery of telomerase, which added other sequences of the nucleotides, was a necessary explanation for cells, as those of germ line, capable of numberless divisions [Greider and Blackburn

1985]. Telomerase was shown to be repressed by regulatory proteins [van Steensel and de Lange 1997].

In cells where telomerase is not active, an infinite number of duplications is impossible for the progressive shortening of the telomere. Before telomeres reach their minimum length, two phenomena are described:

A) Cell senescence

In a cell in "cycling" state, the telomere, whatever its length, oscillates between two phases: "capped" and "uncapped" (by a protein complex). The probability of the uncapped phase is inversely proportional to the relative reduction of telomere length. In the uncapped phase, the cell is vulnerable to the transition to non-cycling state, i.e. to the activation of cell senescence program [Blackburn 2000] (Figure 1).

Cell senescence, which can also be activated by other factors, is determined by a mechanism in which the p53 protein is involved and is characterized by the block of the cell cycle besides a long series of changes in the expression of cellular genes. These changes also include modifications of cellular secretions that cause alterations of the extracellular matrix, inflammation, reduced secretion of important structural proteins such as elastin and collagen, and impairments of the surrounding cells [Fossel 2004].

Cell senescence with its stereotyped and predictable alterations has been described as a "fundamental cellular program" [Ben-Porath and Weinberg 2005].

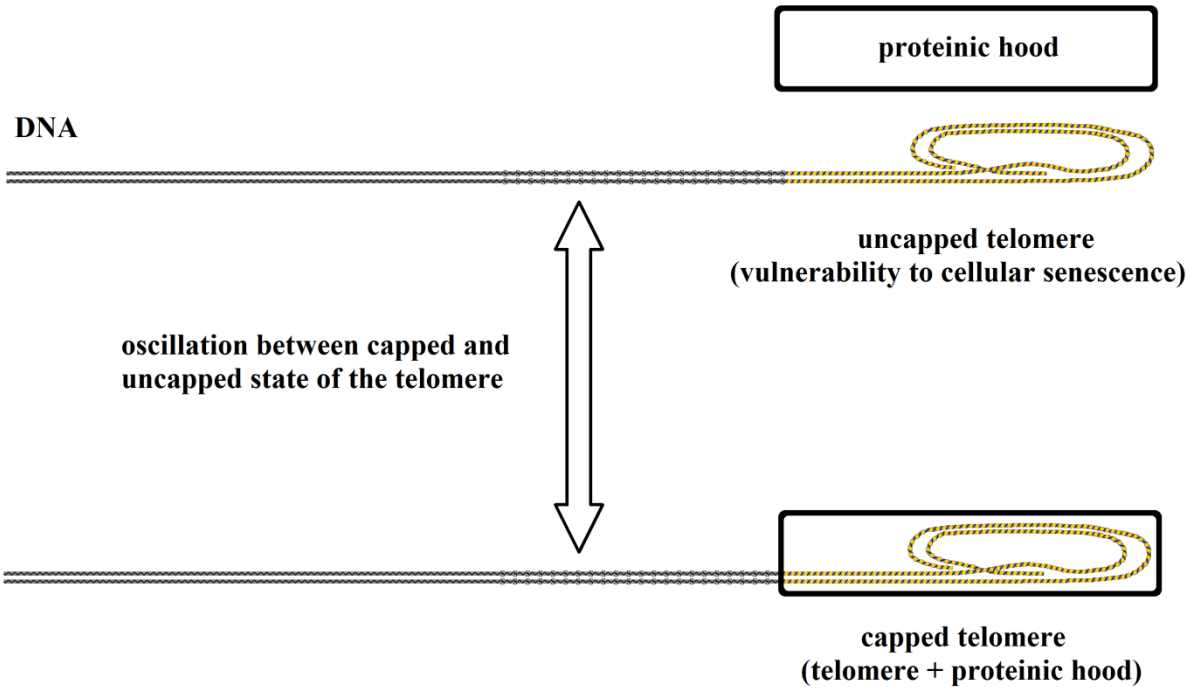


Figure 1 - Cell senescence. The telomere (DNA end part marked by dots) oscillates between two possible states: uncapped or capped by a proteinic hood. As telomere progressively shortens, the probability of being in the uncapped state increases and in this state the chromosome is vulnerable to homologous recombination and so to cell senescence [Blackburn 2000].

B) "Gradual" cell senescence

The progressive shortening of the telomeres has another effect. The telomere is covered (capped) by a protein complex that, as the telomere shortens, hides the subtelomeric DNA and causes transcriptional silencing (Figure 2).

"As the telomere shortens, the hood slides further down the chromosome .... the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity … These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel 2004]

These phenomena (cell senescence and "gradual" cell senescence) progressively affect the mean functioning of the cells in a tissue and the intercellular environment. But, by the activation of telomerase, cell senescence and all related alterations are completely cancelled [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

"Telomerase gene transfection ('telomerization') is an experimental determinant, switching somatic cells from mortal to immortal without disruption of the remainder of gene expression … This process of gene control is central to cell aging and experimental intervention. Resetting gene expression occurs in knockout mice, cloning, and other interventions, permitting us to make sense of how cell senescence causes aging in organisms. ... Cells do not senesce because of wear and tear, but because they permit wear and tear to occur because of an altered gene expression. Telomerization effectively replaces the score, allowing the gene to express their previous pattern. … cells do not senesce because they are damaged, but permit damage because they senesce. Homeostatic processes suffice indefinitely in germ cell lines; they suffice in somatic cells if senescence is abrogated." [Fossel 2004]

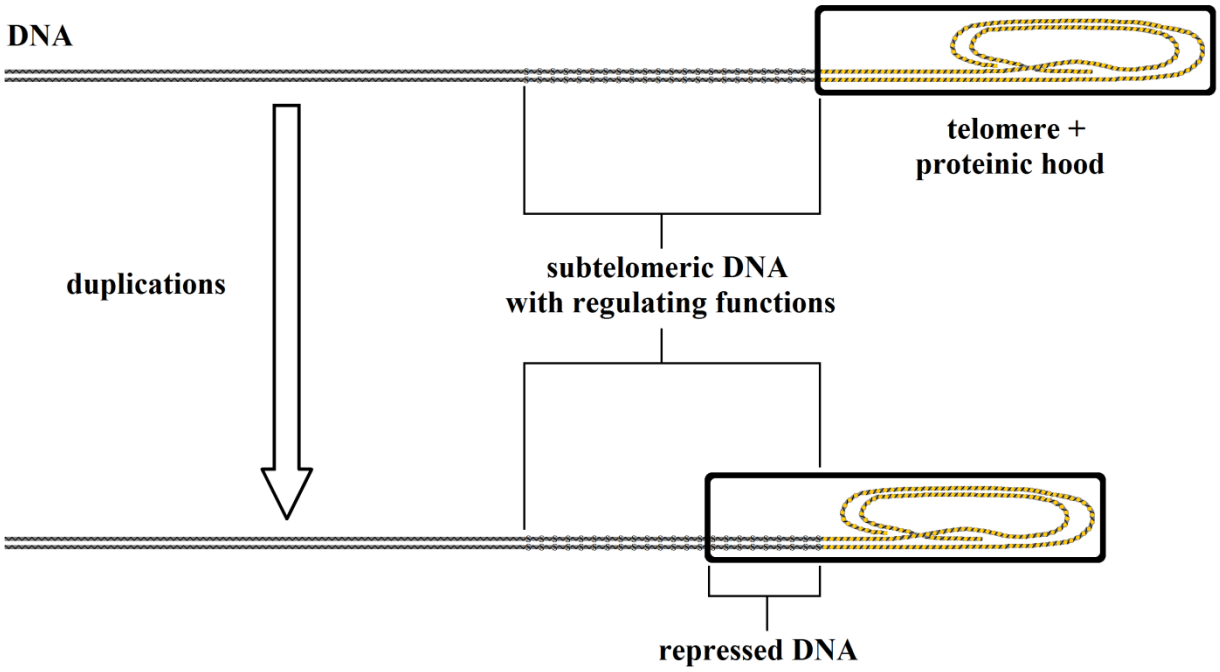


Figure 2 - "Gradual" cell senescence. Telomere (DNA end part marked by dots) is capped by a proteinic hood. As telomere progressively shortens, an increasing part of subtelomeric DNA is also capped by the proteinic hood. It is likely that the subtelomeric DNA has regulating functions and its progressive capping alters this regulation and so the expression of many genes [Fossel 2004].

With the passage of time (and with very different rhythms, varying for cell types and organs), in a tissue there is an increase of the percentage of cells:

- in senescent state;

- having functions more or less affected by the shortening of telomeres and the consequent interference in the subtelomeric region;
- affected by altered secretions of other cells (Figure 3).

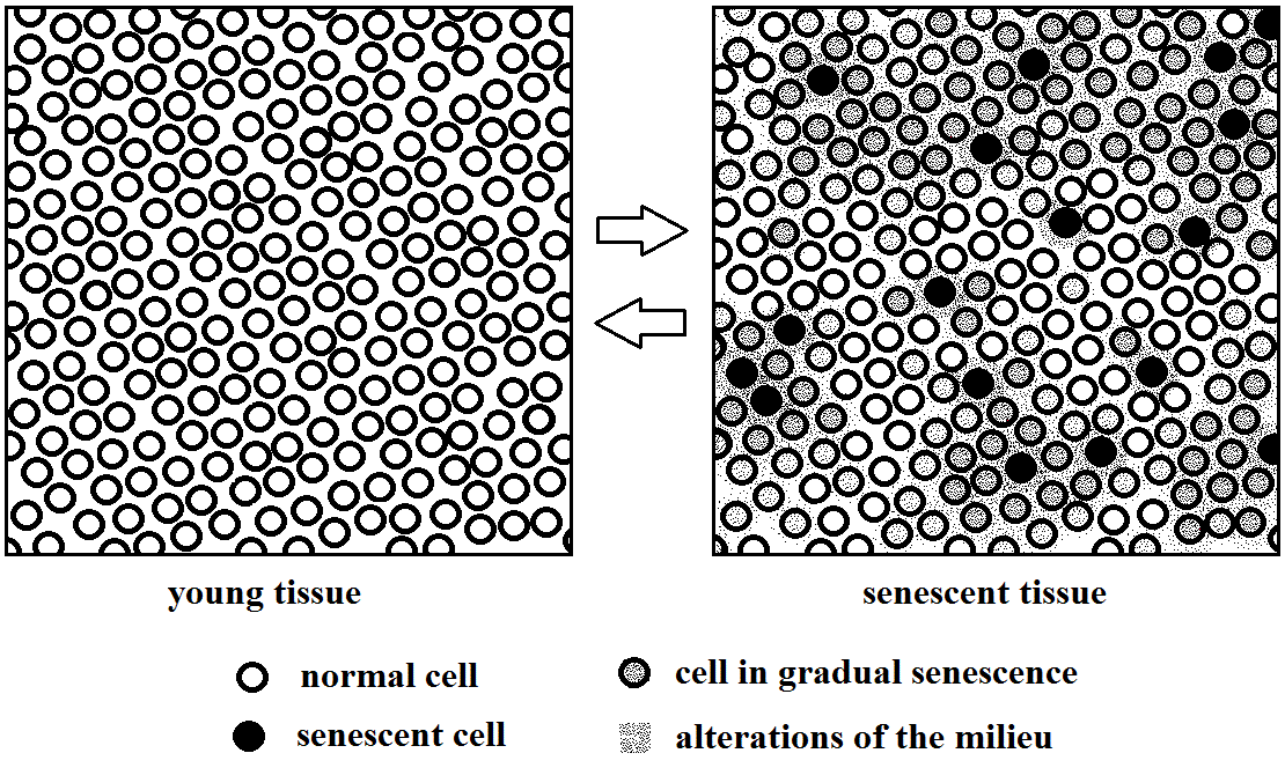


Figure 3 - Schematic interpretation of the transformation of a young tissue into a senescent tissue: "a modicum of cells display varying degrees of senescent change" [Fossel 2004]. The inverse transformation is demonstrated: *in vitro*, for single cells [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999] and, *in vivo*, in aged telomerase-deficient mice [Jaskelioff et al. 2011].

This leads, for each tissue and organ, to the "atrophic syndrome", which is characterized by [Libertini 2009a]:

a) reduced mean cell duplication capacity and slackened cell turnover;
b) reduced number of cells (atrophy);
c) substitution of missing specific cells with nonspecific cells;
d) hypertrophy of the remaining specific cells;
e) altered functions of cells with shortened telomeres or definitively in noncycling state;
f) alterations of the surrounding milieu and of the cells depending on the functionality of the senescent or missing cells;
g) vulnerability to cancer because of dysfunctional telomere-induced instability [DePinho 2000].

Aging in our species

The simple concepts outlined in the previous section permit an easy concise description of what characterizes aging. For brevity, this description – already, in part, expounded elsewhere [Libertini 2009a] - will be outlined here only for some types of cells and tissues.

A) Endothelium

The right functionality of endothelium is fundamental to avoid atherogenesis and its complications. The turnover of endothelial cells is assured by endothelial progenitor cells (EPCs), which derive from bone marrow. EPC number is inversely related to age, reduced by cardiovascular risk factors (cigarette smoking, diabetes, hypertension, hypercholesteremia, etc.), and increased by drugs, such as statins, which protect organ integrity [Hill et al. 2003].

A slackened turnover of endothelial cells increases the probability of endothelial dysfunction and, therefore, of diseases derived from altered blood circulation (cerebral ischemia, cardiac infarctions, and other diseases caused by compromised blood circulation). Moreover, with negative relation, the number of EPCs is a predictor of cardiovascular risk equal to or more significant than Framingham risk score [Hill et al. 2003; Werner et al. 2005].

In the senile state, diseases deriving from a compromised endothelial function increase exponentially in correlation with the age, even if other cardiovascular risk factors are absent [Tallis et al. 1998]. These factors anticipate and amplify the age-related risk [Tallis et al. 1998], while drugs with organ protection qualities, as statins [Davidson 2007], ACE-inhibitors and sartans [Weir 2007a] counter their effects.

B) Heart

An old and deep-rooted belief is that the heart is an organ incapable of regeneration and without cell turnover. On the contrary, "The Heart is a Self-Renewing Organ" [Anversa et al. 2006]: in a normal heart, every day about 3 million myocytes die by apoptosis and are replaced by cardiac stem cells: "the entire cell population of the heart is replaced approximatively every 4.5 years … The human heart replaces completely its myocyte population about 18 time during the course of life, independently from cardiac diseases." [Anversa et al. 2006].

Cardiac stem cells duplicate and differentiate, allowing myocyte turnover, and show age-related telomeric shortening and cell senescence [Leri et al. 2001; Urbanek et al. 2003; Chimenti et al. 2003]. In the old heart there is a global loss of myocytes, with a progressive increase in myocyte cell volume per nucleus [Olivetti et al. 1991]. The decreasing number of myocytes is due the progressive decline in the ability to duplication of cardiac stem cells [Anversa et al. 2006].

The decline of cardiac contractile capacities causes an enlargement of the heart that conceals the underlying atrophy of the contractile cells. So, in apparent contradiction, the heart chambers are dilated and the senile heart, although atrophic as number of cells, is morphologically hypertrophic [Aronow 1998].

"With aging, there is also a progressive reduction in the number of pacemaker cells in the sinus node, with 10 percent of the number of cells present at age 20 remaining at age 75. ... Age-associated left ventricular hypertrophy is caused by an increase in the volume but not in the number of cardiac myocytes. Fibroblasts undergo hyperplasia, and collagen is deposited in the myocardial interstitium." [Aronow 1998]

The heart shows "... some increase in the amount of fibrous tissue and fat in the atrial myocardium with a decrease in the number of muscle fibres, and loss of fibres in the bifurcating main bundle of His and at the junction of the main bundle and its left fascicles, with lesser degrees of loss in the distal bundle branches." [Caird and Dall 1978].

Drugs effective in "organ protection", as ACE-inhibitors, sartans and statins, are effective in the prevention of atrial fibrillation [Jibrini et al. 2008; Fauchier et al. 2008].

C) Skin

"Stratum corneal thickness is unchanged in the elderly although its moisture content and cohesiveness are reduced coupled with an increase in renewal time of damaged stratum corneum. ... Human epidermis is highly proliferative but in a steady-state condition dependent, as are other self-renewing structures, on slowly cycling, undifferentiated stem cells. These stem cells are located within the basal compartment of the epidermis – the nonserrated keratinocytes at the tips of the epidermal rete ridges. Loss of rete ridges and consequent flattening of the dermal-epidermal

junction is a hallmark of intrinsically aged skin. Such flattening results in a reduction in mean surface area of the dermal-epidermal junction. One study has estimated a reduction in mean area of dermal-epidermal junction/mm^2 from 2.6 at age 21 to 40 years to 1.9 at age 61 to 80 years. These changes are accompanied by a reduction in microvilli – cytoplasmic projections from basal keratinocytes into the dermis. ... The rate of epidermal renewal is reduced in the skin of individuals aged 60 years or greater. ... Melanocytes are decreased in number in intrinsically aged epidermis, although the estimates of this decrease vary from study to study according to the methodologies used to quantitate melanocyte numbers. This said, the reduction is in the order of 8 to 20 percent per decade compared to young adult skin. ... The number of Langerhans cells is reduced in intrinsically aged epidermis, ... Gilchrest et al. demonstrated that subjects aged 62 to 86 years had a 42 percent reduction in the number of Langerhans cells in sun-protected skin as compared to young subjects aged 22 to 26 years. ... Numbers of dermal fibroblasts decrease with age ... Aged skin is relatively hypovascular, particularly due to loss of small capillaries that run perpendicular to the dermal-epidermal junction and form capillary loops. This loss is concomitant with the loss of epidermal rete ridges. Blood vessels within the reticular dermis are reduced in number and their walls are thinned. ... There is an approximate 50 percent reduction in numbers of mast cells in intrinsically aged skin. ... Eccrine glands are reduced in number and function in aged skin. ... Age probably reduces and disorganizes the nerve supply of the skin; indeed there is an approximate two-thirds reduction in numbers of Pacinian and Meissner's corpuscles with age. ... Hair, particularly scalp hair, is lost with age in both sexes. ... Nails grow more slowly in the elderly ... The study of aging skin particularly as a consequence of the ready accessibility of cutaneous tissue is one that presents a paradigm for aging of other organs." [Griffiths 1998]

In derma, as a likely consequence of the exhaustion of specific stem cells, a general reduction of all its components (melanocytes, Langerhans cells, dermal fibroblasts, capillaries, blood vessels within the reticular dermis, mast cells, eccrine glands, hair. etc.) is reported and nails grow more slowly [Griffiths 1998].

D) Orofacial Tissues and Organs

"Atrophy of the fascial planes within the eyelids may lead to herniation of the orbital fat into the lid tissue, producing the 'bags under the eyes' frequently seen in the elderly. Atrophy or disinsertion of the aponeurosis of the levator palpebrae muscle, which ordinarily supports the upper eyelid, may cause the opened lid to fail to uncover the pupil, as seen in senile ptosis, despite normal levator muscle function ... Secretory function of the lacrimal glands declines with age ..." [Brodie 1998]

"Structural changes in human oral epithelia with aging include thinning of the epithelial cell layers (e. g., thinning of the lingual epithelial,) diminished keratinization, and simplification of epithelial structure. ... Histologic studies of aging salivary glands show a gradual loss of acinar elements, a relative increase in the proportion of ductal elements, an increase in inflammatory infiltrates, and an increase in fibrofatty tissue." [Devlin and Ferguson 1998]

"The number of taste buds decreases after age 45, resulting in a decrease in taste sensation..." [Reinus and Brandt 1998]

E) Hematopoietic cells

" ... peripheral blood lymphocyte populations do seem to show a significant change in age, with a fall in total numbers. CD4+ T-helper cells, responsible for major histocompatibility complex class II restricted recognition of foreign antigen and subsequent activation of CD8+ T-suppressor, B-lymphocyte, and granulocyte effector cells of the immune response, show an overall decline with age accompanied by a reduction in capacity to produce virgin CD4+ CD45RA T cells. ... Gradual involution of red marrow continues but is especially marked after the age of 70 years when iliac crest marrow cellularity is reduced to about 30 percent of that found in young adults." [Gilleece and Dexter 1998]

In older people, fewer neutrophils arrive at the skin abrasion sites [MacGregor and Shalit 1990]. T lymphocytes proliferative capacity for nonspecific mitogens shows an age-related reduction [Schwab et al. 1985; Murasko et al. 1986].

Age-related functional decline in hematopoietic stem cells is a likely limiting factor for longevity in mammals [Geiger and van Zant 2002].

F) Gastrointestinal System

In aged individuals, various types of stomach cells show progressively shorter telomere, and atrophy of gastric mucosa, with or without Helicobacter pylori infection, is associated with shorter telomeres [Takubo et al. 2010].

"Using postmortem material, Chacko et al. (1969) found that in an Indian population the shape of villi changed on aging. The youngest subjects had finger-shaped villi, but the frequency of broad villi and convolutions increased in specimens from older people. Webster and Leeming (1975a) described similar changes when fresh jejunal specimens from geriatric patients were compared with normal young controls. They found that in the elderly broader villi were more common, and in addition the villi were significantly shorter. ... Andrew and Andrew (1957) noticed an increase in the amount of fibrous tissue between the crypts of Lieberkuhn and a general reduction of cellularity in older mice. ... Lesher, Fry and Kohn (1961), Lesher and Sacher (1968) and Fry, Lesher and Kohn (1961), using autoradiography and tritiated thymidine, showed a prolonged generation time for duodenal crypt cells in old animals and an increased cell transit time (for cells to progress from the crypts to villous tips). In conclusion, the possible expected age changes in the small bowel of man are an increase in broad villi, with a reduction in villous height. These changes may be due to reduced cell production." [Webster 1978]

In the colon, atrophy of the *muscolaris propria* and an increase in the amount of fibrosis and elastin has been shown [Baime et al. 1994].

In each intestinal crypt, there are four to six stem cells that with their intensive duplication activity renew continuously the epithelium of the small intestine [Barker et al. 2007]. These changes, surely due to a declining mitotic activity of crypt stem cells, as hypothesised from a long time [Webster 1978], reduce intestinal functionality and, likely, overall fitness.

G) Skeletal muscle

Age-related muscle atrophy, both in terms of overall muscle bulk and of the size of individual fibers, is well-known [Grimby et al. 1982; Lexell et al. 1988] (Figure 4).

"These changes are to some extent dependent on the fallout of anterior horn cells that occurs with age, but this does not completely explain the process of aging atrophy. In detailed studies it has been shown that the progressive reduction that occurs in muscle volume with aging can be detected from age 25 years and that up to 10 percent of muscle volume is lost by age 50 years. Thereafter the rate of muscle volume atrophy increases, so that by 80 years almost half the muscle has wasted. ... Both reduction in fiber number and fiber size are implicated in the loss of muscle volume." [Cumming 1998]

In Duchenne muscular dystrophy, chronic destruction of myocytes, continually replaced by duplication of stem cells until these are exhausted, has been described [Adams et al. 2001].

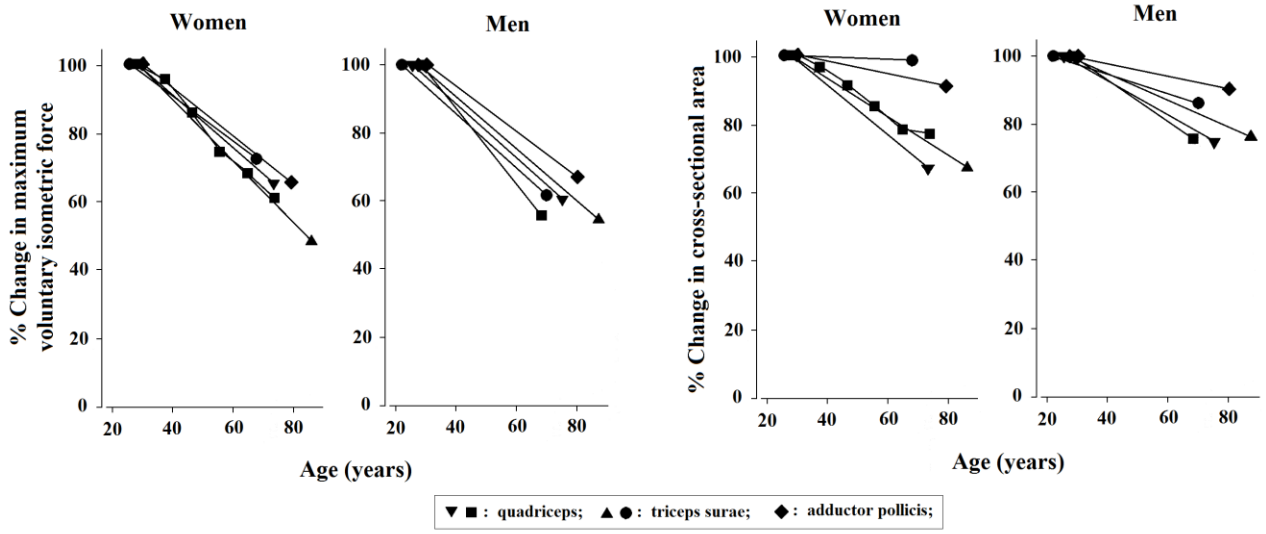


Figure 4 - Age-related decline in maximum voluntary isometric force and in cross-sectional area for various muscles [Young et al. 1984, 1985; Davies et al. 1986; Vandervoort and McComas 1986; Klitgaard et al. 1990; Philips et al. 1992; Rutherford and Jones 1992].

H) Liver

The volume of liver shows an age-related declines [Marchesini et al. 1988], both in proportion to body weight and in absolute values [Wynne et al. 1989]. This reduction has been estimated to be about 37 percent between ages 24 and 91 [Marchesini et al. 1988]. Liver blood flow also declines with age, by about 53 percent between ages 24 and 91 [Marchesini et al. 1988] but, while liver size declines with age, hepatocytes increase in size, unlike in the liver atrophy caused by starvation [Watanabe and Tanaka 1982; David and Reinke 1988].

The chronic destruction of hepatocytes by hepatitis, alcoholism or other factors is a known cause of cirrhosis: by exhaustion of duplication capacities of hepatocyte stem cells, the atrophic syndrome transforms the liver, often with the complication of epatocarcinomas caused by dysfunctional telomere-induced instability [DePinho 2000; Artandi 2002].

I) Pancreatic β-cells

Pancreatic β-cells show turnover [Finegood et al. 1995] and an insufficient substitution of β-cells exhausted by metabolic stress has been suggested as cause of type 2 diabetes mellitus [Bonner-Weir 2000; Cerasi et al. 2000]. Diabetes is a manifestation of Werner syndrome [Martin and Oshima 2000], as a likely consequence of an insufficient replacement of apoptotic β-cells by impaired replication of β-cell stem cells. Diabetes frequency shows an age-related increment [Harris et al. 1987] likely caused by the progressive exhaustion of β-cell turnover.

Drugs that are effective in "organ protection", as ACE-inhibitors and sartans and statins, reduce the risk of diabetes [McCall et al. 2006; Ostergren 2007].

J) Bone

"Once middle age is reached, the total amount of calcium in the skeleton (i.e., bone mass) starts to decline with age ... This is associated with changes in skeletal structure, resulting in it becoming weaker and more prone to sustaining fractures. For example, the bony cortex becomes thinner due to expansion of the inner medullary cavity, the trabecular network disintegrates, and there is an accumulation of microfractures. ... Bone loss in the elderly is largely a result of excess osteoclast activity, which causes both an expansion in the total number of remodelling sites and an increase in the amount of bone resorbed per individual site. .... Bone loss in the elderly is also thought to involve an age-related decline in the recruitment and synthetic capacity of osteoblasts" [Dieppe and Tobias 1998]

“Involutional bone loss ... starts between the ages of 35 and 40 in both sexes, but in women there is an acceleration of bone loss in the decade after menopause. Overall, women lose 35 to 50 percent of trabecular and 25 to 30 percent of cortical bone mass with advancing age, whereas men lose 15 to 45 percent of trabecular and 5 to 15 percent of cortical bone. ... Bone loss starts between the ages of 35 and 40 years in both sexes, possibly related to impaired new bone formation, due to declining osteoblast function.” [Francis 1998].

K) Lungs

“The most important age-related change in the large airways is a reduction in the number of glandular epithelial cells ... the area of the alveoli falls and the alveoli and alveoli ducts enlarge. Function residual capacity, residual volume, and compliance increase. ...” [Connolly 1998] Lung volumes (FEV1, FVC) show an age-related reduction [Enright et al. 1993].

Statins contrast the decline in lung function and their anti-inflammatory and antioxidant properties could explain this effect [Alexeeff et al. 2007]. Alternatively, it could be the consequence of actions on type II alveolar epithelial cells analogous to those on endothelial cells [Hill et al. 2003].

L) Kidneys

“Age-induced renal changes are manifested macroscopically by a reduction in weight of the kidney and a loss of parenchymal mass. According to Oliver, the average combined weight of the kidneys in different age groups is as follows: 60 years, 250 g; 70 years, 230 g; 80 years, 190 g. The decrease in weight of the kidneys corresponds to a general decrease in the size and weight of all organs. Microscopically, the most impressive changes are reductions in the number and size of nephrons. Loss of parenchymal mass leads to a widening of the interstitial spaces between the tubules. There is also an increase in the interstitial connective tissue with age. The total number of identifiable glomeruli falls with age, roughly in accord with the changes in renal weight.” [Jassal et al. 1998]

Microalbuminuria, a reliable marker of nephropathy, is “predictive, independently of traditional risk factors, of all-cause and cardiovascular mortality and CVD events within groups of patients with diabetes or hypertension, and in the general population ... It may ... signify systemic endothelial dysfunction that predisposes to future cardiovascular events” [Weir 2007b].

M) Cell types without turnover

M-1) Photoreceptor cells

Photoreceptor cells (cones and rods) are highly differentiated nervous cells without turnover, but metabolically depending on other cells with turnover, retina pigmented cells (RPCs), which are highly differentiated gliocytes.

Each day, with an extraordinary metabolic activity, every RPC phagocytizes about 10% of the membranes with photopsin molecules of about 50 photoreceptor cells. With the age-related decline of RPC turnover, the deficiency of their function kills the photoreceptors not served [Berger et al. 1999]. This is above all manifested in the functionality of the more sensitive part of the retina, the macula, from which the name “age-related retina macular degeneration” (ARMD) [Fine et al. 2000].

ARMD affects 5%, 10% and 20% of subjects 60, 70 and 80 years old, respectively [Berger et al. 1999], and it is likely that a large proportion of older individuals suffer from ARMD.

Risk factors for endothelial cells as smoking, diabetes, and obesity are risk factors for ARMD too [Klein et al. 2007].

M-2) Neurons of the central nervous system

Neurons are perennial cells but their vitality depends on other cells (e.g., microglia, a type of gliocytes) which show turnover. The hypothesis that Alzheimer Disease (AD) is caused by cell

senescence of microglia cells has been proposed [Fossel 1996, 2004; Flanary 2009; Libertini 2009a].

Microglia cells degrade β-amyloid protein [Qiu et al. 1998; Vekrellis et al. 2000] and this function is known to be altered in AD [Bertram et al. 2000] with the consequent noxious accumulation of the protein.

Telomeres have been shown to be significantly shorter in patients with probable AD than in apparently healthy control subjects [von Zglinicki et al. 2000]. AD could have, at least partially, a vascular aetiology due to age-related endothelial dysfunction [Fossel 2004], but "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." [Fossel 2004]

An interesting comparison between AD and ARMD is possible: both are probably determined by the death of cells with no turnover as a likely consequence of the age-related failure of cells with turnover (Figure 5) [Libertini 2009a]. Moreover, AD shows an age-related increasing frequency as ARMD: it affects 1,5% of USA and Europe population at age 65 years and 30% at 80 [Gorelick 2004] and a centenarian has a high probability of suffering from it.

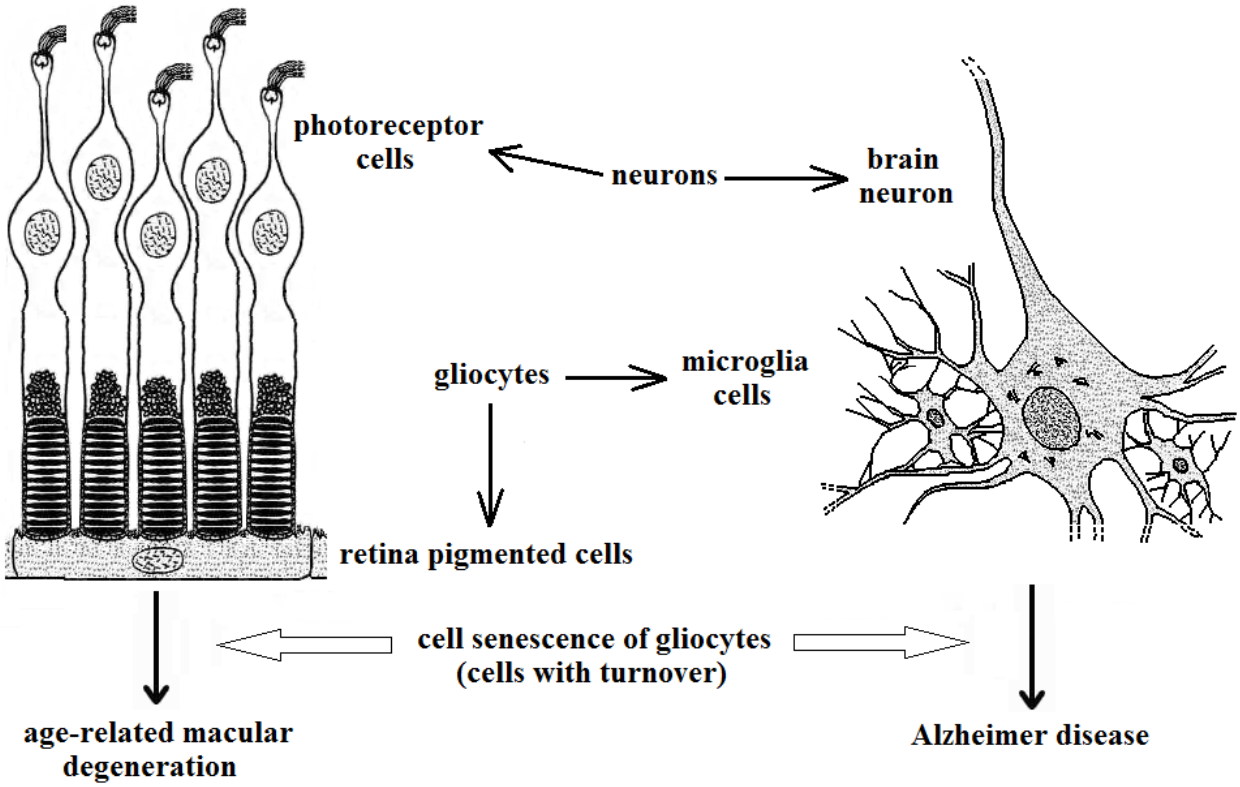


Figure 5 - Some retina photoreceptors and a brain neuron (both specialized neurons) served by two types of differentiated gliocytes (RPCs and microglia cells, respectively). Cell senescence of RPCs and microglia cells cause ARMD and AD, respectively.

M-3) Crystalline Lens

The crystalline lens has no cell in its core, but its functionality depends on lens epithelial cells that show turnover [Tassin et al. 1979]. "Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells" [Martin and Oshima 2000], which is consistent with age-related reduction in growth potential for lens epithelial cell reported for normal human subjects [Tassin et al. 1979].

Statins lower the risk of nuclear cataract, the most common type of age-related cataract [Klein et al. 2006]. This has been attributed to "putative antioxidant properties" [Klein et al. 2006], but could

be the consequence of effects on lens epithelial cells analogous to those on endothelial cells [Hill et al. 2003].

N) Other Organs or Tissues

Telomere dysfunction for cells in replicative senescence, in particular those, mostly epithelial, with higher turnover, is a significant cause of cancer in older individuals [DePinho 2000].

Finally, we must consider the numberless complications for many organs deriving by the progressive impairment of endothelial, neuronal and immunological functions and, in general, by the interlacement of the decline of several functions [Tallis et al. 1998].

Pathology of aging phenomenon

As any function, aging must have its pathology, e.g.:

A) Various non-genetic factors increase apoptotic rates and therefore aging manifestations (see "risk factors" in the previous section).

B) Among various genetic diseases that display aging characteristics, two are particularly interesting: dyskeratosis congenita (DC) and Werner syndrome (WS). In DC, there is an inherited defect in telomerase function and "problems trend to occur in tissues in which cells multiply rapidly – skin, nails, hair, gut and bone marrow ... People with DC, as well as late-generation telomerase-deficient mice, also suffer from a higher rate of cancer. This can likewise be explained by the lack of telomerase, which result in unstable chromosomes – in DC sufferers and the mutant mice, many chromosomes fuse end to end, probably because their telomeres are terminally eroded (de Lange & Jacks 1999)" [Marciniak and Guarente 2001]. In DC, there is no alteration for tissues with no telomerase expression. On the contrary, in WS, there are problems for DNA-recombination process and so for telomere maintaining, and the disease shows alterations for tissues with lower cell turnover and a closer version of normal ageing [Martin and Oshima 2000; Marciniak and Guarente 2001].

These facts are summarized in Table 1.

Table 1 – Manifestations of aging and of its pathologies

Cell type	**Manifestations of aging** [Tallis et al. 1998; Fillit et al. 2010]	**Risk factors and their effects** (see references in "Aging in our species" section)	**Werner syndrome** [Martin and Oshima 2000]	**Dyskeratosis congenita** [Marciniak and Guarente 2001]
Endothelial cells	Atherosclerosis (→ miocardial infarction and other vascular problems)	Smoking, hypertension, hypercholesteremia, diabetes (→ atherosclerosis)	Atherosclerosis	
Alveolar type II cells	Emphysema	Smoking, chronic inhalation of noxious substances (chronic bronchitis, emphysema);		Fibrosis
Cardiac myocytes	Cardiac insufficiency	Myocarditis (→ dilatative cardiomyopathy)		
Epidermis cells	Skin atrophy	Excessive sun exposure (→ photoaging)	Skin atrophy, regional atrophy of subcutaneous tissue, ulcerations in parts exposed to traumas	Abnormal pigmentation, nail dystrophy
Glomerular cells	Renal insufficiency	The same as for endothelial cells (→ renal insufficiency)		
Hepatocytes	Hepatic atrophy	Chronic hepatitis, alcoholism (→ cirrhosis)		Cirrhosis, hepatic carcinoma
Intestinal cells	Intestinal atrophy			Gut disorders
Lens epithelial cells	Cataract	Exposure to radiations (→ cataract)	Cataract	
Microglia cells	AD	The same as for endothelial cells (→ AD)		
Retina pigmented cells	ARMD	The same as for endothelial cells (→ ARMD)		
Myocytes	Muscle atrophy	Specific genetic defects (→ muscular dystrophies)	Muscle atrophy	
Osteoblasts	Osteoporosis		Osteoporosis	
Pancreatic β-cells	Latent or mild diabetes	Hyperalimentation, specific viral infections	Type II diabetes mellitus	
Hair	Progressive baldness		Premature greying and thinning of hair	Alopecia
Testes	Diminished fertility, testicular atrophy		Diminished fertility, premature testicular atrophy	Hypogonadism
Bone marrow	Reduction of various cell types			Failure to produce blood cells
Oral cavity	Atrophy of oral mucosa			Leukoplakia (precancerous oral lesions)

Conclusion

More than a century ago, Weismann proposed that aging was determined by an evolved limitation in the replicative capacities of cells [Weismann 1892]. As well discussed elsewhere

[Kirkwood and Cremer 1982], the famous wrong experiments of Carrel and Ebeling, the uncertainties and contradictions of the same Weismann, and various theoretical objections wiped out the scientific memory of this hypothesis.

In 1959, the great physicist Leo Szilard proposed that, by action of some factors, the somatic cells decrease with age at an accelerating rate and that this was the key factor in ageing [Szilard 1959]. In modern times, this idea has been developed and strengthened by experimental data [Skulachev 2012].

This shows that the importance of the relationship between aging and age-related loss of cellularity has already been pointed out by others. However, the key question is whether this correlation and its causes are in support or against, compatible or incompatible with each of the two opposing paradigms.

Programmed cell death, "gradual" cell senescence and cell senescence program, cell duplication limits (variable, according to cell types and influenced by various physiological and pathological events), cell turnover and its limitations (variable, depending on cell types) are all phenomena genetically determined and regulated (with clear differences among the species).

Some features of these phenomena have no justification in terms of physiological factors other than as aging determinants. In particular, the supporters of old paradigm try to justify the limits in cell replication as a general defense against cancer [Campisi 1997; Wright and Shay 2005].

But:

- species with negligible senescence (i.e., with individuals showing no age-related decay) have no age-related reduction of telomerase activity and no increase in mortality due to cancer [Libertini 2008];
- in the human species, studied under natural conditions, fitness decay/mortality increment (i.e., aging) reaches significant levels without a contemporaneous detectable incidence of cancer mortality. It is untenable that a defense against cancer kills large part of the population before cancer as cause of death becomes detectable [Libertini 2013].

Moreover, the above-said justification is even more unlikely to explain:

- gradual cell senescence and cell senescence program and their damaging effects;
- the regulatory functions of subtelomeric DNA (a condition indispensable for "gradual" cell senescence), i. e. the position of pivotal parts of DNA where they are more vulnerable when telomere shortens.

The mechanisms, genetically determined and regulated, here summarized, are a likely cause of the age-related progressive deterioration of all functions, namely aging. Their existence is predicted by the new paradigm and indeed are essential for its validity.

On the contrary, they are not expected by the old paradigm and are in complete contrast with it.

The explanation of aging through the new paradigm allows:

- a rational and consistent interpretation of the manifestations of aging, its pathologies included (e.g.: AD, ARMD, dyskeratosis congenita, Werner syndrome);
- the prospect of being able to change aging manifestations and even to obtain a full control of aging through scientific procedures that are technically feasible [Libertini 2009a, 2009b]. The exposition and discussion of this last prospect, already briefly expounded elsewhere [Libertini 2009b], is however outside and beyond the limits of time and of topic of this work.

Chapter 7

Libertini G (2015a) Non-programmed versus programmed aging paradigm. *Curr. Aging Sci.* 8(1): 56-68.

Non-programmed versus programmed aging paradigm

Giacinto Libertini

Abstract

There are two opposite paradigms to explain aging, here precisely defined as "age-related progressive mortality increase, i.e. fitness decline, in the wild". The first maintains that natural selection is unable to maintain fitness as age increases. The second asserts that, in particular ecological conditions, natural selection favors specific mechanisms for limiting the lifespan. The predictions derived from the two paradigms are quite different and often opposing. A series of empirical data and certain theoretical considerations (non-universality of aging; great inter-specific variation of aging rates; effects of caloric restriction on lifespan; damage of aging for the senescing individual but its advantage in terms of supra-individual selection; existence of fitness decline in the wild; proportion of deaths due to intrinsic mortality inversely related to extrinsic mortality, when various species are compared; impossibility of explaining the age-related fitness decline as a consequence of genes that are harmful at a certain age; age-related progressive decline of cell turnover capacities; cell senescence; gradual cell senescence) are compared with the predictions of the two paradigms and their compatibility with each paradigm is considered. The result is that the above-mentioned empirical data and theoretical considerations strongly contradict and falsify in many ways all theories belonging to the first paradigm. On the contrary, they are consistent or compatible with the predictions of the second paradigm.

Introduction

Aging, here defined as "increasing mortality with increasing chronological age in populations in the wild" [Libertini 1988], alias "age-related progressive mortality increase, i.e. fitness decline, in the wild", is a phenomenon that is interpreted in two completely opposite ways [Libertini 2008].

The first, here referred to as the "old paradigm", sees aging as due to a variety of damaging factors that, with the passage of time, progressively undermine organism efficiency. Harmful actions are counteracted by natural selection, but this is sufficient only in part, and to a decreasing extent, at older ages. For some hypotheses, natural selection is restrained by pleiotropic genes or by physiological/biochemical contrasting demands.

The second, here referred to as the "new paradigm", sees the gradual decline of vital functions as a genetically programmed phenomenon, i.e. something that is determined and shaped by natural selection because it is advantageous in particular ecological conditions.

The empirical evidence in support of or against the two opposite paradigms has already been discussed previously [Libertini 2008]. Here, I wish to update and widen the discussion in the light of evidence that has subsequently come to light.

In this work, for the old paradigm, the main hypotheses, or category of hypotheses, will be considered:

- *Damage Accumulation hypotheses*. Aging is caused by the accumulation of damage of various kinds. The older hypotheses interpreted aging as caused by mechanical wear or by various types of biochemical damage and/or tissue degenerations, e.g. "wear and tear" without further specifications, mechanochemical deteriorations in cell colloids, changes in specific organs or tissues

(nervous/endocrine/vascular/connective, etc.), accumulation of toxic substances produced by intestinal bacteria, accumulation of various metabolites, effect of cosmic rays, etc. [Comfort 1979].

The newer ones explain aging as a consequence of the accumulation of chemical damage due to DNA transcription errors [Weinert and Timiras 2003], or as caused by oxidative effects of free radicals on the whole body [Harman 1972; Croteau and Bohr 1997; Beckman and Ames 1998; Oliveira et al. 2010], on the mitochondria [Miquel et al. 1980; Trifunovic et al. 2004; Balaban et al. 2005; Sanz and Stefanatos 2008] or on the DNA [Bohr and Anson 1995; Weinert and Timiras 2003].

- *Cessation of Somatic Growth hypothesis*. For organisms with a fixed growth, i.e. growth which ends when a determinate size has been attained, senescence starts when the growth of new tissues stops. Conversely, for species where the growth is without limits, as for many lower vertebrates, there is no age-related fitness decline [Minot 1907; Carrel and Ebeling 1921a, 1921b; Brody 1924; Bidder 1932; Lansing 1948, 1951].

- *Mutation Accumulation hypothesis*. Aging is due to the combined effect of many harmful genes that act late in life and are insufficiently removed by natural selection [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987; Partridge and Barton 1993].

- *Antagonistic Pleiotropy hypothesis*. Aging is caused by genes that are both advantageous in the young or adult stage and disadvantageous in the older ages, and are, therefore, only partially counteracted by natural selection [Williams 1957; Rose 1991].

- *Disposable Soma hypothesis*. Physiological and/or biochemical restrictions limit and hamper the maintenance of an optimal efficiency of maintenance systems at advanced ages. The body, in the allocation of poorly defined limited resources, must choose between higher reproductive capacity and a greater efficiency of maintenance systems. Therefore, the limited resources jeopardize the preservation of an optimum efficiency at advanced ages [Kirkwood 1977; Kirkwood and Holliday 1979].

- *Quasi-Programmed Aging hypothesis* [Blagosklonny 2006]. For this theory, a variation of the Disposable Soma hypothesis: "nature blindly selects for short-term benefits of robust developmental growth ... aging is a wasteful and aimless continuation of developmental growth" [Blagosklonny 2013].

As regards the new paradigm:

- The concept that aging has the hallmarks of an adaptation, i.e. something determined and modulated by natural selection, has been underlined by various Authors [Skulachev 1997; Bredesen 2004; Mitteldorf 2004; Longo et al. 2005]. Skulachev coined [Skulachev 1997] the pregnant neologism "phenoptosis" to define the vast and well-known [Finch 1990] group of phenomena in which an individual sacrifices itself or close relatives by means of mechanisms favored by natural selection at a supra-individual level [Skulachev 1997, 1999a; Libertini 2012a].

- It was Wallace, the co-discoverer of evolution by natural selection, who, in 1865-1870, proposed that death by aging was programmed [Skulachev and Longo 2005]. In 1889, Weissmann, albeit without a clear exposition or sound proof, hinted that aging was beneficial because the death of old individuals was evolutionarily useful, liberating space for the next generation [Weismann 1889; Kirkwood and Cremer 1982]. Moreover, as regards the mechanisms causing aging, he hinted that cell turnover slackened or stopped in the older ages and this determined a loss of functionality for the organs and consequently fitness decline [Kirkwood and Cremer 1982]. He later disowned these revolutionary ideas however [Weismann 1892; Kirkwood and Cremer 1982].

- In 1988 (anticipated in 1983 by a non-peer reviewed book [Libertini 1983]), a theory was put forward, justifying aging as adaptive in terms of kin selection, in spatially structured populations [Libertini 1988]. This hypothesis, which for the first time predicted an inverse relation between extrinsic mortality and the proportion of senescent deaths, was later reaffirmed [Libertini 2006, 2008, 2009a, 2013].

- Other theories underlining an evolutionary advantage for programmed death in spatially structured populations were put forward in 2004 and later on [Travis 2004; Martins 2011; Mitteldorf and Martins 2014].

- In the context of aging interpreted as a programmed phenomenon favored by natural selection, the damage induced by mitochondrial ROS was seen as pivotal mechanism [Skulachev 1999b, 2001; Skulachev and Longo 2005].

- Another theory, which follows Weismann's insight, maintains that aging is favored by natural selection in that it increases the speed of evolution, or evolvability [Goldsmith 2004, 2008a].

- In 2009, aging was explained as an adaptation to limit the spread of diseases, by analogy with Red Queen hypothesis on the adaptive meaning of sex [Mitteldorf and Pepper 2009].

- In 2008, a number of logical common predictions for all aging programmed hypotheses were underlined: A) the existence of species without an age-related increase of mortality; B) in a comparison of different species, an inverse relation between extrinsic mortality and the proportion of senescent deaths; C) the existence of specific aging-causing, genetically determined and modulated mechanisms. Moreover, it was stressed that: (A) would be hardly justified by many non-programmed aging theories; (B) and (C) were in total contrast with them [Libertini 2008].

Discussion

In this section, I will consider a series of theoretical arguments and documented phenomena. Each of them will be weighed against the predictions of the two paradigms, and their compatibility or incompatibility with both paradigms will be examined.

1) Non-universality of aging

Evidence: In his authoritative textbook, Finch reports, in the wild, for many species (including vascular plants, invertebrates and vertebrates) "Indeterminate Lifespans and Negligible Senescence" i.e. a life table without any age-related increase of mortality [Finch 1990]. In some cases, in connection with an age-related increase in body size, which reduces the risk of death due to predation by other species, the mortality rate even decreases at older ages [Vaupel et al. 2004].

Predictions of old paradigm theories: According to the various theories of the old paradigm, aging should be present in all species in which the hypothesized causes are present. The exceptions should be precisely explained, in particular in terms of the correlation between the absence/presence of aging with the absence/presence of the hypothesized cause. The data do not seem to justify the numerous documented exceptions.

As regards the many non-evolutionary older aging hypotheses based on damage accumulation assumptions, it is sufficient to consult the classical documented review of Comfort [Comfort 1979]: the absence of age-related decline shown by many species in the wild is not at all justified or considered by these theories. An exception is the group of theories that explain aging as caused by the cessation of somatic growth. Bidder pointed out that, for many lower vertebrates, there was no age-related mortality increase and suggested that there was "some mechanism to stop natural growth so soon as specific size is reached. This mechanism may be called the regulator ... senescence is the result of the continued action of the regulator after growth is stopped" [Bidder 1932].

As regards "newer" hypotheses, at least for those claiming to fall within evolutionary dynamics, the insufficient investigation of the non-universality of aging and the lack of plausible explanations for it have already been pointed out by others: "The possibility of negligible senescence has not been widely discussed, and may be in conflict with mathematical deductions from population genetics theory" [Finch and Austad 2001].

Predictions of new paradigm theories: According to the new paradigm, when the ecological conditions for the proposed advantage of aging are absent, natural selection always favors individuals with better fitness up until ages when, in the wild, the fraction of surviving individuals

is so small as to render selection ineffective. Therefore, in absence of particular selective circumstances favoring life restraints, the default condition is that of non-aging, i.e. fitness must not show an age-related decline at ages existing in the wild.

On the other hand, a lifespan with programmed limits (i.e. genetically determined and controlled, or influenced according to specific periods, or affected by particular events), within that broad category of phenomena, now generally referred to as "phenoptosis" [Skulachev 1997], described by Finch and known to scientists for some time [Finch 1990], is an evolved condition that requires specific evolutionary advantages, obviously in terms of supra-individual selection.

In short, cases of non-aging, which for the old paradigm constitute a large group of exceptions to the general rule of aging for all species (with strenuous and questionable attempts to justify them), conversely, for the new paradigm constitute the simplest condition, with many exceptions when particular ecological conditions favor this or that kind of phenoptosis.

2) Great inter-specific variation of aging rates

Evidence: Among the species whose individuals age, there is a wide variation in the rate of aging, even within the same phylum. For convenience of reasoning, I would stress that the rate of aging is inversely related to longevity, a parameter necessitating an exact definition, but which I will leave in its imprecise form for this type of reasoning.

Longevity: (A) is related to adult body weight in vertebrates [Bourlière 1957, 1960; Sacher 1959]; (B) is related to adult brain weight in mammals (likely related to the ability of learning) [Sacher 1959; Comfort 1979]; (C) does not appear inversely related to the rate of metabolism (e.g. birds have a high metabolic rate and often a long lifespan) [Comfort 1979].

Predictions of old paradigm theories: For each theory, the rate of aging should depend on the hypothesized cause for the phenomenon. For many non-evolutionary older theories there is clear contradiction or absence of relationship between aging rates and hypothesized causes [Comfort 1979].

Many of the newer evolutionary theories of the old paradigm could be compatible with (A) and (B) (greater body mass and greater capacity for learning imply stronger selective pressures in favor of a greater longevity), but do not seem compatible with (C) [Comfort 1979].

Predictions of new paradigm theories: Longevity must depend on the ecological conditions that favor aging. In addition, in the balance between (supra-individual) benefits and (individual) disadvantages of aging, both a greater body mass and a greater ability to learn increase the disadvantages of a shorter lifespan and therefore (A) and (B) are predicted and justified [Libertini 1988]. On the other hand, (C) is not predicted and is not necessary.

3) Effects of caloric restriction on lifespan

Evidence: For a long time, it has been known that animals raised under conditions of caloric restriction (CR) have a greater longevity than animals with *ad libitum* feeding [McCay et al. 1935; Ribarič 2012; Lee and Min 2013]. It is possible to interpret this evidence as a relation between CR and longevity increase or, alternatively, in the following ways: 1) It is an artificial phenomenon due to the overfeeding of control animals as the normal condition (i.e. that existing in the wild) is CR: "instead of comparing control animals with restricted animals, we are in fact comparing overfed animals with adequately fed ones, and, not surprisingly, the overfed ones die younger." [Austad 2001]; 2) *Ad libitum* feeding is, in effect, hyperalimentation, which reduces longevity by favoring various pathological conditions [Masoro 2005; Ribarič 2012]; 3) The increase in longevity is only a laboratory artifact as CR in the wild would not have the effect of increasing lifespan [Adler and Bonduriansky 2014].

Predictions of old paradigm theories: According to the Disposable Soma (DS) hypothesis, aging is due to the reduced availability of resources that forces an evolutionary choice, that is whether to direct the resources towards reproduction or survival. By favoring reproduction, organism maintenance is reduced and aging is the consequence. It follows that a reduction in

resources should lead to a reduction of longevity and vice versa. The effects of CR appear to be an increase, or at least the non-reduction, in longevity. Whatever the interpretation of the phenomenon, the empirical evidence does not seem to be compatible with the predictions of the DS hypothesis. A special feature of the DS theory has been put forward to solve this contradiction [Kirkwood et al. 2000], but the proposed solution has been criticized as contradictory and insufficient [Mitteldorf 2001].

Predictions of new paradigm theories: For the new paradigm, aging is not dependent on the greater or lesser availability of calories or of other metabolic limiting factors. Accordingly, the effects of CR are not in contradiction with the new paradigm, whatever the interpretation of these effects and the mechanisms that cause them. This does not exclude (on the contrary, it is predicted as likely) that ecological conditions to which a species is not adapted (e.g.: overfeeding) can be harmful and so may reduce longevity [Libertini 2009b].

4) Damage of aging for the senescing individual but its advantage in terms of supra-individual selection

Evidence: Natural observation shows an extraordinary number of phenomena in which an individual, or an immediate blood relative, is clearly sacrificed [Finch 1990]. This proves beyond any doubt that natural selection can favour phenomena that are altogether unjustifiable in terms of strict individual selection.

Predictions of old paradigm theories: Authoritative supporters of the old paradigm, in a prominent journal, maintained that it is unlikely that phenomena harmful to the individual might be favoured by natural selection: "any hypothetical 'accelerated ageing gene' would be disadvantageous to the individual. It is therefore difficult to see how genes for accelerated aging could be maintained in stable equilibrium, as individuals in whom the genes were inactivated by mutation would enjoy a selective advantage" [Kirkwood and Austad 2000]. More recently, this conviction has been confirmed: "The anomalous nature of ageing as a putative adaptation is that it is bad for the individual in which the process is exhibited. An animal that grows to maturity and thereafter reproduces indefinitely has, other things being equal, a greater Darwinian fitness than one that grows to maturity and then survives and reproduces for only a fixed period of time." [Kirkwood and Melov 2011]

Predictions of new paradigm theories: Phenomena in which an individual sacrifices himself, or a direct blood relative, though widely known and described long ago [Finch 1990], have only in recent times been defined under the unifying term "phenoptosis" [Skulachev 1997], with the explicit statement that these phenomena are genetically determined, and harmful to the individual concerned (or to direct blood relatives [Libertini 2012a]). The new paradigm argues that aging is one among many types of phenoptotic phenomena ("slow phenoptosis" [Skulachev 2002b]), and therefore must necessarily be explained in terms of supra-individual selection [Libertini 2012a]. The erroneous exclusion of the possibility that a character may be favored by natural selection because it is harmful at the individual level implies a restrictive and totally unacceptable conception of the mechanisms of natural selection [Libertini 2012a, 2014b].

5) Existence of fitness decline in wild conditions

Evidence: For many animal species, there is a well-documented age-related increase in mortality at ages existing in the wild [Ricklefs 1998; Nussey et al. 2013]. As an example, Figure 1 shows the life table and the mortality of the lion (*Panthera leo*) in natural conditions.

For our species, we have the data from the study of a human population (Ache of Paraguay) under natural conditions. This study shows that the fractions of surviving individuals at the ages of 65, 70 and 75 years, were 27%, 20% and 12%, respectively. Excluding individuals who died before they were twenty years old, the survivors at ages 65, 70 and 75 years, were 42%, 32% and 18%, respectively (see Figure 2) [Hill and Hurtado 1996].

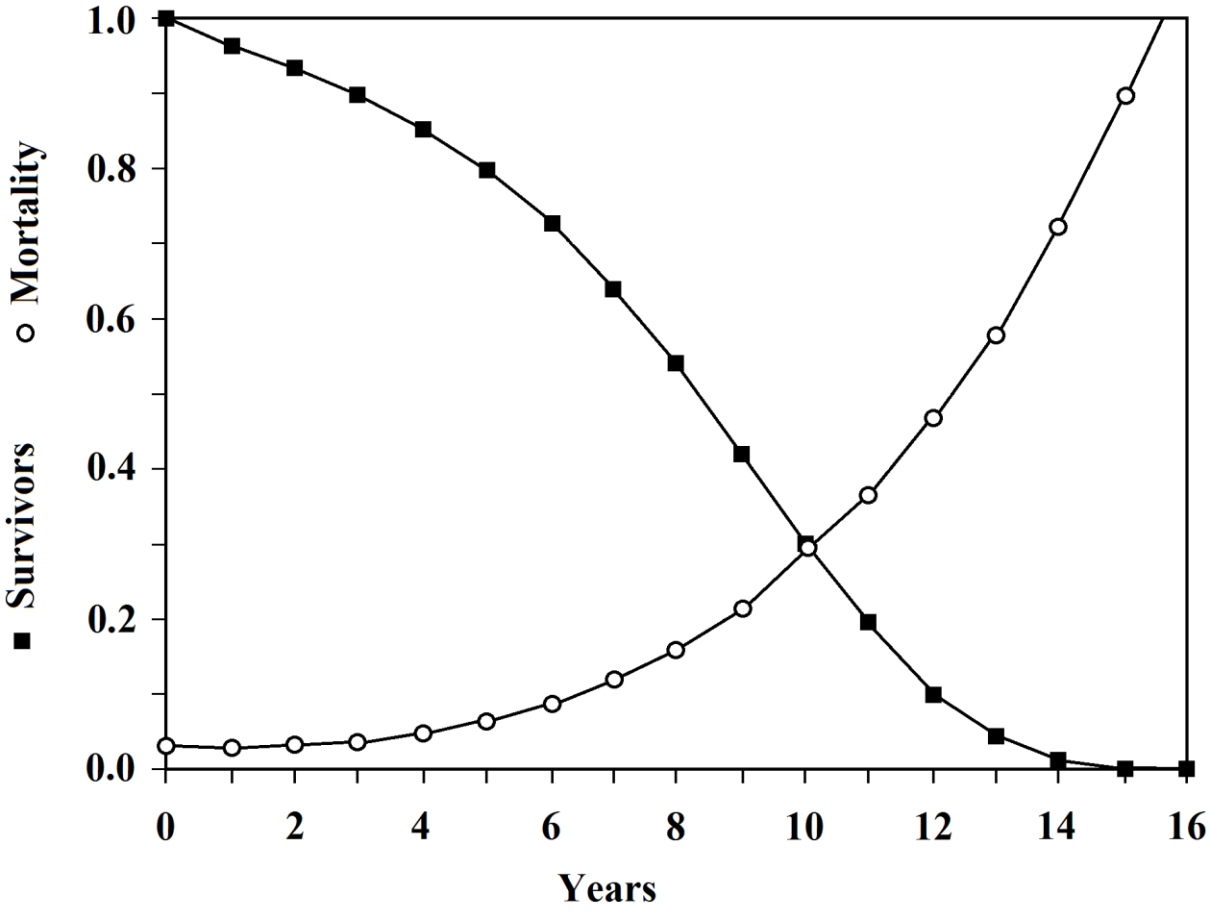


Figure 1 - Life table and mortality of *Panthera leo* in the wild (Data from [Ricklefs 1998]).

Predictions of old paradigm theories: In a 2000 Nature paper, influential supporters of the old paradigm, maintained the impossibility of an evolutionary advantage of any kind in aging because: "there is scant evidence that senescence contributes significantly to mortality in the wild ... As a rule, wild animals simply do not live long enough to grow old ... Therefore, natural selection has limited opportunity to exert a direct influence over the process of senescence" [Kirkwood and Austad 2000].

The concept has been reaffirmed over subsequent years: "Data on age-related mortality patterns in wild animal populations reveal that, in many species, individuals rarely survive to ages when senescent deterioration becomes apparent ..." [Kirkwood 2005]; "senescence-associated increases in age-related mortality are far from ubiquitous, and ..., even where they are observed, they contribute only to a relatively small fraction of deaths within the population, ..." [Kirkwood and Melov 2011].

Predictions of new paradigm theories: Thirteen years later, one of the Authors of the 2000 Nature paper [Kirkwood and Austad 2000], along with other Authors, says and documents the opposite: "The recent emergence of long-term field studies presents irrefutable evidence that senescence is commonly detected in nature. We found such evidence in 175 different animal species from 340 separate studies." [Nussey et al. 2013]

In any case, the aforementioned objection regarding the absence of aging in the wild could be coherent within the restricted conception of aging erroneously limited to the existence of individuals with extreme functional decay, that is to say with levels of fitness reduced to arbitrarily established minimal values, which - by definition - are incompatible with survival. But, aging has been defined as the age-related progressive decay of functions and therefore cannot be identified with the extreme outcome of this decay.

Empirical data show that mortality increase (i.e. fitness decline) is well documented in the wild, and therefore is strongly influenced (i.e., opposed or favored) by natural selection. Moreover, if we limit the discussion to our own species, in wild conditions the fractions of individuals in advanced stages of senescence are certainly remarkable, and this is another reason for which the concept of an *a priori* ineffectiveness of selection on aging is unacceptable.

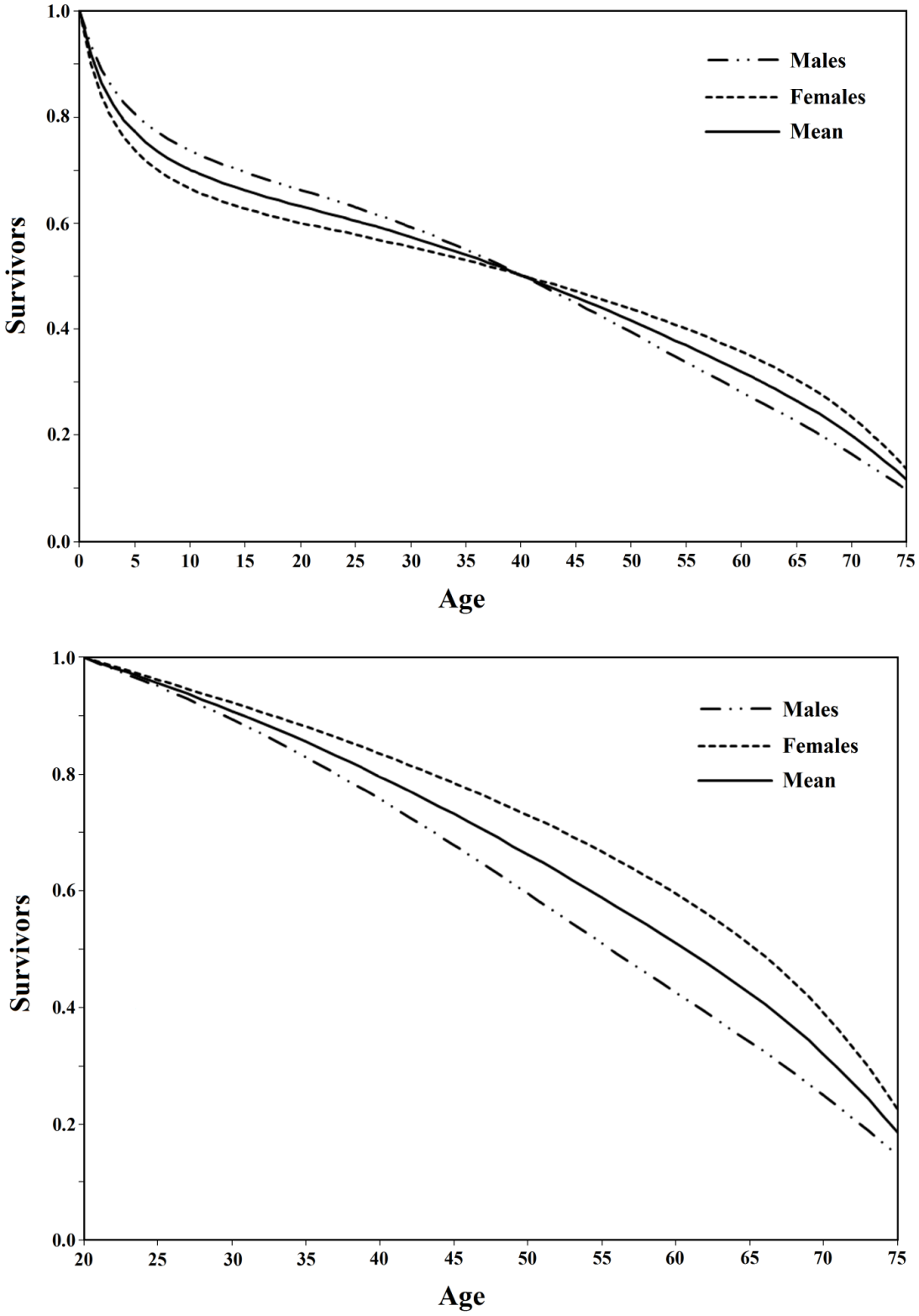


Figure 2 - Life table of *Homo sapiens* in wild conditions: A) whole population; B) limited to 20 years old survivors. Data from Ache population (Paraguay) [Hill and Hurtado 1996].

6) Proportion of deaths due to intrinsic mortality inversely related to extrinsic mortality, in a comparison of species

Evidence: For species that demonstrate age-related mortality increase (i.e. aging) in wild conditions, an inverse relation between extrinsic (or environmental) mortality and the proportion of deaths due to the age-related mortality increase has been well documented [Ricklefs 1998]. This relationship is confirmed by the inclusion of data from a human population studied in wild conditions [Libertini 2013].

Predictions of old paradigm theories: A direct relationship is plainly predicted: "The principal determinant in the evolution of longevity is predicted to be the level of extrinsic mortality. If this level is high, life expectancy in the wild is short, the force of selection attenuates fast, deleterious gene effects accumulate at earlier ages, and there is little selection for a high level of somatic maintenance. Consequently, the organism is predicted to be short lived even when studied in a protected environment. Conversely, if the level of extrinsic mortality is low, selection is predicted to postpone deleterious gene effects and to direct greater investment in building and maintaining a durable soma" [Kirkwood and Austad 2000]. The contradiction between old paradigm hypotheses and the above-mentioned inverse relationship observed is clearly stated by Ricklefs, who, after reporting his data - a fatal blow against ingrained beliefs -, makes a feeble attempt at saving the DS hypothesis only [Ricklefs 1998].

Predictions of new paradigm theories: If aging is a programmed phenomenon, a paradoxical inverse relationship was predicted long ago [Libertini 1983, 1988], well before Ricklefs' data [Ricklefs 1998] were published. This inverse relationship is also predicted by a model that shows aging to be advantageous in spatially structured populations [Mitteldorf and Martins 2014]. It has been stressed that this inverse relation is implicitly a general prediction of programmed aging theories and that there is a clear contradiction with the predictions of non-programmed aging hypotheses: "adaptive hypothesis ... appears indispensable to explain the observed inverse correlation between extrinsic mortality and the proportion of deaths due to intrinsic mortality" [Libertini 2008]; "this complementary relationship between background death and evolved senescence is characteristic of adaptive theories of aging. A high background death rate leads to a *longer* evolved life span. This contrasts with classical theories, in which a high background death rate leads to a *shorter* evolved life span." [Mitteldorf and Martins 2014]. However, no explanation compatible with old paradigm theories has been proposed.

7) Impossibility of explaining age-related fitness decline as a consequence of genes that are harmful at a certain age

Evidence: This argument has already been discussed elsewhere [Libertini 1988], but it is useful and necessary to reassert and better expound it in this work, as it has never been disproved and is essential for the acceptability of the Mutation Accumulation (MA) hypothesis.

Let us consider a harmful gene (C), which reduces fitness by a value *s*, and its neutral allele (C'). If *v* is the mutation frequency of C' in C, and the frequency of the reverse mutation is considered, for the sake of simplicity, to be insignificant, it is possible to calculate the equilibrium frequency between the new mutations C' -> C and the elimination of C by natural selection. It is also possible to calculate the frequency of the phenotypic expression of the gene (P_e) both in the case that C is recessive:

$$P_e = v/s \quad (1)$$

and in the case that C is dominant:

$$P_e \approx v/s \quad (2)$$

The achievement of these formulas is explained in detail elsewhere [Libertini 2009b].

By defining a "t-gene" as a hypothetical gene that is harmful, by a value *s*, at time t and neutral in the preceding ages, as a t-gene damages only the fraction of survivors at time t (Y_t), natural selection lowers its frequency in function of ($s\ Y_t$) and the formulas (1) and (2) become:

$$P_e \approx v/(s\ Y_t) \quad (3)$$

Now, let us consider a population with a constant mortality at each age (i.e., a non-aging population) and verify whether the action of a considerable number of t-genes may determine a curve similar to that of a species with an age-related increase in mortality.

The base curve of the population is given by the equation:

$$Y_{t+1} = Y_t\ (1 - \lambda) \quad (4)$$

where: Y_x = survivors at time x; λ = death-rate.

Now, if we suppose m t-genes acting at time t, as many genes at time t+1, and so on, each with harm equal to s (for the sake of calculation simplicity assumed to be equal for all t-genes), the survivors at any time t+1 will be:

$$Y_{t+1} = Y_t\ (1 - \lambda - m\ s\ P_e) \approx Y_t\ (1 - \lambda - m\ v/Y_t) \quad (5)$$

The equation (5) does not include the value of *s*, which is therefore irrelevant, and, as the value of *v* is assumed to be small, the decrease in Y at each unit of time will be sensible only when the value of Y_t is small.

Figure 3 shows a hypothetical life table with a constant mortality and the modifications caused by a large number of hypothetical t-genes.

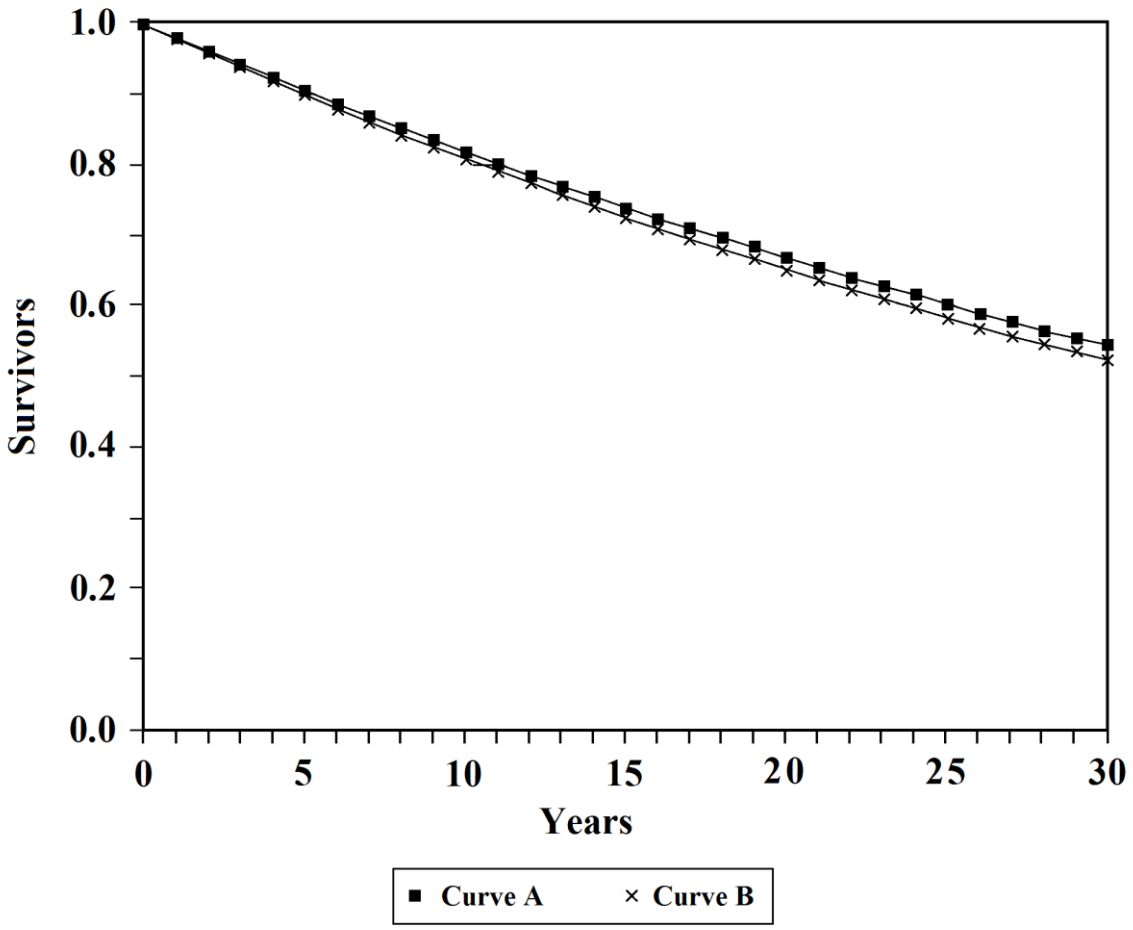


Figure 3 - Curve A: ideal life table obtained by formula 4, with λ = .02. Curve B: effects on curve A by a great number of t-genes, obtained by formula 5, with λ = .02; m= 1000; v= .000001.

Figure 4 shows: A) part of the life table of a species (*Panthera leo*) studied in natural conditions; B) the same life table without the increase in mortality due to intrinsic mortality and thus with the constant extrinsic mortality only; C) curve B plus the effects of a large number of hypothetical t-genes. In both figures, it is evident that the effects of the hypothetical t-genes do not cause curves which are comparable to that of a species that ages.

Predictions of old paradigm theories: For the MA hypothesis, aging would be caused by the cumulative effects of many t-genes.

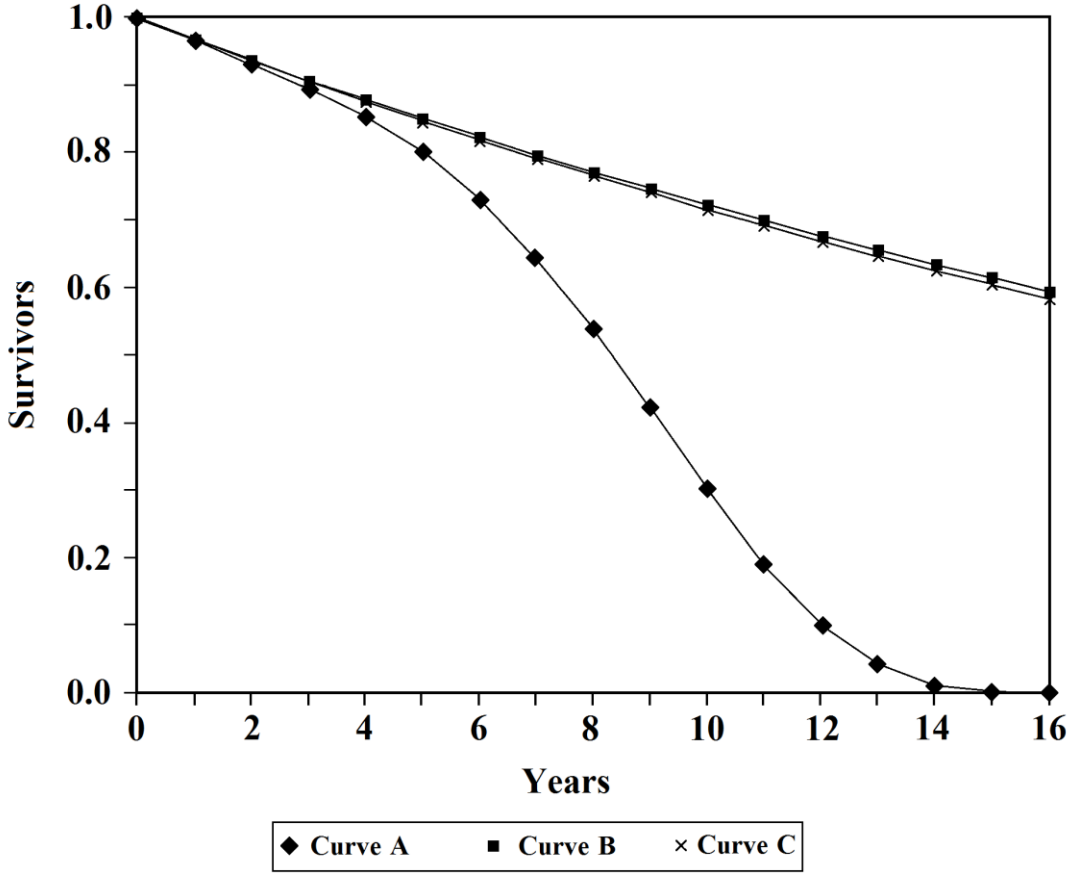


Figure 4 - Hypothetical effects of a great number of t-genes on the life table of a real species. Curve A, life table in the wild of *Panthera leo*, with mortality described by Weibull's equation ($m_t = m_0 + \alpha\, t^{\beta}$), using the values $m_0 = .032$; $\alpha = .000252$; $\beta = 3$; from [Ricklefs 1998]. Curve B, hypothetical, shows the same life table without the age-related increment of mortality, i.e. with a constant mortality ($m_0 = .032$). Curve C, hypothetical, shows the effects on curve B of a great number of t-genes (m= 1000; v= .000001;).

Predictions of new paradigm theories: The argument proposed by the MA hypothesis is totally unacceptable for ages existing in the wild and therefore cannot be considered a plausible explanation for aging. As an interesting corollary, natural selection - by definition - cannot delete a t-gene that would exert its action at ages not existing in the wild. It follows that a species that does not show any mortality increase in the wild, might under artificial conditions and at ages successive to those existing in the wild show an age-related mortality increase due to t-genes that cannot be eliminated by natural selection. This theoretical prediction, already formulated before [Libertini 1988], concerns a phenomenon that should be clearly distinguished from aging.

8) Age-related progressive decline of cell turnover capacities

Evidence: In normal conditions, in vertebrates, cells die continuously as a result of various types of programmed cell death (PCD). The most studied type of PCD is apoptosis, first observed in hepatocytes [Kerr et al. 1972], but well documented in many other organs and tissues [Libertini 2009a]. Other types of PCD are the keratinization of epidermis cells and subsequent detachment, the detachment of cells from mucosas, the phagocytosis of erythrocytes and of osteocytes, etc. Continuous cell death (50-70 billion/day [Reed 1999]) is balanced by the continuous duplication of stem cells with rhythms that vary for each cell type and organ [Richardson et al. 2014]. At the one extreme, cells of intestinal epithelium are renewed in 3-6 days [Alberts et al. 2014], while heart myocytes in about 4.5 years [Anversa et al. 2006] and osteocytes in about 10 years [Alberts et al. 2014].

The few cell types that are not subject to cell turnover (e.g., neurons of the central nervous system and retina photoreceptors) are strongly dependent on other cells that undergo turnover and that actively renew the critical parts of the cells without turnover [Libertini 2009a].

However, cell turnover declines as one grows older due to known limitations in cell replication, first demonstrated by Hayflick in his seminal work [Hayflick and Moorhead 1961].

Aging may be described as the result of the gradual decline of cell turnover, resulting in a progressive atrophy of all tissues and organs [Libertini 2009a, 2014a], associated with the increase of the percentage of cells in cell senescence and in gradual cell senescence (s. below). In any case, cell turnover and its gradual decline are clearly subjected to a genetic regulation that is certainly very complex and sophisticated.

Predictions of old paradigm theories: Aging is not explained as a progressive slowdown in cell turnover. For the old paradigm, the limits in cell turnover, which are clearly genetically determined and modulated and determine an age-related fitness decline, cannot be explained as being caused by the accumulation of harmful effects and so must have a different acceptable explanation. The only proposed explanation is that these cell limits defend the organism from cancer [Campisi 1997; Wright and Shay 2005].

This justification, put forward and/or accepted by authoritative scholars, does not explain the existence of species without age-related fitness decline (species with negligible senescence), which show no age-related decline in telomerase activity and no age-related increase in cancer mortality [Libertini 2008]. Moreover, for our species studied in the wild, fitness decline – i.e. aging - kills almost all individuals before cancer cases become a detectable cause of death and it is unlikely that a defense against cancer may kill before the disease begins to be lethal [Libertini 2013]. Other strong objections to the above-mentioned justification for the limits in telomerase action, and consequently in cell turnover, have been underlined with clear conclusions: "The hypothesis that telomerase is restricted to achieve a net increase in lifespan via cancer prevention is certainly false. Were it not for the unthinkability of the alternative – programmed death – the theory would be dead in the water." [Mitteldorf 2013]

Predictions of new paradigm theories: The new paradigm predicts and, indeed, absolutely requires aging to be genetically determined and regulated. Therefore, the above-mentioned phenomena, which gradually reduce fitness, are not in conflict with the new paradigm but rather are essential to its plausibility [Libertini 2008].

9) Cell senescence

Evidence: Cells pass from a cycling state, in which they can duplicate, to a non-cycling state, where cells cannot duplicate, through the random activation of a mechanism with a probability inversely proportional to the reduction of telomere length [Blackburn 2000].

The inactivation of replication capacities is part of a specific complex mechanism, cell senescence, which is characterized by predictable and stereotyped modifications and is considered a "fundamental cellular program" [Ben-Porath and Weinberg 2005].

In the senescent state, cells are characterized by complex alterations of transcriptome, with many cell functions compromised, including the cell secretions in the intercellular matrix and the

consequent damage to other cells and to the functionality of the tissues/organs of which the cells are part [Campisi and d'Adda di Fagagna 2007]. Among other things, cell senescence results in a lower resistance to oxidative substances and in an accumulation of oxidative damage. But it is worth pointing out that the damage caused by oxidation is a consequence and not the cause of cell senescence [Fossel 2004]. Moreover, it is well established that cell senescence and all its manifestations, including oxidative damage, are totally reversible by activating the enzyme telomerase [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998].

Predictions of old paradigm theories: For the old paradigm, aging is caused by the accumulation of various types of damage in many locations (depending on the various assumptions of the hypotheses). The fact that a cell changes from the condition of cycling state/non-senescence (no damage evident) to non-cycling/senescent state (damage of many types) as a consequence of the activation of a specific program is completely unexpected. Equally unforeseen is that this program is completely reversible with the total disappearance of the damage caused by cell senescence and the perfect reactivation of replication capacities. Moreover, cell senescence is activated in somatic cells and not in germline cells and this means that the mechanism is not an inevitable feature of living cells or an inevitable consequence of replications, but a sophisticated mechanism that cannot be the consequence of damage accumulation and that requires specific selective advantages to justify its existence.

These phenomena are in clear and complete contradiction with the predictions of old paradigm theories. Cell senescence absolutely needs a justification, other than that of its unlikely attributed anticancer powers, in order to nullify this contradiction.

Predictions of new paradigm theories: The new paradigm predicts and, indeed, absolutely requires aging to be genetically determined and regulated. Therefore, the above-mentioned phenomena, which gradually reduce fitness, are not in conflict with the new paradigm but rather are essential to its plausibility.

Some of these concepts are illustrated in fig. 5.

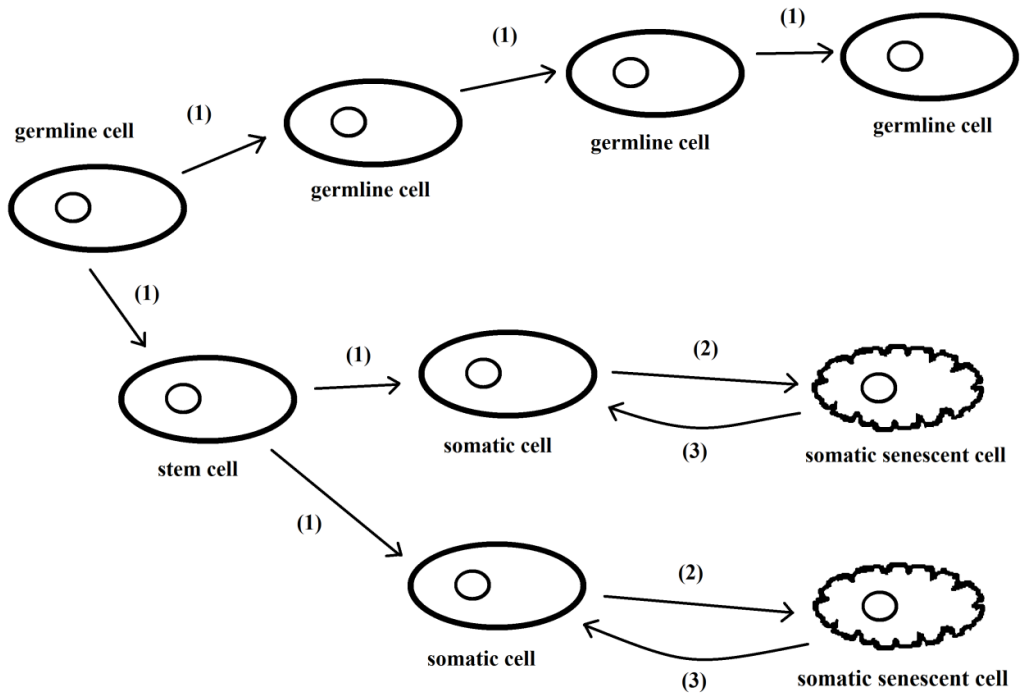


Figure 5 - Germline and stem cells duplicate (1) and do not change into senescent cells. On the contrary, somatic cells are subject to cell senescence phenomenon (2). This difference is not easily explainable if cell senescence is caused by damaging factors. Analogously, the complete reversibility of cell senescence (3), by activation of telomerase, is explainable only if cell senescence is a programmed phenomenon. This evidence is in clear contrast with the old paradigm.

10) Gradual cell senescence

Evidence: In yeast (*Saccharomyces cerevisiae*), telomerase is always active and telomere length does not decrease with duplications [D'Mello and Jazwinski 1991; Smeal et al. 1996]. Moreover, there is an asymmetric division between mother and daughter cells: while the mother lineage may continue only for a limited number of generations and there is a progressive decline in the ability to withstand stress [Jazwinski 1993], daughter ("budding") yeast cells divide indefinitely [Maringele and Lydall 2004].

In mother cells, extrachromosomal ribosomal DNA circles (ERCs) accumulate at each duplication [Sinclair and Guarente 1997], "several lines of evidence suggest that accumulation of ERCs is one determinant of life span", and, in proportion to the number of duplications, increasing metabolic alterations, definable as cell senescence, are evident [Lesur and Campbell 2004].

These alterations are a likely consequence of ERCs accumulation which interferes with gene expression of critical parts of subtelomeric DNA. As a matter of fact, yeast dna2-1 mutants, with abnormalities in DNA duplication and thus increased rates of ERCs accumulation, show precocious alterations of gene expression. In particular, transcriptome of older wild-type individuals of mother lineage are similar to those of young mother individuals of dna2-1 mutants [Lesur and Campbell 2004].

In yeast, tlc1Δ mutants, which are telomerase-deficient mutants, both mother and daughter cells show telomere shortening and individuals of daughter cell lineages, which have no ERCs accumulation, have a transcriptome similar to that of older wild-type individuals of mother lineage, and of individuals of dna2-1 mutants [Lesur and Campbell 2004]. It is possible that in tlc1Δ mutants, telomere shortening causes the sliding of a telomere heterochromatin hood which interferes with subtelomeric DNA, while in wild-type yeast subtelomeric DNA is somehow repressed by ERCs.

In multicellular eukaryotic organisms, in proportion to the number of duplications there is an increasing probability of replicative senescence and an increasing alteration in the expression of many genes, i.e. an alteration of the transcriptome, which compromises overall cell functionality and has deleterious consequences on the extracellular matrix and on other cells that are physiologically interdependent. All this is certainly in relation to the relative shortening of telomere (Fossel's "cell senescence limited model") [Fossel 2004].

"One model of telomere-gene expression linkage is an altered chromosomal structure (Ferguson et al., 1991), such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome (Fossel, 1996; Villeponteau, 1997; Wright et al., 1999). As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) ... the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity (Aparicio and Gottschling, 1994; Singer et al., 1998; Stevenson and Gottschling, 1999). These silenced genes may in turn modulate other, more distant genes (or sets of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel 2004].

Recent results confirm the influence of telomere length on subtelomeric DNA: "Our results demonstrate that the expression of a subset of subtelomeric genes is dependent on the length of telomeres and that widespread changes in gene expression are induced by telomere shortening long before telomeres become rate-limiting for division or before short telomeres initiate DNA damage signaling. These changes include up-regulation and down-regulation of gene expression levels." [Robin et al. 2014]

In short, in yeast and in multicellular eukaryotic organisms, subtelomeric DNA is of pivotal importance for overall cell functionality and is vulnerable to inactivation as a consequence of

telomere shortening or of ERCs accumulation around the telomere. Excluding the possibility of an absurd evolutionary illogicality, this positional vulnerability must be somehow explained in terms of natural selection.

This evidence and the deduced concepts are illustrated in figure 6.

Predictions of old paradigm theories: If aging is opposed by natural selection, it is quite illogical – or rather unlikely - that delicate parts of the DNA with general regulatory functions, will be placed in the position most exposed to the consequences of telomere shortening, as the sliding of the telomeric hood on the subtelomeric segment (or the ERCs accumulation, in yeast) dysregulates genes that are critical for cell functions.

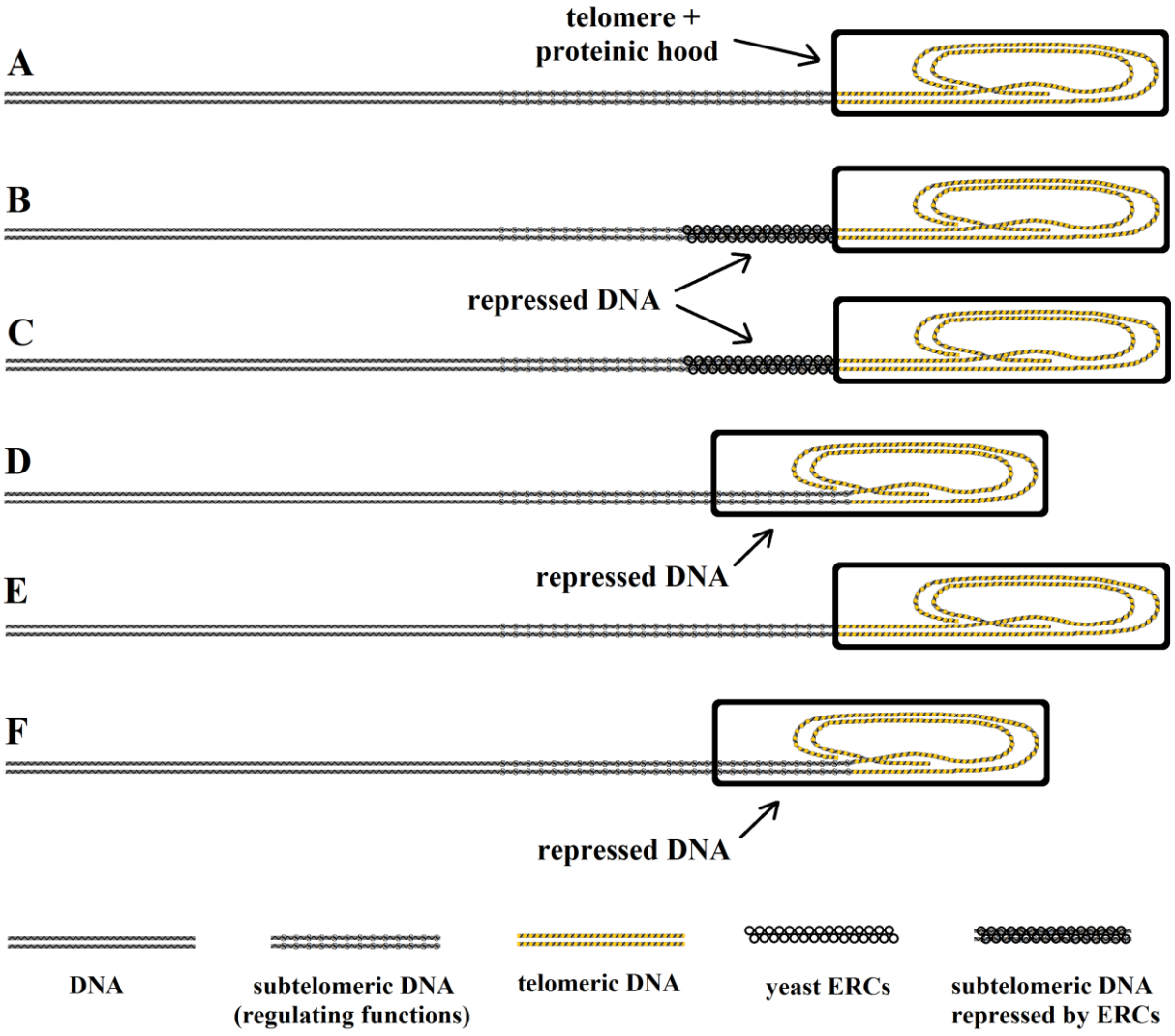


Figure 6 - A) yeast, normal stock, daughter lineage; B) yeast, normal stock, old individuals of mother lineage; C) yeast, dna2-1 mutants, young individuals of mother lineage; D) yeast, tlc1Δ mutants, daughter lineage; E) multicellular eukaryotes, normal stock, germinal line; F) multicellular eukaryotes, normal stock, somatic line. In A and E, telomeres are not shortened and subtelomeric DNA is not repressed. In B and C, subtelomeric DNA is repressed by the accumulated ERCs. In D and F, telomere are shortened and the subtelomeric DNA is repressed by the proteinic hood.

Predictions of new paradigm theories: The gradual impairment of cellular functions in relation to telomere shortening, or to ERCs accumulation in yeast, which are phenomena based on genetically determined mechanisms, is perfectly compatible with the new paradigm and indeed represents a further element of sophistication of the system. As regards the thesis that this could be

part of a hypothetical general defense against cancer, see what has been said in the previous subsection. Moreover, in unicellular species, such as yeast, cancer is by definition impossible, but in these species, which show aging in the mother lineage cells, there is a similar mechanism in which the vulnerability of the subtelomeric DNA segment, which is crucial for the general functioning of the cell, has not been countered by natural selection. Incredibly, although the common ancestors of mammals and yeast date back over 600 million years ago [Minkoff 1983], (i) the vulnerability of the subtelomeric segment, (ii) its crucial importance for the functionality of the entire cell, (iii) the progressive damage to this segment in relation to cell doublings (in the mother lineage cells for the yeast and in the cells in which telomerase is not active for mammals) are highly conserved features, although they are clearly harmful in individual terms.

Conclusion

1 - The absence of an age-related mortality increase in many species requires, for non-programmed aging hypotheses, specific justifications, which are inexistent (with the sole exception of the Cessation of Somatic Growth hypothesis). This does not exclude the possibility that specific explanations for each case may exist, but until they are found, the absence thereof should be considered as a point against non-programmed aging hypotheses. The Cessation of Somatic Growth (CSG) hypothesis is an exception, as it justifies non-aging conditions in species with continuous somatic growth.

2 - In a comparison of the species, in particular within the same phylum, the great variability in aging rates should be in direct relation to the strength of the hypothesized causes for aging in the various hypotheses. But, in many cases, the theories of the old paradigm do not demonstrate a direct relationship between aging rates and the hypothetical causes and, furthermore, in some cases we find the opposite relationship [Comfort 1979]. In this case too, the CSG hypothesis is an exception, as it could justify aging rate variety as related to growth differences.

3 - The effects of caloric restriction on longevity, that is to say, its observed increase or at least non-increase, are in clear contrast with the predictions of the Disposable Soma hypothesis.

4, 5 - Old paradigm theories are based on two assumptions which are totally groundless: A) aging does not exist in natural conditions; B) a character that is disadvantageous in individual terms cannot be favored by natural selection. These assumptions would result in the impossibility of a programmed aging theory and in the absolute necessity for an explanation for aging to fall within the old paradigm. As the two concepts are blatantly wrong, it follows that an age-related fitness decline cannot be explained without some form of evolutionary advantage. Therefore, the two alleged arguments against the new paradigm and in support of the old paradigm become the opposite, i.e. arguments against the old paradigm and in support of the new paradigm.

Among the older theories, the CSG hypothesis does not claim the absence of senescence in the wild and so it cannot be criticized on this point.

6 - The prediction of a direct relationship between extrinsic mortality rates and proportions of deaths due to aging is intrinsic to all old paradigm theories while the opposite is expected from new paradigm theories [Libertini 2008]. The evidence [Ricklefs 1998] is against the old paradigm and in support of the new one. Moreover, there is the complete lack of an explanation compatible with old paradigm theories, which seem to ignore or gloss over the topic.

7 - The existence of a strong, unrefuted, theoretical demonstration [Libertini 1988], against the seemingly indisputable theorem that aging is wholly, or at least partially, a consequence of natural selection that weakens as survivors decline with age, is an insuperable blow to the Mutation Accumulation hypothesis. There is, perhaps, a concealed awareness of this weakness which is the basis for the origin of the Antagonistic Pleiotropy and Disposable Soma hypotheses, the first of which assumes the existence of genes advantageous at lower ages and disadvantageous at higher ages, and the second which postulates insuperable limits in the availability of ill defined resources. The supporters of these two hypotheses fail to demonstrate the existence of pleiotropic genes or of

limited resources. In addition, the above-mentioned hypotheses do not explain at all the great variability of aging rates in a comparison of species, meaning that there is probably an implicit assumption that pleiotropic genes and limiting factors are variable depending on the greater or lesser longevity (an *ad hoc* unacceptable hypothesis). However, it is necessary to stress that hypotheses based on postulates, or on multiple layers of postulates, cannot be considered scientific.

8, 9, 10 - Progressive decline in cell turnover capacities, gradual cell senescence and cell senescence, while fully compatible and, indeed, necessary for the new paradigm, are totally incompatible with old paradigm theories, unless a sound justification of their existence, other than that of their being part of the aging mechanism, is put forward. The hypothesis that they are a general defense against cancer is untenable. Moreover, for gradual cell senescence, even the aforementioned explanation could not justify why DNA parts with fundamental regulatory effects on cell functions are localized in the subtelomeric portion, which is the most vulnerable in proportion to the number of replications. Besides, such localization is phylogenetically very old and is also present in unicellular species, in which cancer is impossible.

These considerations are summarized in Table 1.

In short, old paradigm hypotheses prove to be entirely untenable and of historical value only, while the new paradigm is clearly compatible with the empirical data and the theoretical arguments.

Table 1 - Correspondence between empirical data/theoretical arguments and the various theories

	DA	**CSG**	**MA**	**AP**	**DS**	**QPA**	**New Paradigm**
1) Non-universality of aging	No/-	Yes	No/-	No/-	No/-	No/-	Yes
2) Great inter-specific variation of aging rates	No/-	Yes	No/-	No/-	No/-	No/-	Yes
3) Effects of caloric restriction on lifespan	-	-	-	-	No	-	Yes
4) Damage of aging for the senescing individual but its advantage in terms of supra-individual selection	No	-	No	No	No	No	Yes
5) Existence of fitness decline in wild conditions	No	Yes	No	No	No	No	Yes
6) Proportion of deaths due to intrinsic mortality inversely proportional to extrinsic mortality, in a comparison of species	No	No	No	No	No	No	Yes
7) Impossibility of explaining age-related fitness decline as a consequence of genes that are harmful at a certain age	-	-	No	-	-	-	Yes
8) Age-related progressive decline of cell turnover capacities	No	No	No	No	No	No	Yes
9) Cell senescence	No	No	No	No	No	No	Yes
10) Gradual cell senescence	No	No	No	No	No	No	Yes

Abbreviations: DA=Damage Accumulation hyp.; CSG = Cessation of Somatic Growth hyp.; MA=Mutation Accumulation hyp.; AP=Antagonistic Pleiotropy hyp.; DS=Disposable Soma hyp.; QPA=Quasi-Programmed Aging hyp.;
No = not explained or predicted by the hypothesis or in contrast with its predictions;
- = irrelevant for accepting/rejecting the hypothesis;
Yes = predicted by the hypothesis or compatible with it.

Chapter 8

Libertini G (2015b) Phylogeny of Aging and Related Phenoptotic Phenomena, *Biochem. (Mosc.)* 80(12), 1529-46.

Phylogeny of aging and related phenoptotic phenomena

Giacinto Libertini

Abstract

The interpretation of aging as adaptive, i.e. as a phenomenon genetically determined and modulated, and with an evolutionary advantage, implies that aging, as any physiologic mechanism, must have phylogenetic connections with similar phenomena.

This paper tries to find the phylogenetic connections between vertebrate aging and some related phenomena in other species, especially within those phenomena defined as phenoptotic, i.e. involving the death of one or more individuals for the benefit of other individuals.

In particular, the aim of the work is to highlight and analyze similarities and connections, in the mechanisms and in the evolutionary causes, between: 1) proapoptosis in prokaryotes and apoptosis in unicellular eukaryotes; 2) apoptosis in unicellular and multicellular eukaryotes; 3) aging in yeast and in vertebrates; and 4) the critical importance of the DNA subtelomeric segment in unicellular and multicellular eukaryotes.

In short, there is strong evidence that vertebrate aging has clear similarities and connections with phenomena present in organisms with simpler organization. These phylogenetic connections are a necessary element for the sustainability of the thesis of aging explained as an adaptive phenomenon, and, on the contrary, are incompatible with the opposite view of aging as being due to the accumulation of random damages of various kinds.

Introduction

Aging, here precisely defined as "increasing mortality with increasing chronological age in populations in the wild" ("IMICAW" [Libertini 1988]), is observed in many species under natural conditions [Deevey 1947; Laws and Parker 1968; Spinage 1970, 1972; Finch 1990; Holmes and Austad 1995; Ricklefs 1998; Nussey et al. 2013], our species included [Hill and Hurtado 1996]. The definitions "actuarial senescence in the wild" [Holmes and Austad 1995] and "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [Kirkwood and Austad 2000] are synonyms of the above said descriptive definition of aging. On the contrary, statements as: "Ageing, or senescence, results from a waning of the force of natural selection with respect to the age of gene effects" [Martin and Oshima 2000] or "aging is caused ... by evolved limitations in somatic maintenance, resulting in a build-up of damage." [Kirkwood 2005] do not describe aging in a neutral way but are only short expressions of not proven hypotheses about aging (see below).

In fact, aging is interpreted in two very different ways [Libertini 2008], which, for their opposite numberless implications and for the importance of the subject, deserve to be considered incompatible paradigms in the meaning of the term "paradigm" proposed by Kuhn [Kuhn 1962].

The first ("old paradigm") includes a vast patchwork of disparate hypotheses that try to explain aging as the inevitable consequence of damaging factors that progressively jeopardize fitness [Minot 1907; Carrel and Ebeling 1921a, 1921b; Brody 1924; Bidder 1932; Lansing 1948, 1951; Medawar 1952; Williams 1957; Hamilton 1966; Edney and Gill 1968; Harman 1972; Kirkwood 1977; Comfort 1979; Kirkwood and Holliday 1979; Miquel et al. 1980; Mueller 1987; Rose 1991; Partridge and Barton 1993; Bohr and Anson 1995; Croteau and Bohr 1997; Beckman and Ames 1998; Weinert and Timiras 2003; Trifunovic et al. 2004; Balaban et al. 2005; Blagosklonny 2006,

2013; Sanz and Stefanatos 2008; Oliveira et al. 2010]. In the older theories, damage accumulation is conceived without considering any evolutionary mechanism, with the implicit incorrect assumption that aging and natural selection act in two different contexts. The newer theories, or at least some of them, try to take into account the mechanisms of natural selection, which, according to them, would be able to counteract damaging factors only partially and to an age-related decreasing extent, also as a consequence of pleiotropic effects and contrasting physiological necessities.

The second paradigm ("new paradigm") includes hypotheses that define and interpret aging as a physiological phenomenon, i.e. a phenomenon that, in spite of its undoubted disadvantages for the aging individual, is determined and modulated by natural selection as being evolutionarily advantageous in terms of supra-individual selection [Weismann 1889; Kirkwood and Cremer 1982; Libertini 1983, 1988, 2006, 2009a, 2012a, 2013; Skulachev 1997, 1999a, 1999b, 2001; Bredesen 2004; Goldsmith 2004, 2008a; Mitteldorf 2004; Travis 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf and Pepper 2009; Martins 2011; Mitteldorf and Martins 2014], although for some hypotheses only in particular conditions [Libertini 1988; Mitteldorf and Martins 2014].

The new paradigm sees aging as a particular type of phenoptosis (see definition below), a term coined in 1997 by Skulachev [Skulachev 1997]. Subsequently, the same author, to emphasize the peculiarity of the phenomenon within the phenoptotic universe, coined for aging the definition of "slow phenoptosis" [Skulachev 2002b].

The concept of phenoptosis may be shortly defined as "the programmed death of an individual" [Skulachev 1999a], or described more extensively: "Phenoptosis is the death of an individual caused by its own actions or by actions of close relatives (siblicide; in particular, the parent-caused death of an offspring or filial infanticide) and not caused primarily by accidents or diseases or external factors, which is determined, regulated or influenced by genes favoured by natural selection." [Libertini 2012a]. It includes a large and very heterogeneous category of phenomena, most well-known and documented for a long time [Finch 1990], but not appreciated, before Skulachev's definition, in their entirety and in terms of their important implications [Libertini 2012a].

For the new paradigm, aging is a physiological phenomenon and must necessarily have, like every other phenomenon of this nature: a) a normal function (or physiology); b) pathological alterations in specific cases; c) evolutionary causes; and finally, d) a phylogeny.

In this paper, I will not discuss or reiterate the arguments or the evidence in support of the new paradigm and against the old paradigm, which have already been debated elsewhere [Libertini 2008, 2015a], nor a description of aging physiology and pathology, already expounded in general in other works [Libertini 2009a, 2009b, 2014a].

The aim of this paper is only to indicate, or at least to hypothesize, phylogenetic aspects of aging in relation to other similar or related phenoptotic phenomena.

It is opportune to stress that the definition of phenoptosis includes a wide and heterogeneous set of phenomena that do not necessarily involve the same evolutionary advantages or a single monophyletic origin. In fact, by choosing among many phenoptotic phenomena, as examples:

1) Endotokic matricide, a particular phenomenon shown by some invertebrates, where maternal death is obligatory in reproduction: "the young kill their mother by boring through her body wall" or cannibalizing her body [Finch 1990];

2) Cryptic female choice [Loisiel et al. 2008], i.e. non-pathologic miscarriages to eliminate before birth offspring with lesser antigen variability and possible reduced resistance to infections [Apanius et al. 1997];

3) Semelparity and sudden death after reproduction shown by many species of Anguilliformes, Salmoniformes, dasyurid marsupials, rodents, and plants [Finch 1990];

4) Aphagy in adult insects: "Aphagy from defective mouthparts or digestive organs is very common during the adult phases of insects ... and is *the* limiting factor in the adult lifespan of many short-lived species. This phenomenon is, inarguably, programmed senescence. ..." [Finch 1990];

5) Bacterial suicide activated by phage infection [Raff 1998];

6) Filial infanticide [Hausfater and Hrdy 1984];

it is quite unlikely that these phenomena can be explained on the basis of common evolutionary advantages and/or with a single phylogenetic origin.

Thus, the phylogenetic investigation will be restricted to the relationships and similarities between aging and certain types of phenoptotic phenomena that appear to have a probable common phylogenetic origin, with or without a common evolutionary advantage.

Prokaryote world

Phenoptosis is well-documented among prokaryotes, e.g.:

1) Bacterial suicide activated by phage infection "thereby curtailing viral multiplication and protecting nearby *E. coli* from infection" [Raff 1998]; "In *E. coli*, three suicide mechanisms that are activated by the appearance of a phage in the cell interior have been described" [Skulachev 2003];

2) Mass suicide of bacterial phytoplankton as defense against viruses [Lane 2008];

3) In *E. coli*, the "built-in suicide module" that is activated by antibiotics [Engelberg-Kulka 2004].

The mechanisms that activate phenoptosis in bacteria have been defined as "proapoptosis" and proposed as phylogenetic precursors of apoptosis in eukaryotes [Hochman 1997], because they share various features with apoptosis: "Several key enzymes of the apoptotic machinery, including the paracaspase and metacaspase families of the caspase-like protease superfamily, apoptotic ATPases and NACHT family NTPases, and mitochondrial HtrA-like proteases, have diverse homologs in bacteria, but not in archaea. Phylogenetic analysis strongly suggests a mitochondrial origin for metacaspases and the HtrA-like proteases, whereas acquisition from Actinomycetes appears to be the most likely scenario for AP-ATPases. The homologs of apoptotic proteins are particularly abundant and diverse in bacteria that undergo complex development, such as Actinomycetes, Cyanobacteria and alpha-proteobacteria, the latter being progenitors of the mitochondria." [Koonin and Aravind 2002].

In the prokaryote world, phenoptosis is not at all a curiosity limited to a few rare cases but appears to be a very common occurrence that determines spectacular mass suicide [Lane 2008].

Intrinsic to the definition of phenoptosis and therefore necessary to explain "programmed death in bacteria" [Lane 2008; Skulachev 2003] is that these phenomena are favored by natural selection.

In prokaryotes, the main causes of selective pressure in favor of phenoptosis appear to be: 1) defense against infections by phages [Raff 1998; Lane 2008]; 2) the elimination of somehow impaired individuals that take away resources from other individuals: "Most bacterial species actually do not live as planktonic suspensions in vivo but form complex biofilms, tightly knit communities of cells. From this perspective, programmed death of damaged cells may be beneficial to a multicellular bacterial community." [Lewis 2000]

In both cases, it is essential to envisage mechanisms of kin or group selection (as already proposed by others: "As most plankton in a bloom are near identical genetically, from the perspective of their genes, a die-off that creates enough scorched earth to stop the viral advance can make sense" [Lane 2008]), despite the old theoretical anathema against group selection by Maynard Smith [Maynard Smith 1964, 1976].

Regarding the type of selection that would favor these phenomena, it is important to stress that for kin selection, which is well-known and accepted [Hamilton 1964, 1970; Trivers 1971; Trivers and Hare 1976], if a species is divided into demes, each consisting of closely related individuals or even with monoclonal origin, the distinction between kin selection and group selection thins or disappears.

In fact, kin selection calculates the inclusive fitness of a C gene that acts in the individual defined as 1, and that has also some consequences for the fitness of other individuals (2, 3, ... n) in which the probability of the existence of C is equal to the coefficient of kinship (r). In each generation, C is favored by natural selection if the variation of the inclusive fitness is positive, i.e. if:

$$\sum_{x=1}^{n} (S_x \cdot P_x \cdot r_x) > 0 \quad (1)$$

where S_x = advantage/disadvantage for the individual x (-1 >= S_x <= +1); P_x = reproductive value of individual x (0>= P_x <= 1); r_x = coefficient of kinship between individual x and individual 1 (0>= r_x <= 1).

In cases where the gene acts only on individual 1, since by definition $r_1 = 1$, the formula (1) becomes:

$$S_1 \cdot P_1 > 0 \quad (2)$$

which is the classic formula for individual selection.

Now, let us consider a species divided into monoclonal demes and subjected to a catastrophic event. In such cases, for each of them, if there is no sacrifice of any individual, there is a disadvantage for every individual equal to *S*.

On the contrary, if, by action of the C gene, among n individuals having the C gene, some (n_d) sacrifice themselves and die (S_d = -1) while the survivors (n_s) have an advantage S_s, as in bacteria a constant reproductive value at any age can be assumed (P_x=1) and as in a monoclonal deme r_x is always equal to 1, the C gene will be favored by natural selection if:

$$\sum_{x=1}^{n_d} S_d + \sum_{x=1}^{n_s} S_s > S \cdot n \quad (3)$$

that is:

$$n_d \cdot S_d + n_s \cdot S_s > S \cdot n \quad (4)$$

formula that is a development of (1).

In cases where the deme is composed of several clones (1, 2, ... z), if C exists in all the individuals of clone 1, it will exist in clone x with a probability equal to the coefficient of kinship of the individuals of clone x with those of clone 1 (r_x), and gene C is favored by selection if:

$$(n_{1,d} \cdot S_d + n_{1,s} \cdot S_s) + (n_{2,d} \cdot r_2 \cdot S_d + n_{2,s} \cdot r_2 \cdot S_s) \ldots . + (n_{z,d} \cdot r_z \cdot S_d + n_{z,s} \cdot r_z \cdot S_s) > S \quad (5)$$

where, in a clone x, $n_{x,d}$ are the individuals that sacrifice themselves and $n_{x,s}$ the survivors.

It should be noted that kin selection formula (1) has been transformed into a formula that describes a type of group selection; thus, there is no insurmountable distinction between individual selection, kin selection and group selection.

Unicellular eukaryiotic world

For the sake of brevity and to propose evidence and arguments based on sound grounds, in this section the discussion will be limited to a single but well-studied eukaryotic unicellular species, i.e. yeast (*Saccharomyces cerevisiae*).

In this species, a phenomenon that closely resembles apoptosis of multicellular eukaryotes is a relatively recent finding [Madeo et al. 1997]. In particular, the phenomenon was shown to be elicited by the overexpression of a factor that triggered apoptosis (mammalian BAX) [Ligr et al. 1998], while the overexpression of another factor that inhibited apoptosis (human Bcl-2) appeared to delay the processes leading to it [Longo et al. 1997].

The similarity between this phenomenon and apoptosis in multicellular eukaryotes is corroborated by a growing body of evidence. This implies that the two types of phenomena deserve the same name and, moreover, suggests a common phylogenetic origin [Madeo et al. 1999; Longo et al. 2005; Kaeberlein et al. 2007]: "... since the first description of apoptosis in a yeast (*Saccharomyces cerevisiae*) strain carrying a CDC48 mutation ..., several yeast orthologues of crucial mammalian apoptotic proteins have been discovered ..., and conserved proteasomal, mitochondrial, and histone-regulated apoptotic pathways have been delineated ..." [Büttner et al. 2006]

In yeast, apoptosis is triggered or favored by: a) harmful chemical alterations to the habitat [Madeo et al. 1999]; b) a decrease in nutrients [Granot et al. 2003]; c) unsuccessful mating [Büttner et al. 2006]; and d) killer toxins that are secreted by competing yeast tribes [Büttner et al. 2006].

A crucial fact, analogous to what happens in multicellular eukaryotes, is that when a yeast cell dies by apoptosis, its parts are not harmful to other individuals and, on the contrary, are usefully phagocytosed or absorbed by other cells, which, consequently, "are able to survive longer with substances released by dying cells" [Herker et al. 2004].

Yeast apoptotic patterns have been explained as adaptive because they appear to be useful for the survival of the deme [Skulachev 2002a, 2003; Fabrizio et al. 2004; Herker et al. 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf 2006]. The adaptive interpretation of mass suicide by apoptosis appears plausible in many cases if a yeast species is divided into small demes, each consisting of one or a few clones. A different case is suicide by apoptosis triggered by toxins that are secreted by enemy yeast tribes, where the abovementioned adaptive mechanism is clearly exploited by competitors [Büttner et al. 2006].

So far it is possible to notice evident analogies between prokaryotic mass phenoptosis through proapoptotic mechanisms and yeast mass phenoptosis through apoptosis, and in both cases group selection is a likely evolutionary cause of these phenomena. But, yeast shows more sophisticated mechanisms and something other that, as we shall see, is connected with phenomena shown by multicellular organisms.

Yeast reproduction occurs by asymmetric division into two cells, one defined as "mother" and the other as "daughter". Cells of the daughter lineage show no limit to reproduction, while those of mother lineage can reproduce only a limited number of times; Jazwinski found a limit of 25-35 duplications in about 3 days [Jazwinski 1993]. As the number of duplications increases, there is a growing vulnerability to apoptosis and replicative senescence [Laun et al. 2001; Herker et al. 2004; Büttner et al. 2006; Fabrizio and Longo 2008], and this explains how in particular stress conditions part of the population dies and the other survives.

However, this is more than a sophisticated mechanism to select a list of the individuals that must sacrifice themselves if necessary (with a priority for sacrifice proportional to the number of previous duplications in mother lineage).

In yeast, mother lineage cells show, proportionally to the number of duplications, besides increasing susceptibility to replicative senescence and apoptosis, increasing metabolic alterations [Laun et al. 2001; Lesur and Campbell 2004; Herker et al. 2004; Büttner et al. 2006; Fabrizio and Longo 2008]. The consequent age-related death rate increase follows an exponential dynamic [Laun et al. 2007], similar to that shown by individuals of many multicellular species [Ricklefs 1998; Nussey et al. 2013]. Wild yeast cells of the mother lineage show a decline in fitness and an increase in mortality that is proportional to the number of duplications, so this phenomenon is somehow within the concept of aging ("increasing mortality with increasing chronological age in populations in the wild" [Libertini 1988]). It should be noted that while it is possible to say that yeast ages, this is inappropriate for bacteria.

Now, it is essential to consider how this happens in yeast.

Eukaryotic cells, both of unicellular and of multicellular species, unlike prokaryotes that have circular DNA, have linear DNA. It is well-known that, at each replication, DNA polymerase leaves out part of the terminal section of linear DNA (the telomere) and the molecule becomes shorter

[Olovnikov 1971; Watson 1972]. The progressive shortening of DNA leads to duplication impairment and, so, it was predicted that an indispensable but hypothetical enzyme restored the lost part of the telomere [Olovnikov 1973]. The enzyme (telomerase) was subsequently discovered [Greider and Bleckburn 1985].

In yeast, telomerase is always active and at any duplication faithfully restores the length of the DNA molecule. Therefore, yeast cells of both mother and daughter lineage show no telomere length decrease at each replication [D'Mello and Jazwinski 1991; Smeal et al. 1996; Maringele and Lydall 2004].

This indicates that the metabolic alterations and the vulnerability to apoptosis and replicative senescence shown, proportionally to the number of divisions, by the individuals of the mother lineage are due to another mechanism that has been identified.

In yeast mother cells of the wild-type, particular molecules, i.e. extrachromosomal ribosomal DNA circles (ERCs), accumulate proportionally to the number of duplications [Sinclair and Guarente 1997] and "several lines of evidence suggest that accumulation of ERCs is one determinant of life span" [Lesur and Campbell 2004].

In this regard, two yeast mutant types show interesting data. The mutants of *dna2-1* type suffer from anomalous DNA replication and so manifest increased rates of ERC accumulation, which causes precocious alterations in gene expression. In short, in mother lineages, young individuals of these mutants have a transcriptome that is similar to those of older individuals of normal yeast [Lesur and Campbell 2004].

Another type of mutant yeast, *tlc1Δ* mutants, which is telomerase deficient, show telomere shortening both in mother and daughter cells. Moreover, older individuals belonging to daughter cell lineages, which – as normal strains - have no ERC accumulation, manifest an overall expression of genes, i.e. a transcriptome, similar to that of mother lineage older individuals of normal strains and of mother lineage young individuals of *dna2-1* mutants [Lesur and Campbell 2004].

As we shall see below for multicellular eukaryotes, it is possible that in yeast mutants that are telomerase deficient, as in cells of multicellular eukaryotes with inactive telomerase, the shortening of the telomere causes the sliding of a heterochromatin hood over the telomere and this interferes with critical parts of subtelomeric DNA.

From evidence largely based on experiments in yeast: "One model of telomere-gene expression linkage is an altered chromosomal structure ..., such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome .... As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) or the hood shortens (as the telomere is less capable of retaining heterochromatin). In either case, the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity .... These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel 2004].

While in *tlc1Δ* yeast mutant the silencing of subtelomeric DNA could be a consequence of telomere shortening, in non-mutant yeast cells of the mother lineage, silencing could be caused by progressive ERC accumulation that covers and inhibits the subtelomeric region (for further considerations about gradual subtelomeric silencing and the related metabolic alterations, see the next section, i.e. the subsection "gradual" cell senescence).

Regarding the increasing susceptibility to apoptosis and replicative senescence, in proportion to duplication number, a mechanism analogous to that found in multicellular eukaryotes could be proposed (see the next section, i.e. the subsection "Cell senescence").

However, these data raise an important question. A mechanism that causes a differential resistance to apoptosis and therefore establishes a kind of priority list for individual sacrifice in case

of need is perfectly consistent with the logic of group selection that appears to favor mass phenoptosis if this is useful for the survival of the deme.

On the contrary, the fact that the same mechanism (or something related) progressively impairs cellular metabolism and the vulnerability to replicative senescence, and thus fitness, does not appear to be necessary for the purposes of possible sacrifice and, therefore, is not explained by the abovementioned group selection. We must therefore assume: (i) an obligatory link between increasing vulnerability to apoptosis and progressive metabolic impairment (and likewise the critical importance of the DNA subtelomeric region), (ii) or that there is an evolutionary alternative explanation.

In fact, Büttner et al. already suggested that "apoptosis coupled to chronological and replicative aging limits longevity that would maintain ancient genetic variants within the population and, therefore, favor genetic conservatism." [Büttner et al. 2006]

This is not at all a new hypothesis.

Well before the acquisition of the above reported data from yeast, it was proposed that, for species subject to K-selection (see definition below) [Pianka 1970], an age-related fitness decline, i.e. aging, would be adaptive [Libertini 1988, 2006]. The original papers should be read, but here a short exposition may be useful.

Within any species, the speed of diffusion of any gene G depends both on its advantage (S) over an allele that is assumed neutral and on the generation time, i.e. the reciprocal of the "mean duration of life" (= $1/ML$; see fig. 1).

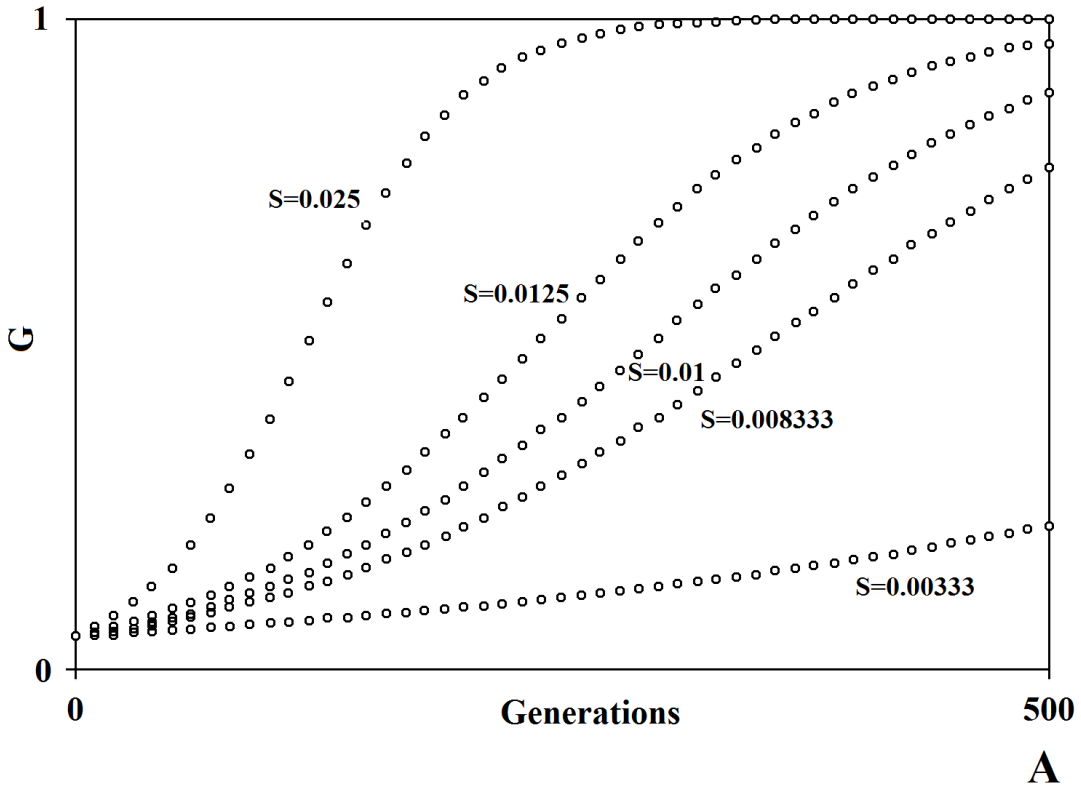
Now, a hypothetical gene C that brings about the untimely death of the individual I in which C is present, and therefore reduces its ML and determines a disadvantage S', quickens the diffusion of any advantageous gene G in the individual I' that replaces I. If I' is relative to I, C will be increased in its frequency by natural selection, if:

$$r \cdot \sum_{x=1}^{n} (S_x) \cdot (1/ML_C - 1/ML_{C'}) - S' > 0 \qquad (6)$$

where: ML_C indicates the ML of individuals having gene C and $ML_{C'}$ is the ML of those having a neutral allele C'; $\Sigma(S_x)$ is the summation of the advantages of n favorable genes G spreading within the species; $-S'$ is the disadvantage of a shorter ML; and r indicates the mean coefficient of kinship between I and I'.

Three brief annotations: (i) formula (6) is another development of the general formula (1) for kin selection; ii) if G is a harmful gene, C accelerates its elimination; iii) kin selection as an explanation for aging should not be confused with its use to explain the survival of post-reproductive individuals, e.g. as suggested elsewhere [Lee 2008].

This hypothesis was proposed for multicellular eukaryotes, but for its application to unicellular eukaryotes there is no opposing theoretical argument against it. Büttner et al. do not propose alternative evolutionary explanations besides the above-mentioned suggestion [Büttner et al. 2006], which may be considered a short enunciation of the hypothesis described [Libertini 1988], in particular if, for yeast, we suppose ecological life conditions of the K-selection type. On the other hand, the above described adaptive interpretation of aging applied to yeast may be considered within Büttner et al.'s suggestion formulated in terms of kin selection.



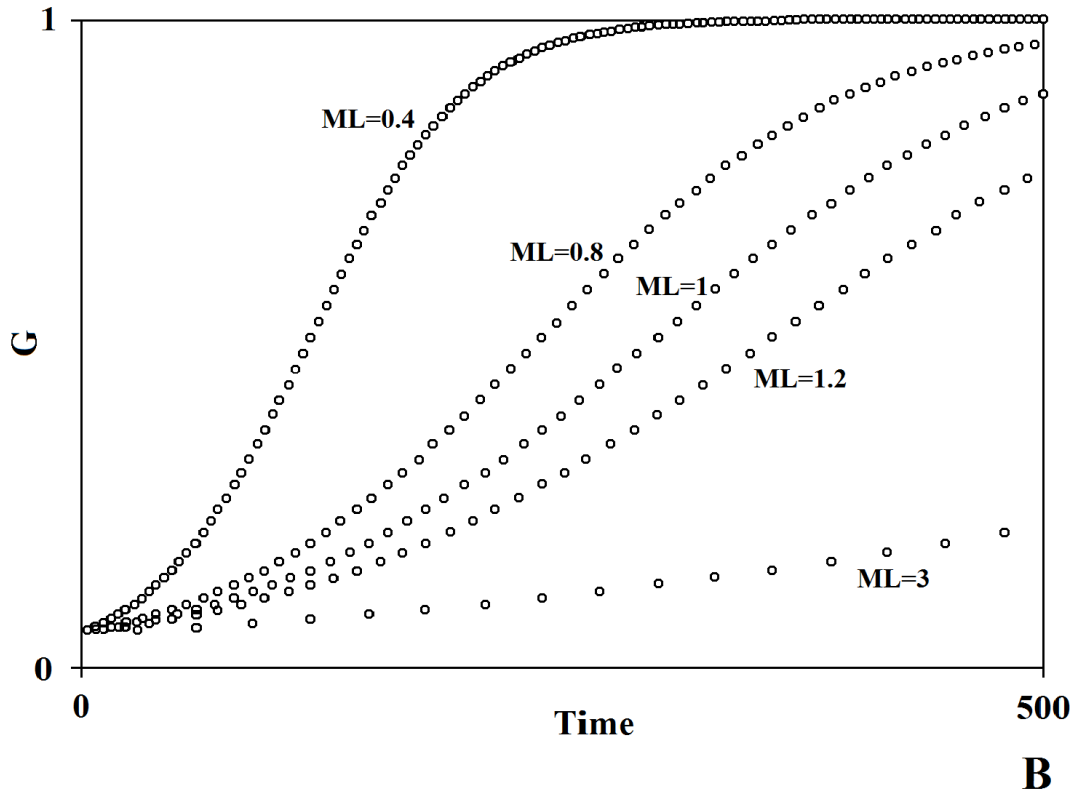


Figure 1 - A) Diffusion of a gene in relation to the value of *S*; B) Diffusion of a gene in relation to ML variation. An increase/decrease in *S* or of the inverse in the *ML* value have the same results on the spreading speed of a gene within a species (figure redrawn from [Libertini 1988]).

Lewis, in his criticism of this hypothesis, argues against the "suggestion that yeast cells provide a precedent for programmed death" [Lewis 2000], proposed by others [Sinclair et al. 1998], by the

following remark: if a mother lineage yeast cell dies after the 25-35 duplications reported in laboratory conditions [Jazwinski 1993], the presence of a single individual with the greatest possible number of duplications among 2^{25}-2^{35} (= $3.36 \cdot 10^{7}$-$3.44 \cdot 10^{10}$) descendants appears unlikely and so its death would be insignificant for any adaptive theory of programmed death.

However, Lewis' argument misses the pivotal point: the death at the last possible duplication of a single individual among countless others is a very rare or impossible event, while the increasing and progressive probability of apoptosis - proportionally to the number of duplications -, plus the differences in fitness (i.e. mortality rates) and in the capability of having offspring in the comparison between "younger" and "older" individuals, is real: ("in a population of [yeast] cells the lifespan distribution follows the Gompertz law" [Laun et al. 2007], i.e. an age-related progressive increase of mortality; "The probability that an individual yeast cell will produce daughters declines exponentially as a function of its age in cell divisions or generations (Jazwinski et al., 1998)." [Lesur and Campbell 2004]). If, in the wild, the death of "older" individuals significantly reduces the *ML* of yeast and thus causes a quicker generation turnover, Lewis' argument is trifling for the hypothesis that yeast duplication-related increasing mortality has a favorable selective value. However, Lewis' argument is very interesting as it echoes an analogous objection against programmed aging hypotheses for multicellular organisms, which will be debated in the next section.

Another possible criticism is that, in yeast, generations follow one another within a few days, a very fast rate when compared with that of species such as ours, and therefore the reduction of a few days of yeast *ML* might appear irrelevant. But this objection does not consider that the adaptive aging theory proposed in 1988 [Libertini 1988] (illustrated in fig. 1), is based on the relative acceleration of the evolution rate and not on the absolute value of it. For example, if the *ML* of a species passes from a value t to a value $t/2$, the spreading rate of a gene within a species doubles, both if t = 30 years and if t = 5 days (and if t has any other value).

The transition from unicellular to multicellular eukaryotes

The transition from a unicellular and monoclonal eukaryotic deme to a multicellular eukaryotic organism with undifferentiated or minimally differentiated cells likely means a gradual transition and not a drastic break.

In a monoclonal deme composed of unicellular individuals, the sacrifice of an individual by apoptosis is clearly a phenoptotic phenomenon explainable in terms of group selection. In a multicellular individual with undifferentiated cells, it is possible to define the sacrifice of some cells, e.g. by apoptosis, as a phenomenon distinct from phenoptosis but the difference of this sacrifice in the comparison with the apoptosis-phenoptosis of an individual in a monoclonal deme of single-cell individuals is small and difficult to define.

As, in multicellular species, the cells of an individual increase their differentiation and, in particular, as a fundamental divide, when reproductive function is entrusted solely to some differentiated cells, apoptosis differs markedly in its meaning from apoptosis-phenoptosis of unicellular organisms and acquires its distinct functions in the context of the more complex organization of a multicellular organism with differentiated cells and organs.

Multicellular eukaryotic world with differentiated cells

Multicellular eukaryotes show a series of phenomena that are important for our discussion.

1) Programmed cell death

In addition to apoptosis, multicellular organisms show various kinds of programmed cell death (PCD), e.g.: keratinization of epidermis or hair cells, osteocyte phagocytosis by osteoclasts,

detachment of cells from the internal walls of body cavities, and erythrocytes, which are specialized cells that lose their nucleus and are subsequently removed by macrophages.

For the first time, apoptosis was described and clearly differentiated from necrosis in multicellular eukaryotes organisms in a study of the normal liver [Kerr et al. 1972]. While necrosis is cell death determined by acute cellular damage, apoptosis may be defined as an ordered form of cell self-destruction. It is ubiquitous in eukaryotic species [Longo et al. 2005] and, similarly to what occurs in the yeast [Herker et al. 2004], a cell that dies by apoptosis does not harm other cells; cell fragments are removed by phagocytes in an orderly manner and do not elicit an inflammatory response [Erwig and Henson 2008].

PCD by apoptosis, which is selectively triggered at specific times and for specific cells, is indispensable for a series of functions that necessarily represent a development and an adaptation of the original functions in single-celled organisms: morphogenetic mechanisms (neural development in embryo [Nijhawan et al. 2000], wound healing [Greenhalgh 1998], etc.), lymphocyte selection [Cohen 1993; Opferman 2008], removal of infected or damaged cells [Tesfaigzi 2006; White 2006], etc.

In vertebrates, apoptosis occurs in many tissues and organs [Pontèn et al. 1983; Dremier et al. 1994; Finegood et al. 1995; Migheli et al. 1997; Prins and O'Rahilly 1997; Benedetti et al. 1998; Spelsberg et al. 1999; Cardani and Zavanella 2000; Harada et al. 2000; Héraud et al. 2000; Pollack and Leeuwenburgh 2001, Sutherland et al. 2001; Xia et al. 2001] and is an essential fact for cell turnover in healthy organs [Wyllie et al. 1980; Lynch et al. 1986; Israels and Israels 1999; Medh and Thompson 2000].

Short telomeres and inactivated telomerase increase the probability of apoptosis [Ozen et al. 1998; Holt et al. 1999; Seimiya et al. 1999; Ren et al. 2001; Fossel 2004].

2) Limits to cell duplication capacity and cell turnover

In healthy organ and tissues, to ensure normal cell turnover, continuous cell death by apoptosis and other PCD types must be balanced by substitution with an equal number of cells from the duplication of specific stem cells.

Before the 1960s, Nobel laureate Alexis Carrel's old thesis of unlimited duplication capacity in non-germline cells of multicellular organisms was undisputed [Carrel and Ebeling 1921a, 1921b], but, at the same time, the so-called Hayflick limit of cell duplication capacity had been demonstrated *in vitro* [Hayflick and Moorhead 1961; Hayflick 1965] and then *in vivo* [Schneider and Mitsui 1976]. This limit is documented for many cell types [Rheinwald and Green 1975; Bierman 1978; Tassin et al. 1979], is in inverse relation with the age of the individual [Martin et al. 1970] and, approximately, in direct relation with the longevity of the species [Röhme 1981]. As mentioned in a previous section, this limit is the consequence of the incomplete duplication of the telomere by polymerase.

Telomeres, highly conserved repetitive sequences of DNA [Blackburn and Gall 1978; Moyzis et al. 1988; Blackburn 1991], shorten at each cell duplication [Harley et al. 1990], but telomerase, if active, elongates telomere with each replication. This explains why germline cells have unlimited duplication capacity [Greider and Blackburn 1985]. If telomerase is inactive, duplications cause telomere shortening and a cell culture or a tissue shows a reduction in its duplication capacity [Yu et al. 1990], while a cell with activated telomerase becomes capable of unlimited duplications [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

Telomerase activity is regulated by specific proteins [van Steensel and de Lange 1997] and is active without restrictions in immortal human cell lines [Morin 1989]. On the contrary, for most cell types, telomerase activity is limited, likely in inverse proportion to the cell turnover rate. In fact, it is well-known that the cell turnover rate is quite variable according to cell, tissue and organ type: “bone has a turnover time of about ten years in humans” [Alberts et al. 2013] and “the heart is replaced roughly every 4.5 years” [Anversa et al. 2006], but “cells [of the intestinal epithelium] are

replaced every three to six days" [Alberts et al. 2013] (for other data about cell turnover rhythms, see [Richardson et al. 2014]). This implies that the various types of stem cells must allow the regulation of telomerase activity that varies enormously according to cell type. So, any limitation in the activity of telomerase must be genetically determined and finely modulated and cannot be the result of insurmountable biochemical restrictions.

In multicellular eukaryotes, in short, the limits of cell replication are determined and modulated by restrictions in telomerase activity.

This is different from what happens in yeast, where, as already emphasized, telomerase is always active and the progressive limitation in the ability to duplicate is determined by the accumulation – which is proportional to the number of duplications and happens only in the mother lineage - of particular molecules (ERCs) over the subtelomeric segment.

However, it has been also pointed out that for yeast *tlc1Δ* mutants, which suffer from telomerase inactivation, cells of the daughter lineage, which have no accumulation of ERCs, show telomere shortening and limitations in their ability to duplicate and other alterations such as those caused by ERC accumulation in mother lineage cells (Fig. 2). The similarities between yeast *tlc1Δ* mutants and the cells of multicellular organisms are impressive and have allowed us to envisage a common phylogenetic relationship.

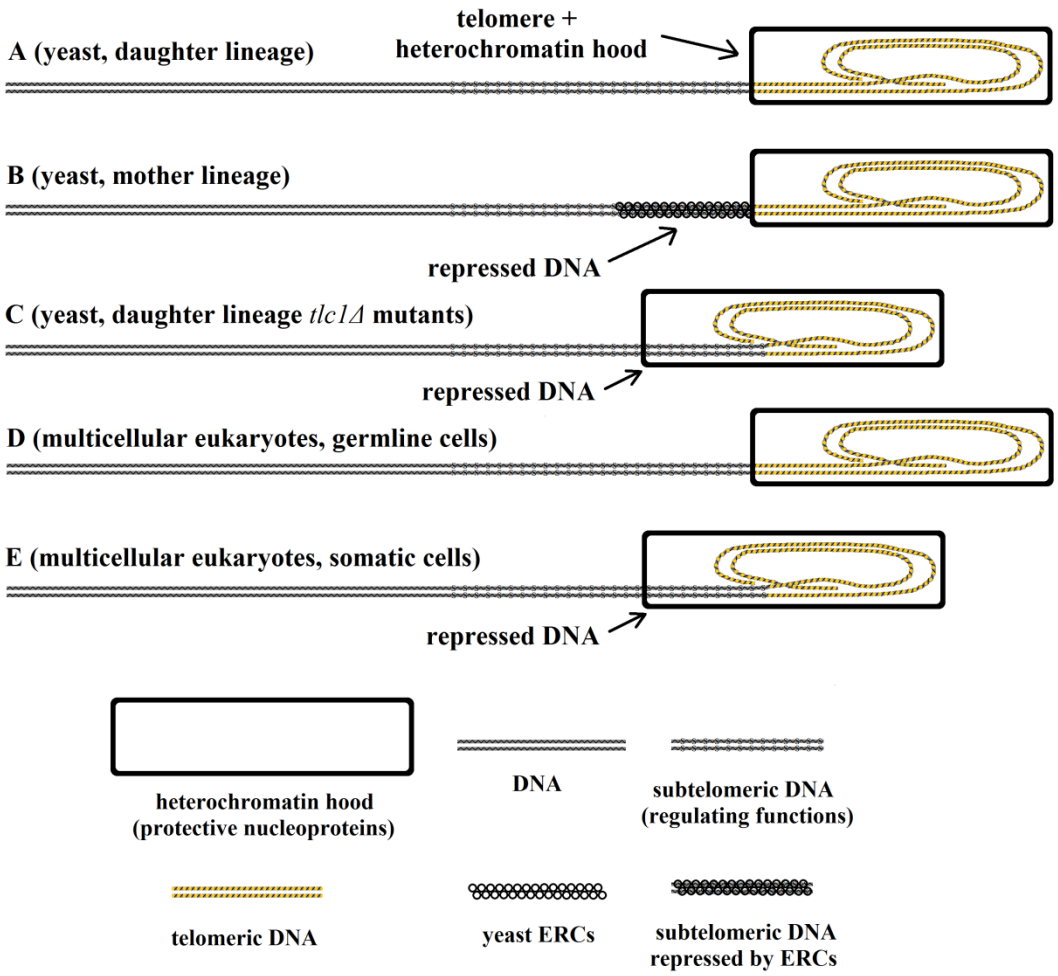


Figure 2 (modified and redrawn from fig. 6 of [Libertini 2015a]) - In A and D, telomeres are not shortened and the subtelomere (subtelomeric section of DNA molecule) is not repressed. In C and E, telomere are shortened and the subtelomere is repressed due to protein hood sliding. The "symbols" in the first line are used in the subsequent figures.

3) Cell senescence

In a cell culture, replicative senescence, i.e. the final incapability to cell duplication, was shown to be a progressive reduction in the growth potential of a cell culture, related to telomere length reduction, and not a sudden contemporaneous event for all cells [Pontèn et al. 1983; Jones et al. 1985].

According to Blackburn's model [Blackburn 2000], a protein hood caps the telomere, which oscillates between "uncapped" and "capped" conditions: the first state is susceptible to passage to replicative senescence, i.e. non-cycling conditions, while the duration of the other state is directly related to telomere length (fig. 3). Even if cells have activated telomerase and maintain telomeres at the maximum length, with each division, a small percentage of them should pass into the non-cycling state [Blackburn 2000].

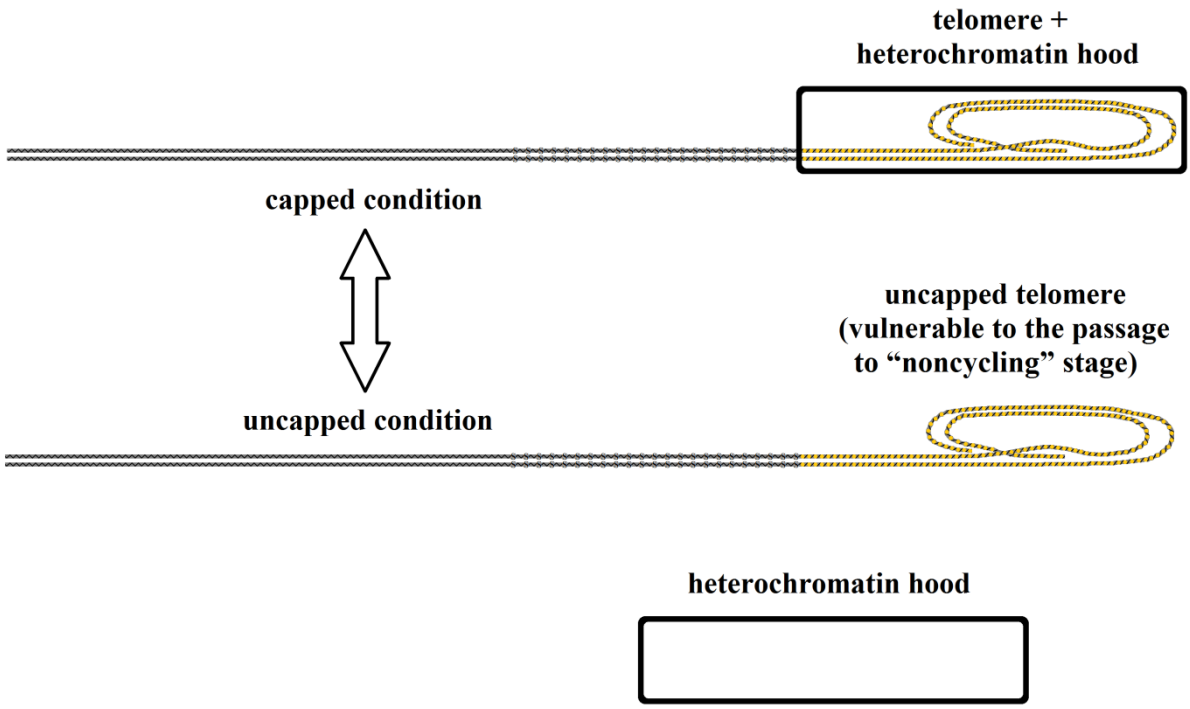


Figure 3 - Telomeres oscillate between the "capped" and "uncapped" conditions. The probability of uncapped telomeres increases in proportion to telomere shortening. A non-protected telomere is a free end of the DNA molecule and is susceptible to end-to-end joining that blocks cell replication.

For a population of cells with inactive telomerase and telomeres at their maximum length, a progressive decline of the replication capacity, proportional to the number of duplications, has been demontrated. Moreover, stem cells, unlike germ cells, should show levels of telomerase activity only partially able to preserve telomere length [Holt et al. 1996] and, therefore, they cannot indefinitely replace the elements eliminated by PCD for cell populations in renewal [Fossel 2004].

The absolute length of telomeres is not constantly or strictly related to the life span of a species, e.g. a) the hamster and the mouse have long telomeres [Slijepcevic and Hande 1999], but they age more precociously than humans who have shorter telomeres; b) in rodents, there is no relationship between telomerase activity and maximum lifespan [Gorbunova et al. 2008].

In connection with the mean number of cell duplications in a tissue or a cell culture, there is a growing probability of cell senescence, which has been indicated as a "fundamental cellular program" [Ben-Porath and Weinberg 2005] and is characterized by the modified expression of many genes, in a way that compromises cell functions, and by replicative senescence (i.e.,

Blackburn's “noncycling state” [Blackburn 2000]). A senescent cell has harmful consequences both on the extracellular matrix and on other cells that are physiologically interdependent or physically nearby. Cell senescence (replicative senescence, its main characteristic, included), certainly derives somehow from relative telomere shortening (Fossel’s “cell senescence limited model”) [Fossel 2004].

4) “**Gradual**” **cell senescence**

Telomere shortening influences the expression of subtelomeric DNA. This phenomenon has been known for some time and has been called the “telomere position effect” [Gottschling et al. 1990], but I prefer the definition of “ ‘gradual’ cell senescence” to this quite prudent expression. Apart from the references reported in the section dedicated to unicellular eukaryotes, a recent paper [Robin et al. 2014] confirms this phenomenon: “Our results demonstrate that the expression of a subset of subtelomeric genes is dependent on the length of telomeres and that widespread changes in gene expression are induced by telomere shortening long before telomeres become rate-limiting for division or before short telomeres initiate DNA damage signaling. These changes include up-regulation and down-regulation of gene expression levels.”, and highlights that telomere shortening, by repressing subtelomeric DNA, modifies gene expression even for distant non-subtelomeric parts of DNA.

Additionally, the likelihood of a mechanism of this type for multicellular eukaryotes as well is discussed at length by Fossel (see pp. 45-56 in [Fossel 2004]; a scheme of the phenomenon is illustrated in fig. 4).

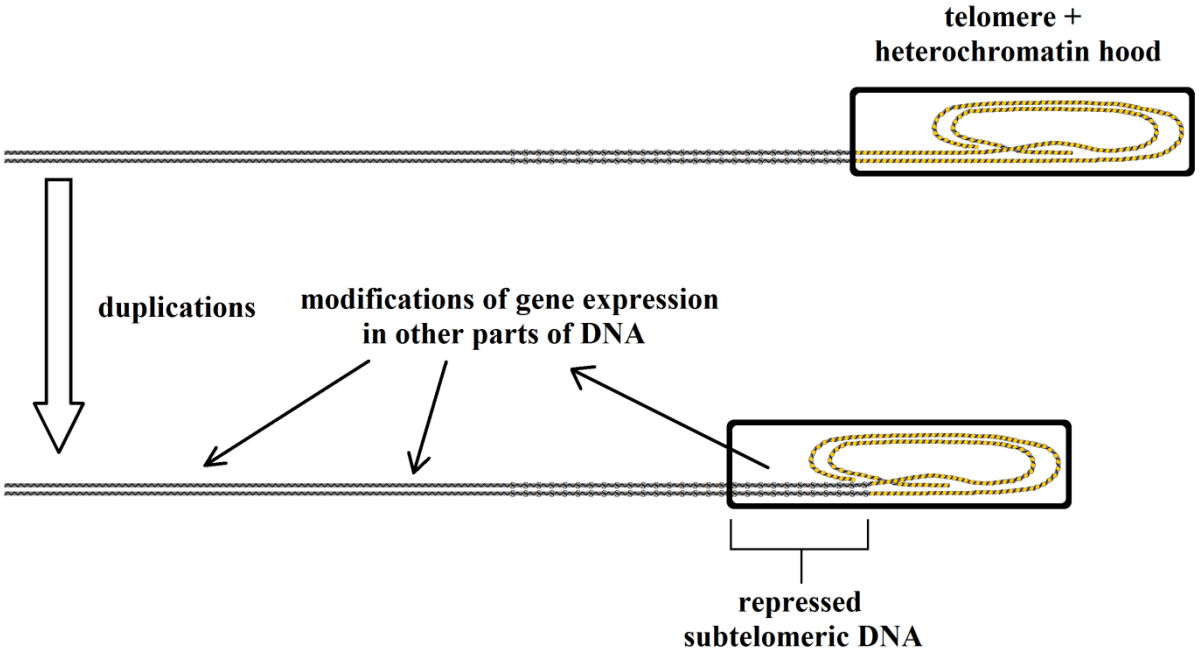


Figure 4 - Telomeres shorten at each replication and repress an increasing portion of subtelomeric DNA. This allows for alterations in gene expression in different and distant parts of the DNA molecule.

The capping nucleoproteins of cell senescence [Blackburn 2000] and the heterochromatin “hood” of “gradual” cell senescence [Fossel 2004] are very probably the same thing as: 1) they cover necessarily the same end part of DNA molecule; and 2) the activation of telomerase and the consequent lengthening of the telomere determine the reversal of all the characteristics of cell senescence [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

5) Relationship between aging and relative telomere shortening and not with absolute telomere length

For germline cells and for the somatic cells of a donor from which a cloned animal is originated, resetting the telomere clock is indispensable before the first cell duplication [Fossel 2004]. The initial telomere length must be established in the reset phase because, with each following telomere shortening, the probability of cell senescence will increase. In the "reset" phase, the absolute value of the "telomere length is irrelevant" [Fossel 2004]. For example, two *Mus* strains, with a telomere length of 10 and 20 kb, respectively, have equal life spans and patterns in the timing of cell senescence; the same has been demonstrated for cloned animals derived from somatic cells with shortened telomeres and their donor animals [Fossel 2004]. In the "reset" phase, an appropriate modelling of the heterochromatin hood based on telomere length could justify the related timing of "gradual" cell senescence and of cell senescence in spite of the different lengths of their telomeres (fig. 5).

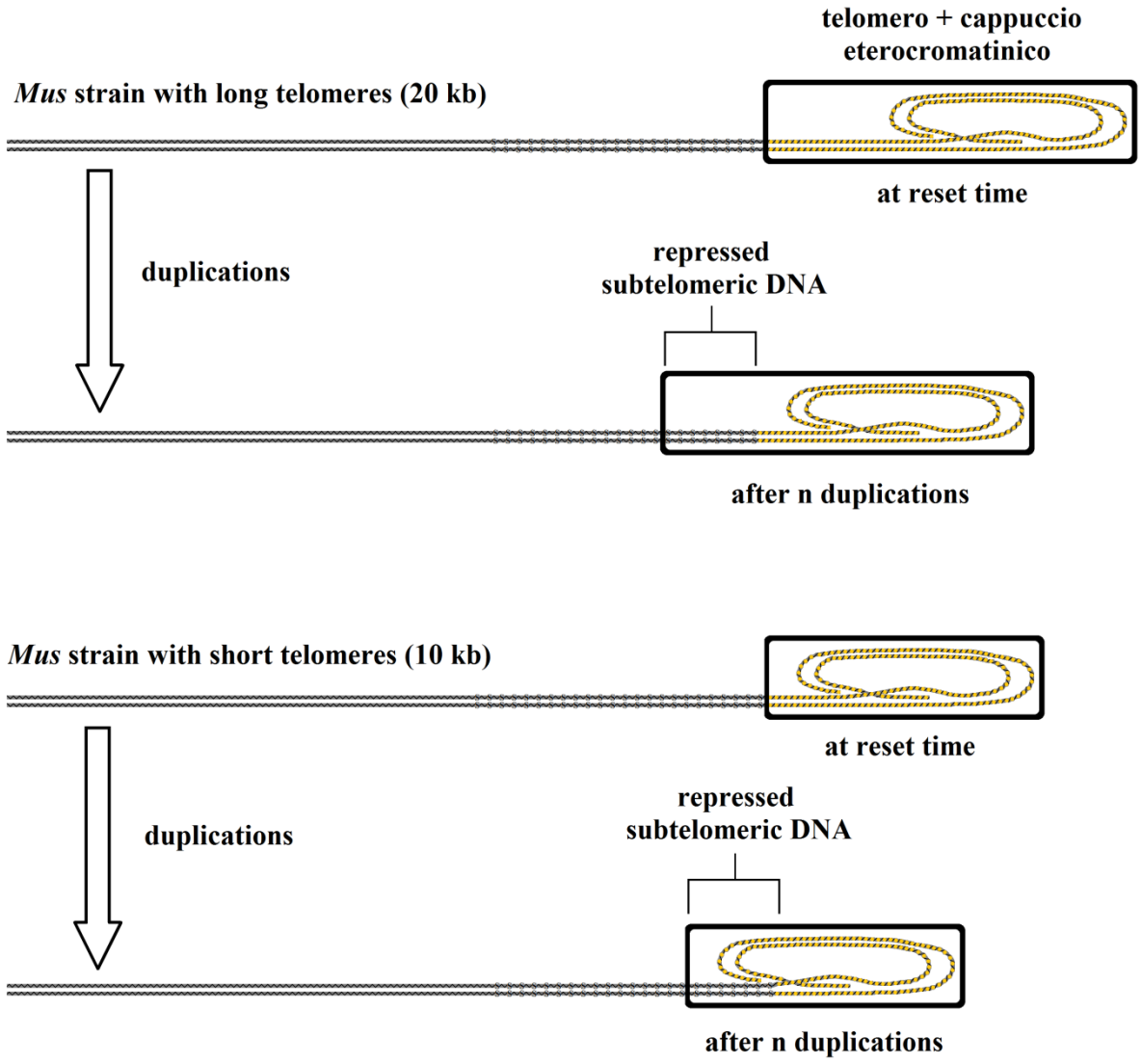


Figure 5 - When the telomere clock is reset, the heterochromatin hood is likely shaped in proportion to the telomere length and should have the same size during all cell life. Replication related telomere shortening determines the sliding of the abovesaid heterochromatin hood over the subtelomeric DNA with the above-mentioned negative consequences on cell functions and on the equilibrium between the capped/uncapped telomere state. This hypothetical model may explain the irrelevancy of initial telomere length as regards the consequences of its subsequent shortening [Fossel 2004].

Mice and other animals show a shorter life span, despite much longer telomeres in comparison with our species [Slijepcevic and Hande 1999] and a baseline activity of telomerase in most somatic cells [Prowse and Greider 1995]. (But, in mice microglia cells, it has been observed that telomeres shorten with age and "the low levels of telomerase activity present may be preferentially recruited to maintain the shortest telomeres while allowing the longer ones to shorten more rapidly" [Flanary 2003].) Moreover, in telomerase knockout (mTR-/-) mice, characterized by genetically inactivated telomerase, we see that only after four [Herrera et al. 1999] to six [Blasco et al. 1997] generations, when telomeres become very shortened, fertility and viability are jeopardized, but in organs with high cell turnover, the dysfunctions appear in early generations [Lee et al. 1998; Herrera et al. 1999] (in any case, the fitness reduction caused by this alterations should be considered in relation to wild conditions and not in the artificial protected conditions of the laboratory). The model of fig. 5, as developed in fig. 6, could easily explain this apparently paradoxical phenomenon.

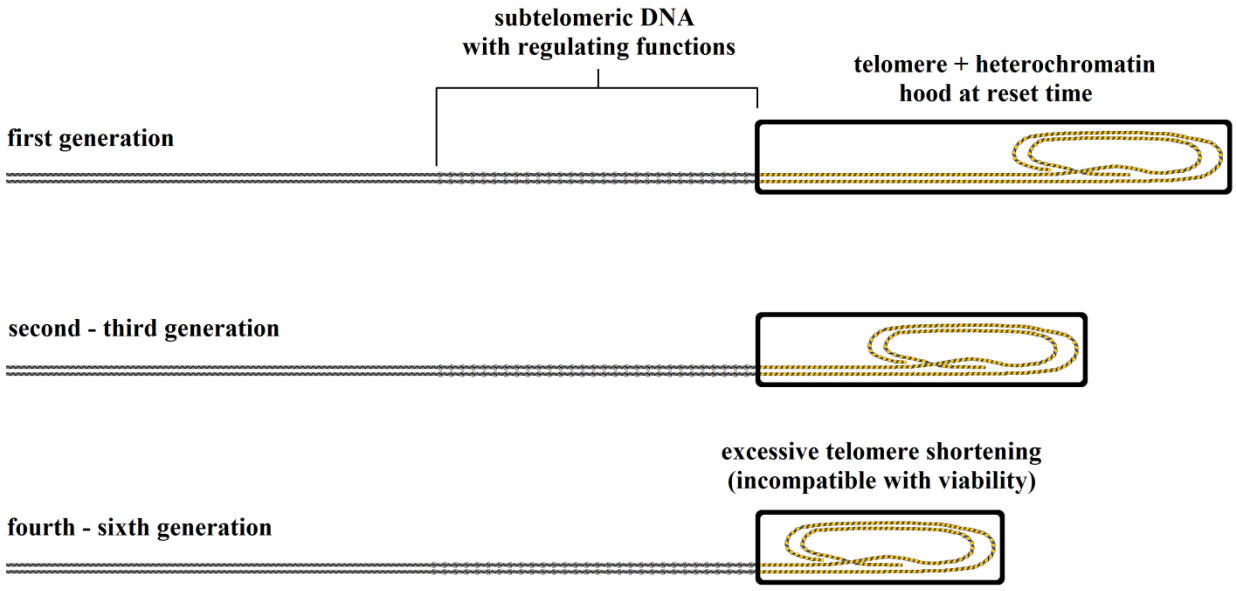


Figure 6 - In knockout mice, heterochromatin hood length, which is defined in the reset phase, must be proportional to telomere length. Afterwards, the heterochromatin hood slides over subtelomeric DNA and progressively represses it, but this is not influenced by the length of the hood. If the telomere, in the reset phase, is exceedingly shortened, the mechanism is jeopardized and the viability of the cell is lost.

The evidence highlighted before (with its falsely misleading apparent contradictions) leads to an immediate crucial consideration. If:

a) the telomere length in the reset phase (provided that it is not below a critical level) does not affect longevity;

b) aging is proportional to telomere shortening and

c) telomere shortening is directly related to the gradual repression of subtelomeric DNA;

the trivial conclusion is that for the greater or lesser longevity we should investigate the relationship not with telomere length but with the length and other properties of the subtelomeric segment.

This hypothesis, restricted to the length of the subtelomeric region, is illustrated in fig. 7 and is compatible with the evidence and the arguments expounded above and illustrated in figs. 2-6. Moreover, we should recall that in yeast, which has a fixed telomere length, aging is caused solely by ERC accumulation over the subtelomeric segment.

An implication of this hypothesis is that no necessary correlation between telomere length and longevity is predicted; in fact, a correlation is contradicted by the evidence [Slijepcevic and Hande 1999; Gorbunova et al. 2008].

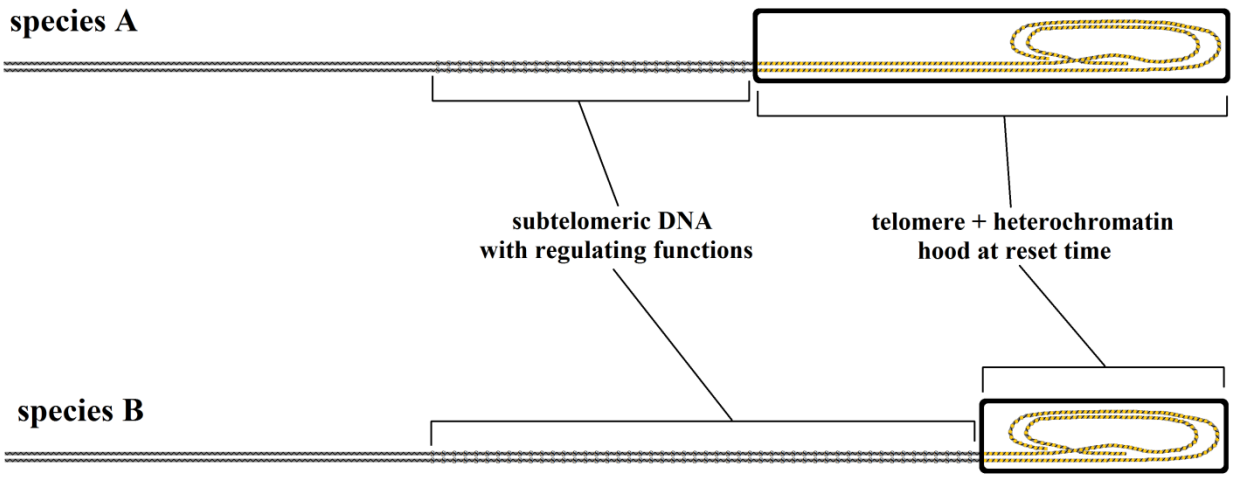


Figure 7 - Species A has longer telomeres and shorter subtelomeric segments than species B. For species A, telomere shortening causes a greater relative impairment of the subtelomeric area and this should result in earlier aging.

In short, subtelomeric DNA has been shown to have both essential importance for cell functions and a position vulnerable to repression by telomere shortening itself. If we exclude inexplicable evolutionary contradictions, this coincidence may be justified only as something favored by selection to determine "gradual" cell senescence and cell senescence. A likely interpretation of the evolution of the system that includes telomeres, telomerase, subtelomeric DNA and cell senescence is proposed below.

A suggested and very probable cause for age-related fitness decline is the progressive slowing down of cell turnover, which means a progressive prevalence of PCD on cell substitution by stem cell duplication (Fossel's "cell senescence general model of aging" [Fossel 2004; Libertini 2006]) coupled with an increasing fraction of cells more or less altered by "gradual" cell senescence and cell senescence [Fossel 2004; Libertini 2014a].

In support of this hypothesis, in some species (rockfish and lobsters), both mortality rate and telomere length do not vary with the age [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998].

There is evidence for an evolutionary advantage of the age-related mortality increase phenomenon [Libertini 2008, 2015a], which in its more advanced manifestations, common in artificially protected conditions, is usually defined as "aging", a term that is inaccurate in this context [Libertini 2006]. A theory, identical to that mentioned above to explain "gradual" cell senescence and cell senescence in yeast, justifies this fitness decline, or mortality increase, as evolutionarily advantageous, in terms of supra-individual selection, by a mechanism based on kin selection that, due to a faster generation turnover, allows a quicker diffusion of any advantageous mutations. In accordance with the theory, this advantage exists only when there is K-selection (i.e., species divided into demes that are composed of kin individuals and living in saturated habitats where only the disappearance of an individual allows the existence of a new individual) [Libertini 1988, 2006].

An objection against this hypothesis, is that "As a rule, wild animals simply do not live long enough to grow old. Therefore, natural selection has limited opportunity to exert a direct influence over the process of senescence." [Kirkwod and Austad 2000]. This criticism, analogous to Lewis'

argument for yeast and as mentioned above, misses an essential point: the absence in wild conditions of "old" individuals (e.g., for *P. leo*, individuals that are older than 15 years) is irrelevant. Individuals of *P. leo* with an age below 15 years are "not old" individuals according to Kirkwood and Austad's concept [Kirkwood and Austad 2000], yet they show increasing mortality at ages existing under natural conditions: this significantly reduces the *ML* with a consequent quicker generation turnover and the proposed selective advantage.

"Senescence reduces average life span ... by almost 80% when m_0 = 0.01 yr^{-1}" [Ricklefs 1998]. For the individuals that survived the high mortality of the first life phases, in eight mammal species studied in the wild, the ratio between *ML* with age-related increasing mortality (wild condition) and *ML* without the mortality increase (hypothetical condition) has been calculated to be in the range of 2.5-5. If we do not disregard the individuals that died in the first life phases, the ratio has been shown to be in the range of 1.55-3.21 [Libertini 1988].

Moreover, the study of a human population under wild conditions [Hill and Hurtado 1996; Libertini 2013] has shown that: (i) survival for 60- and 70-year old individuals was about 30% and nearly 20%, respectively, and (ii) the proportion of *ML* reduction was considerable and, so, undoubtedly subjected to natural selection (fig. 8).

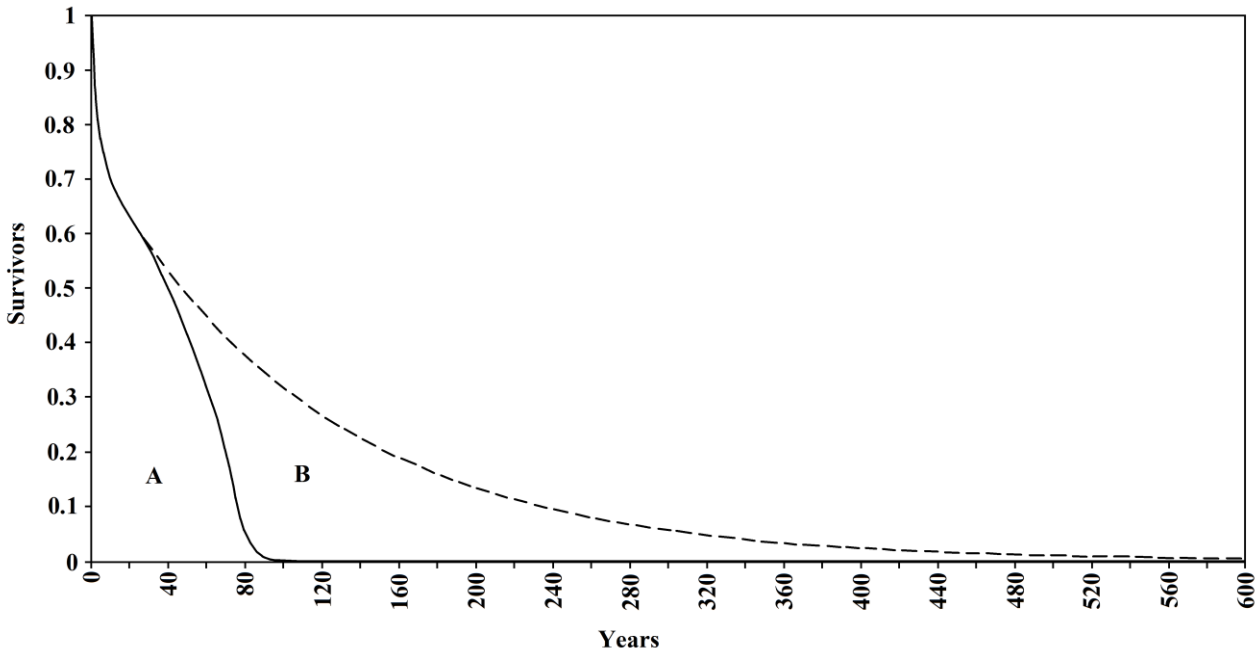


Figure 8 - Area A (delimited by the continuous line): life table of Ache in the wild, data from [Hill and Hurtado 1996], figure – modified - from [Libertini 2013]; Area B (delimited by the dashed line): hypothetical life table without age-related increasing mortality. The proportion of senescent death (P_s, as defined by Ricklefs [Ricklefs 1998]) is given by the ratio between B and A+B.

In short, in the wild, *ML* reduction determined by age-related fitness decline is not at all negligible, although the equivalents of centenarians or older indiviuals for animals are most likely non-existent under wild conditions.

Conclusion

The main points of the above-mentioned evidence and arguments can be summarized as follows:

a) Prokaryote mass phenoptosis by proapoptosis and unicellular eukaryote mass phenoptosis by apoptosis, in particular cases, are favored by similar supra-individual selective mechanisms and likely have a common phylogenetic origin [Hochman 1997].

b) For both types of phenomena, a mechanism that activates the suicide pattern is indispensable only in a fraction of the population and should be proportional to necessity (e.g., the scarcity of a critical resource). Yeast have evolved an effective mechanism depending on the number of preceding duplications and on ERC accumulation over subtelomeric DNA that creates a kind of ranking for individuals destined to sacrifice themselves [Herker et al. 2004; Büttner et al. 2006; Laun et al. 2007; Fabrizio and Longo 2008].

c) Apoptosis in multicellular eukaryotic species has an evident phylogenetic relationship with unicellular eukaryotic apoptosis [Longo and Finch 2003] but the evolved clock is based not on the accumulation of ERCs but on telomere shortening due to restrictions in telomerase activity analogously to the case of yeast *tlc1Δ* mutants in which telomerase is inactive [Fossel 2004]. In both cases, the damaging effects on the cell are caused by the progressive repression of subtelomeric DNA, which is clearly a pivotal aspect of ageing mechanisms.

d) In multicellular eukaryote organisms with undifferentiated cell functions, if we consider each individual as a clone that has all cells with the same genes, apoptosis of a cell may be regarded as caused by mechanisms analogous to kin selection. In multicellular eukaryote organisms, as cells assume different functions and, in particular, with the limitations of reproductive function to some specific cells, this interpretation is inappropriate and the multicellular individual is subjected to natural selection as a single entity.

e) In multicellular eukaryote organisms, apoptosis assumes a long series of functions (morphogenetic mechanisms, lymphocyte selection, removal of damaged or infected cells, etc.), which are unquestionably a derived function, as they are impossible in unicellular organisms.

f) In yeast, apoptosis is essential for the phenomenon summarized in (a), but, moreover, the phenomena defined as cell senescence and "gradual" cell senescence cause a duplication-related progressive fitness decline, which may be defined "aging". This appears to contrast "genetic conservatism" [Büttner et al. 2006], and might be explained in the same way as aging in multicellular organisms [Libertini 1988, 2006] (see next paragraph).

g) In eukaryotic multicellular organisms, PCD (by apoptosis and other types of PCD), cell senescence, "gradual" cell senescence, and limits to cell duplication capacity appear to be elements of a highly sophisticated system that is essential both for cell turnover (i.e. continuous renewal of the organism) and for age-related progressive fitness decline [Fossel 2004; Libertini 2006]. This decline, i.e. aging, could be explained as a powerful system to accelerate evolution by means of supra-individual selection, which is possible only under conditions of K-selection [Libertini 1988, 2006].

h) Aging in yeast has been proposed as adaptive [Büttner et al. 2006]. For multicellular eukaryotes, aging as an adaptive phenomenon is excluded by the prevailing gerontological paradigm [Kirkwood and Austad 2000], but this is contrasted both by empirical evidence and theoretical arguments [Libertini 1988, 2006, 2008, 2015a; Skulachev 1997; Goldsmith 2003; Longo et al. 2005; Mitteldorf 2006].

i) The limits to cell duplication capacity determined by the telomere-telomerase system are currently justified as a general defensive mechanism against cancer [Campisi 1997, 2003; Troen 2003; Wright and Shay 2005] but evidence and strong arguments contradict this hypothesis [Fossel 2004; Libertini 2008, 2013; Milewski 2010; Mitteldorf 2013], e.g.: the secretion by senescent cells of substances that increase both mutation rates and oncogenic risk [Parrinello et al. 2005; Coppé et al. 2008]. The obstinate affection by the advocates of non-adaptive aging theory to the hypothetical against-cancer role of telomerase restrictions is likely caused by the absence of any explanation compatible with the non-adaptive hypotheses and as a consequence of philosophical bias [Milewski 2010]. "The hypothesis that telomerase is restricted to achieve a net increase in lifespan via cancer prevention is certainly false. Were it not for the unthinkability of the alternative – programmed death – the theory would be dead in the water." [Mitteldorf 2013]

In short, aging shows clear phylogenetic connections regarding both underlying physiological mechanisms, and evolutionary causes. This represents a further set of arguments and evidence in support of the paradigm that interprets aging as a phenoptotic phenomenon, i.e. a type of death that is genetically determined and modulated with specific evolutionary causes.

Chapter 9

Libertini G, Ferrara N (2016b) Possible Interventions to Modify Aging. *Biochem. (Mosc.)* 81, 1413-28.

Possible interventions to modify aging

Giacinto Libertini, Nicola Ferrara

Abstract

The programmed aging paradigm interprets aging as a function favored by natural selection at a supra-individual level. This function is implemented, according to the telomere theory, through mechanisms that operate through the subtelomere-telomere-telomerase system. After reviewing some necessary technical and ethical reservations and providing a concise description of aging mechanisms, this work considers interventions that could lead to the control of some highly disabling characteristics of aging, such as Alzheimer's and Parkinson's syndromes and age-related macular degeneration, and afterwards to a full control of aging up to a condition equivalent to that of the species defined as "with negligible senescence". The various steps needed for the development of such interventions are described along general lines.

Introduction

Aging, defined as "increasing mortality with increasing chronological age in populations in the wild" [Libertini 1988], also known as "actuarial senescence in the wild" [Holmes and Austad 1995], or "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [Kirkwood and Austad 2000], is observed in the wild for many species [Libertini 1988; Finch 1990; Ricklefs 1998; Nussey et al. 2013], our species included [Hill and Hurtado 1996].

The theories that try to explain aging are many [Comfort 1979; Medvedev 1990; Libertini 2015b] and, in general, belong to one of only two very different general interpretations [Libertini 2008, 2015a], which for their important and opposite implications deserve the definition of paradigms in the meaning proposed by Kuhn [Kuhn 1962].

The first, or "old", paradigm includes various hypotheses that justify aging as due to inevitable damaging factors that are insufficiently contrasted by natural selection for various reasons. The second, or "new", paradigm, includes theories that explain aging as a specific function, or program, which is favored by natural selection, at the supra-individual level, under particular conditions [Libertini 2015a, 2015b]. The new paradigm interprets aging as a particular type of phenoptosis [Skulachev 1997] ("programmed death of an individual" [Skulachev 1999a]), a concept that includes many different phenomena, known for a long time [Finch 1990], but not considered in their entirety and for their important implications until recent times [Libertini 2012a].

In this paper, the evidence in support of the new paradigm and against the old paradigm [Libertini 2008, 2015b] and a less concise description of aging physiology [Libertini 2014a] is not expounded for brevity. This work tries to propose feasible interventions in the aging process that are consequent to the main concept of the new paradigm: indeed, if aging is a function, i.e. a physiological program that is genetically determined and regulated, and provided that aging mechanisms are sufficiently known, it should be possible to conceive possible modifications of this program that could hamper or even cancel age-related fitness decline. In contrast, this is not at all likely if, as proposed by the old paradigm, aging is an inevitable consequence of the cumulative effect of various damaging factors that act on many cellular and organismal processes [Libertini 2009b].

Within the new paradigm, the theory that explains aging as a consequence of telomere shortening and the consequent: (i) progressive inhibition of cell functions and (ii) cell senescence (Fossel's "cell senescence general model of aging" [Fossel 2004; Libertini 2009a, 2014a]) will be defined as "telomere theory" of aging and described in brief in the next section. Afterwards, some possible effective methods to modify aging will be proposed.

Premises

- Technical reservations

The methods that subsequently will be expounded require in various steps the modifications of DNA segments by appropriate means. Currently, the technique that seems most feasible regarding both cost and feasibility, in comparison with other means such as ZFNs (zinc finger nucleases) and TALENs (transcriptional activator-like effector nucleases), is the so-called CRISPR-CAS9 (clustered regularly interspaced short palindromic repeat–CRISPR-associated nuclease 9) technique [Harrison et al. 2014; Zhang et al. 2014].

With regard to this technique or about any other possible means, in particular for the possible necessity of inserting longer DNA segments, a critical element is the accuracy and reliability of DNA modifications associated with this method [O'Geen et al. 2015; Chandrasegaran and Carroll 2016]. Moreover, recent papers have shown ways to greatly ameliorate the precision of the CRISPR-CAS9 technique [Kleinstiver et al. 2016; Slaymaker et al. 2016].

This criticality varies in a fundamental way according to the type of experiment:

- Level 1: For example, if we operate on yeast cells or on cultured cells or on animals, possible errors in DNA changes reduce the certainty and the accuracy of the results but do not involve consequences of pathological relevance;
- Level 2: If we act on human individuals no longer in the reproductive stage and suffering from diseases for which a cure is desperately sought, the imprecision of the changes could reduce the effectiveness of the results or even confer negative effects on the health of the affected subject as a consequence of unexpected DNA alterations, but will have no effect on genetic heritage;
- Level 3: In contrast, by operating on human subjects who may reproduce, the desired genetic modifications in germ line cells (in addition to the ethical reservations of the next point) could be accompanied by unexpected and undesired changes in other segments of DNA molecules that will be transmitted to subsequent generations.

These considerations imply that the degree of reliability of the possible techniques to modify DNA (i) is not critical for experiments of level 1, (ii) is critical and preliminary for experiments or treatments of level 2, (iii) is extremely critical and also strongly preliminary for experiments or actions of level 3.

The observations outlined here, in very general terms, should always be considered for the possible actions discussed in this paper. They will not be repeated but shall be considered as always implied in any proposal that will be formulated.

- Ethical reservations

Any test or treatment or action on humans, or on living beings in general, requires an ethical evaluation; all the more for work on genetic information that will be transmitted to subsequent generations. For the proposals set out in this work, we need two kinds of ethical evaluations, quite different from each other.

The first type relates to the possible dangers to the health of those affected by the experiments or by the proposed actions. In general, for this type of hazard, it is essential first of all to conduct a series of experiments at the level of cultured cells and/or on other species. Only at a later stage will experiments on individuals suffering from serious and incurable diseases and not in condition to reproduce be admissible.

The second type of ethical evaluations is radically different. According to the prevailing conception, i.e. the old paradigm, aging results from the cumulative effect of various degenerative processes and so the care of one or more (or even all) aspects of aging is only the cure for various diseases. According to this paradigm, there is no particular ethical problem in procedures that contrast the aging process. Conversely, if the aging process is conceived of as a genetically determined and regulated phenomenon, an idea that is implicit to a detailed description of the mechanisms that carry it into effect and is a prerequisite for the proposals that afterwards will be here formulated, it is no longer possible to conceive aging as a sum of diseases or, on the whole, as a complex disease. By defining a disease as an alteration of a normal physiological process, it follows that aging may be "modified" but not "cured" because aging is a normal physiological process and not a disease. It would be different if aging, although normal, was equated to a medical condition due to its discomforts and impairments, but this different definition of disease should also be carefully evaluated in ethical terms.

Therefore, actions that modify aging require a particular ethical evaluation. Even more, possible actions that change the rates and manifestations of the aging process by gene modifications that will be transmitted to subsequent generations, as they are changes to human nature, need a very particular ethical evaluation that is beyond the scientific limits and falls within the ethical, religious, philosophical and political fields. Consequently, the possibility of modifying aging, regardless of technical feasibility and of possible dangers associated with the procedures, needs assessments and decisions that are beyond mere scientific competence.

Mechanisms of aging according to the telomere theory

Aging mechanisms in *S. cerevisiae*

S. cerevisiae (yeast), a unicellular species, shows some phenomena that may be somehow considered as aging, if it is precisely defined, as aforesaid, "increasing mortality with increasing chronological age in populations in the wild" [Libertini 1988].

In fact, each yeast cell reproduces by division into two cells, "mother" and "daughter" cells, which are not perfectly equal. The daughter cell is identical to the parent cell, while the cells of the mother lineage can reproduce only a limited number of times (in 3 days, for about 25-35 duplications [Jazwinski 1993]). In proportion to the number of duplications, two phenomena are observed: i) increasing metabolic alterations [Laun et al. 2001; Herker et al. 2004; Lesur and Campbell 2004; Büttner et al. 2006; Fabrizio and Longo 2008]; ii) growing vulnerability to replicative senescence and apoptosis [Laun et al. 2001; Herker et al. 2004; Büttner et al. 2006; Fabrizio and Longo 2008]. These phenomena are a likely explanation of why some individuals die and others survive under particular conditions of stress. However, in the mother lineage, the death rate increases with an exponential dynamic as a function of the number of duplications [Laun et al. 2007]. This is similar to the age-related increase in mortality shown by many multicellular species [Ricklefs 1998; Nussey et al. 2013] and may be considered within the concept of aging, as underlined before.

In yeast, DNA is linear, as for all eukaryotic cells, and not circular as for prokaryotes. In each replication, the enzyme DNA polymerase does not duplicate a small terminal part of the DNA molecule (the telomere) [Olovnikov 1971; Watson 1972]. The necessity of an enzyme able to restore telomere integrity was predicted in 1973 [Olovnikov 1973] and some years later the existence of this enzyme (telomerase) was demonstrated [Greider and Blackburn 1985].

In yeast, telomerase is always perfectly active both in mother and daughter cells and there is no reduction in telomere length with each duplication [D'Mello and Jazwinski 1991; Smeal et al. 1996; Meringele and Lydall 2004].

In mother cells, the metabolic alterations and the vulnerability to replicative senescence and apoptosis, which increases after each duplication, are caused by a particular mechanism that is not related to telomere shortening, as in multicellular eukaryotes (see below). In yeast mother cells, proportionally to the number of replications, there is the accumulation of particular molecules,

defined as extrachromosomal ribosomal DNA circles (ERCs) [Sinclair and Guarente 1997]; "several lines of evidence suggest that accumulation of ERCs is one determinant of life span" [Lesur and Campbell 2004].

Specifically, a particular type of yeast mutant indirectly shows that the accumulation of ERCs interferes with the action of that part of the DNA molecule adjacent to the telomere, the subtelomere, and that in these mutants interference is analogous to what happens in eukaryotic multicellular organisms, where subtelomere repression is a consequence of telomere shortening due to telomerase inactivity. In fact, *tlc1Δ* mutants, whose telomerase is inactive, show telomere shortening both in mother and daughter cells. However, the cells of the daughter cell lineage, although they show no ERC accumulation like normal strains, manifest all the alterations of mother lineage cells with an equal number of duplications. In particular, the overall expression of genes, defined as the transcriptome, is similar [Lesur and Campbell 2004].

Experiments in yeast have allowed us to hypothesize that: "One model of telomere-gene expression linkage is an altered chromosomal structure (Ferguson et al., 1991), such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome (Fossel, 1996; Villeponteau, 1997; Wright et al., 1999). As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) ... the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity (Aparicio and Gottschling, 1994; Singer et al., 1998; Stevenson and Gottschling, 1999). These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [Fossel 2004] (p. 50).

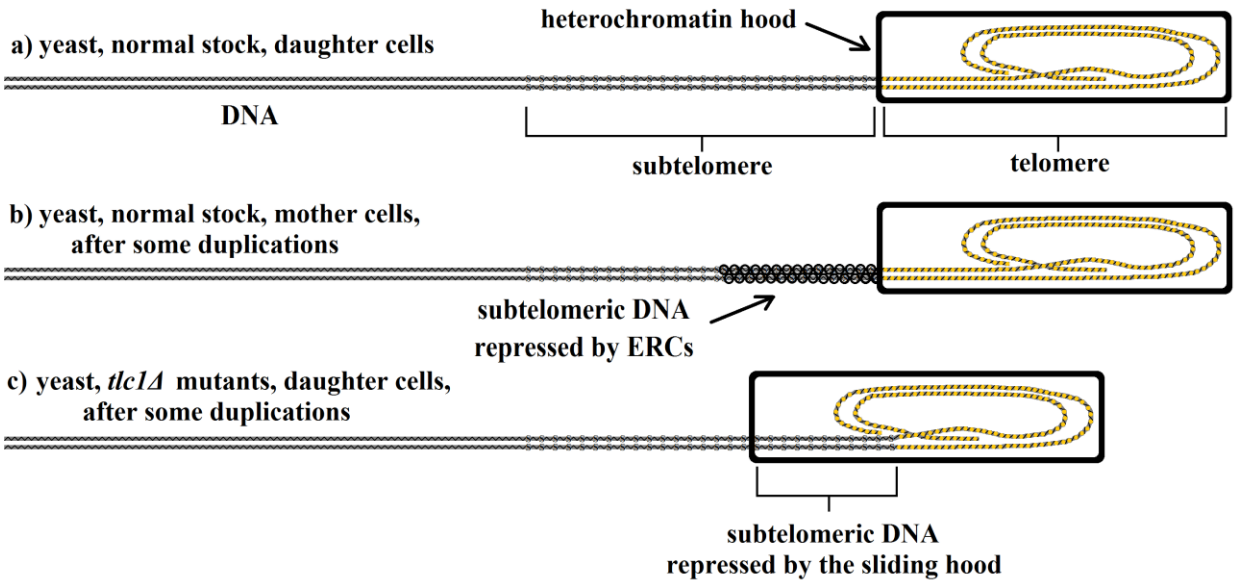


Figure 1 - In yeast: a) normal stock, daughter lineage, the telomere is not shortened and the subtelomere is not repressed; b) normal stock, mother lineage, after each duplication, the telomere is not shortened but the subtelomere is progressively repressed by ERC accumulation; c) *tlc1Δ* mutants, daughter lineage, after each duplication, the telomere is shortened and the subtelomere is repressed by sliding of the heterochromatin hood and not by ERC accumulation.

The gradual repression of subtelomeric DNA, caused by the accumulation of ERCs in normal yeast or by telomere shortening and heterochromatin hood sliding in yeast *tlc1Δ* mutants, has been known from some time and defined as the "telomere position effect" [Gottschling et al. 1990]. It has regulatory effects on gene expression in distant parts of the DNA molecule [Robin et al. 2014]. Perhaps, due to its effects, it is better to substitute the neutral expression "telomere position effect" with "gradual cell senescence", which has a descriptive and interpretative value [Libertini 2015b].

These concepts are illustrated in Figure 1.

Regarding the passage from a normal state to replicative senescence and apoptosis, possible analogies with the cell senescence program (replicative senescence + gradual cell senescence in the highest degree) in multicellular eukaryotes have been proposed (see below).

Aging mechanisms in multicellular eukaryotes

- "Gradual" cell senescence

In vertebrates, in cells where telomerase is inactive or partially active, the telomere shortens with each duplication and the sliding of the heterochromatin hood progressively represses the subtelomere. In contrast, in cells where telomerase is perfectly active (e.g. germ line cells), the telomere does not shorten and the subtelomere is not repressed [Fossel 2004]. The first case is analogous to that of yeast *tlc1Δ* mutants, daughter lineage cells, while the second case is analogous to that of normal daughter lineage cells (Figure 2).

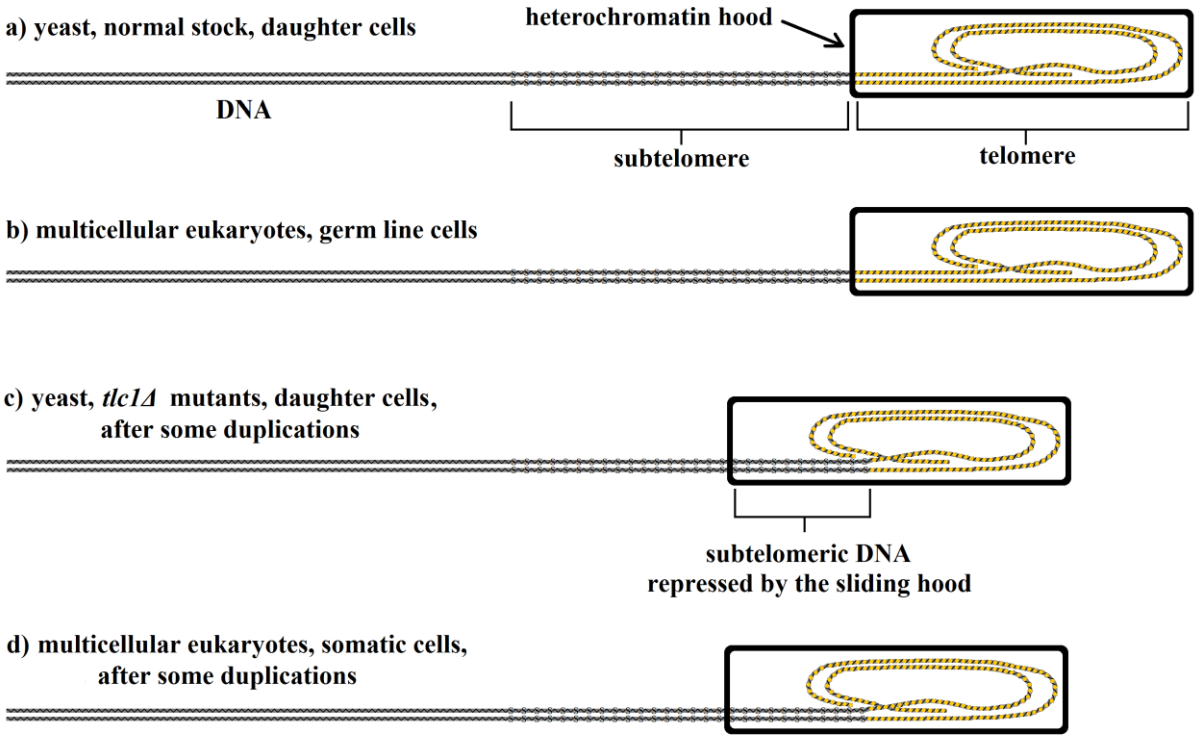


Figure 2 - a and b: analogy between normal yeast, daughter lineage, and germ line cells in vertebrates (or, in general, in multicellular eukaryotes); c and d: analogy between yeast *tlc1Δ* mutants, daughter lineage, and vertebrate somatic cells.

Regarding the alterations to subtelomere regulatory actions caused by the progressive sliding of the heterochromatin hood, it is possible to hypothesize that the subtelomere has a series of

regulatory sequences ("r"), which carry on their actions on distant parts of the DNA, and, one after the other, are progressively covered and repressed by the heterochromatin hood (Figure 3).

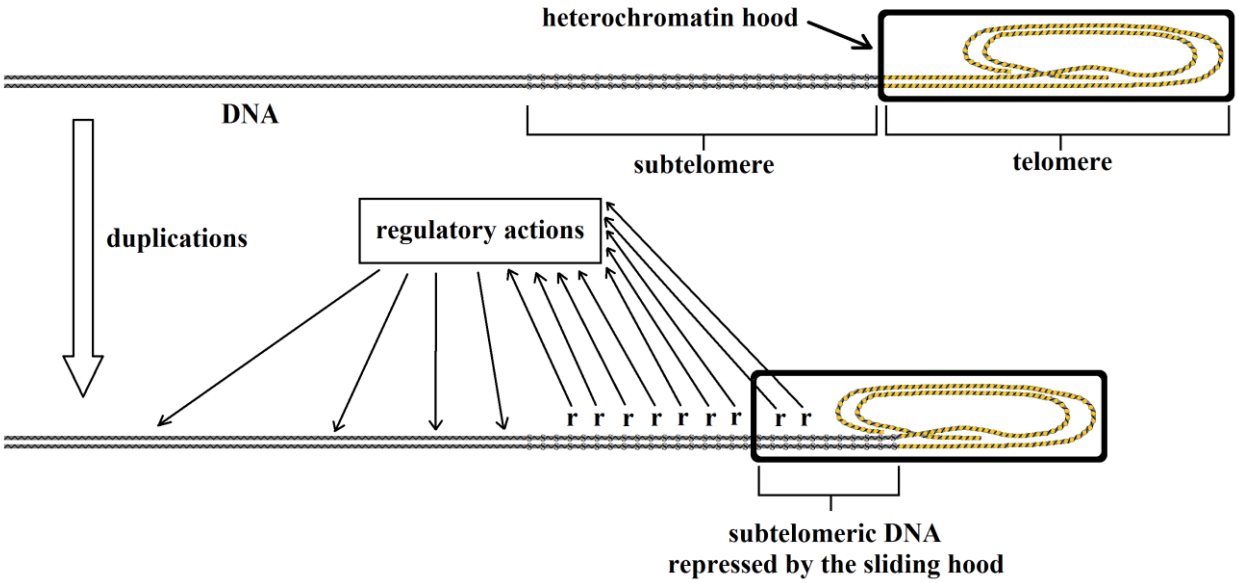


Figure 3 - The telomere shortens with each replication and the heterochromatin hood slides over the subtelomere, repressing an increasing portion of subtelomeric DNA, which probably means the repression of the hypothetical "r" sequences. This allows for alterations in gene expression in different and distant parts of the DNA molecule (modified and redrawn from fig. 4 of [Libertini 2015b]).

The hypothesis of the existence of "r" sequences is supported by the structure of the subtelomere, which has an "unusual structure: patchworks of blocks that are duplicated" [Mefford and Trask 2002]. Moreover: "A common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences." [Torres et al. 2011]

This "long arrays of tandemly repeated satellite sequences", an "unusual structure" that characterizes the telomere and is apparently without meaning and useless, therefore would be a general regulator of cell functions progressively inhibited by the knob of telomere shortening (or, in yeast, of ERCs accumulation) and so this could be the pivotal mechanism of aging.

According to this interpretation, the subtelomere might be defined as that part of DNA that is contiguous to the telomere and has general regulatory functions. One of its ends is easily defined by the beginning of telomere monotone sequence, while the other end is where repression is at the highest possible level before the obligatory triggering of the cell senescence mechanism caused by excessive telomere shortening.

- "replicative senescence", "cell senescence program"

A simplistic hypothesis could be that the cell becomes unable to duplicate (replicative senescence) only when telomere shortening reaches a critical value. However, the growth potential of a cell culture shows not an abrupt collapse of duplication capacities, i.e. contemporary replicative senescence for all cells after a certain number of duplications, but a progressive decrease related to telomere length reduction [Pontèn et al. 1983; Jones et al. 1985]. A brilliant explanation for this phenomenon was proposed by Blackburn [Blackburn 2000].

A protein hood (likely the same aforementioned heterochromatin hood [Libertini 2015b]) caps the telomere, and there is a continuous oscillation between two telomere states: "uncapped" and "capped". The first state, whose duration is related to telomere shortening, is vulnerable to the

passage to replicative senescence ("noncycling" state), while the other state is resistant to replicative senescence. Even with activated telomerase and telomeres at the maximum length, with each division there is a short period of uncapped state and a small percentage of cells shows replicative senescence [Blackburn 2000].

As the percentage of time in which the telomere is uncapped and vulnerable is related to the progressive subtelomere repression, and as this repression is related to the gradual reduction of the relative telomere length (see before) and not to the initial length of the telomere (see next section), it is proper to assume that the oscillation between capped and uncapped states is somehow regulated by subtelomeric repetitive sequences, which might be the same before defined as "r". In Figure 4, these sequences are indicated with the letter "r".

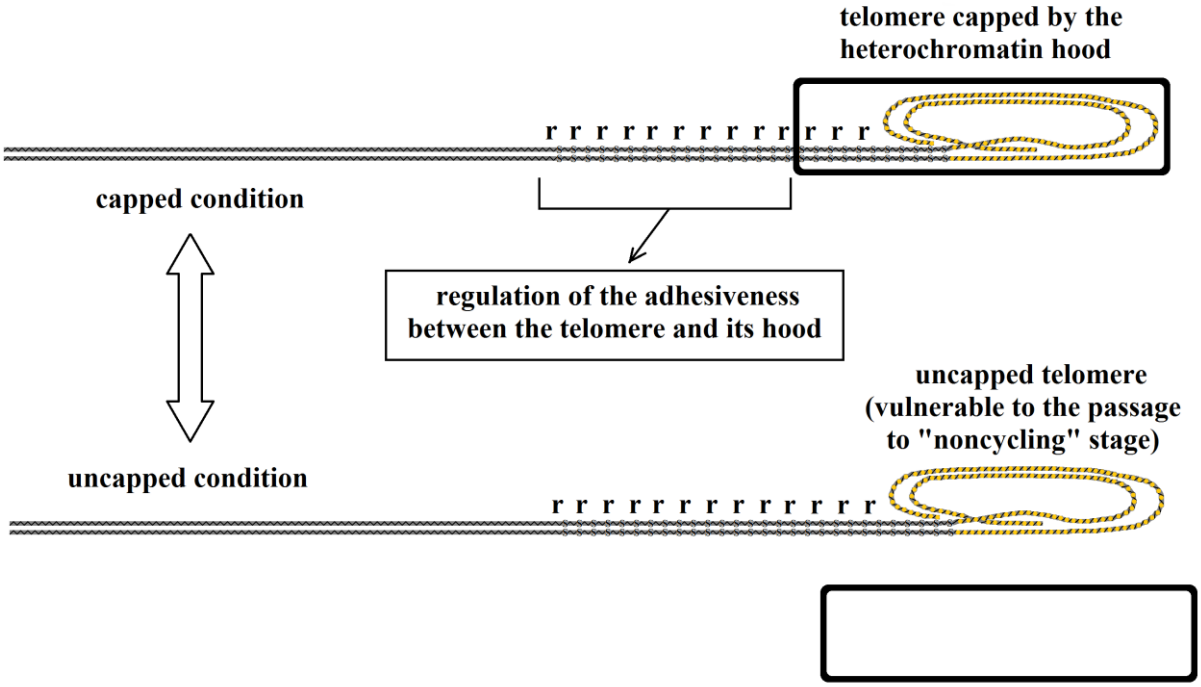


Figure 4 - Telomeres oscillate between the "capped" and "uncapped" conditions. The probability of uncapped telomeres increases in proportion to subtelomere repression of hypothetical repetitive sequences ("r") that regulate the degree of adhesion between the heterochromatin hood and the telomere. In the uncapped state, the non-protected telomere is a free end of the DNA molecule and is susceptible to end-to-end joining that blocks cell replication [Blackburn 2000].

Stem cells, which, unlike germ line cells, show telomerase activity that only partially preserves telomere length [Holt et al. 1996], are therefore limited in their replacement capacities of the elements eliminated by cell turnover [Fossel 2004].

The cellular phenomenon described by Blackburn as the shift to a "noncycling state", i.e. replicative senescence, in fact is part of so-called "cell senescence", well-defined as a "fundamental cellular program" [Ben-Porath and Weinberg 2005]. It is characterized both by replicative senescence and by the altered expression of many genes in a way that jeopardizes cell functions, cellular secretions included - and thus also the extracellular matrix and those cells physiologically dependent on the altered cells or simply nearby - up to the maximum degree that may be caused by gradual cell senescence. As the transition to the altered physiological conditions determined by cell senescence, and the reverse passage - by the activation of telomerase [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999] - towards a condition in which all cellular functions are intact, is a bidirectional on/off transformation, this type of cell alteration has been defined also as "on/off senescence" [Libertini 2012b].

- Absence of a relationship between longevity and initial telomere length or telomerase activity

In the comparison between species, an easy prediction, however falsified by the evidence, is a direct relation between longevity and initial telomere length (i.e., that in the germ cell) and, similarly, between longevity and the degree of telomerase activity.

However, many facts disprove these predictions:

a) hamsters and mice have longer telomeres than our species but age more precociously [Slijepcevic and Hande 1999];

b) among rodents, there is no relationship between telomerase activity and maximum lifespan [Gorbunova et al. 2008];

c) mice show a baseline activity of telomerase in most somatic cells [Prowse and Greider 1995], but have limited longevity;

d) two *Mus* strains, the first with a telomere length of 20 kb and the other of only 10 kb, show equal life spans and the same timing patterns of cell senescence [Fossel 2004];

e) analogously, cloned animals derived from somatic cells, which have shortened telomeres, show the same senescence timing of donor animals [Fossel 2004];

f) in telomerase knockout (mTR-/-) mice, which have genetically inactivated telomerase, only after four [Herrera et al. 1999] to six [Blasco et al. 1997] generations, when telomeres are very shortened, it is possible to see, at least in artificially protected laboratory conditions, that fertility and viability are jeopardized. However, in organs with high cell turnover, the alterations are found in early generations [Lee et al. 1998; Herrera et al. 1999], so, fitness would likely be reduced under natural conditions.

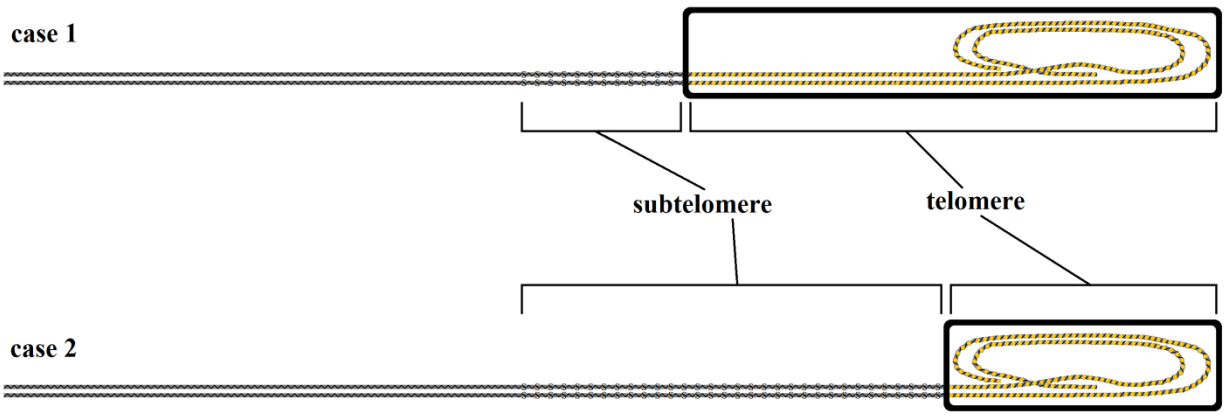


Figure 5 - In case 1, there are shorter subtelomeres and longer telomeres than in case 2. After a certain number of duplications, by assuming that telomeres are equally shortened at each generation in the two cases, in case 1 the shorter subtelomeres are more impaired by the sliding of the heterochromatin hood, which should lead to earlier aging. If we assume for case 1 also a greater mean telomerase activity, which contrasts telomere shortening, this may be insufficient to compensate for greater subtelomeric repression. This model is an easy explanation for phenomena a, b, and c.

These phenomena, which are apparently inexplicable if you want to assign an exclusive role in longevity determination to telomere length, on the contrary are completely explicable if it is assumed that in the germ cell, in a phase - defined as the “reset” phase - before the first cell replication, the heterochromatin hood is formed with a size proportional to telomere length. In this reset phase, regarding the longevity, the absolute “telomere length is irrelevant” [Fossel 2004], provided of course that telomere length is not less than a critical size [Fossel 2004]. Afterwards, in

proportion to the number of duplications and so to the progressive shortening of the telomere (if it is not elongated by telomerase), there is progressive sliding of the heterochromatin hood, and thus proportional repression of the subtelomere, which causes the manifestations of gradual cell senescence and increases the probability of cell senescence, i.e. replicative senescence plus gradual cell senescence in the highest degree.

For this model, it is necessary that: i) the heterochromatin hood must have a fixed length in all the cells of the organism; ii) the telomere elongation by telomerase up to the initial length must somehow have the hood length as a limiting factor. It is certainly necessary to study in depth the mechanisms underlying these phenomena, which must be phylogenetically very old.

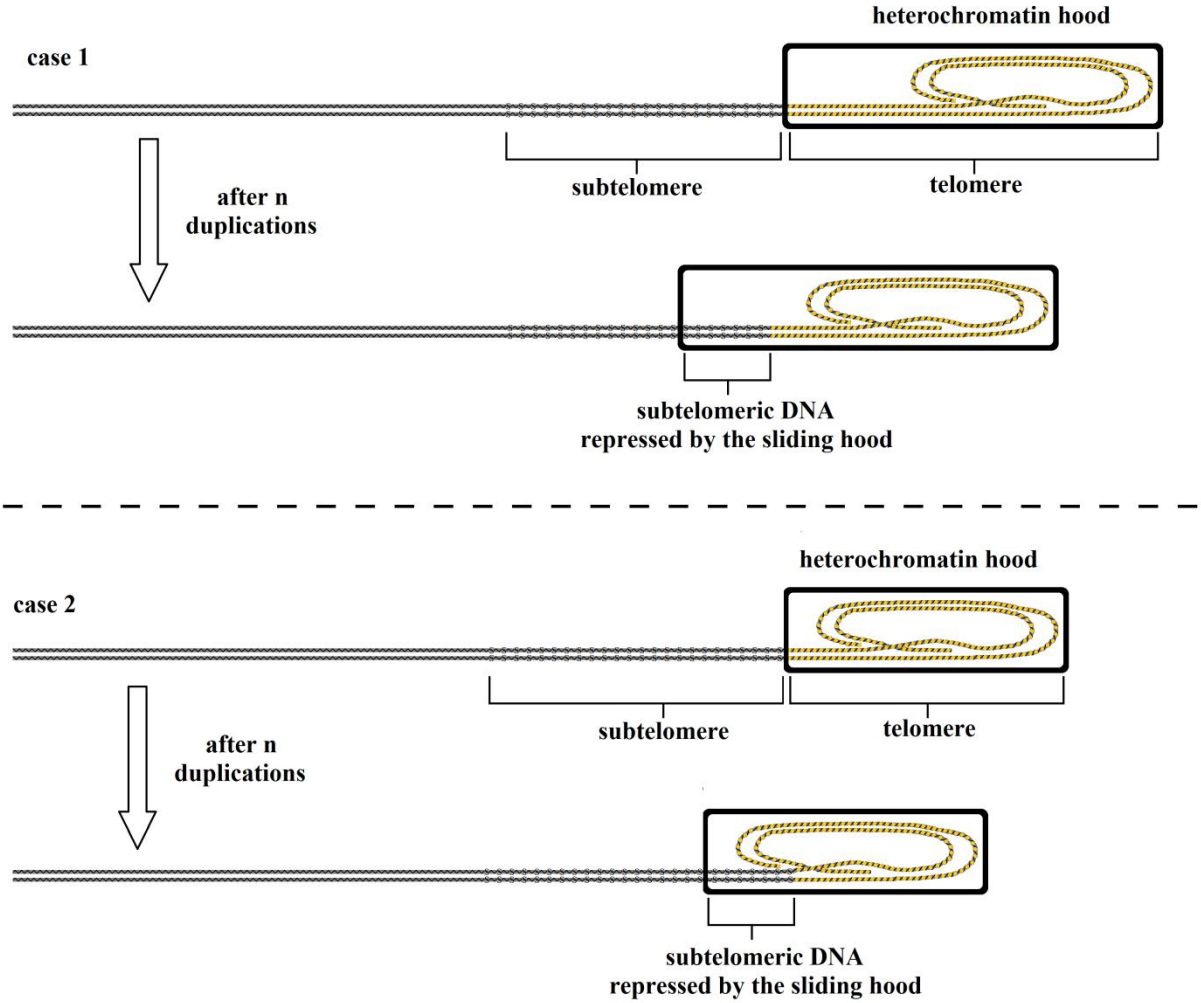


Figure 6 - Case 1: *Mus* strain with 20 kb telomeres; case 2: *Mus* strain with 20 kb telomeres (or case 1: donor animals; case 2: cloned animals). In case 1, cells have longer telomeres and heterochromatin hoods than in case 2 cells, but the longevity is the same: the progressive gradual cell senescence and the increasing probability of cell senescence activation are not a function of telomere absolute initial length but of progressive subtelomere repression, caused by relative telomere shortening. This model explains phenomena d and e.

In fact, as described above, in yeast, with an evolutionary history that diverged from that of our species at least at the beginning of Cambrian era, i.e. about 600 million years ago [Minkoff 1983]: (i) in the normal stocks, at each duplication, each cell restores telomere length on the basis of the heterochromatin hood size, which is then kept fixed despite the succession of generations; (ii) in the

daughter cells of *tlc1Δ* mutants, where telomerase is inactive, the telomere shortens with each division, but the hood remains at a fixed length and represses the subtelomere by sliding on it.

All this indicates that, as regards longevity, the critical element is not the absolute initial length of the telomere, but the progressive inhibition of subtelomeric DNA caused by telomere shortening [Fossel 2004; Libertini 2015b]. The aforementioned phenomena (a-f) are illustrated and interpreted in Figure 5 for phenomena a, b and c; in Figure 6 for d and e; and in Figure 7 for f.

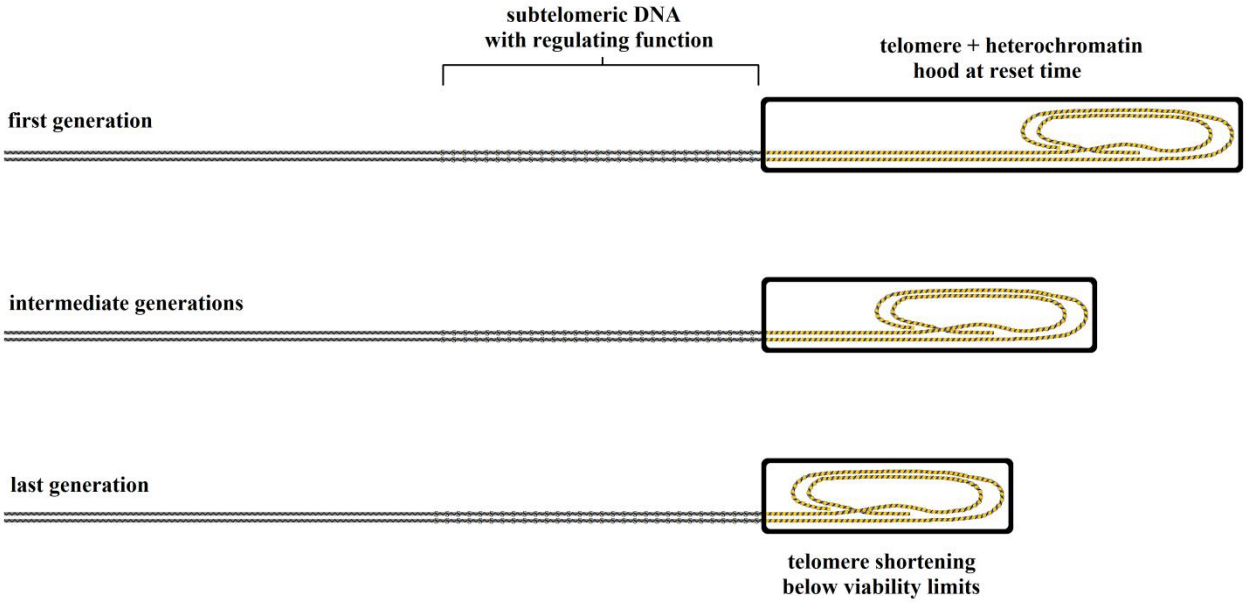


Figure 7 - In mice with telomerase genetically inactivated, the length of the heterochromatin hood, modeled on the telomere in the reset phase, and that of the telomere in the first generation are equal to that of normal stocks. As telomerase is inactive, in each subsequent generation, the initial length becomes shorter, up to fourth to sixth generation when the telomere shortens below viability limits. With each generation, the subtelomere length is unvaried, because it is not influenced by telomerase. Within each generation, with each cell duplication, the telomere shortens and the subtelomere is progressively inhibited by sliding of the heterochromatin hood. So, as shown in the previous figure, this genetic repression is a function of relative telomere shortening and not a function of the absolute initial telomere length [Fossel 2004]. This explains phenomenon f (modified and redrawn from fig. 6 of [Libertini 2015b]).

- Consequences of gradual cell senescence and cell senescence

The effects of gradual cell senescence and cell senescence (replicative senescence + gradual cell senescence in the highest degree) on the organism as a whole, considered under natural conditions, are the likely roots of all aging manifestations, as already proposed and explained elsewhere [Fossel 2004; Libertini 2009b, 2014a].

In short, we have an "atrophic syndrome" of all tissues and organs, which is the consequence of: (i) an age-related increase in the percentage of cells with altered functions, to varying degrees, due to gradual cell senescence and cell senescence; (ii) an age-related decline in the speed and completeness of cell turnover due to the progressive increase in the percentage of stem and somatic cells in replicative senescence; (iii) perennial cells, i.e. cells without turnover (e.g. almost all neurons), are compromised and die by the phenomena i-ii in satellite cells (glial cells), which show turnover and are essential for their functionality, causing problems that are considered distinct diseases (Alzheimer's disease, Parkinson's disease, age-related macular degeneration, etc. [Libertini and Ferrara 2016a]); (iv) the consequent (to phenomena i-iii) reduction in functional cells in a tissue or organ, partially replaced by non-functional cells, and the related decline in tissue or organ functionality [Libertini 2009b, 2014a].

The related alterations reduce fitness, i.e. the ability to survive under natural conditions. In contrast, under protected conditions, even a remarkable fitness reduction can be compatible with

survival, but, at older ages, the progressive worsening of the above-mentioned tissue/organ alterations becomes lethal even under artificial conditions of greatest protection, which are non-existent in the wild [Libertini 2009b, 2013].

- Distinction between aging and diseases whose frequency and severity increases with age

It is essential to make a clear distinction between the physiological process of aging and a number of diseases that are virtually absent under natural conditions [Libertini 2009b, 2013] and often increase in frequency and severity over the years (e.g. hypertension, type 2 diabetes, vascular diseases, various types of cancer, etc. [Libertini 2009b]).

These diseases, as a rule, are due to changes in the ecological niche (eating habits, lifestyles, pollutants in the environment, etc.) to which the species is not adapted and which are therefore harmful [Libertini 2009b]. Some of these alterations, here defined as "risk factors", are countered, at least in part, by medications or other measures ("protective factors").

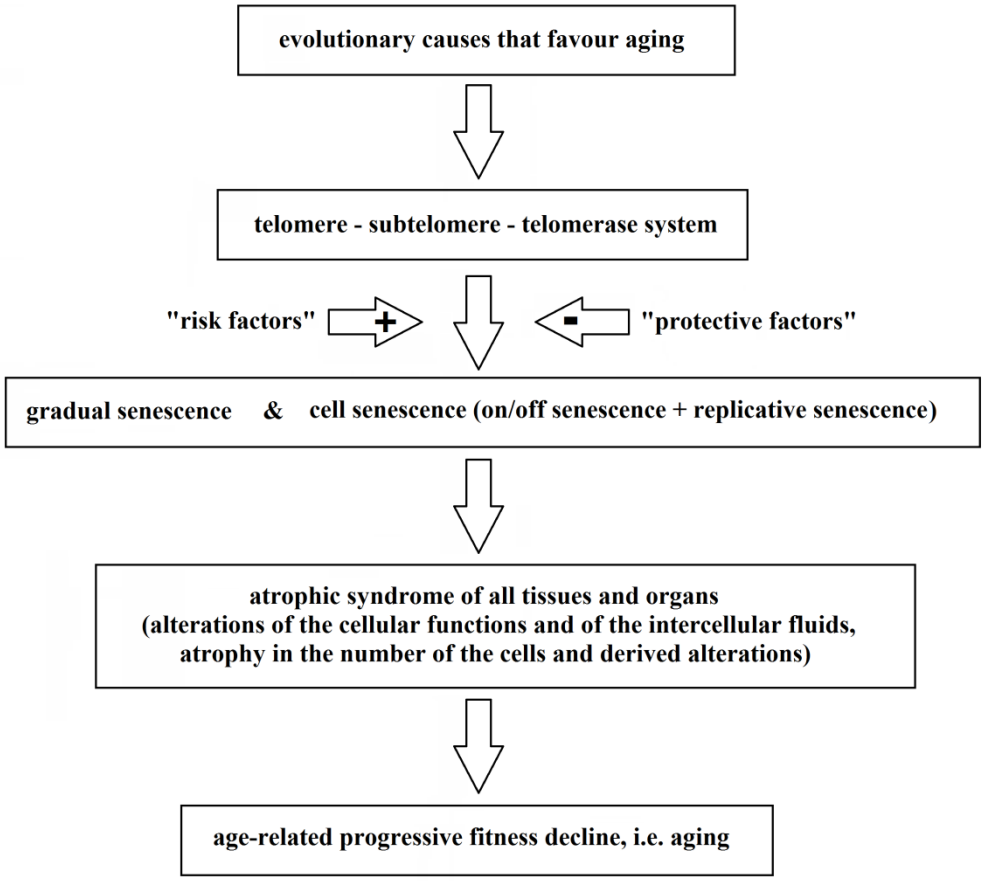


Figure 8 - A scheme of the aging process. Evolutionary causes are not discussed in this work.

Surely, it is possible to propose alternative interpretations of the aging process that might be compatible with the programmed aging paradigm (e.g., Olovnikov's hypothetical "chronomeres" and "printomeres" as pivotal parts of aging mechanisms [Olovnikov 2003, 2015]), but we think that our explanation is more consistent with evidence.

The distinction between the aforesaid diseases and the aging process is that these problems are, at least partially, preventable or curable by the appropriate restoration of the correct ecological niche or by opportune drugs, while, on the contrary, aging, in principle - being a physiological phenomenon - is not contrasted by preventive, protective or curative actions. Lifestyles and environmental conditions that are ideally perfect to preserve health allow the achievement of physiological, i.e. normal, aging but cannot block or reverse aging [Libertini 2009b]. However,

"risk factors" may cause an acceleration of the aging process; "protective factors" may contrast this acceleration and give the false impression of contrasting aging while they only counteract pathologically altered aging. A specific example is the gradual weakening of endothelial function, which is a major cause of vascular diseases and can be measured by counting endothelial progenitor cells (EPCs) [Hill et al. 2003; Werner et al. 2005]. EPC number, which is a predictor of cardiovascular disease as reliable as the Framingham score [Wilson et al. 1987], decreases over the years and is also reduced by risk factors. A healthy lifestyle or the action of various drugs can restore the normal EPC number for the age but do not block the reduction related to age [Hill et al 2003].

These concepts are summarized in Figure 8.

Possible interventions

The aim of this work is to search for feasible methods to slacken, block or reverse the normal mechanisms of aging. It must be stressed that many common diseases caused by alterations in the ecological niche, or by other factors: (i) are not part of the normal process of aging, even if their frequency and seriousness may often be age-related; (ii) can be prevented or treated with appropriate measures; and (iii) are not part of this goal.

For our species, in individuals who are in the best physical condition and free from any disease that could endanger their fitness, there is a progressive age-related fitness decline, which starts from about 30 years: "No one would consider a man in his thirties senile, yet, according to athletic records and life tables, senescent is rampant during this decade." [Williams 1957]. In fact, this decline may somehow be quantified by observing the world speed records for each age group (Figure 9).

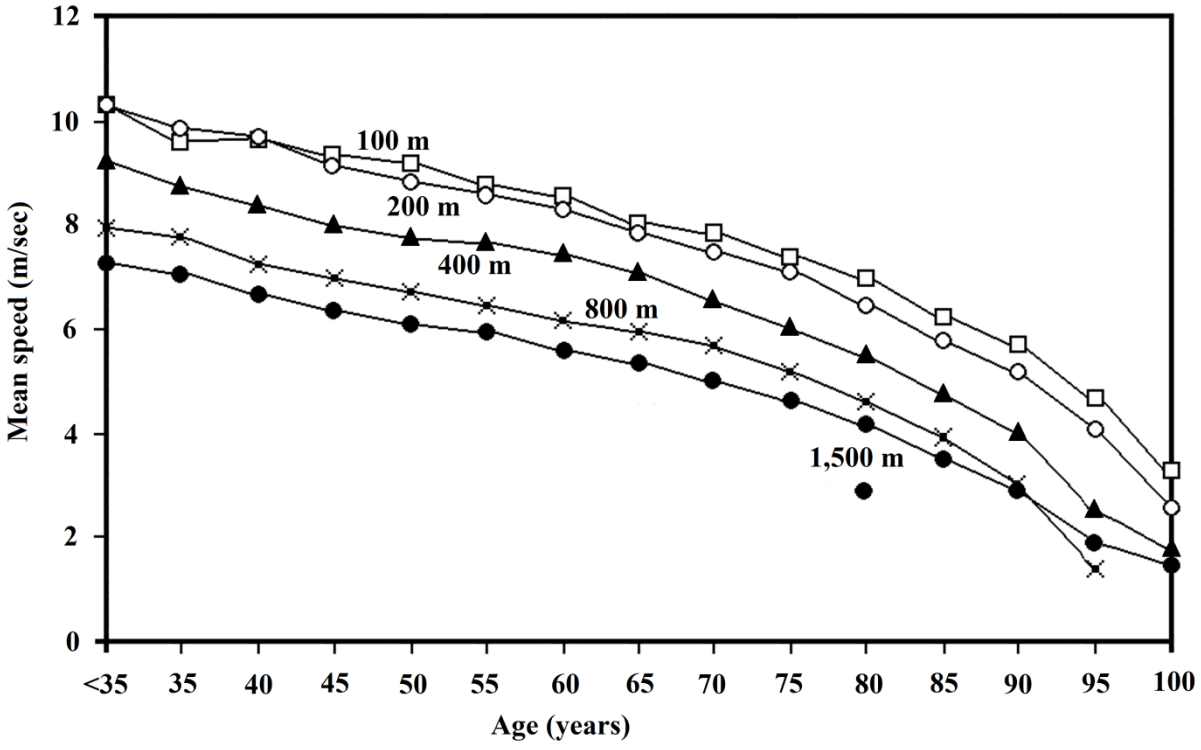


Figure 9 - A quantification of age-related fitness decline based on data from modern populations, namely on speed records for various distances in individual younger than 35 [World records in athletics 2017] and in other age groups [World record in athletitcs for age groups 2017]. From the beginning of adulthood to an age of about 30-35 years, we observe the best performance, i.e. the greatest fitness, and this is the period with the lowest mortality observed under natural conditions [Hill and Hurtado 1996].

Methods to reverse aging should transform the curve of fitness decline into a straight horizontal line, i.e. a fitness or mortality rate that is constant at any age. The search for these methods will be based exclusively on the interpretation of the aging mechanisms outlined above.

- Method 1: Telomerase activation

The evaluation of aging mechanisms and also the experiments carried out so far suggest immediately that the simplest and best way to contrast aging is telomerase activation in order to bring telomere length to the initial one, defined by the size of the heterochromatin hood.

Well-known and already mentioned experiments on cultured cells have shown since 1998 that telomerase activation is able to completely reverse the features of cell senescence, namely to totally rejuvenate cells with the markers of cell senescence [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1999].

At the tissue level, in vitro-aged fibroblasts with "substantial alterations in gene expression" were treated with telomerase and then "assessed by incorporation into reconstituted human skin", which showed no biological difference with skin obtained from young fibroblasts [Funk et al. 2000].

At the organismal level, in aged mice with artificially blocked telomerase, telomerase reactivation shows a marked reversal of degenerative manifestations, even for the nervous system [Jaskelioff et al. 2011]. Moreover, in one- and two-year-old normal mice, telomerase expression, induced by adeno-associated viruses carrying the mouse telomerase reverse transcriptase, delays aging and increases longevity (increase in median lifespan of 24 and 13%, respectively) without increasing cancer risk [Bernardes de Jesus et al. 2012].

The use of telomerase reactivation to control aging is, however, wrongly hampered by a formidable non-technical difficulty originating in the assumptions of the old paradigm, which is still the dominant thesis although its validity as a scientific theory is strongly opposed by evidence and theoretical arguments [Libertini 2015a]. In fact, according to the old paradigm, genetically determined and regulated mechanisms that progressively impair fitness simply cannot exist. Therefore, the subtelomere-telomere-telomerase system, concisely described in the previous section and supported by strong evidence, for the old paradigm, cannot have aging as an evolutionary motivation but must absolutely have another reason that justifies its existence. The only motivation that has so far been proposed to justify these mechanisms is the old one that interprets them as a general defense against cancer [Campisi 1997; Wright and Shay 2005]. Replicative senescence would constitute an effective obstacle to cancer proliferation. Thus, aging would be just a side effect of this defense, in a sort of terrible evolutionary trade-off between the problems of aging and the need to defend the organism from cancer [Campisi 2000], in excellent compatibility with the ideas maintained by some old traditional evolutionary hypotheses about the evolutionary causes of aging (antagonistic pleiotropy theory [Williams 1957; Rose 1991]; disposable soma theory [Kirkwood 1977; Kirkwood and Holliday 1979]).

However, several strong arguments are against the aforesaid explanation, as in part already explained elsewhere [Libertini 2009b, 2013]:

a) There are animal species without any age-related fitness decline ("animals with negligible senescence" [Finch 1990]) and old individuals of such species (e.g. rainbow trout and lobster) have under natural conditions the same telomerase activity shown by young individuals [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998], but there is no age-related increased vulnerability to cancer, as their constant mortality at any age shows;

b) Gradual cell senescence and cell senescence cause a progressive weakening of immune system efficiency [Fossel 2004], and this increases vulnerability to cancer and so cancer incidence [Rosen 1985];

c) Shortened telomeres cause DNA instability and this increases the probabilities of cancer onset [DePinho 2000; Artandi 2002; Artandi and DePinho 2010];

d) In eukaryotic unicellular species such as yeast, replicative senescence and apoptosis, not caused by shortened telomeres but by ERC accumulation, are well-documented [Jazwinski 1993; Laun et al. 2007; Fabrizio and Longo 2007], but cannot be a defense against cancer, as it is impossible in unicellular species;

e) Gradual cell senescence, i.e. the existence of critical regulatory sequences in subtelomeric DNA that are gradually repressed as a consequence of telomere shortening, is an implausible defense against neoplastic cell proliferation;

f) In mice, the selective elimination of senescent cells ($p16^{Ink4a+}$ cells) contrasts several age-dependent changes, delays the progression of malignant diseases and increases lifespan [Baker et al. 2016] and this is against the possibility that senescent cells are a defense against cancer.

g) In humans studied in the wild: (i) the survivors at ages 60 and 70 were approximately 30% and 20%, respectively; (ii) cancer cases were not reported and only in a few older individuals (> 70 years) there was the possibility that death was caused by cancer. In the same population, the age-related increase in mortality, i.e. aging, was evident starting from the thirties. The hypothesis that the mechanisms underlying this increment of mortality could be a defense against a rare disease which shows its deadly effects at later ages is clearly illogical [Libertini 2013];

h) Telomerase activation, which is a common feature in cancer, as it is subsequent to and does not precede cancer onset, must be considered a cancer aggravating phenomenon and not a cause of it [Fossel 2004];

It must be stressed that with the rejection of the aforesaid explanation for cell aging mechanisms as a defense against cancer, the old paradigm loses a valuable last trench and becomes even more untenable. This explains historically and psychologically, but does not justify scientifically, the tenacious defense of this explanation, which is actually a heavy unjustified brake against the use of telomerase to contrast aging.

However, in overcoming this difficulty, the main way to slow aging is clearly telomerase activation and the subsequent restoration of the telomere to its initial length [Fossel 2015].

One possibility is the use of drugs that are capable of such action. Certain substances, called astragalosides, have shown some action in reactivating telomerase [Harley et al. 2011; Harley et al. 2013], but are remarkably expensive and their effect is limited [Fossel 2015].

However, the technique that appears to be more effective and more feasible in a short time is telomerase activation by telomerase reverse transcriptase (TRT) introduced into the organism using an adenovirus as a vector, as in an experiment carried out with significant positive results in mice and already mentioned in this work [Bernardes de Jesus et al. 2012].

This must necessarily be accomplished through several phases:

1) New experiments on animals to study: (i) the best techniques to introduce TRT into the organism with the highest standards of efficiency and security; (ii) further details on the results, also as a function of the age of the individual when the technique is applied;

2) First experiments on elderly human subjects suffering from diseases that are seriously debilitating and are part of aging process, such as Alzheimer's disease, Parkinson's disease, age-related macular degeneration, etc. (excluding precocious cases likely due to genetic defects or to harmful lifestyles), and possibly in individuals not in a condition to reproduce, to avoid possible transmission of an altered genome;

3) Experiments on subjects with less disabling diseases, or even on individuals with reproductive capacity if the possibility of genome alteration has been excluded;

4) Experiments for the treatment of subjects suffering from other diseases that may be considered a consequence of aging process acceleration;

5) Experiments for the treatment of healthy elderly individuals, i.e. simply to rejuvenate them.

Method 1 would allow for the rejuvenation of the organism with the limit that any irreversible changes (for example: macro-structural alterations, reduction in stem cell number) would not be correctable. Therefore, it should be applied early enough (e.g. before the age of 40) and repeated

after relatively frequently (e.g. 10 years). Clearly these are reasonable assumptions that require due confirmation.

Method 2 – Subtelomere modification

It is possible to envisage a different method based on genetic modifications to subtelomeres such as to increase the age at which fitness decline begins to manifest (i.e., about 30 years in our species [Williams 1957]). For example, if it were possible to increase this age from 30 to 60 and if, in conjunction with this method, we apply method 1 at the age of 60 and then every 30 years, we would lengthen the period of biological youth without any treatment and also minimize possible irreversible changes at the age when method 1 is applied.

Now, let us see the method which is proposed here.

Some abbreviations are necessary for a more concise exposition:

- The telomere is composed of a monotonous repetition of a motif (TTAGGG in vertebrates), that will be indicated with "<m>". So, if the motif is repeated n times, the telomere will be described as "$<m>^n$";
- That part of the subtelomere which is next to telomere and may be defined as the telomere-subtelomere junction; it will be indicated with "<J>";
- All the remaining part of the subtelomere will be indicated with "<ST>". According to these abbreviations, the terminal part of a chromosome will be indicated with: $<ST><J><m>^n$;
- The abbreviation "<NS>" means a neutral sequence, i.e. a sequence that has no negative or positive action on the remaining part of the DNA molecule or on any genetic or cellular function;
- The abbreviations "<c1>" and "<c2>" indicate two sequences with distinct marker codes without any identical sequence in the genome.

It is necessary to specify, *inter alia*, that:

- The study of the <J> sequence is preliminary to the development of the method. It is essential that the <J> sequence is, or is delimited, such that there is no identical sequence in the genome;
- Likewise, the definition of one or more neutral sequences (<NS>) will be preliminary.

The objective of method 2 is to achieve the insertion of one or more sequences <NS> between <ST> and <J>, namely to get the following structure of the terminal part of chromosome:

$<ST><J><m>^n \Rightarrow <ST><NS>^y<J><m>^n$,

where y is an integer arbitrarily chosen according to convenience.

To achieve this result, by using a technique such as CRISPR-CAS9, the following procedure might be feasible:

1) Substitution of <J> with the sequence <c2><NS><c1>:
$<ST><J><m>^n \Rightarrow <ST><c2><NS><c1><m>^n$,

2) Substitution of <c1> with <J>:
$\Rightarrow <ST><c2><NS><J><m>^n$,

3) Substitution of <c2> with the sequence <c1><NS>:
$\Rightarrow <ST><c1><NS>^2<J><m>^n$,

4) Substitution of <c1> with the sequence <c2><NS>:
$\Rightarrow <ST><c2><NS>^3<J><m>^n$,

5) Repetition of the steps 3 and 4 until <NS> is repeated y times:
$\Rightarrow <ST><c1 \text{ or } c2><NS>^y<J><m>^n$,

6) Removal of <c1 or c2>:
⇨ <ST><NS>^{y}<J><m>^{n},
which is the required result.

We should note that each sequence that marks where an operation must be performed (e.g. <J> in 1) is always destroyed by the action, so that it cannot be executed more than once.

This change would have effect both on the individual on which it is performed, and for individuals of the following generations if it has been accomplished in germ cells.

From a theoretical point of view, it would be useless to lengthen the subtelomere beyond the maximum length for which the subtelomere can be inhibited by telomere shortening before the telomere reaches a critical minimum length and cell senescence is obligatorily triggered.

This method has an important advantage. As it takes place on the subtelomere and not on the telomere, thus, experiments would be easily feasible in a *S. cerevisiae* animal model that "ages" as a consequence of subtelomere inhibition not caused by telomere shortening but by progressive ERC accumulation. As optimal result, in a strain of *S. cerevisiae* modified by this method, the maternal lineage cells should stop their replications after a number of duplications greater than the 25-35 limit found by Jazwinski [Jazwinski 1993].

After acquiring a good mastery of the techniques to be employed, the method should be applied on a multicellular animal model with short longevity (e.g. mice) and afterwards on animals whose longevity and physiology are closer to our species (e.g. pigs).

As for method 1, with the reservations expressed in the Introduction (in particular for the following step c), the subsequent steps may be conceived with the accompanying application (possibly repeated) of method 1:

a) application on human subjects who are unable to procreate and suffer from serious diseases such as Alzheimer's disease, Parkinsons's disease, age-related macular degeneration, etc.; the possible success of this approach, particularly for diseases like Alzheimer's and Parkinson's, would also allow a decisive discrimination between their interpretation as coherent and critical parts of the aging mechanisms [Libertini and Ferrara 2016a] and other interpretations related to biochemical events and functional disorders of the same diseases (e.g.: [Femminella et al. 2014; Femminella et al. 2016; Pagano et al. 2015; Pagano et al. 2016]).

b) application on elderly subjects who are unable to procreate but are healthy;

c) modification of the germ cells of healthy individuals.

Method 3 –Telomere elongation without the use of telomerase

By operating after the reset phase, telomerase can lengthen the telomere to the maximum length allowed by the heterochromatin hood. By using an alternative method on germ cells, it is possible to envisage telomere elongation up to the desired length. In both cases, since the heterochromatin hood is modeled in the reset phase according to telomere length, this does not affect longevity. But if the subtelomere has been modified by method 2, this would allow more telomere shortening before the telomere length reaches a critical level. In short, the combined use of method 2 and method 3 would lead to a longer period before reaching the stage at which fitness begins to decline and there is a need to resort to method 1 for restoring fitness.

So, if the goal is to achieve telomere elongation by adding y repeats of the sequence <m>^{f} (where y and f are integers arbitrarily chosen according to convenience), namely:
<ST><J><m>^{n} ⇨ <ST><J><m>$^{n+yf}$,
the following procedure might be feasible.

1) Substitution of <J> with the sequence <c1><m>^{f}<c2>:
<ST><J><m>^{n} ⇨ <ST><c1><m>^{f}<c2><m>^{n},

2) Substitution of <c1> with <J>:
⇨ <ST><J><m>^{f}<c2><m>^{n},

3) Substitution of <c2> with the sequence <m>^{f}<c1>:
⇨ <ST><J><m>^{2f}<c1><m>^{n},

4) Substitution of <c1> with the sequence <m>^{f}<c2>:
⇨ <ST><J><m>^{3f}<c2><m>^{n},

5) Repetition of the steps 3 and 4 until the sequence <m>^{f} is repeated y times:
⇨ <ST><J><m>^{yf}<c1 or c2><m>^{n},

6) Removal of <c1 or c2>:
⇨ <ST><J><m>$^{n+yf}$,
which is the required result.

Figure 10 illustrates by way of example the three methods outlined here.

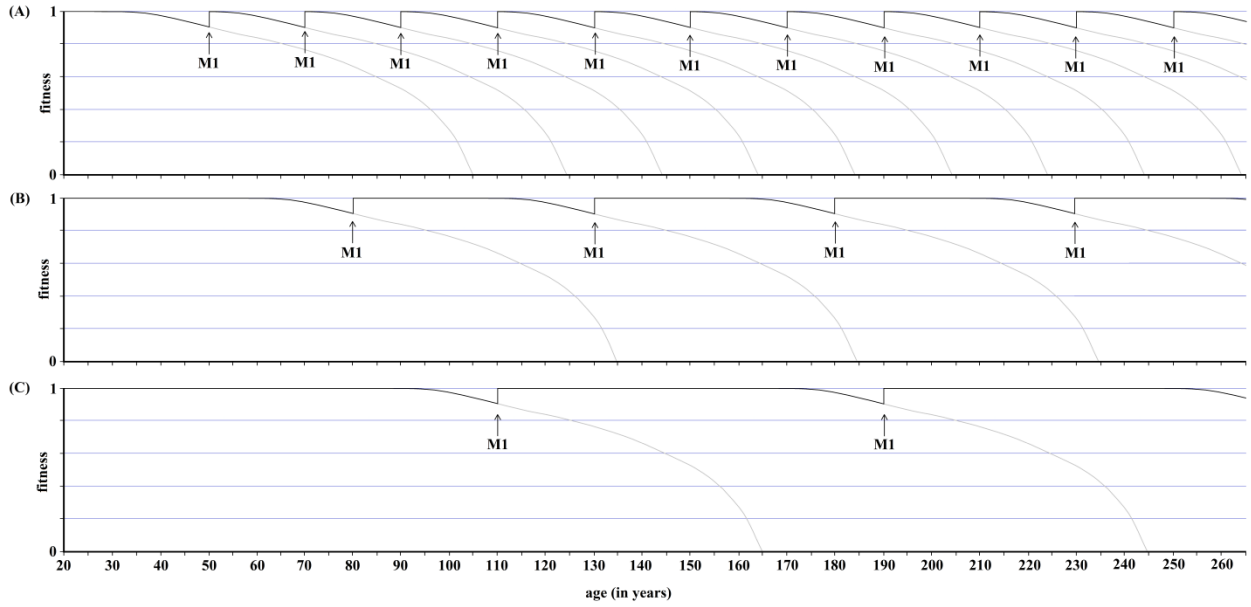


Figure 10 - A: Method 1 (M1) is applied several times, the first at age 50 and then every 20 years; B: the subtelomere has been lengthened by method 2, and then method 1 is applied several times, the first at age 80 and then every 50 years; C: the subtelomere has been lengthened by method 2 and the telomere by method 3, and then method 1 is applied several times, the first at age 110 and then every 80 years. The grey lines show fitness decline when method 1 is not applied.

Conclusion

Clearly, what is proposed in the last section might be considered exceedingly speculative.

However, the purpose of this work is precisely to speculate what would be possible to perform to slacken or block the aging process, starting from the necessary condition of the truthfulness of the new paradigm - not discussed here - and of what is known about the subtelomere-telomere-telomerase system as a result of a considerable amount of evidence.

In no way, will this be accepted by those who reject *a priori* the new paradigm, but the same are requested to consider what is proposed here by accepting the new paradigm only as a working

hypothesis, i.e. the revolutionary idea that aging is a physiological phenomenon determined and regulated by genes and therefore in principle modifiable.

Chapter 10

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Aging of perennial cells and organ parts according to the programmed aging paradigm

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Abstract

If aging is a physiological phenomenon - as maintained by the programmed aging paradigm -, it must be caused by specific genetically determined and regulated mechanisms, which must be confirmed by evidence. Within the programmed aging paradigm, a complete proposal starts from the observation that cells, tissues and organs show continuous turnover: As telomere shortening determines both limits to cell replication and a progressive impairment of cellular functions, a progressive decline in age-related fitness decline (i.e., aging) is a clear consequence.

Against this hypothesis, a critic might argue that there are cells (most types of neurons) and organ parts (crystalline core and tooth enamel) that have no turnover and are subject to wear or manifest alterations similar to those of cells with turnover.

In this review, it is shown how cell types without turnover appear to be strictly dependent on cells subjected to turnover. The loss or weakening of the functions fulfilled by these cells with turnover, due to telomere shortening and turnover slowing, compromises the vitality of the served cells without turnover. This determines well-known clinical manifestations, which in their early forms are described as distinct diseases (e.g. Alzheimer's disease, Parkinson's disease, age-related macular degeneration, etc.).

Moreover, for the two organ parts (crystalline core and tooth enamel) without viable cells or any cell turnover, it is discussed how this is entirely compatible with the programmed aging paradigm.

Introduction

Aging, which is here precisely described and defined as "increasing mortality [i.e. decreasing fitness] with increasing chronological age in populations in the wild," [Libertini 1988] alias "actuarial senescence in the wild" [Holmes and Austad 1995], is widely documented [Nussey et al. 2013].

There are two mutually incompatible interpretations of aging [Libertini 2015a], which for their opposite and important implications are certainly paradigms in the meaning proposed by Kuhn [Kuhn 1962].

The "old paradigm" describes aging as the random age-related overlapping of many degenerative processes, which, in principle, might be partially retarded and contrasted but never entirely tamed [Libertini 2015a]. In contrast, the "new paradigm", or programmed aging paradigm, explains aging as a physiological phenomenon, favored by evolution in terms of supra-individual natural selection [Libertini 2015a], i.e. a particular type of phenoptosis [Skulachev 1997] alias "the programmed death of an individual." [Skulachev 1999a] This implies that aging is the outcome of specific genetically determined and regulated mechanisms, and therefore, in principle, it might be entirely tamed [Libertini 2009a].

The discussion about the evidence and the arguments that are in support or against each of the two paradigms is debated in another paper [Libertini 2015a] and is not the topic of the present review.

For our goals, it will suffice to say that evidence and arguments appear to be clearly in support of the new paradigm and in contrast with the old paradigm [Libertini 2015a], though the opposite paradigm remains the prevalent idea [Kirkwood and Melov 2011].

The aforementioned mechanisms that determine a progressive age-related fitness impairment have been described in brief in another paper [Libertini 2014] and here only a brief mention of them will be given.

The various cell types that constitute a vertebrate organism are subject to various kinds of programmed cell death (PCD), which are balanced by an equivalent proliferation of stem cells. The replication of these cells is subject to genetically determined and regulated limitations, due to telomerase inhibition and therefore to restrictions in telomere length restoration [Libertini 2009a]. Telomere shortening leads to an increasing probability of the complete blocking of cell duplication capacity plus a wide impairment of cell functions [Fossel 2004], i.e. cell senescence [Ben-Porath and Weinberg 2005], and also to a progressive impairment of cell functions, i.e. gradual cell senescence [Fossel 2004; Libertini 2014, 2015b]. The progressive limitation for stem cells in replacing cells eliminated by PCD leads to a gradual slowing of cell turnover. This, together with the effects of cell senescence and gradual cell senescence, progressively determines an atrophic syndrome for all organs and tissues that is characterized by:

"a) reduced mean cell duplication capacity and slackened cell turnover;

b) reduced number of cells (atrophy);

c) substitution of missing specific cells with non-specific cells;

d) hypertrophy of the remaining specific cells;

e) altered functions of cells with shortened telomeres or definitively in noncycling state;

f) alterations of the surrounding milieu and of the cells depending from the functionality of the senescent or missing cells;

g) vulnerability to cancer because of dysfunctional telomere-induced instability ..." [Libertini 2014a]

A fair objection against this mechanism, in particular regarding its ability to explain all aging features, is that this would be contradicted by the existence of cell types and organ parts that are not subject to renewal but show aging alterations as cell types and organs that are subject to cell turnover.

In this regard, in an aforementioned paper [Libertini 2014a], a partial and short answer has already been given, but given the importance of the subject, it is necessary that this answer is deepened and enriched here with further elements.

Discussion

Neurons

A neuron, in general, is connected to many other neurons. According to one estimate, the human brain has about 10^{11} neurons interconnected by 10^{14} synapses [Williams and Herrup 1988]. This means that, on average, a neuron has 1000 connections with other neurons. For a hypothetical neuron turnover, analogous to that of other cell types, the imaginary unlikely mechanism should also precisely restore, for each neuron, all the preexistent links with the other neurons, to avoid the loss of neuron function deriving from wrong connections.

This explains why – with few exceptions [Horner and Cage 2000; Zhao et al. 2008] – there is no neuron turnover unlike the continuous renewal of other cell types. But this contrasts with the need to maintain the full functionality of the neurons, and of the nervous tissue in general, despite the passage of time.

These two seemingly incompatible requirements are satisfied by a mechanism that may be understood in its principles starting from the study of the most accessible and well-studied type of cells in the central nervous system, namely retina photoreceptor cells.

Photoreceptor nervous cells

The retina is an extroversion of the brain and the first processing of data detected by photoreceptor cells (cones and rods) is carried out in it. These cells are highly differentiated nervous cells, without turnover like almost all neurons, and are metabolically dependent on other cells with turnover, specifically the cells of retina pigmented epithelium (RPE cells), which are highly differentiated gliocytes with turnover. The tops of photoreceptor cells lean on RPE. Each day, 10% of photoreceptor cell membranes, on which photopsin molecules lie, are phagocytized by an RPE cell and substituted, by the photoreceptor cell, with an equal amount of new membrane. Each RPE cell serves 50 cones or rods and, therefore, each day an RPE cell metabolizes photopsin membranes of about five cones or rods, demonstrating a very high metabolic activity. Photoreceptor cells cannot survive without the macrophagic activity of RPE [Fine et al. 2000; Jager et al. 2008].

With the age-related decline of RPE turnover, in RPE cells there is an accumulation of damaging substances, such as A2E, a vitamin A-derived breakdown product [Sparrow 2003]. The death of RPE cells, also through the action of these substances, causes holes in the RPE layer, when the cell is not substituted by a new cell, and the deficiency of their function kills the photoreceptors not served [Berger et al. 1999].

Above all, this is manifested in the functionality of the most sensitive part of the retina, the macula – where the accumulation of A2E is most abundant [Sparrow 2003; Ablonczy et al. 2012] –, from which the name "age-related macular degeneration" (AMD) comes [Fine et al. 2000].

With particularly abnormal deficiencies of RPE cells, AMD arises at lower ages and is considered a distinct disease, while at later ages its frequency increases exponentially and must be considered a feature of the senile state. In fact, AMD affects 5%, 10% and 20% of subjects 60, 70 and 80 years old, respectively [Berger et al. 1999], and it is likely that a large proportion of older individuals suffer from AMD.

There is an association between AMD and unhealthy lifestyles [Mares et al. 2011] that reduce the number of endothelial progenitor cells, a likely consequence of a quicker cell turnover of endothelial cells [Hill et al. 2003], and that could have an analogous harmful effect on RPE: Risk factors for endothelial cells, and so for cardiovascular diseases, such as smoking, diabetes and obesity are also risk factors for AMD [Klein et al. 2007].

Moreover, while "The retina, with its high oxygen content and constant exposure to light, is particularly susceptible to oxidative damage" [Chong et al. 2007], the meta-analysis of 12 studies did not show that antioxidant supplements prevented early AMD [Chong et al. 2007].

Brain neurons and Alzheimer's disease

As photoreceptor cells (specialized types of neurons without turnover) depend on other cells (RPE cells, specialized gliocytes with turnover), other types of neurons – such as those of the cerebral cortex, basal nuclei and other parts of the central nervous system – appear to depend on other types of gliocytes. If this is true, cell senescence and gradual cell senescence of these gliocytes should cause pathologies analogous to AMD.

Indeed, neurons of the central nervous system are perennial cells but their vitality depends on other cells (e.g. microglia, a type of gliocyte) that exhibit turnover. Microglia cells degrade β-amyloid protein [Qiu et al. 1998; Vekrellis et al. 2000; Miners et al. 2008] and this function is known to be altered in Alzheimer's disease (AD) [Bertram et al. 2000] with the consequent noxious accumulation of the protein.

Therefore, replicative senescence and gradual cell senescence of these gliocytes should cause pathologies similar to AMD. Without the key example of AMD, it was previously hypothesized that AD was dependent upon the decline in function of these particular gliocytes determined by telomere shortening [Fossel 1996, 2004]: "One function of the microglia (Vekrellis et al., 2000) is degradation of β-amyloid through insulin-degrading enzyme (IDE), a function known to falter in Alzheimer disease (Bertram et al., 2000)" (p. 233), "telomere lengths of circulating monocytes can

serve as an independent predictor in at least vascular dementia (von Zglinicki et al., 2000b)" (p. 235) [Fossel 2004].

The hypothesis that AD is caused by cell senescence of microglia cells has been reproposed by others [Flanary 2009; Libertini 2009a, 2009b].

As for AMD, there are precocious familial cases of AD that are considered distinct diseases with genetic causes [Fossel 2004]. Disregarding these cases, AD frequency increases exponentially with age: 1.5% at the age of 65 and 30% at 80 [Gorelick 2004], with a very high probability that centenarians are affected by it.

AD could have, at least partially, a vascular etiology due to age-related endothelial dysfunction [Fossel 2004], but "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." [Fossel 2004]

Discarding the simplistic deduction that AD is only a consequence of vascular dysfunction, it is likely that there is a common pathogenetic mechanism: endothelial dysfunction caused by insufficient endothelial progenitor cells in the first case [Hill et al. 2003], and microglia dysfunction caused by insufficient microglia progenitor cells in the second. In both cases, the telomere-telomerase system is the primary causal factor and cardiovascular/AD risk factors accelerate telomere failure, whereas "protective" drugs counter these effects.

In fact, telomeres have been shown to be significantly shorter in patients with probable AD than in apparently healthy control subjects [von Zglinicki et al. 2000]. Moreover, there is an association between AD and cardiovascular risk factors [Vogel et al. 2006; Rosendorff et al. 2007]. Drugs, such as statins, ACE inhibitors and sartans, which are all considered "protective drugs" for cardiovascular diseases, are effective against AD [Vogel et al. 2006; Ellul et al. 2007].

As for AMD, in contrast to the hypothesis that the primary cause of AD is the accumulation of β-amyloid and tau protein, attempts by pharmaceutical societies to counter or eliminate the effects of these substances have been a large therapeutic failure for AD [Abbott 2008]. In particular, drugs or even vaccines that attempted to counter the formation of β-amyloid have provided disappointing results [Abbott 2008]: "Post-mortem analyses showed that almost all the patients had stripped-down amyloid plaques, despite most of them having progressed to severe dementia before they died." [Abbott 2008]

Attempts to treat the cognitive alterations of AD, in the hope that this could stop the disease, have been another huge failure [Hill et al. 2003; Ballard et al. 2009]. Antipsychotic drugs appear to increase the long-term risk of mortality [Ballard et al. 2009] while cholinesterase inhibitors (e.g. donezepil, galantamine, and rivastigmine) and N-Methyl-D-aspartate receptor antagonist (e.g. memantine) "are marginally effective at best." [Abbott 2008]

Brain neurons and Parkinson's disease

Parkinson's disease (PD) is a degenerative disorder of the central nervous system characterized by the accumulation inside neurons of alpha-synuclein (AS), a protein that forms inclusions known as Lewy bodies [Davie 2008; Schulz-Schaeffer 2010]. Dementia with Lewy bodies (DLB), which is classified as a Parkinson-plus syndrome [Nuytemans et al. 2010], is a primary parkinsonism with additional features [Samii et al. 2004]. In multiple system atrophy, a rare genetic disease, AS accumulates in oligodendrocytes [Sturm and Stefanova 2014].

PD, DLB and multiple system atrophy are defined as synucleinopathies for the accumulation of the aforementioned protein, while AD is defined as a tauopathy for the accumulation of tau protein in the form of neurofibrillary tangles [Galpern and Lang 2006], but clinical and pathological manifestations overlap between these types of neuropathies [Aarsland et al. 2009].

PD is considered mainly a disease affecting the motor system, as it shows typical movement disorders, but it also has various nonmotor symptoms, such as sensory deficits [Barnett-Cowan et al. 2010]. Dementia, the most typical manifestation of AD, is present at advanced stages of PD, while neurofibrillary tangles are present in brains suffering from PD [Galpern and Lang 2006].

However, senile plaques and neurofibrillary tangles, which are characteristic of AD, are uncommon in PD without dementia [Dickson 2007].

The risk of dementia in PD patients is two to six times that of the whole population [Caballol et al. 2007; Jankovic 2008] and increases with the duration of the disease [Caballol et al. 2007].

Behavior and mood alterations (e.g. apathy, depression, anxiety, etc.) are common symptoms in PD patients with dementia and, in PD cases without cognitive impairment, are more common than in the general population [Jankovic 2008].

Only AD is more frequent than PD among the neurodegenerative disorders [de Lau and Breteler 2006; Yao et al. 2013]. PD frequency is about 0.3% in industrialized countries and increases from 1% in individuals older than 60 to 4% in the population older than 80 [de Lau and Breteler 2006], with a mean age of onset of around 60; however, in 5–10 % of cases, it begins before 50 years of age [Samii et al. 2004].

"PD and DLB are common neurodegenerative diseases in the population over the age of 65. About 3% of the general population develops PD after the age of 65, whereas about 20% of all diagnosed dementia patients have DLB (McKeith, 2004; Dorsey et al., 2007). In both disorders movement and cognition, as well as mood and autonomic function are severely affected. Diagnosis to distinguish PD and DLB is very difficult, because of the overlap of symptoms and signs (Henchcliffe et al., 2011)." [Brück et al. 2015]

PD symptoms are a clear consequence of cell death in the pars compacta region of the substantia nigra, which causes a greatly reduced activity of dopamine secretion, although the mechanism by which the neurons are lost is debated [Obeso et al. 2008].

In PD evolution, in a first pre-clinical phase, Lewy bodies are shown in the olfactory bulb, pontine tegmentum and medulla oblongata. Later, they appear in the substantia nigra, in some areas of the midbrain and basal forebrain, and finally in the neocortex [Davie 2008]. In these places, there is neuronal degeneration, but it has been proposed that Lewy bodies could be a protection against harmful factors and not the cause of cell death [Obeso et al. 2010; Schulz-Schaeffer 2010]. However, in demented AD individuals, Lewy bodies are largely present in cortical areas and "reduced AS clearance is involved in the generation of AS inclusions in DLB and PD ..." [Brück et al. 2015]

"Glial cells are important in supporting neuronal survival, synaptic functions and local immunity ... However, glial cells might be crucial for the initiation and progression of different neurodegenerative diseases, including ASP ..." [Brück et al. 2015]

"... microglial cells contribute to the clearance of debris, dead cells and AS thereby supporting neuronal survival. But on the other hand, microglial cells can get over activated in the course of the disease and might contribute to disease initiation and progression by enhancing neurodegeneration through elevated oxidative stress and inflammatory processes." [Brück et al. 2015]

These elements suggest that the activation of microglial and astroglial cells could be a reaction to AS accumulation, which, in turn, would be caused by the loss of trophic functions of specific gliocytes, in particular those dedicated to axon trophism. These gliocytes could be astrocytes [Morales et al. 2015] and the decline of their function would be a consequence of their decline in turnover, as is the case for the decline of RPE cells and the subsequent AMD.

The metabolic syndrome is an important risk factor for PD [Zhang and Tian 2014]. A study has demonstrated that high skinfold thickness in midlife is associated with PD [Abbott et al. 2002]. Another study found that obesity in middle age increases the risk of future dementia independently of other conditions, and perhaps adiposity works together with other risk factors to increase neurodegenerative disease [Whitmer et al. 2005]. In addition, some evidence shows that body mass index is associated with a risk of PD and that the effect is graded and independent of other risk factors [Hu et al. 2006].

In aging, hyperglycemia is also associated with PD through damage to the central nervous system, a consequence of long-term exposure to glucose [Hu et al. 2007; Tomlinson and Gardiner

2008]. Epidemiologic studies suggest that previous type 2 diabetes is also a risk factor for developing PD [Mercer et al. 2005].

Hyperhomocysteinemia, a risk factor for endothelial dysfunction [Woo et al. 1997], has been shown to be involved in neurodegenerative disorders, such as AD and PD [Kruman et al. 2000].

Statin use appears to lower the risk of PD [Gao et al. 2012; Friedman et al. 2013; Undela et al. 2013]. Captopril, an angiotensin-converting enzyme inhibitor protects nigrostriatal dopamine neurons in PD animal models [Lopez-Real et al. 2005; Sonsalla et al. 2013].

Strangely, smoking, a risk factor for AMD [Klein et al. 2007], appears to lower PD risk [de Lau and Breteler 2006].

Olfactory receptor cells

Olfactory receptor cells (ORCs) are an example of neurons with turnover (see below).

In the upper part of the nasal cavity, the odor perception takes place through ORCs, which are specialized neurons that "have a single dendrite that extends to the apical surface of the epithelium and ends in a terminal knob, which has many small cilia extending into the mucosa. A single axon projects through the basal side of the epithelium through the lamina cribrosa to terminate in the olfactory bulb. Each of the receptor neurons expresses one of a family of over 1000 olfactory receptor proteins ... The neurons are surrounded by glial-like cells, called sustentacular cells. Other cells in the epithelium contribute to the continual production of the new receptor neurons ..." [Bermingham-McDonogh and Reh 2011]

The continuous renewal of the ORCs in healthy adult individuals is well documented [Maier et al. 2014].

"The ongoing genesis of olfactory receptor cells is common to all vertebrates (see Graziadei and Monti Graziadei, 1978, for review) and the rate of production is quite high. The production of new olfactory receptor cells is critical to the maintenance of this system, as the olfactory receptor cells only last a few months. The rate of production of new olfactory receptor cells is balanced by their loss so that a relatively stable population of these receptors is maintained." [Bermingham-McDonogh and Reh 2011]

In a healthy, undamaged olfactory epithelium, the continuous renewal of ORCs is ensured by slowly cycling stem cells (globose basal cells) and by transit-amplifying progenitor cells (horizontal basal cells) [Caggiano et al. 1994; Huard et al. 1998; Chen et al. 2004; Leung et al., 2007; Iwai et al 2008]. This model of cell turnover is analogous to that of other cell types (e.g. epidermis) [Watt et al. 2006].

ORC turnover is: (i) necessary, as these cells are very exposed to external insults, and (ii) simple, as each neuron has a single dendrite and a single axon. Therefore, unlike most neurons, ORCs undergo cell turnover and an age-related slowing of such turnover should impair the olfactory function. This does not exclude the possibility that the age-related slowing of the turnover for (i) glial cell satellites of ORCs, (ii) neurons of the olfactory bulb, or (iii) other olfactory areas in the central nervous system may contribute to a decline in olfactory function.

About half of the individuals between 65 and 80 years old suffer from evident olfactory dysfunction [Doty et al. 1984; Duffy et al. 1995; Murphy et al. 2002], and olfactory impairment increases with age [Schubert et al. 2012].

Hyposmia is often a precocious sign of PD and an early and constant characteristic both of AD and of DLB [Factor and Weiner 2008]. Olfactory dysfunction is a symptom present in AD, in PD dementia and in other forms of dementias [Barresi et al. 2012]. Estimated numbers of olfactory dysfunction may be as high as 100% in AD [Duff et al. 2002] and 90% in PD [Doty 2012].

Hearing neurons

The organ of Corti, in the cochlea (inner ear), for its hearing function is equipped with auditory cells, which are differentiated neurons, divided into an internal row (inner hair cells) and some external rows (outer hair cells), which are connected to specialized neurons (spiral ganglion

neurons). There are 15,500 hair cells and 35,000 neurons in each cochlea of a newborn [Wong and Ryan 2015]. Both of these types of cells, like most neurons, are perennial cells [Wong and Ryan 2015]: "... no epithelial maintenance has been described for the hair cells of the cochlea of mammals, though hair cell addition and repair occur in lower vertebrates ..." [Maier et al. 2014]

Age-related hearing loss, or presbycusis, is well known – >50% in individuals older than 60 – [Zhan et al. 2010] - and may be aggravated by other factors, such as noise exposure, diabetes or hypertension [Wong and Ryan 2015]. However, even in healthy animals reared in silence, presbycusis is still observed [Sergeyenko et al. 2013; Yan et al. 2013].

There is an association between hypacusia and incident dementia [Lin et al. 2011a]. The incidence of PD was shown to be 1.77 times more likely in patients with hearing loss than in those without hearing loss [Lai et al. 2014], and hearing impairment is a common feature in idiopathic PD [Vitale et al. 2012].

Neuropathies: a synthesis

Neurons, in their multiple differentiated forms, appear to be perennial cells that, in turn, require auxiliary cells, namely differentiated forms of gliocytes, with some exceptions for their perennial status (e.g. ORCs). These gliocytes are not perennial cells but are subject to turnover.

The slowdown in the turnover of the above-mentioned gliocytes gradually impairs the function of the served neurons to the point of their death.

Furthermore, the factors for other cell types that, in general, appear to accelerate the turnover or otherwise accelerate the achievement of the limits in duplication capacity (e.g. diabetes, overeating, unhealthy lifestyles, etc., or in brief "risk factors") should generally also accelerate this impairment. In contrast, the factors for other cell types that, in general, appear to have the opposite effects (e.g. "protective drugs", healthy lifestyles, etc., or in brief "protective factors"), similarly should slow this decline.

These concepts place in the same category a number of troubles (AD, PD, DLB, AMD, presbycusis, age-related hyposmia) that are generally considered distinct diseases while they appear to be all caused by physiological cell turnover decline (i) of ORCs for age-related hyposmia or (ii) of satellite gliocyte cells for the other troubles) (Fig. 1). The term "trouble" instead of "disease" has been used to highlight the fact that they are features of a physiologic phenomenon – aging – and not specific pathologies.

The data reported above separately for each trouble may be summarized as follows:

- For all the aforementioned troubles (excluding a small percentage of cases with early onset and clear genetic origin), the relationship between age of onset, rate of aggravation and other characteristics of aging in tissues/organs that undergo turnover;
- The relationship between aggravation and/or increased incidence of each of these troubles and risk factors for other types of tissues/organs subject to turnover;
- Conversely, the relationship between slowdown/lower incidence of each trouble and protective factors for other types of tissues/organs that undergo turnover.

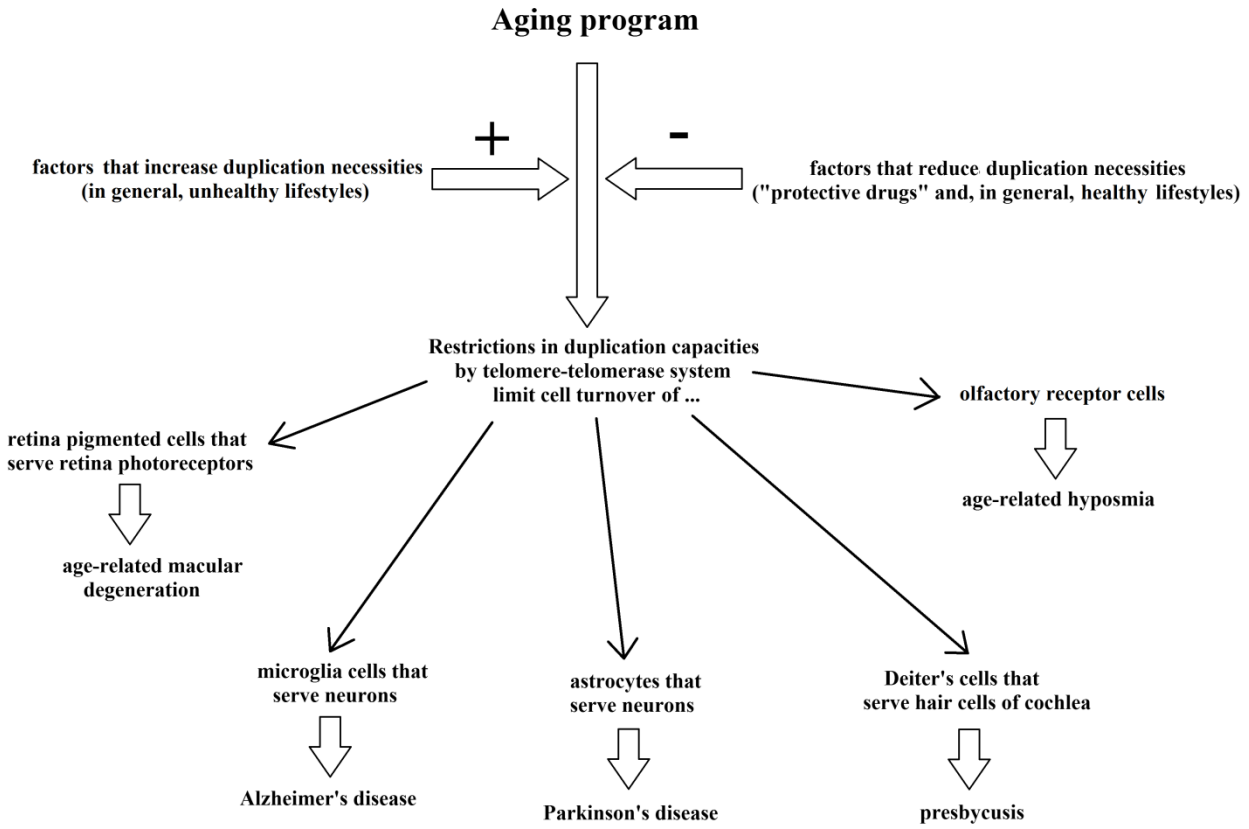


Figure 1 - Scheme for aging of neuron types that are perennial cells as a consequence of turnover decline of satellite gliocytes. The turnover decline of olfactory receptor cells is the main direct cause of olfactory function decline.

Eye crystalline lens

The lens has three main parts: the lens capsule, the lens epithelium, and the lens fibers. The lens capsule forms the outermost layer of the lens, and the lens fibers form the bulk of the interior of the lens. The lens capsule, a smooth, elastic and transparent basement membrane, completely surrounds the lens; it is composed of collagen and is synthesized by the lens epithelium [Forrester et al. 1996]. The lens epithelium is only on the anterior side of the lens between the lens capsule and the outermost layer of lens fibers. The cells of the lens epithelium regulate most of the homeostatic functions of the lens [Candia 2004].

Lens epithelium cells are also progenitors for new lens fiber cells that are constantly produced in the embryo, fetus, infant, and adult. After birth, the lens continues to grow, and the new lens fiber cells are added as outer layers in the germinative zone, which is in the equatorial area of the lens epithelium. After the transformation in lens fiber cells, the lens epithelial cells elongate, detach from the capsule and epithelium cells, begin to synthesize crystallin protein and, finally, lose their nucleus, becoming mature lens fiber cells [Forrester et al. 1996].

The change of lens shape, which determines a greater or lesser dioptric power and therefore allows the correct image focusing on the retina, is a function of the differential contraction of the ciliary muscle [Forrester et al. 1996].

The lens, and its capacity for accommodation, is subject to three types of age-related alterations, of which only the first depends on cell turnover:

1) Cell turnover decline of the lens epithelial cells

The crystalline lens has no nucleated cell in its core and its functionality, in particular the transparency, depends on lens epithelial cells that undergo turnover [Tassin et al. 1979].

"Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells" [Martin and Oshima 2000], which is consistent with the age-related reduction in growth rate of lens epithelial cells described in healthy human subjects [Tassin et al. 1979].

Smoking and diabetes, which are risk factors for cardiovascular diseases [Hill et al. 2003] are risk factors for cataracts, as well [Delcourt et al. 2000].

Statins lower the risk of nuclear cataract, which is the most common type of age-related cataract [Klein et al. 2006]. This could be explained by their "putative antioxidant properties" [Klein et al. 2006], but it is more rational to suppose that their effects on lens epithelial cells are analogous to those on endothelial cells [Hill et al. 2003].

2) Lens enlargement with progressive loss of accommodation ability

The progressive age-related lens enlargement, due to the slow proliferation of lens epithelial cells in the equatorial area, progressively reduces the accommodation capacity of the lens for near vision. This sight reduction (presbyopia), with the consequent fitness alteration, happens to humans at ages existing in the wild. In fact, at the age of 50, when presbyopia is the normal condition, surviving individuals represent about 40% in a population in wild conditions [Hill and Hurtado 1996]. Since a higher or lower growth rate of crystalline lens is genetically regulated, the fitness reduction determined by presbyopia is an element of the aging process that is not dependent on the slowing of cell turnover but, at the same time, not resulting from uncontrollable "wear and tear" phenomena.

3) Chemical alterations of lens proteins

"In the lens proteins (crystallins) of big whales, spontaneous isomerization of L-amino acids to D-amino acids occurs at any age. Crystallins are synthesized during formation of lens and originally contain, as any othe r proteins, only L-amino acids. Crystallins are practically not replaced during entire life of the whale. L-D isomerization is not encoded by genomes and it is a chemical property of amino acids. Fortunately, this process is very slow (2% per 10 years). However, after 200 years, 40% of L-amino acids are already isomerized to D-amino acids in crystallins, an event strongly affecting the spatial structure of these proteins and apparently their unique ability to be absolutely transparent for the visible light. If such a process results in formation of cataract, it may lead to blindness which should make impossible the life of old whale in the ocean. He should die, such a death being age-dependent. And nevertheless it cannot be regarded as slow phenoptosis." (Vladimir Skulachev, observation as editor-in-chief for [Libertini 2012a]). Clearly, this age-related biochemical alteration must be classified within the "wear and tear" category, but the possible formation of cataracts, a deadly fitness alteration, could happen at ages rarely or never existing in natural conditions and therefore cannot be contrasted or shaped by natural selection.

Teeth

A species is defined as monophyodont, diphyodont or polyphyodont when in its life cycle it has one, two or many successive sets of teeth, respectively [Buchtova et al. 2012]. In our species, which is diphyodont, a set of deciduous teeth (milk teeth) is replaced by a new set of permanent (adult) teeth, which has no subsequent turnover.

Many reptiles, such as geckos and crocodiles, most toothed fishes and other vertebrates are polyphyodont [Fuenzalida et al. 2000; Gaete and Tucker 2013]. Most mammals are diphyodont, such as our species, but others (e.g. elephants, kangaroos and manatees) are polyphyodont. However, mammalian ancestors (Therapsida) were polyphyodont [D'Emic et al. 2013], and this suggests that the limit of two sets of teeth is an adaptation.

In our species, as regards the teeth and the adjacent anatomical areas, the various components of the pulp, the gums and the bone that supports the teeth are subject to turnover like identical cells in other parts of the body. The enamel, however, is not subject to turnover and is therefore subject to wear, which clearly increases with age.

This is different from the turnover of the other parts of the body and might seem to be evidence in support of the ancient belief that aging is simply the result of the accumulation of excessive wear and damage of various kinds.

In contrast to this interpretation, we should first consider what happens in natural conditions and not limit ourselves to inferences based on what happens in modern artificial conditions.

In natural conditions, phenomena such as dysodontiasis, caries, periodontal disease, and their complications, which affect, to varying degrees, the vast majority of "civilized" individuals and lead to the loss of the teeth or, in general, to their impaired function, are uncommon phenomena [Price 1939].

The monumental book of Price, with its exceptional photographic documentation, demonstrates unequivocally that people living in primitive conditions do not have the dental disease of civilized man [Price 1939]. Some excerpts from this book [Price 1939]:

"Another important source of information regarding the Aborigines of Australia was provided by a study of the skeletal material and skulls in the museums at Sydney and Canberra, particularly the former. I do not know the number of skulls that are available there for study, but it is very large. I examined many and found them remarkably uniform in design and quality. The dental arches were splendidly formed. The teeth were in excellent condition with exceedingly little dental caries." (Ch. 10)

"... there are some excellent collections of skulls in museums in Peru, with the skulls in position where they can be readily studied for the shape of the dental arches. When we have in mind that from 25 to 75 per cent of individuals in various communities in the United States have a distinct irregularity in the development of the dental arches and facial form, the cause and significance of which constitutes one of the important problems of this study, the striking contrast found in these Peruvian skulls will be seen to constitute a challenge for our modern civilizations. In a study of 1,276 skulls of these ancient Peruvians, I did not find a single skull with significant deformity of the dental arches." (Ch. 13)

"Several studies have been made dealing with the incidence of dental caries or tooth decay among these ancient cultures. The author of "Bird Islands of Peru" (MURPHY, R. C. Bird Islands of Peru. New York, Putnam, 1925) states that in his examination of fifty mummies in succession he found only four with a tooth with dental caries. This again is in striking contrast to our modernized communities in which from 95 to 100 per cent of all the members of a community group suffer from dental caries. I have shown in connection with the Indians of the western coast of Canada that in six highly modernized communities where the Indians were using white man's foods, 40 per cent of all the teeth had been attacked by dental caries. A similar high percentage was found in the Indians now living in Florida. The ancient burials in southern Florida revealed apparent, complete immunity. These were pre-Columbian burials." (Ch. 13)

Therefore, under natural conditions, the only permanent dentition is quite sufficient for the whole of life and is not a limiting factor for the duration of life.

Certainly, in natural conditions, for a person who lived, say, 200 years, this unique permanent dentition would be a limiting factor for his lifespan. But the only permanent dentition is adaptive for the duration of life found in natural conditions, so there are no selective pressures in favor of solutions that provide for the substitution (turnover) of the permanent dentition. In cases where the duration had been greater, the solution would have been as easy as is clearly indicated in many species, in which increased tooth wear requires a more or less rapid renewal of teeth. The elephant has six dentitions [Shoshani 2000], and alligators can replace up to 50 times their teeth [Wu et al. 2013]: The number of replacements is specific enough for the duration of their lives. Other species with strong wear have multiple substitutions, unlimited in their number [Fuenzalida et al. 2000; Gaete and Tucker 2013], or they exhibit a continuous growth of the teeth as they wear out (e.g. rodents [Single et al. 2001]).

Conclusion

The criticisms against the programmed aging paradigm based on the existence of perennial cells or organ parts not subjected to turnover are clearly overcome by the evidence discussed above. Moreover, this evidence supports and documents further the existence, predicted by the programmed aging paradigm as indispensable, of genetically determined and regulated mechanisms that gradually reduce organism fitness.

Additional elements and insights emerge from the evidence expounded earlier:

1) The factors (e.g. harmful foods and inhalations, unhealthy lifestyles, exposure to harmful factors, etc.) that damage cells and thus accelerate cell turnover, appear to accelerate and anticipate the physiological process of aging.

2) In contrast, the reduction or avoidance of these "risk" factors and the use of "protective drugs" (e.g. statins, ACE inhibitors, sartans) reduce the risk both of the troubles caused by the slackened turnover of cell types subject to turnover (e.g. arteriosclerosis due to endothelial turnover failure, senile emphysema due to alveolocyte turnover failure, etc.) and disorders related to cells or structures not subject to turnover but that suffer from slackened turnover of cells that are trophic to them (e.g. AD, PD, AMD, etc., caused by the failure of their specific trophic differentiated gliocytes).

3) The complete avoidance of the risk factors does not slow down or eliminate aging with its physiological rhythms in optimal conditions.

4) In general, disregarding the particular cases where a genetic alteration is the cause of a precocious form, the difference between the physiological form of a trouble due to aging and the precocious forms of the same trouble (in this case definable as diseases), apart from the anticipating causes, is mainly (or only) in the onset time and the speed of worsening of its manifestations. Therefore, it is difficult practically to distinguish between the precocious forms (due to specific and modifiable risk factors) and physiological forms (which are part of the aging phenomenon and cannot be modified by the avoidance of risk factors or by the use of protective drugs).

5) The above-mentioned physiological forms of these diseases and aging in general are directly or indirectly dependent on telomere shortening. It has been well known from 1998 that telomerase activation elongates telomeres, restores cell duplication capacities and erases all the manifestations of cell senescence [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998], i.e. all the manifestations of cell senescence and gradual cell senescence. In the search for methods to slow or reverse aging, a preliminary step should be to find a cure for troubles, such as AD, PD and AMD that are highly debilitating or deadly. This could be the best way to show the validity and the usefulness of the approach and the methodology proposed. For several years, countering AD by telomerase reactivation has been suggested [Fossel 1996, 2004]. The same proposal was formulated for AMD [Libertini 2009b]. In this article, it is useful to point out that it would be useful for PD, presbycusis and age-related hyposmia, as well. As regards AMD, it is interesting to note that in the first experiment that showed how the manifestations of cell aging are fully reversible, this was demonstrated for retina RPE cells (and for another cell type): "two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced staining for beta-galactosidase, a biomarker for senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine." [Bodnar et al. 1998]

A final general consideration may be useful. The criticisms against a theory may serve to falsify the hypothesis and determine its rejection if it is wrong. However, if the theory is correct, the criticisms lead to insights that confirm it and open the way for further developments. The intelligent criticism that the programmed aging paradigm could be invalidated by the existence of perennial cells or organ parts appears to be overcome by the evidence, and this, moreover, leads to proposals

that could be of extreme importance, both for remedies for widespread and debilitating disorders and for a valid, not unrealistic or utopian, approach to controlling aging.

Chapter 11

Libertini G (2017a) The Feasibility and Necessity of a Revolution in Geriatric Medicine, *OBM Geriatrics* 1(2), doi: 10.21926/obm.geriat.1702002.

The feasibility and necessity of a revolution in geriatrics

Giacinto Libertini

Abstract

Nowadays, geriatrics is mainly the treatment by palliative methods of the disorders that characterize senile decay. This is perfectly compatible with the prevailing view that aging is the inevitable result of multiple degenerative processes that can only partially be treated as they are in themselves inevitable and irreversible.

This interpretation of aging clashes with a mass of data and arguments that, conversely, indicate aging as a specific physiological function, favoured by supra-individual natural selection and genetically determined and modulated. Under this concept, it is possible to modify or even cancel aging by actions on its primary mechanisms. This is entirely different from the current interventions of geriatrics that act only on the effects of such mechanisms.

The goal of having complete control of aging may appear utopian; however, it is quite rational and feasible if we consider the already proven reversibility of aging at the cellular level and in some in vivo models. The method for achieving this objective by no means contrasts the countless alterations that characterize aging. On the contrary, it is the control of the telomere-subtelomere-telomerase system, which appears to be the general determining factor and regulator of aging. This system appears quite easily controllable by actions on telomerase activity, or even, as suggested recently, along with actions on telomere and subtelomere structure.

In a first step, these actions must be mainly addressed in the care of some aging manifestations, such as Alzheimer's disease, Parkinson's disease and age-related macular degeneration, which are particularly harsh and harmful due to the suffering and the economic burden that they cause. Effective solutions to these diseases will be the first pivotal step for a revolution in geriatric medicine, which is now possible and also necessary. This revolution will have implications and developments that will extend well beyond the boundaries of geriatrics.

Introduction

It is inconceivable to discuss geriatrics, i.e., the prevention and care of the manifestations of senile decay, without precise scientific knowledge of what aging is. First, a clear definition of aging that does not contain, clearly or covertly, preconceived ideas about its primary causes is indispensable.

A neutral definition of aging is: "increasing mortality [i.e., fitness decline] with increasing chronological age in populations in the wild" [Libertini 1988], which may be well summed up by the expression "actuarial senescence" [Holmes and Austad 1995] if the necessary specification "in the wild" is added.

Definitions of aging, such as: "a persistent decline in the age-specific fitness components of an organism due to internal physiological degeneration." [Rose 1991], "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [Kirkwood and Austad 2000], and "age-dependent decline in physiological function, demographically manifest as decreased survival and fecundity with increasing age" [Flatt and Schmidt 2009], are also acceptable if "evident physiological degeneration" is not considered a synonym of "increasing mortality", as an increased death rate may also occur with slight fitness reduction without any evident physiological degeneration. The question is not negligible. Indeed, if, under natural conditions, rare individuals

with overt physiological alterations are sought, i.e., in a condition definable as a "state of senility" [Williams 1957], aging will be a rarity in the wild ("aging is extremely difficult to observe in the natural habitats of most organisms" [Rose 1991]), and so it would be irrelevant for natural selection [Kirkwood and Austad 2000].

This is an old and large error: "Comfort is severely critical of Weissmann's theory, and offers in its place the theory that senescence is selectively irrelevant. He argues (e.g., 1956: 39) that senescence is outside the developmental program that concerns natural selection, since almost no wild organisms ever attain the senile stage. I believe that this theory is incorrect. Its fallacy lies in the confusion of the process of senescence with the state of senility ... No one would consider a man in his thirties senile, yet according to athletic records and life tables, senescent is rampant during this decade." [Williams 1957] Indeed, a recent review has documented evidence of aging, defined as increasing mortality with chronological age in populations in the wild, in 175 species [Nussey et al. 2013]. Moreover, the existence of species with evident aging under natural conditions, including humans [Hill and Hurtado 1996], has been known for a long time [Libertini 1988; Finch 1990; Ricklefs 1998]. Thus, aging cannot be considered irrelevant for natural selection.

There are many theories about the causes of aging [Comfort 1979; Medvedev 1990; Libertini 2015b], which can be divided into two opposing categories of interpretations [Libertini 2008, 2015a], worthy of being defined as paradigms, in the sense proposed by Kuhn [Kuhn 1962] for their diversity and importance of their implications.

The "non-programmed aging" paradigm maintains that: (i) aging is harmful to the individual and so is unlikely to be favoured by natural selection ("any hypothetical 'accelerated ageing gene' would be disadvantageous to the individual. It is therefore difficult to see how genes for accelerated ageing could be maintained in stable equilibrium, as individuals in whom the genes were inactivated by mutation would enjoy a selection advantage." [Kirkwood and Austad 2000]); (ii) aging is the effect of many inevitable damaging factors that, for various reasons, natural selection may counter only partially [Kirkwood and Austad 2000]. Within this paradigm, the mechanisms of natural selection in older theories are disregarded or scarcely considered [Comfort 1979], while some more recent theories hypothesize that the damaging factors are insufficiently opposed by natural selection because: (i) in the wild there are few "old" individuals and so natural selection against aging is weak (mutation accumulation hypothesis) [Medawar 1952; Hamilton 1966]; (ii) there are genes with pleiotropic effects, i.e., advantageous in the young and deleterious in "older" individuals (antagonistic pleiotropy hypothesis) [Williams 1957; Rose 1991]; (iii) there are limits determined by other physiological necessities (disposable soma hypothesis) [Kirkwood 1977; Kirkwood and Holliday 1979]. For this paradigm, aging is only a useful term to summarize many distinct phenomena and not a unitary phenomenon. Therefore, as this paradigm is nowadays dominant, in the current international classifications of diseases (ICD9 [ICD-9-CM 2016] and ICD10 [ICD-10 2016]) there is no distinct code for aging, which is an impossible cause of death in the statistics of the World Health Organization [World Ranking Total Deaths 2014].

On the contrary, the "programmed aging" paradigm maintains that aging is a physiological phenomenon, i.e., something genetically determined and regulated that is favoured by supra-individual natural selection [Libertini 2015a]. For this thesis, aging is certainly harmful to the individual but, in principle, a gene that is harmful to the individual may be favoured by natural selection at a supra-individual level. This is widely accepted for the many types of phenoptosis ("programmed death of an organism" [Skulachev 1999b]), a large category of well-known phenomena [Finch 1990], although without a unifying definition until recently [Libertini 2012a]. Within the programmed aging paradigm, there is an interpretation of aging, in terms of supra-individual selection, that explains it as beneficial in populations spatially separated in demes, and in conditions of K-selection [Pianka 1970] as it accelerates the spread of any gene within the population. This was described by a kin selection mechanism [Libertini 1988] or using population models [Travis 2004; Martins 2011; Mitteldorf and Martins 2014]. When the hypothesized conditions that favour aging are absent, the existence of non-aging species is expected, and it is well

known species exist that show no age-related fitness decline, defined prudentially as species with "negligible senescence" [Finch 1990]. For this paradigm, aging is a distinct single phenomenon, but its countless manifestations conceal this, as well as many different manifestations of a disease (e.g., diabetes) could mask its unity if we disregard its single origin.

These concepts are illustrated in Figure 1.

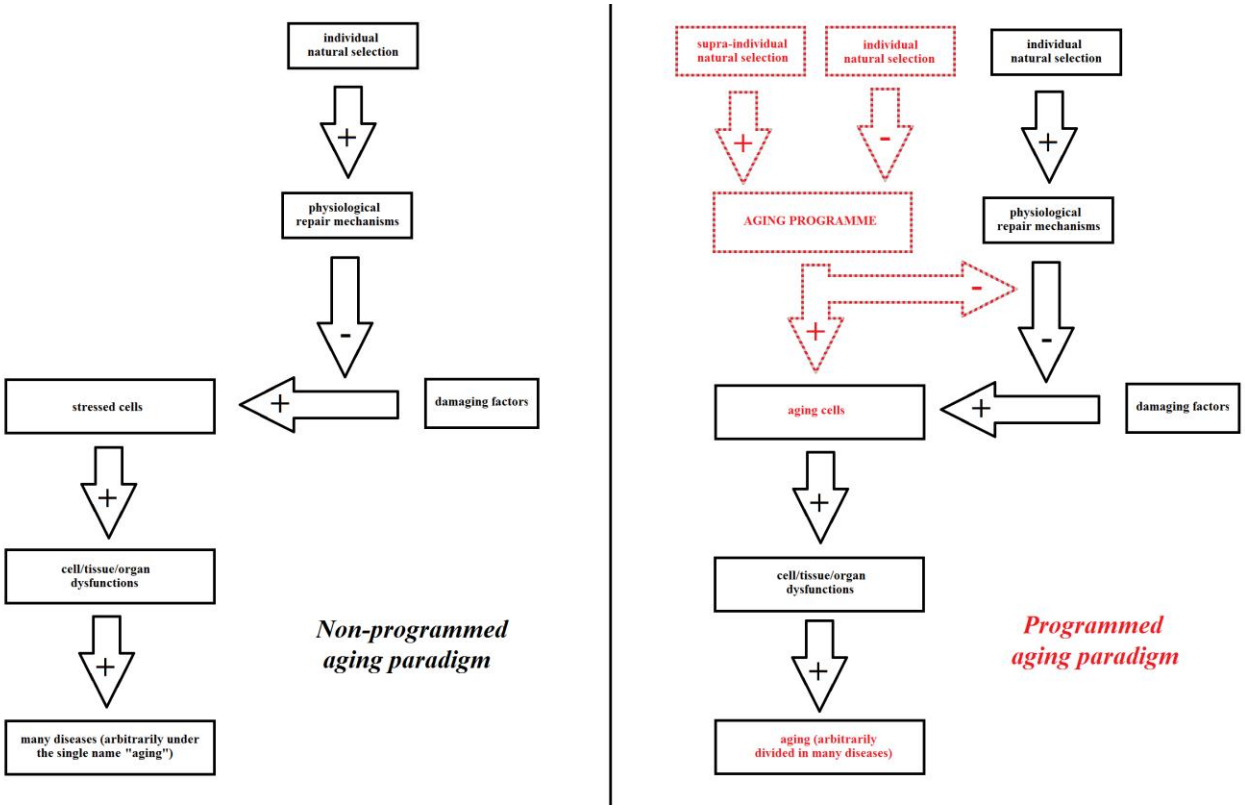


Figure 1 - Interpretation of aging according to the non-programmed aging paradigm (on the left) and to the programmed aging paradigm (on the right).

The main difference between the two paradigms is as follows:

--- The "non-programmed aging" paradigm, since it explains aging as being caused by several harmful factors, does not expect the existence of genetically determined and regulated mechanisms that cause aging: "... had aging evolved in a non-adaptive way, it would have been impossible to provide justification for any well-defined program" [Lenart and Bienertová-Vašků 2016]. Indeed, such mechanisms, if any, would prove that the paradigm is false. However, if this paradigm is true, any action against aging should attempt to minimize the effects and consequences of unavoidable damaging factors.

--- On the contrary, the "programmed aging" paradigm expects the existence of these mechanisms, and their absence would demonstrate that the paradigm is false. However, if this paradigm is true any action against aging should try to modulate differently or block the aforesaid mechanisms.

It is clear that this difference is essential for possible actions aimed at countering aging. Therefore, the deepening of the mechanisms expected by the programmed aging thesis is well within the objectives of this work. In contrast, other issues are not directly relevant to this goal and will therefore be omitted. In particular, we will not debate the evidence and arguments in support of the programmed aging thesis and/or against the opposite view, already given elsewhere [Libertini 2015a], nor the phylogeny of aging [Libertini 2015b]. The pathology of aging, outlined elsewhere [Libertini 2009a, 2009b, 2014a], will be summarily described where necessary.

The immediate objection to this approach is that the choice to follow only the programmed aging paradigm is questionable and biased. However, the answer is simple and straightforward: in this paper, the aforementioned thesis is considered as a working hypothesis, while in many other papers the opposite view is accepted, implicitly or explicitly, as a working hypothesis. Consistent with the scientific method, beyond the theoretical arguments and the evidence collected so far, the results will show which is the most fruitful working hypothesis.

Aging machinery: the telomere theory

Regarding the aging mechanisms, although other descriptions have been proposed (e.g.: [Skulachev 1999a; Olovnikov 2003, 2015; Skulachev and Longo 2005; Goldsmith 2008a]), here only the description that appears largely as the most homogeneous and supported by evidence, for brevity defined as "telomere theory", will be considered and described in its essentials. Other works [Fossel 2004; Libertini 2009a, 2015a; Libertini and Ferrara 2016a] will be referred in order to provide a more detailed description and a more extensive list of the references on which it is based.

- The system telomere-subtelomere-telomerase

At each end of the chromosomal DNA molecule, there is a region with a repetitive sequence called the telomere. This sequence is TTAGGG in humans and other mammals [Moyzis et al. 1988], and is highly conserved, as it is present in many phylogenetically distant species [Blackburn 1991].

Between the telomere and the main part of the DNA molecule, there is another region, the subtelomere (functionally defined below), with an "unusual structure: patchworks of blocks that are duplicated" [Mefford and Trask 2002]. "A common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences." [Torres et al. 2011].

The telomere is covered by a heterochromatin hood [Fossel 2004] (Figure 2).

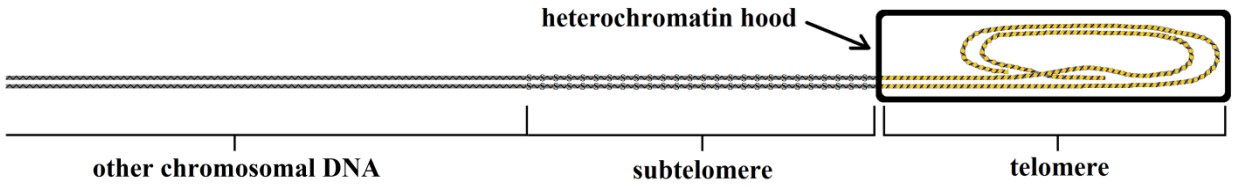


Figure 2 - Scheme of telomere, subtelomere, and heterochromatin hood on telomere.

With each replication, a small part of the telomere is not duplicated by the DNA polymerase [Olovnikov 1971; Watson 1972]. As an excessive shortening would jeopardize cell vitality, the existence of an enzyme with the capacity of restoring the unduplicated part was predicted [Olovnikov 1973]. This enzyme, telomerase, was identified in 1985 [Greider and Blackburn 1985], thus explaining the capacity of stem and germ line cells to duplicate many or unlimited times. Later, telomerase activity was shown to be repressed by specific regulatory proteins [van Steensel and de Lange 1997]. Moreover, for many cell types there is an age-related progressive shortening of telomeres [Takubo et al. 2010] and in animal species studied under natural conditions there is association between telomere length of an individual and its life expectancy [Haussmann et al. 2005; Pauliny et al. 2006; Bize et al. 2009].

This explained the limits of cell duplication capacities demonstrated in vitro [Hayflick and Moorhead 1961; Hayflick 1965; Rheinwald and Green 1975; Bierman 1978; Tassin et al. 1979] and in vivo [Schneider and Mitsui 1976], the inverse relationship between duplication capacities and age among different individuals [Martin et al. 1970], and the direct relationship between duplication capacity and longevity among different species [Röhme 1981].

However, in a cell culture where each cell had an equal number of previous replications, each cell ceased to duplicate itself at a random time but with an increasing probability related to telomere shortening. This caused a progressive decrease in the average capacity of duplication of the culture, but not a contemporary inability to duplicate in all cells after a certain number of replications [Pontèn et al. 1983; Jones et al. 1985]. This phenomenon was later explained [Blackburn 2000]: the telomere is "capped" by the aforesaid heterochromatin hood but, at some times, for a duration related to telomere shortening, the hood is temporarily detached and the telomere is "uncapped". In this phase, the cell is vulnerable to the triggering of "cell senescence", a "fundamental cellular program" [Ben-Porath and Weinberg 2005] that blocks the cell replication capacity ("replicative senescence"). Moreover, even when the telomere is shortened in the least, there is a short "uncapped" phase and so a small probability of triggering cell senescence.

Another phenomenon is consequent on telomere shortening. When the telomere shortens, the heterochromatin hood slides over the subtelomere and this determines the transcriptional silencing of the subtelomere covered part [Fossel 2004]. This silencing effect, known as the "telomere position effect" [Gottschling et al. 1990] and defined also as "gradual cell senescence" [Libertini 2015b], determines an altered functioning of genes "over long distances" in the chromosome [Robin et al. 2014]. Moreover, there is an effect on many cell functions, including cellular secretions (e.g., collagen, elastin, etc.), and thus there are alterations of the intercellular matrix, inflammation and damage to other cells [Fossel 2004]. Gradual cell senescence allows a functional definition of the subtelomere: one end is where the telomere begins (junction subtelomere-telomere) while the other end is the farthest subtelomere section repressed by the greatest sliding of the telomere hood [Libertini and Ferrara 2016a].

These phenomena indicate that the subtelomere has important regulatory functions (i) on many cell functions; and (ii) on the "capped"/"uncapped" alternation, i.e., on the probability of triggering the cell senescence program. This is compatible with the repeated sequences present in the subtelomere, and the hypothesis that they have the aforesaid regulatory functions and are progressively silenced by the sliding of the telomere hood (Figure 3).

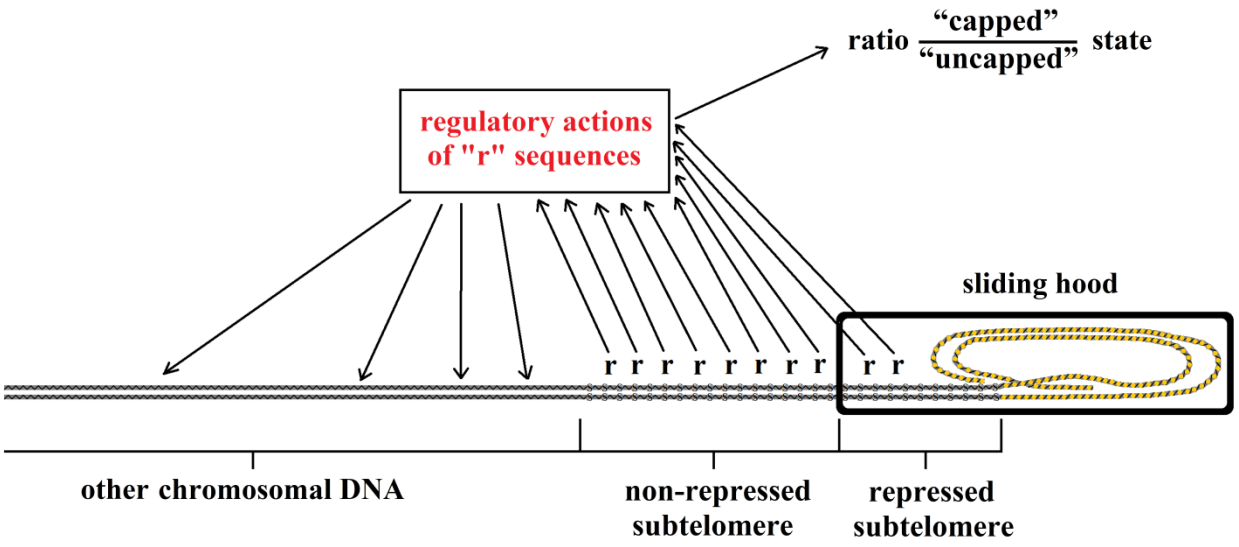


Figure 3 - Scheme of telomere sequences ("r") with regulatory actions and of their repression by the sliding of telomere hood.

- Absence of correlation between longevity and telomere length of germ line cells

A simple expectation is that, in different species, longevity should be related to telomere length of germ line cells ("initial telomere length") and/or to the intensity of telomerase activity. However, the following evidence has clearly disproved this expectation:

(i) mice and hamsters have long telomeres but age precociously [Slijepcevic and Hande 1999];

(ii) in different rodent species, longevity and telomerase activity are not related [Gorbunova et al. 2008];

(iii) two *Mus musculus* strains with different telomere lengths showed the same life spans and timing patterns of cell senescence [Fossel 2004];

(iv) donor animals and cloned animals, derived from the somatic cells of the donors, had different telomere lengths (shorter in the cloned animals), but showed the same senescence timing [Fossel 2004];

(v) in protected laboratory conditions, mice with telomerase inactivated by actions on genes showed jeopardized fertility and viability only after four to six generations, i.e., when there was a considerable telomere shortening [Blasco et al. 1997; Herrera et al. 1999]. However, as alterations were found in early generations in organs with high cell turnover [Lee et al. 1998; Herrera et al. 1999], it is likely that, in the wild, fitness would be compromised even in the first generation;

(vi) mice had limited longevity, but showed a baseline telomerase activity in most somatic cells [Prowse and Greider 1995].

A detailed exposition of a possible explanation for these phenomena, which are apparently inconsistent with the telomere theory, has been presented elsewhere [Libertini and Ferrara 2016a], and for brevity will not be repeated here. However, in summary:

(i) the germ cell – in a period definable as the "reset" phase – forms the heterochromatin hood with a size proportional to the greater or lesser length of the telomere. Regarding the longevity, the absolute "telomere length in this phase is irrelevant" [Fossel 2004], excluding the case when telomere length is below a critical value [Fossel 2004];

(ii) the heterochromatin hood necessarily has a fixed length in all cells of the organism that, by duplication, come from the germ cell;

(iii) with each duplication, the telomere shortens and the sliding of the heterochromatin hood gradually represses the adjacent subtelomeric region;

(iv) the critical factor is not the absolute initial telomere length but the "relative" telomere shortening and so the fraction of repressed subtelomere [Fossel 2004];

(v) a greater telomerase activity would slow down telomere shortening, and thus result in slower aging, but this could be balanced, or more than balanced, by a shorter subtelomere, which means a greater fraction of repressed subtelomere. In such conditions, it is possible that species exist with longer telomeres and greater telomerase activity, yet with smaller longevity, such as in mice.

It is important to consider the yeast (*Saccharomyces cerevisiae*), a unicellular species with some characteristics that may be considered as aging. Each yeast cell divides into two cells, which are defined as "mother" and "daughter" cells. While the cells of the daughter lineage are identical to the parent cell, those of the mother lineage can reproduce only a limited number of times (about 25-35 duplications [Jazwinski 1993]). In relation to the number of duplications, there are (i) increasing metabolic alterations [Laun et al. 2001; Herker et al. 2004; Lesur and Campbell 2004; Büttner et al. 2006; Fabrizio and Longo 2008]; and (ii) growing vulnerability to death by apoptosis and to replicative senescence [Laun et al. 2001; Herker et al. 2004; Büttner et al. 2006; Fabrizio and Longo 2008], phenomena that may easily explain the mortality differences among the single yeast cells under particular conditions of stress. However, for the cells of the mother lineage, the mortality rate increases with an exponential dynamic related to the number of duplications [Laun et al. 2007], i.e., similar to the age-related increase in mortality, alias aging, shown by many multicellular species

[Ricklefs 1998; Nussey et al. 2013]. Thus, these phenomena may be considered within the concept of aging, as previously defined [Libertini 1988].

In both mother and daughter lineage cells, telomerase is always active and there is no telomere shortening with each duplication [D'Mello and Jazwinski 1991; Smeal et al. 1996; Maringele and Lydall 2004]. However, in the cells of the mother lineage, proportional to the number of duplications, there is the accumulation over the subtelomere of particular molecules (extrachromosomal ribosomal DNA circles, or shortly ERCs [Sinclair and Guarente 1997]), which progressively alter cell viability [Lesur and Campbell 2004]. In yeast *tlc1Δ* mutants, telomerase is inactive and telomeres shorten with each replication both in mother and daughter lineages. The cells of the daughter lineage, although they do not have the ERC accumulation of the mother cells, show the same alterations and the same overall expression of genes, i.e., the transcriptome, as mother lineage cells with the same number of duplications [Lesur and Campbell 2004]. An interesting analogy must be highlighted: (i) the daughter cells of the normal strains are similar to those of the germ line of multicellular eukaryotes, i.e., active telomerase, no telomere shortening, no sliding of the heterochromatin hood over the subtelomere, no repression of the subtelomere, no alteration of cell functions; while (ii) the daughter cells of *tlc1Δ* mutants are similar to non-germ line cells of multicellular eukaryotes after some duplication, i.e., reduced or absent telomerase activity, telomere shortening, sliding of the heterochromatin hood over the subtelomere, repression of part of the subtelomere, and altered cell functions (Figure 4).

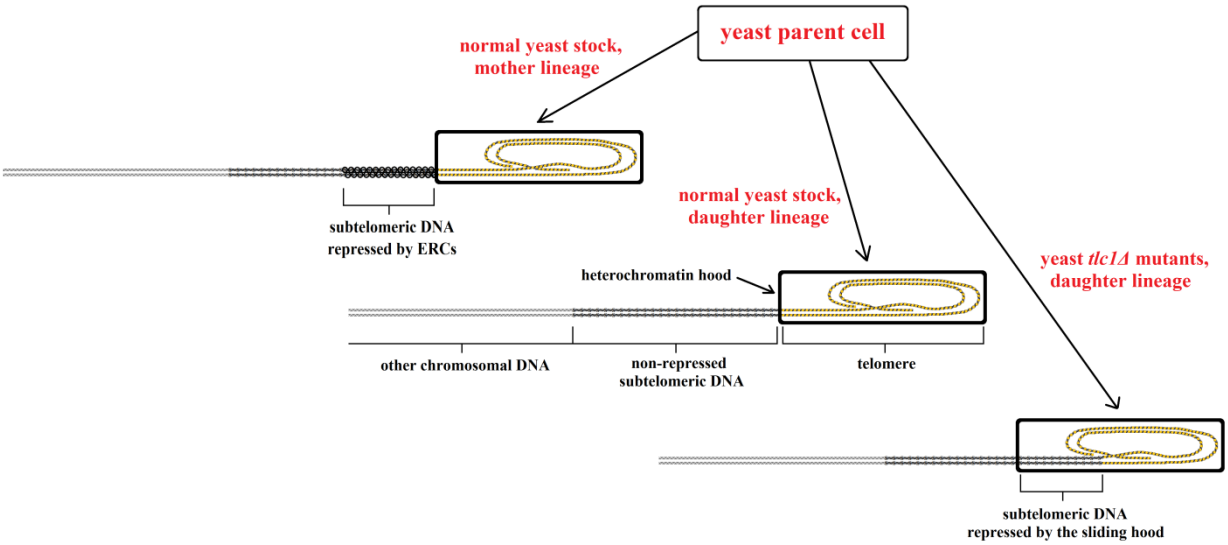


Figure 4 - In normal yeast stocks, the condition of daughter cells are similar to those of germ line cells of multicellular eukaryotes, while in yeast *tlc1Δ* mutants, it is similar to that of non-germ line cells of multicellular eukaryotes after some duplication.

- Cell turnover

In the absence of accidental events that cause cell necrosis (trauma, ischaemia, infection, etc.), in general, cells die by programmed cell death (PCD), e.g., (i) the detachment of cells from the intestine and other internal walls of body cavities; (ii) the keratinization and detachment of the epidermis and hair cells; (iii) osteocytes eliminated by osteoclasts; (iv) erythroblasts transformed into erythrocytes and later removed by macrophages; (v) apoptosis, an orderly process of self-destruction where cell debris are removed and reused without damage to other cells.

Apoptosis, a phenomenon unknown until quite recently, was described for the first time in normal epatocytes [Kerr et al. 1972]. It is phylogenetically ancient (e.g., a form of apoptosis is observable in *S. cerevisiae* [Kaeberlein et al. 2007; Laun et al. 2007]), and, disregarding other

functions, in vertebrates it is essential for cell turnover in normal adult organs [Wyllie et al. 1980; Lynch et al. 1986; Medh and Thompson 2000], as observed for many tissues/organs (e.g., gliocytes [Pontèn et al. 1983], liver [Benedetti et al. 1988], thyroid [Dremier et al. 1994], pancreatic β-cells [Finegood et al. 1995], adipocytes [Prins and O'Rahilly 1997], skeletal muscle [Migheli et al. 1997; Pollack and Leeuwenburgh 2001], bone [Spelsberg et al. 1999], cartilage [Héraud et al. 2000], kidneys [Cardani and Zavanella 2000], biliary epithelial cells [Harada et al. 2000], prostate [Xia et al 2001], and lung type II alveolar epithelial cells [Sutherland et al. 2001]).

Cell death, caused by apoptosis and other types of PCD, is a continuous phenomenon that is balanced by an equivalent production of new cells by duplication of stem cells that are specific for each cell type. The pace of this continuous cell turnover varies greatly depending on cell type and tissue [Richardson et al. 2014]. For example, osteocytes are renewed approximately every 10 years [Alberts et al. 2014] and heart myocytes have a turnover of about 4.5 years [Anversa et al. 2006], while cells of the intestinal epithelium are not older than 3-6 days [Alberts et al. 2014]. The total number of cells that die and are replaced is impressive. An estimate is that, in one year, it is approximately equal to the mass of the whole organism and that every day 50-70 billion cells die and are substituted [Reed 1999].

- The atrophic syndrome

For cells, the progressive telomere shortening with each duplication determines, as described above and summarized as follows:

(i) an increasing gradual cell senescence; and

(ii) an increasing probability of the activation of cell senescence (replicative senescence and gradual cell senescence in the highest degree).

By considering the effects of telomere shortening on cell functions and cell turnover, there is:

(i) an increase in the number of cells with functions altered in varying degrees by the gradual cell senescence;

(ii) an increase in the number of cells in cell senescence, i.e., without replicative capacities and with gradual cell senescence in the highest degree;

(iii) a reduction in the number of stem cells that can replicate, and so a reduction of cell turnover;

(iv) a reduced number of cells (atrophy);

(v) a substitution of missing specific cells with nonspecific cells;

(vi) a compensatory hypertrophy of the remaining specific cells;

(vii) alterations of the intercellular fluids and substances;

(viii) alterations of the cells depending on the functionality of the missing cells or of the cells in gradual cell senescence;

(ix) greater vulnerability to cancer because of dysfunctional telomere-induced instability [DePinho 2000];

(x) a general decline of the functions of cells/tissues/organs, i.e., progressive fitness decline, alias aging.

This condition has been defined as "atrophic syndrome" [Libertini 2009a, 2009b, 2014a] and describes the anatomical and functional progressive alterations that characterize aging. The description of the "atrophic syndrome", which contrasts with the interpretation of the non-programmed aging paradigm, is analogous to that expressed by others: "The accumulation of dysfunctional cells, together with a limited regenerative capacity of tissues, is thought to determine the age-related decline of body organs ... Dysfunctional cells, usually characterized by the presence of short telomeres ..." [Bernardes de Jesus and Blasco 2012]

The schematic description of the atrophic syndrome for cell types with turnover ("direct aging") is illustrated in Figure 5.

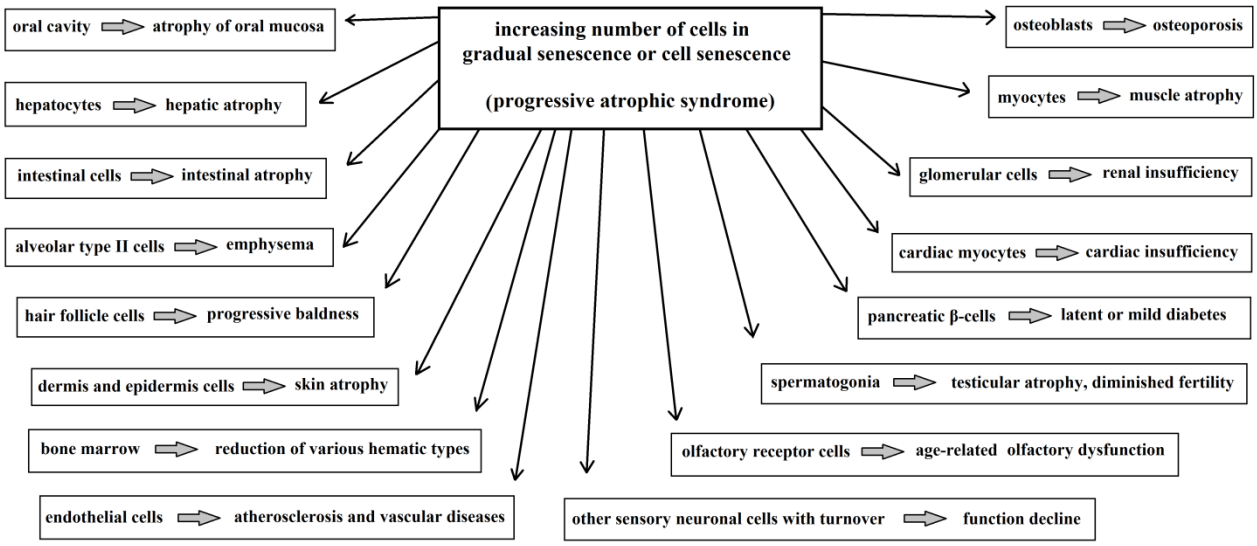


Figure 5 - Telomere theory: atrophic syndrome for cell types with turnover ("direct aging").

- Cell turnover and perennial cells

The general rule of cell turnover has some important exceptions.

The neurons of the central nervous system (CNS), with some exceptions [Horner and Cage 2000, Zhao et al. 2008], do not show cell turnover. This is due to these neurons being connected to other neurons in a variable manner by many synapses (an estimate is that in a human brain there are 10^{11} neurons connected by 10^{14} synapses [Williams and Herrup 1988], which means an average of 1,000 synapses for each neuron). The replacement of these neurons with new elements would require an unlikely faithful restoration of the connections of the replaced neurons. Conversely, where this problem does not exist, turnover does occur for the neurons. For example, olfactory receptor cells, which are specialized neurosensory neurons, receive by a single dendrite a molecular signal from the external environment, which is transmitted to other neurons by a single axon [Bermingham-McDonogh and Reh 2011], and for these cells the turnover is well known [Maier et al. 2014].

However, neurons without turnover depend on other cells that are subject to turnover for their vitality. For example, retinal photoreceptor cells need the macrophagic activity of retinal pigmented epithelium cells, which are highly differentiated gliocytes and show turnover [Fine et al. 2000; Jager et al. 2008]. The decline of these satellite cells determines the accumulation of damaging substances, such as A2E, a breakdown product derived from vitamin A [Sparrow 2003], the death of photoreceptors, and therefore progressive manifestations of age-related macular degeneration [Berger et al. 1999; Fine et al. 2000].

The genesis of Alzheimer's disease has also been explained by the decline of satellite glial cells [Fossel 2004; Flanary 2009; Libertini 2009a, 2009b; Libertini and Ferrara 2016a] and likewise has been proposed for Parkinson's disease and senile presbycusis [Libertini and Ferrara 2016a].

A particular case, analogous to that of perennial cells and which has been already discussed elsewhere [Libertini and Ferrara 2016a], is that of the crystalline lens. The lens lacks nucleated cells in its core and its functionality, including transparency, depends on lens epithelial cells that show turnover [Tassin et al. 1979]. In healthy human subjects, the age-related reduction of duplication capacity of lens epithelial cells is well known [Tassin et al. 1979], and "It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells" [Martin and Oshima 2000].

The schematic description of the atrophic syndrome for cell types and organ parts without turnover ("indirect aging") is summarized in Table 1 and Figure 6.

Table 1 - Atrophic syndrome for cell types and organ parts without turnover ("indirect aging")

A	B	C
Functional cells or organ part (without turnover)	Satellite cells of A (with turnover)	Diseases caused by the declining turnover of B
SNC neurons	Microglia cells	Alzheimer's disease
SNC neurons	Astrocytes	Parkinson's disease
Photoreceptors	Retina pigmented epithelium cells	Age-related macular degeneration
Auditory neurons	Deiter's cells	Presbycusis
Crystalline lens core	Lens epithelium cells	Cataracts

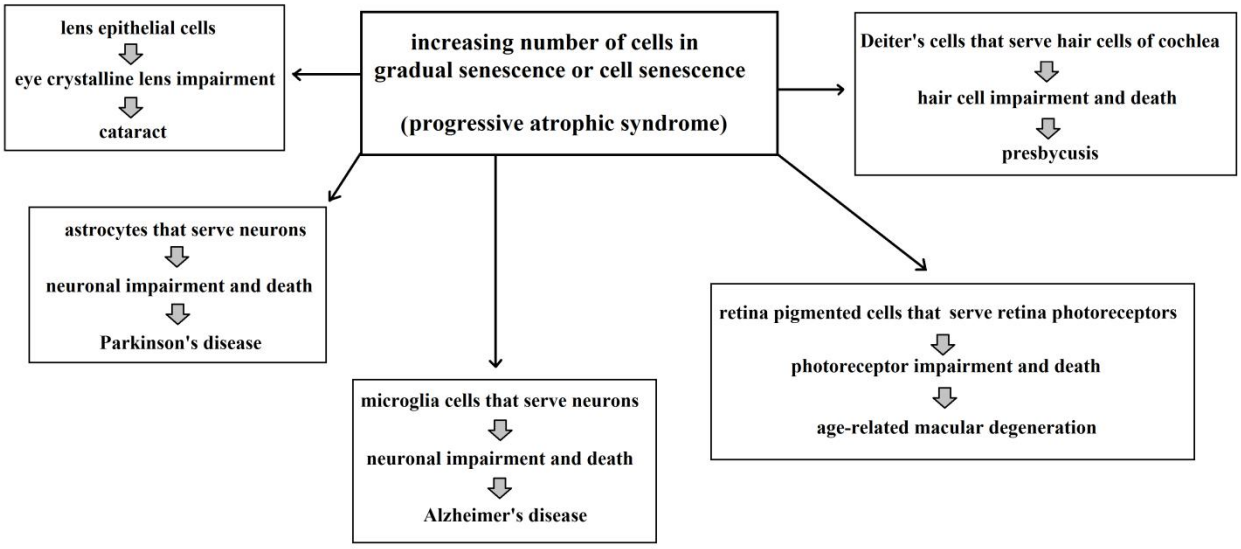


Figure 6 - Telomere theory: atrophic syndrome for cell types and organ parts without turnover ("indirect aging").

A detailed description of the atrophic syndrome for each tissue or organ is beyond the limits and scope of this work. A partial and brief description has been exposed in other papers [Libertini 2009a, 2009b, 2014a]. Only a few concise descriptions will be presented here as examples.

--- **Liver**. It is known that liver volume shows an age-related decline, estimated to be about 37% between the ages of 24 and 91 [Marchesini et al. 1988], both in absolute values and in proportion to body weight [Wynne et al. 1989]. In aged individuals, the size of the remaining hepatocytes increases, while hepatocyte size declines in liver atrophy caused by starvation [Watanabe and Tanaka 1982; David and Reinke 1988].

--- **Heart**. In contrast with old and deep-rooted convictions, the heart is a self-renewing organ. Every day, in a normal human heart, about 3 million myocytes die by apoptosis and are replaced by cardiac stem cells: "the entire cell population of the heart is replaced approximately every 4.5 years … The human heart replaces completely its myocyte population about 18 time during the course of life, independently from cardiac diseases." [Anversa et al. 2006]. The cardiac stem cells allow myocyte turnover and show age-related telomeric shortening and cell senescence [Leri et al. 2001; Urbanek et al. 2003; Chimenti et al. 2003]. The heart shows an age-related decline in the number of myocytes and an increase in cell volume per nucleus in the remaining myocytes [Olivetti et al. 1991]. The loss of myocytes causes a decline in cardiac dynamic capacities and this causes an enlargement of the heart, i.e., a morphological hypertrophy, which conceals the underlying atrophy in the number of contractile cells [Aronow 1998]. "With aging, there is also a progressive reduction in the number of pacemaker cells in the sinus node, with 10 percent of the number of cells present at age 20 remaining at age 75. ... Age-associated left ventricular hypertrophy is caused by an increase

in the volume but not in the number of cardiac myocytes. Fibroblasts undergo hyperplasia, and collagen is deposited in the myocardial interstitium." [Aronow 1998]

--- **Skin**. In older individuals, (i) the dermal-epidermal junction, where the stem cells for the epidermis are, is flattened; (ii) all the components of the derma (dermal fibroblasts, melanocytes, mast cells, Langerhans cells, eccrine glands, capillaries, Pacinian and Meissner's corpuscles, hair, etc.) decrease in number; and (iii) nails are reported to grow more slowly [Griffiths 1998].

--- **Endothelium**. Endothelial cells show cell turnover by effect of stem cells that are known as endothelial progenitor cells (EPCs) and derive from primary stem cells in the bone marrow. The number of EPCs decreases with age and by action of "cardiovascular risk factors" (diabetes, cigarette smoking, hypercholesteremia, hypertension, etc.), while it is increased by statins and other drugs [Hill et al. 2003]. A reduced number of EPCs slackens endothelial cell turnover and jeopardizes endothelial function, and this alters blood circulation, causing diseases such as cardiac infarction or cerebral ischemia. The decline in EPC number is a reliable cardiovascular risk index, equivalent to the Framingham risk score [Hill et al. 2003; Werner et al. 2005]. In older individuals, excluding the effects of cardiovascular risk factors, diseases caused by altered endothelial function increase exponentially [Tallis et al. 1998].

- Reversibility of aging manifestations

If aging is caused by the accumulation of inevitable damage of various kinds, as maintained by the non-programmed aging paradigm, in principle it should be irreversible. On the contrary, if aging is determined by mechanisms that are genetically determined and modulated, as maintained by the programmed aging paradigm, in principle it could be regulated or even cancelled. In clear support of the second thesis and against the opposite thesis, are several examples: (i) in vitro, telomerase activation cancels the cellular biochemical alterations of aging and the incapacity to replicate [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de Lange and Jacks 1998]; (ii) in experiments on humans, skin obtained from cultivating fibroblasts of young subjects and the skin from fibroblasts of old subjects with reactivated telomerase were indistinguishable [Funk et al. 2000]; (iii) in aged mice in which telomerase was genetically blocked, telomerase reactivation reversed the manifestations of aging, even those of the nervous system [Jaskelioff et al. 2011]; and (iv) telomerase reactivation, in one- and two-year-old normal mice, delayed the manifestations of aging and increased the lifespan [Bernardes de Jesus et al. 2012].

- Telomere-subtelomere-telomerase system and cancer

For the non-programmed aging paradigm, the existence of genetically determined and modulated mechanisms, which cause progressive fitness decline, i.e., the telomere-subtelomere-telomerase system, is impossible as they would be certainly eliminated by natural selection. In any case, they are incompatible with this paradigm [Kirkwood and Melov 2011]. Against these convictions, the evidence of the aforesaid mechanisms, as expounded above, appears indisputable, though their existence is still implicitly disregarded [Bredesen 2004], or clearly denied, both in principle and ignoring precise previous descriptions [Libertini 2009a, 2009b], e.g.: "Aging is not and cannot be programmed. ... Programmed theories neither specify nor predict mechanisms of death." [Blagosklonny 2013]; "... biological aging can be defined as the random, systemic accumulation of dysfunctional molecules that exceeds repair or replacement capacity ... aging is not a programmed process, it is not governed directly by genes. On the contrary, aging is a stochastic process." [Hayflick 2016].

However, authoritative scholars (who have contributed to the detection of these mechanisms by remarkable works) think that to justify their existence, there is the absolute necessity of an important function for them that must be different from that of lowering fitness and must overcome, in terms of natural selection, the clear damage caused by their action. The only proposed explanation that is compatible with the non-programmed aging paradigm suggests that they are a general defence against cancer, as replicative senescence would slacken or even block cancer

proliferation [Campisi 1997; Wright and Shay 2005]. A deadly evolutionary trade-off between the need for defence against cancer and aging manifestations was therefore hypothesized [Campisi 2000], and this was compatible with two old popular theories (antagonistic pleiotropy theory [Williams 1957; Rose 1991] and disposable soma theory [Kirkwood 1977; Kirkwood and Holliday 1979]).

However, this proposal is untenable as already expounded elsewhere [Libertini 2009b, 2013; Mitteldorf 2013; Libertini and Ferrara 2016a]. In short,

(i) in yeast, gradual cell senescence, replicative senescence and vulnerability to apoptosis, caused by ERC accumulation, which has the same effects of telomere shortening in multicellular eukaryotes, are well-documented [Jazwinski 1993; Fabrizio and Longo 2007; Laun et al. 2007]. However, this cannot defend from cancer onset, as cancer is impossible in unicellular species such as yeast.

(ii) Gradual cell senescence weakens cells and the whole organism. Moreover, when cell senescence is activated, in contrast to what happens in yeast, there is also a greater resistance to apoptosis [Childs et al. 2014] (in yeast, a greater vulnerability to apoptosis increases the death rate; in multicellular eukaryotes a greater resistance to apoptosis of senescent cells weakens the organism and so increases the death rate. Therefore, for the aim of eliminating aged individuals the effect is the same). These phenomena are implausible as defences against cancer: (a) in mice, when senescent cells are selectively eliminated, there is an increased lifespan, fewer age-dependent changes, and the progression of cancerous diseases is delayed [Baker et al. 2016]; (b) an increasing number of cells in gradual cell senescence and replicative senescence progressively weakens the efficiency of the immune system [Fossel 2004], and this should increase vulnerability to cancer and cancer incidence [Rosen 1985].

(iii) Animal species that show no age-related fitness decline ("animals with negligible senescence" [Finch 1990]) cannot have any age-related increasing vulnerability to neoplastic disease, as proved by their constant death rates at any age. However, under wild conditions, old individuals of these species have the same telomerase activity as young individuals [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998].

(iv) Shortened telomeres cause DNA instability that increases the probabilities of cancer [DePinho 2000; Artandi 2002; Artandi and DePinho 2010].

(v) In a study on a human population in the wild, (a) there was an age-related increase in mortality that began at age 30 (equivalent to that observed in modern populations [Williams 1957]); (b) the survivors were approximately 30% at age 60 and 20% at age 70; and (c) no cases of cancer were reported and sporadic deaths by cancer were only possible in individuals older than 70 years [Hill and Hurtado 1996]. It appears illogical that the mechanisms causing an age-related increase in mortality beginning at age 30 could be a defence against a rare disease found in much older individuals [Libertini 2013].

vi) As telomerase activation, a common feature in cancer, is subsequent and does not precede cancer onset, it cannot be considered a cause of cancer and should only be considered a characteristic of cancerous cells that aggravates the disease [Fossel 2004].

Therefore: "The hypothesis that telomerase is restricted to achieve a net increase in lifespan via cancer prevention is certainly false. Were it not for the unthinkability of the alternative – programmed death – the theory would be dead in the water." [Mitteldorf 2013]

However, the rejection of the hypothesis that the telomere-subtelomere-telomerase system is a defence against cancer does not exclude the possibility that an improper telomerase activation could be carcinogenic in particular conditions [González-Suárez et al. 2001; González-Suárez et al. 2005].

- Pathology of aging

If aging is a function, alterations of this function, viz. specific diseases, are foreseeable. Indeed, there are two main categories of diseases that affect aging:

1) Diseases caused by genetic alterations

These are rare but of great interest for studying aging. Two syndromes, *dyskeratosis congenita* (DC) [Dokal 2000] and Werner's syndrome (WS) [Martin and Oshima 2000], are particularly interesting in this regard.

The DC autosomal dominant form is caused by a defect in the gene that encodes the RNA part of telomerase [Vulliamy et al. 2001], while the X-linked form of DC shows low levels of telomerase and shorter than normal telomeres [Mitchell et al. 1999]. "Problems tend to occur in tissues in which cells multiply rapidly – skin, nails, hair, gut and bone marrow – with death usually occurring as a result of bone-marrow failure." [Marciniak and Guarente 2001]

WS is due to the dysfunction of a helicase type of the RecQ family that determines a dysfunction of somatic cells in the cycling state [Yu et al 1996]. In WS, cells show high somatic mutation rates [Fukuchi et al. 1989] and a reduced replication capacity [Martin et al. 1970]. In consequence of the abnormality in DNA metabolism, there is an atrophic syndrome for non-high turnover cells and tissue [Martin and Oshima 2000].

The differences between the two syndromes have been carefully outlined [Marciniak and Guarente 2001] and later discussed in the context of the programmed aging paradigm [Libertini 2009a]. In short, DC and WS may be considered two model cases of segmental progeria, i.e., the altered functionality of only a fraction of cell phenotypes [Fossel 2004], and it is likely that a non-segmental, i.e., total, progeria is incompatible with life.

Table 2 – Relationships between some aging problems and some "risk factors" or "protective drugs".

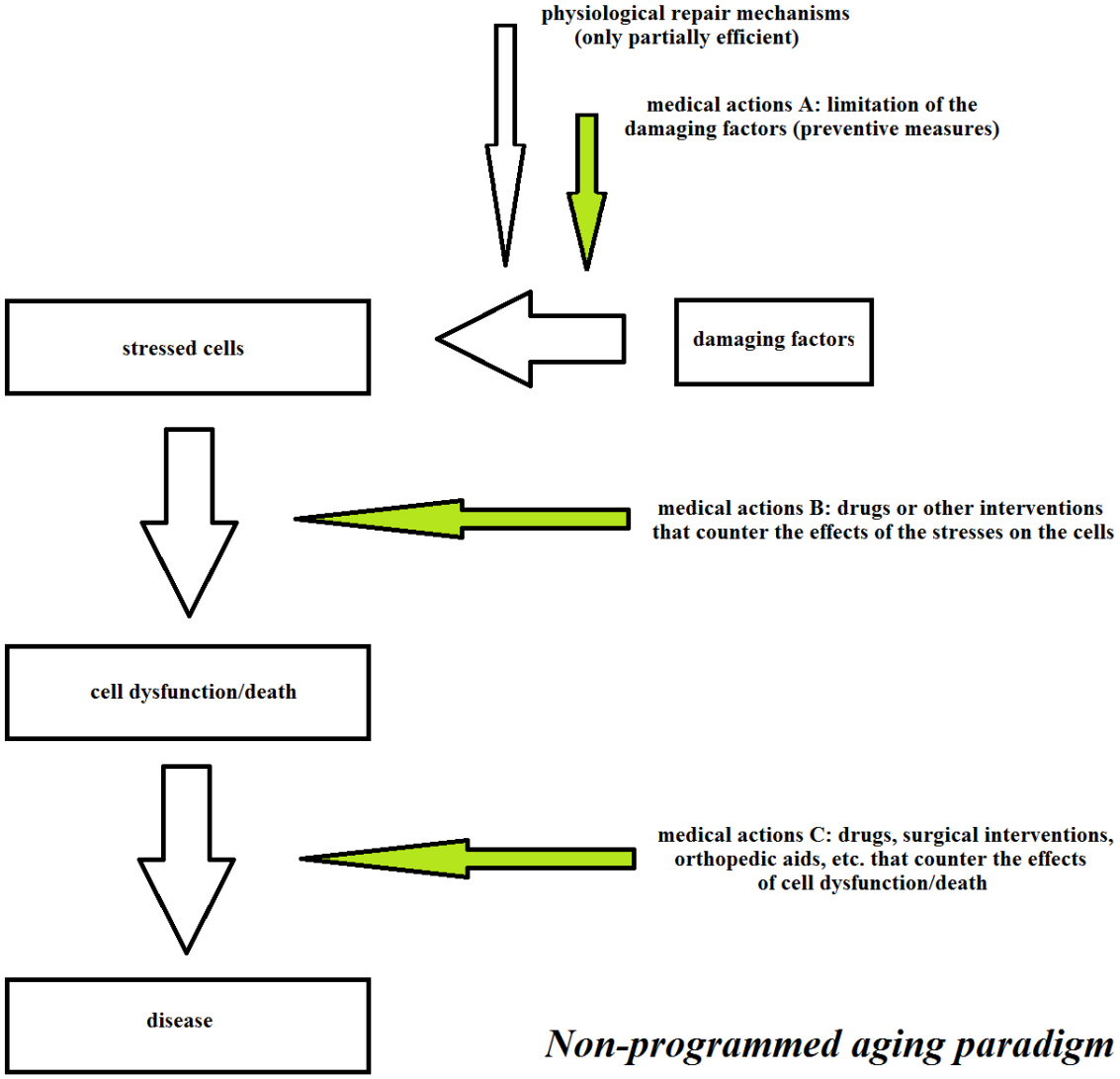
Problems	Cell turnover of the specific cells	Risk increased (+) or lowered (-) or unaltered (/) by							Protective effect by:	
		Age	Diabetes	Obesity/ dislipemia	Hypertension	Smoke	Alcohol moderate use	Alcohol abuse	Statins	ACE-i, sartans
Endothelial dysfunction	Yes	+[1]	+[2]	+[3]	+[4]	+[5]	-[6]	+[7]	+[8]	+[9]
AMD	No	+[10]	+[11]	+[12]	+[13]	+[14]	/[15]	/[16]	+?[17]	[18]
AD	No	+[19]	+[20]	+[21]	+[22]	+[23]	-[24]	+[25]	+[26]	+[27]
PD	No	+[28]	+[29]	+[30]	+[31]	-[32]	-[33]	+[34]	+[35]	+?[36]

Notes: 1-5) [Hill et al. 2003; Wilson et al. 1987]; 6) [Roerecke and Rehm 2014; Gardner and Mouton 2015; de Gaetano et al. 2016]; 7) [Gardner and Mouton 2015; de Gaetano et al. 2016]; 8) [Walter et al. 2002; Su 2015]; 9) [Su 2015]; 10) [Rudnicka et al. 2012]; 11-14) [Klein et al. 2007; Mares et al. 2011]; 15) [Armstrong and Mousavi 2015]; 16) [Klein et al. 2010]; 17) [Gehlbach et al. 2015]; 18) No specific study; 19) [Gorelick 2004]; 20) [Rosendorff et al. 2007; Baglietto-Vargas et al. 2016; Rani et al. 2016; Saedi et al. 2016; Vicente Miranda et al. 2016]; 21) [Rosendorff et al. 2007; Wanamaker et al. 2015]; 22) [Qiu et al. 2005; Rosendorff et al. 2007; Michel 2016]; 23) [Rosendorff et al. 2007; Durazzo et al 2014]; 24) [Campdelacreu 2014; Ilomaki et al. 2015]; 25) [Rosendorff et al. 2007; Campdelacreu 2014]; 26) [Vogel et al. 2006; Ellul et al. 2007; Wanamaker et al. 2015]; 27) [Vogel et al. 2006; Ellul et al. 2007; Yasar et al. 2016]; 28) [de Lau and Breteler 2006; Pringsheim et al. 2014]; 29) [Hu et al. 2007; Tomlinson and Gardiner 2008; Zhang and Tian 2014; Vicente Miranda et al. 2016]; 30) [Abbott et al. 2002; Hu et al. 2006; Zhang and Tian 2014]; 31) [Zhang and Tian 2014]; 32) [Li et al 2016]; 33) [Ishihara and Brayne 2005]; 34) [Eriksson et al. 2013]; 35) [Gao et al. 2012; Friedman et al. 2013; Undela et al. 2013; Sheng et al. 2016]; 36) [Lopez-Real et al. 2005; Sonsalla et al. 2013].

2) Diseases caused by unhealthy lifestyles

Unhealthy lifestyles (e.g., alcohol abuse, cigarette smoking and alimentary habits that cause hypertension, diabetes mellitus, and obesity), definable overall as "risk factors", appear to increase cell replication necessities and so accelerate aging. Conversely, healthy lifestyles and the use of drugs with organ protection qualities ("protective drugs"), definable overall as "protective factors", appear to reduce cell replication necessities and to counter the aging accelerating effects of the "risk factors" [Libertini 2009b]. As a specific example, we have previously highlighted that there is an inverse relationship between the number of EPCs and age and/or "cardiovascular risk factors" (i.e., factors defined previously as risk factors), while EPC number is increased by statins and other

protective drugs [Hill et al. 2003]. These relationships are observable for many other aging manifestations. In Table 2, for brevity, they are considered in comparison with endothelial dysfunction only for age-related macular degeneration (AMD), Alzheimer's disease (AD), and Parkinson's disease (PD).



Figures 7 - Traditional interpretation of the diseases that afflict the elderly. The concepts that underlie the present scheme are obtainable in any traditional textbook of geriatric medicine (e.g., [Fillit et al. 2010]).

- Differences between non-programmed and programmed aging paradigms in the definition of aging problems

For the "non-programmed aging" paradigm, an elderly individual suffers, to varying degrees, from many different diseases, which are non-exhaustively listed in short in Figures 5 and 6. They are caused by various inevitable harmful factors, although some harmful factors may coincide for different diseases. These illnesses are not a unique entity and therefore the term "aging", commonly used to define them together, must be considered as a lexical convenience that does not mean a scientific acceptance of their unit. The manifestations of each of these diseases may be anticipated and aggravated by further damaging factors ("risk factors") to varying degrees, while the prevention of these risk factors and special medicines ("protective drugs") may prevent, restrict or neutralize such additional damage. If we exclude any aggravating factor, these distinct diseases may be cured

and counteracted only partially and with illusory and temporary effects, because they are caused by factors that are intrinsic to organismal physiology, and inevitable and inexorable in their actions. Any cure can only try to limit the damage suffered by cells and tissues, or, at a later stage, to limit or compensate for the damage that has occurred. This conception is summarized in Figure 7.

As an example referring to a single disease, Figure 8 illustrates the current interpretation, prevention and care of Alzheimer's disease (AD).

For this illness, regarding "medical actions B" (see Figure 8), pharmaceutical societies have failed to cure or stop the clinical manifestations of AD by the elimination of β-amyloid and tau protein. In particular, drugs or vaccines against the formation and accumulation of β-amyloid have been disappointing [Abbott 2008]. A study showed that a vaccine could eliminate β-amyloid plaques and/or avoid their formation, without any positive clinical effects: "Seven of the eight immunised patients who underwent post-mortem assessment, including those with virtually complete plaque removal, had severe end stage dementia before death ... Although immunisation with Abeta(42) resulted in clearance of amyloid plaques in patients with Alzheimer's disease, this clearance did not prevent progressive neurodegeneration" [Holmes et al. 2008]. Another study has shown that aducanumab, a human monoclonal antibody, was effective in eliminating β-amyloid plaques, though the clinical results were uncertain [Sevigny et al. 2016].

Regarding "medical actions C", (i) the neurological symptoms of AD are treated by acetylcholinesterase inhibitors (e.g., donezepil, galantamine, rivastigmine, tacrine), N-Methyl-D-aspartate receptor antagonist (e.g., memantine), and other drugs [Waite 2015; Mendiola-Precoma et al. 2016], but "Current therapies for Alzheimer's disease do not modify the course of disease and are not universally beneficial." [Waite 2015]; (ii) The treatment of the cognitive alterations of AD is disappointing and, moreover, there is an increased long-term risk of mortality in relation to the use of antipsychotic drugs [Ballard et al. 2009].

In short, "The only drugs available for Alzheimer's patients aim to treat symptoms ... They are marginally effective at best." [Abbott 2008].

In any case, for the non-programmed aging paradigm, both the countless diseases that altogether constitute aging and the precocious and/or aggravated forms of the same diseases that are caused by unhealthy lifestyles or by genetic alterations, are always considered as individual diseases, and so their treatment is always considered a cure.

For the programmed aging paradigm, the interpretation of the same problems is quite different.

Aging is interpreted as a unique physiological phenomenon that manifests itself in various ways, depending on (i) the tissue or organ concerned; (ii) the previous events of the life; and, (iii) partially, random factors (see the triggering of cell senescence). Aging is a physiological phenomenon and, if "disease" is defined as an alteration of the normal condition, the manifestations of aging cannot be considered diseases nor can they be "cured": their treatment would only be the modification of a physiological phenomenon. On the contrary, if we define "disease" differently, namely by considering "disease" also as something that is physiological but causes suffering and/or death, aging can be considered a disease and becomes the object of treatment. However, this different definition implies an evaluation that is ethical, or more generally non-scientific, because by this definition a possible treatment that lengthens the life beyond the physiological limits would be considered a medical treatment and not a modification of the human nature.

The cases of genetic abnormalities or unhealthy lifestyles that aggravate and/or accelerate aging, or one or some of its manifestations, are different. For example, in a centenarian, some physiological manifestations of aging that may be considered as Alzheimer's disease, age-related macular degeneration, etc., are normal, and cannot be considered diseases in its usual definition. Conversely, if analogous or worsened symptoms of the same kind occur at a less old age, because of genetic abnormalities or unhealthy lifestyles, they can be considered as diseases (Alzheimer's disease, age-related macular degeneration, etc.) in every sense and treated as such. In this case, therefore, the aforementioned ethical problem does not arise.

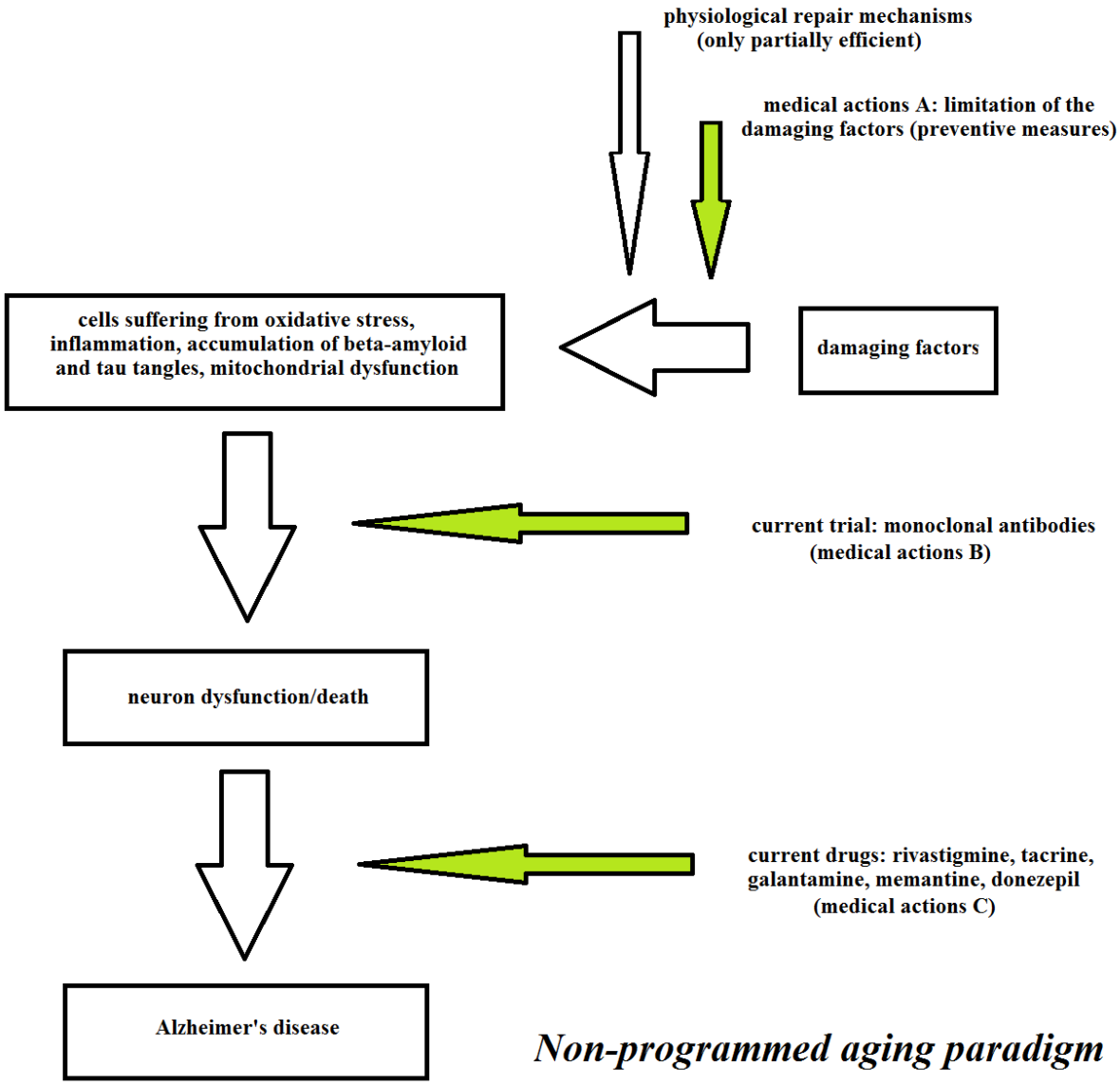


Figure 8 - Current interpretation, prevention and care of Alzheimer's disease.

This is important because, without any ethical objection, in a first phase it will be possible to search for the treatment of such diseases with methods that, when properly developed and tested, at a later stage can lead to the slowing or even to the complete control of aging, actions for which an ethical problem exists.

Apart from these considerations, for the programmed aging paradigm, the scheme for a tissue or organ in which the main cells show turnover, and its failure ("direct aging") is illustrated in Figure 9. The scheme for a tissue or organ in which the main cells do not show turnover while indispensable satellite cells show turnover, and its failure ("indirect aging") is illustrated in Figure 10. The medical actions A, B and C are the same as for the non-programmed aging paradigm, but B and C are considered palliative or supplemental to the medical actions A, which are important, and to the "medical" actions D that are the primary and decisive actions. I say "medical" actions D instead of medical actions D, because, as before said, they are not, *strictu sensu*, medical acts.

However, in the next section, since the programmed aging paradigm in its description defined as "telomere theory" has been considered the working hypothesis of this paper, only "medical" actions D will be discussed.

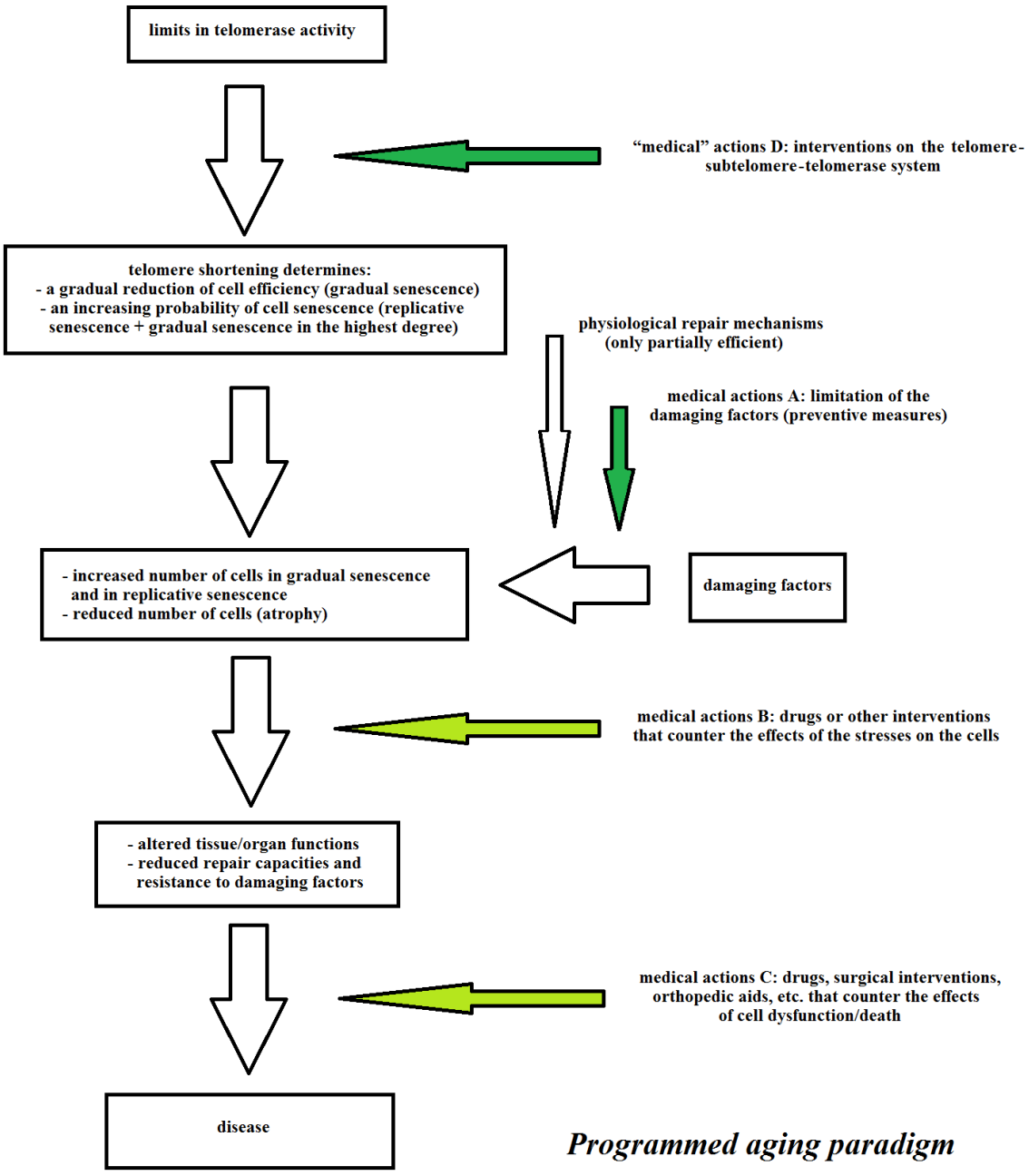


Figure 9 - Telomere theory: a scheme for "direct aging". Medical actions A are important and "medical" actions D are essential, while B and C are palliative or supplemental.

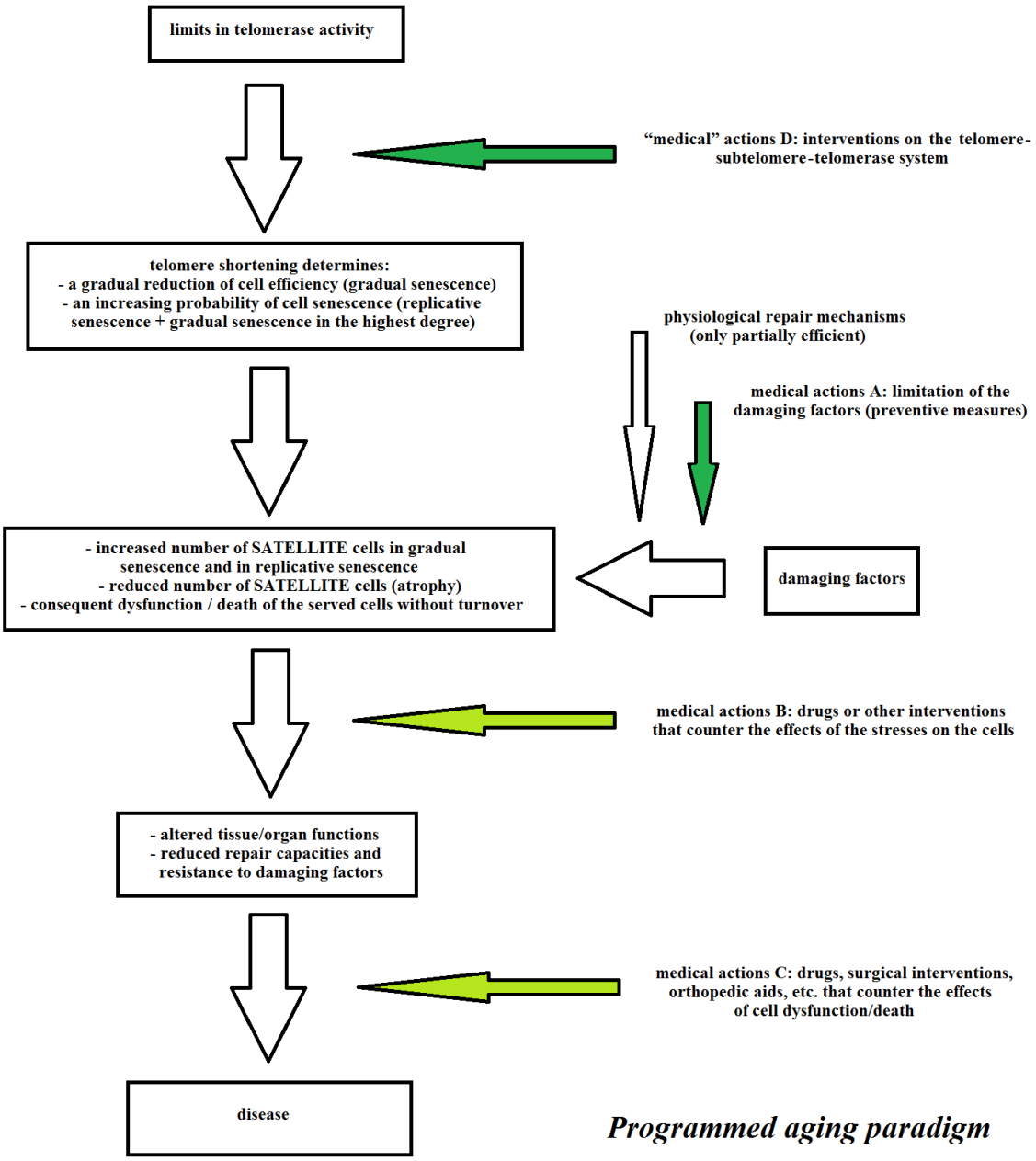


Figure 10 - Telomere theory: a scheme for "indirect aging". Medical actions A are important and "medical" actions D are essential, while B and C are palliative or supplemental.

Possible treatment for aging

Three main general approaches are here considered.

1) Telomerase activation by drugs

It is possible that opportune drugs might stimulate or restore telomerase activity, so that telomeres could slacken their shortening or even be restored to their initial lengths [Fossel 2015]. Astragalosides have shown a limited capacity in reactivating telomerase [Harley et al. 2011; Harley et al. 2013], but are quite expensive and with limited efficacy [Fossel 2015].

A more general objection to this approach is that the existence of drugs that, in vivo, are free from harmful effects and effective in restoration of telomerase activity is not assured. The research in this direction would also require large investments, and take time without leading to any success.

Other approaches with a greater chance of success in a reasonable time should therefore be preferred.

2) Telomerase activation by genetic methods

As shown in an experiment carried out in mice with positive outcomes, telomerase may be easily activated by telomerase reverse transcriptase with the use of an adenovirus as vector [Bernardes de Jesus et al. 2012]. The adenovirus did not integrate into the DNA of the cell and so there was a loss of telomerase expression in highly proliferating cells. This avoided any risk of cancerous proliferation: "we have recently shown by using a gene therapy strategy with non-integrative adeno associated virus (AAV), that re-activation of telomerase in adult or old mice results in delayed aging and significant lifespan extension in the absence of increased cancer susceptibility ... A single telomerase (TERT) treatment of WT mice with these vectors was sufficient to rescue the age-dependent decline and to delay normal mouse physiological aging ... In this experimental setting, median lifespan was extended by up to 24% in 1-year-old mice, and by 13% in animals of 2 years of age." [Bernardes de Jesus et al. 2012]

This type of technique (telomerase activation) could be applied in successive experimental phases:

a) treatment of elderly subjects without reproductive capacity and suffering from severe and/or disabling diseases for which there is no effective cure (e.g., Alzheimer's disease, Parkinson's disease, age-related macular degeneration). In this case, there is no ethical problem, as discussed above, and its successful outcome would open the way for subsequent phases.

b) Treatment of elderly subjects without reproductive capacity, and suffering from less disabling diseases or from the aforesaid diseases in a more precocious phase.

c) Treatment of healthy elderly subjects, also with reproductive capacity, i.e., simply to rejuvenate them. In this case the ethical problem exists and needs a precise discussion and a decision.

The theoretical limit of this approach is that, even supposing periodical treatments, the progressive loss of primary stem cells would not be cancelled by telomerase reactivation (the possible cause of the difference of outcomes in one-year and two-year-old mice in the above cited work [Bernardes de Jesus and Blasco 2012]). Therefore, aging would continue with a slackened pace that would be inversely related to the intensity and frequency of telomerase reactivation treatments.

3) Telomerase activation by genetic methods associated with modifications of subtelomeres and telomeres

A different method, which would overcome this theoretical limit and amplify the power of telomerase activation, has already been proposed elsewhere [Libertini and Ferrara 2016a], so it will only be described here in brief. It is necessary to refer to the original work for details and for the strong ethical issues that the method implies, as it proposes genetic changes in germ line cells, i.e., before the period that has been defined as the "reset phase":

A) elongation of the telomere by adding further TTAGGG sequences;

B) insertion of a neutral nucleotide sequence (i.e., without any effect on other parts of the genome), between the subtelomere and the telomere-subtelomere junction ("J").

The genetic modification obtained by A would allow a greater number of duplications before the telomere reaches a critical size that would not allow further shortening.

The genetic modification obtained by B would allow a greater number of duplications (i.e., a greater relative telomere shortening) before the telomere hood begins to repress the regulatory sequences of the subtelomere.

Actions A and B, combined with periodic telomerase activation treatment, would allow indefinite extension of life, with a minimal or absent progressive loss of primary stem cells, as long as the repressive action of the telomere hood is blocked by telomere lengthening before the regulatory sequences of the subtelomere begin to be repressed.

These actions are illustrated in Figure 11.

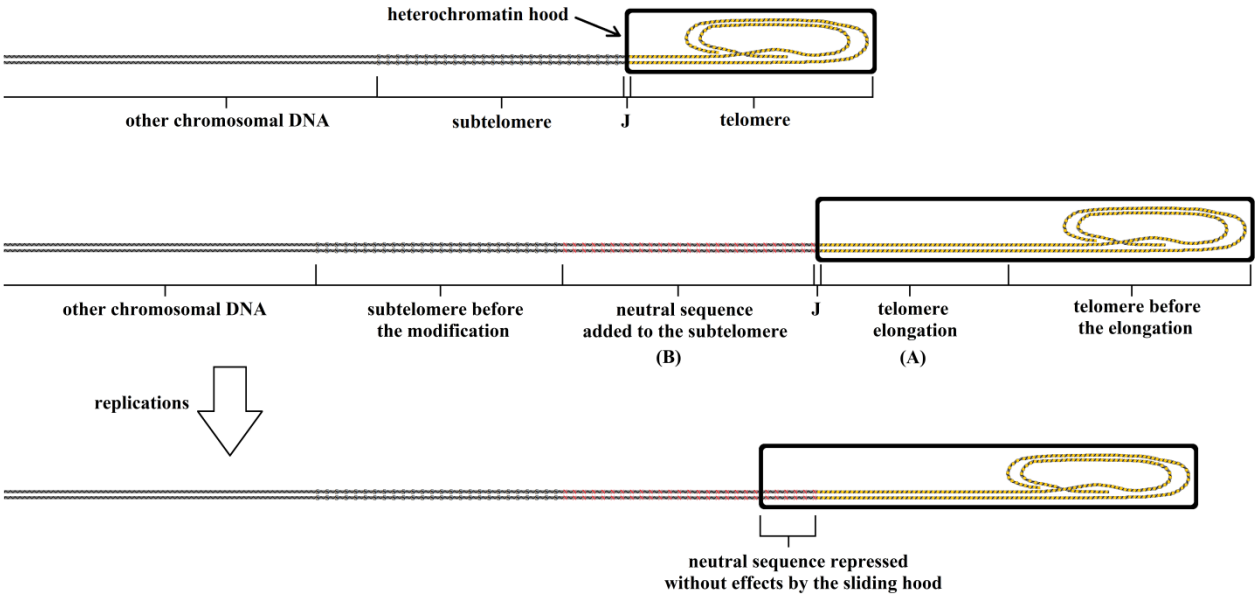


Figure 11 - The telomere is elongated before the formation of the telomere hood in the "reset phase". The greater length of the telomere allows a greater number of duplications before the telomere reaches a critical size that does not allow further shortening. The addition of a neutral sequence between the subtelomere and J allows a greater number of duplications before the subtelomere begins to be repressed by the telomere hood.

Two points must be highlighted:

1) The methods proposed here require great reliability and precision, ideally to perfection, for the techniques used in DNA modifications. The best technique that is currently available, also in economic terms, is the CRISPR-CAS9 (clustered regularly interspaced short palindromic repeat–CRISPR-associated nuclease 9) technique [Harrison et al. 2014; Zhang et al. 2014], with noteworthy recent improvements [Kleinstiver et al. 2016; Slaymaker et al. 2016], which have overcome previously known imperfections of the method [O'Geen et al. 2015; Chandrasegaran and Carroll 2016].

2) As expounded in a preceding section, the mother lineage cells of yeast (*S. cerevisiae*) always have the same telomere length with each replication and there is no sliding of the telomere hood over the subtelomere, while there is ERC accumulation over the subtelomere. This progressively represses the subtelomere, so altering cell functions and limiting the number of duplications to a maximum of 25-35 times [Jazwinski 1993]. Using yeast as an animal model, it could be easy to insert a neutral sequence between the subtelomere and J and verify if, as expected, this determines no progressive alteration in cell functions and an increase in the maximum number of duplications.

Conclusion

Modern geriatric medicine is unable to act on the primary causes of aging and seeks only to treat the symptoms and complications. It can prolong life, but elderly persons are more or less severely disabled in the extra years obtained by its action. For geriatrics, repeating the drastic judgment of Abbott for the treatment of AD, before quoted [Abbott 2008], it is possible to say that the only drugs and medical interventions available for senescent patients aim to treat symptoms and are only marginally effective at best.

This indicates that a radical change of geriatrics is necessary in order to effectively counter the burden of suffering caused by aging.

Regarding the possibility of such a change, it appears real and feasible to use the programmed aging thesis as a working hypothesis in its interpretation defined as "telomere theory". However, it is indispensable to abandon the old notion that aging is a maladaptive characteristic that is only partially corrigible [De Grey 2005] and to embrace the new notion that aging is "a specific biological function" [Skulachev 1997], which allows a more optimistic approach to possible effective treatment [Libertini 2009a, 2009b].

Of course all this will not convince many, and perhaps a large majority will consider the concepts expounded here as inconsiderate and utopian. Perhaps, the current situation is a "cusp of history", as happily defined by Fossel: "Great ideas pivot on the Janus'd cusp of history: looking forward they are obviously foolish, looking backward they appear foolishly obvious. We are doubly blind" [Fossel 2004]. However, if these theories are not unreasonable, they would constitute a scientific revolution or a paradigm shift in the sense defined by Kuhn [Kuhn 1962].

Furthermore, if the paradigm shift allows the choice to effectively apply techniques to increase the lifespan or make it indefinite, this will mean a huge change in our civilization [Libertini 2009a]. Thus, it would not be an exaggeration to consider this as a general great revolution.

As in every revolution, even in the best possible, not everything will change for the better. The greatest danger is indicated in ancient Greek wisdom. The gods were conceived by the ancient Greeks as immortal beings and the worst sin was to be guilty of ὕβρις, which is the search for being like the gods with impious unforgivable arrogance and pride. Perhaps the wisest choice would be to not change anything, but unknown lands are irresistible to humans. However, the choice among the possible options is not in the field of science and is not within the topic of this work.

Chapter 12

Libertini G (2017b) Programmed Aging Paradigm and Aging of Perennial Neurons. In: Ahmad SI (ed) *Aging. Exploring a Complex Phenomenon*. CRC Press, London & New York, pp. 91-115.

Programmed aging paradigm and aging of perennial neurons

Giacinto Libertini

Abstract

The frequency of some diseases including Alzheimer's disease, Parkinson's disease, and age-related macular degeneration that primarily afflict the elderly is increasing as life expectancy increases. There is no treatment that is able to stop or revert these diseases, which are often severely disabling and even fatal, but only symptomatic or palliative cures. This provides the impetus for a different interpretation of such diseases that might produce effective treatments.

Aging is explained in two completely opposite ways that, for the full diversity of the mechanisms proposed and their implications, deserve the definition of paradigms. According to the first paradigm, aging results from the accumulation of heterogeneous damages. According to this interpretation, the aforementioned diseases are the consequence of the progressive inevitable impairment of physiological mechanisms, and we may only attempt to counteract the cumulative damage. On the contrary, according to the second paradigm, aging is a physiological phenomenon, that is genetically programmed and regulated, and with the exception of particular cases, the above-mentioned diseases are actually the expression of the physiology of this phenomenon.

For the telomere theory, which is part of the programmed aging paradigm, telomere shortening determines (i) the progressive impairment of cell functions (gradual cell senescence) and (ii) the increasing frequency of the activation of a cellular program, cell senescence, which causes replicative senescence and gradual senescence to the maximum degree. Since cells, and consequently tissues and organs, show a continuous physiological turnover, the progressive increase of stem cells in replicative senescence causes the gradual decline of cell turnover, and this, coupled with the increased number of cells compromised by gradual senescence, determines the progressive decline of the functions of tissues, organs and the organism in general.

For neuronal cell types that are perennial (i.e., show no cell turnover), the decline of their functions appears justified by the progressive failure of the specialized gliocytes that are subject to cell turnover and are essential for neuronal vitality. Consequently, the rational way to combat the above-mentioned diseases is not to contrast their manifestations but the reactivation of satellite cell turnover.

Introduction

- The problem

Alzheimer's disease (AD), Parkinson's disease (PD), and Age-related Macular Degeneration (AMD) are degenerative diseases of the nervous system that manifest in the accumulation of specific substances (β-amyloid and tau protein for AD, α-synuclein for PD, A2E for AMD) and a progressive impairment of psychomotor (AD, PD) or visual (AMD) functions [Weiner and Lipton 2009; Holz et al. 2013; Lim 2013; Pahwa and Lyons 2013; Husain and Schott 2016].

These diseases primarily affect elderly people and, as a result of the increase in life expectancy, there is a proportionate increase in the percentage and number of affected people. In the USA, approximately 96% of the 5.3 million Americans suffering from AD are over 65 years of age [Alzheimer's Association 2015]. The worldwide, absolute number of people with dementia and over 65 years old was estimated or predicted at 24.3 million in 2001, 42.3 in 2020 and 81.1 in 2040

[Rizzi et al. 2014]. AD frequency increases progressively with age so that the frequency is 1.5% at the age of 65, which becomes 30% at 80 [Gorelick 2004], leaving a very high probability that a centenarian will suffer from AD.

Excluding AD, PD is the most frequent of the neurodegenerative disorders [de Lau and Breteler 2006; Yao et al. 2013]. In industrialized countries, its frequency is approximately 0.3% of the whole population, 1% of the population older than 60, and 4% of the individuals older than 80 [de Lau and Breteler 2006]. The mean age of onset is around 60; however, PD manifestations begin before 50 years of age in 5-10% of cases [Samii et al. 2004]. "Meta-analysis of the worldwide data showed a rising prevalence of PD with age (all per 100,000): 41 in 40 to 49 years; 107 in 50 to 59 years; 173 in 55 to 64 years; 428 in 60 to 69 years; 425 in 65 to 74 years; 1087 in 70 to 79 years; and 1903 in older than age 80." [Pringsheim et al. 2014]

The estimated frequency of AMD is 1.4% at 70 years of age, 5.6% at age 80 and 20% at age 90 [Rudnicka et al. 2012], which implies that a centenarian is a probable AMD sufferer. The projected number of AMD patients in 2020 is 196 million, increasing to 288 million in 2040 [Wong et al. 2014].

In general, there are only symptomatic or palliative cures for these illnesses that do not appear able to block their basic pathogenetic mechanisms:

1) AD is treated by cholinesterase inhibitors (donepezil, galantamine, rivastigmine), memantine, souvenaid [Waite 2015], psychotropic drugs, etc., which aim to relieve its neurological and psychiatric manifestations and by anti-amyloid or anti-tau drugs, which try to limit the accumulation of the substances that are considered to cause the disease. However, "Current therapies for Alzheimer's disease do not modify the course of disease and are not universally beneficial." [Waite 2015]

2) PD is treated by pharmacotherapy, functional stereotaxic neurosurgery, physiotherapy, etc., all symptomatic cures, but "All treatments available until 2016 are of symptomatic nature. No therapy is currently available that slows down the progression of PD or even to prevent its manifestation." [Oertel and Schulz 2016]

3) There is no approved therapy for the atrophic (dry) form of AMD, while the costly treatment with anti-vascular endothelial growth factors (anti-VEGFs) contrast effectively the rapid evolution of the neovascular (wet) form [Azad et al. 2007; Nowak 2014], but do not block or revert it.

For the growing number of people affected and for the impairments caused by these diseases, the human, social and economic costs are progressively growing and becoming unsustainable, especially for the cases of dementia. In the USA: "Total payments in 2015 for health care, long-term care and hospice services for people age 65 years with dementia are expected to be $226 billion." [Alzheimer's Association 2015]

In general, these diseases are described as without a known cause or origin, although many details of their pathogenetic mechanisms have been elucidated. AMD is defined as a "retinal disease with an unprecised etiopathogenesis" [Nowak 2014]. PD "is a chronic, progressive neurological disease ... The molecular mechanisms underlying the loss of these neurons still remain elusive." [Blesa et al. 2015] For AD: "The etiological mechanisms underlying these neuropathological changes remain unclear, but are probably caused by both environmental and genetic factors." [Reitz and Mayeux 2014]

These data indicate the gravity of the problem and the absence of an effective strategy to prevent and treat these illnesses. This serious and disastrous situation is a strong incentive to seek a completely new approach that could enable the understanding of the aetiology of these diseases in order to develop and implement effective measures of prevention and treatment. In this work, a different interpretation of these illnesses is discussed to obtain a necessary premise for the achievement of such an ambitious and seemingly unrealistic goal.

- Non-programmed and programmed aging paradigm

AD, PD, AMD and other age-related illnesses of the nervous system (presbycusis, age-related hyposmia, age-related deficits of other sensory abilities), disregarding the early cases due to "risk factors" (see below for the definition) or to genetic defects, are diseases whose frequency and severity is clearly related to age. Therefore, it appears logical that the nature of their connections with aging must be clarified for their proper understanding (and vice versa a better understanding of their origins may lead to a better understanding of aging mechanisms).

Therefore, it is an imperative preliminary to understand what aging is and the relationship between the mechanisms of aging and the mechanisms underlying these diseases.

It is necessary to specify that:

- A comprehensive discussion about aging is outside the scope of this work. Only a few main points will be outlined while the appropriate papers will be indicated for a more detailed exposition;

- The interpretation here will be reaffirmed that the nature and mechanisms of aging are different and in clear contrast with the contradictory ideas generally accepted as a coherent (!) explanation for aging. However, such a different interpretation allows a unified and consistent view of the aforesaid diseases. Moreover, it will clarify why the current therapeutic approaches fail and will allow the proposition of a rationale for the development of effective treatment.

A necessary premise is to state a definition of aging that is descriptive and not vitiated by a preconceived explanation of this phenomenon. Therefore, aging will be defined precisely, in a neutral descriptive way, as "increasing mortality [= decreasing fitness] with increasing chronological age in populations in the wild" [Libertini 1988]. This phenomenon, which has been documented for 175 different animal species [Nussey et al. 2013], may be also more concisely defined as "actuarial senescence" in reference to animals studied "in the wild" [Holmes and Austad 1995].

Aging is explained in two general ways, which are completely different and mutually incompatible and so deserve the definition of opposite "paradigms" [Libertini 2015a] according to the meaning that was proposed by Kuhn [Kuhn 1962].

The first paradigm, which could be defined as the "non-programmed aging paradigm", or briefly "old paradigm", explains aging as the effect of many degenerative phenomena, insufficiently contrasted by natural selection, which inexorably and inevitably cause the accumulation of random damages over time, as proposed by mutation accumulation [Medawar 1952; Hamilton 1966], antagonistic pleiotropy [Williams 1957; Rose 1991], disposable soma [Kirkwood 1977; Kirkwood and Holliday 1979], and other theories [Libertini 2015b]. According to this paradigm, in principle, we could slow down and fight aging manifestations by using appropriate drugs and measures without the possibility to stop or reverse them [Libertini 2015a].

The second paradigm, which could be defined as the "programmed aging paradigm", or briefly "new paradigm", interprets aging as a physiological phenomenon that:

- is evolutionarily favoured in terms of supra-individual natural selection [Libertini 2015a];

- is a particular type of phenoptosis [Skulachev 1997; Libertini 2012a], i.e., "programmed death of an individual" [Skulachev 1999a];

- is the result of specific mechanisms that are genetically determined and regulated, and therefore, in principle, might be modified up to a complete control of aging [Libertini 2009a, 2009b];

- has its specific pathological forms [Libertini 2009a, 2009b, 2014a];

- has its specific phylogeny [Libertini 2015b].

A discussion about the arguments and the evidence that support or contrast each of the two paradigms has been proposed in another study where arguments and evidence appear to be against the old paradigm and support the new paradigm [Libertini 2015a].

Although the old paradigm is still the prevalent idea [Kirkwood and Melov 2011], the new paradigm will be considered a working hypothesis for the aims of this work. Accepting the interpretation of the new paradigm as a working hypothesis, will provide an easy and unifying

interpretation of AD, PD, AMD, and other diseases, which may be profitable for the goal of the formulation of appropriate therapies. On the contrary, the current concepts based on the old paradigm have led to the current situation of an insufficient understanding of these diseases and of ineffective therapies. The reader will be able to judge whether this approach is more consistent and efficacious.

- Short description of the telomere theory

The new paradigm absolutely requires the existence of specific mechanisms that determine a progressive fitness reduction. These mechanisms, which are documented by a long series of authoritative studies and can be summarized under the term "telomere theory", for the sake of brevity cannot be explained here in detail. Therefore, only a brief description will be provided here, referring to a more detailed description from additional references [Libertini 2009a, 2014a; Libertini and Ferrara 2016b].

During every replication cycle, the DNA molecule requires the telomerase enzyme in order to complete the duplication of a repetitive nucleotide sequence at its terminal regions, the telomere. In cells where telomerase is inactive, or partially active, any duplication results in telomere shortening. As the telomere is covered by a heterochromatin hood with a fixed length, the gradual telomere shortening causes the sliding of the hood on the adjacent part of the DNA molecule, the subtelomere, which so is progressively inhibited. This inhibition has two main effects:

1) the progressive alteration of the regulatory functions of the subtelomere that determines a progressive alteration of numberless cell functions, a phenomenon here defined as "gradual cell senescence" or just as "gradual senescence" [Fossel 2004; Libertini 2014a, 2015b].

2) an increase in the probability of activation of a particular cell program, the cell senescence, which is characterized by the blockage of cell replicative capacities and by the alterations of gradual senescence to its maximum degree [Ben-Porath and Weinberg 2005]. The activation of this program is a random function with a probability that grows in every cell with the shortening of the telomere [Blackburn 2000] and so is likely related to the progressive inhibition of the subtelomere [Libertini and Ferrara 2016b] (Fig. 1).

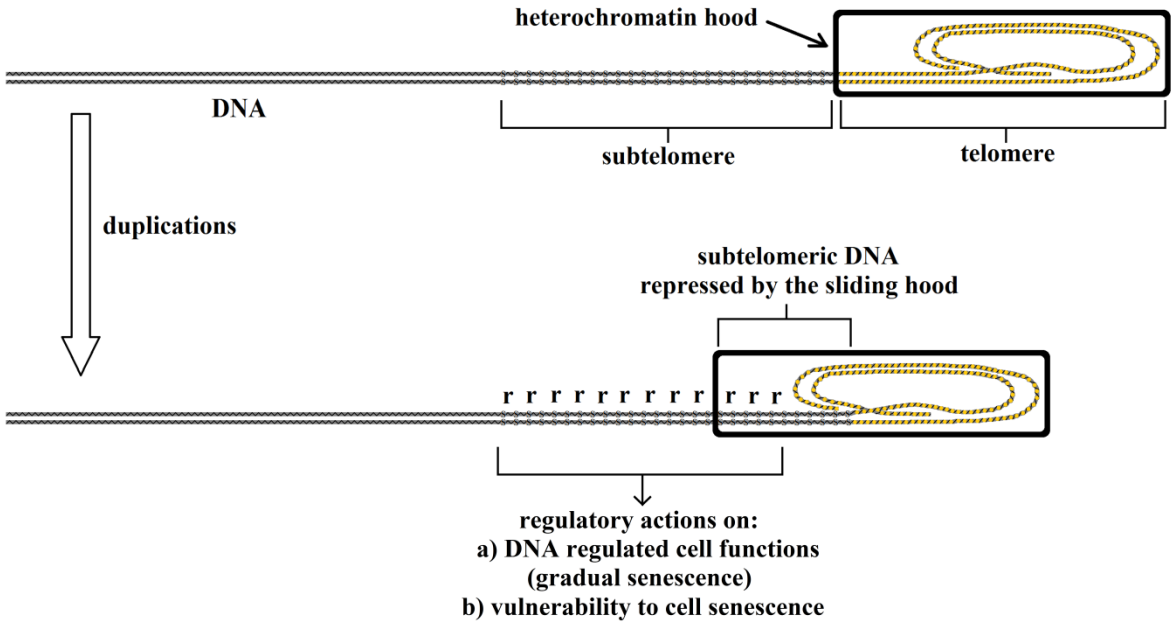


Figure 1 – A scheme of the progressive subtelomere inhibition caused by the sliding of the telomeric hood (modified and redrawn from Figure 4 in [Libertini 2015b]).

It has to be highlighted that, in plain contrast with the tenets of the old paradigm, the aforesaid cell alterations are not caused by irreversible degenerative phenomena, as it has been well demonstrated by experiments in which telomerase reactivation determined the regression of all the aforesaid phenomena [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998].

The majority of cell types in vertebrates undergo a continuous cell turnover. In a young individual, there is a perfect balance between the effects of various types of programmed cell death (apoptosis, keratinization of hair or epidermal cells, detachment of intestine cells, osteocytes phagocytized by osteoclasts, etc.) and the duplication of the opportune stem cells. As telomeres shorten, there is an increase in the number of cells in cell senescence state, resulting in a gradual decrease in cell renewal capacity. This deficit, combined with the growing number of cells in various degrees of gradual senescence, leads to what has been defined as "atrophic syndrome" [Libertini 2009a], characterized by:

"1) reduced mean cell duplication capacity and slackened cell turnover;

2) reduced number of cells (atrophy);

3) substitution of missing specific cells with nonspecific cells;

4) hypertrophy of the remaining specific cells;

5) altered functions of cells with shortened telomeres or definitively in noncycling state;

6) alterations of the surrounding milieu and of the cells depending from the functionality of the senescent or missing cells;

7) vulnerability to cancer because of dysfunctional telomere-induced instability ..." [Libertini 2014a]

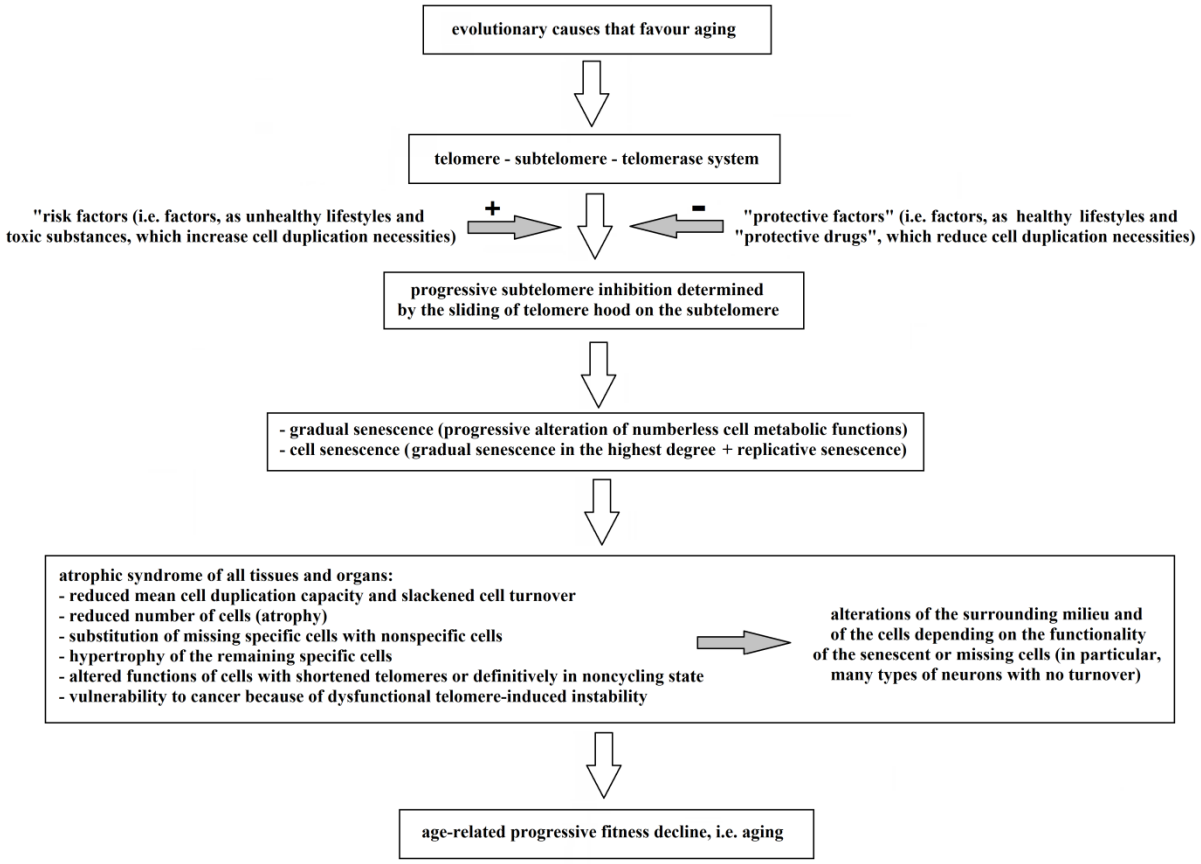


Figure 2 – A scheme of the aging process. The discussion of the evolutionary causes is omitted in this work.

Factors, such as unhealthy lifestyles (which cause diabetes mellitus, hypertension, obesity, etc.), toxic substances (e.g., cigarette smoke, alcohol abuse), which may be defined on the whole as "risk

factors", increase cell duplication necessities and so accelerates the aging process. On the contrary, factors, such as healthy lifestyles, drugs that have organ protection qualities ("protective drugs"), which may be defined on the whole as "protective factors" reduce cell duplication capacities and neutralize the negative effects of the "risk factors" [Libertini 2009b] (Fig. 2).

These mechanisms appear readily applicable to all cell types that are subject to cell turnover, and this could easily explain a large part of the aging phenomenon [Libertini 2009b]. This, however, would not seem to relate to cells not subject to turnover, such as the majority of the neurons. However, a careful reading of the synthetic description of the atrophic syndrome shows (see the point 6 in the description of the atrophic syndrome) that if a perennial cell is dependent for its functionality on other cells subject to turnover, the numerical and functional decline of these "satellite" cells may well explain the concomitant numerical and functional decline of the perennial cells [Libertini and Ferrara 2016b].

This discussion is deepened in the present analysis.

Aging of neurons

- Types of neurons

There are many different neuronal cell types. For the purpose of this work, a preliminary distinction between two opposite extremes is necessary:

1) Certain types of specialized neurosensory neurons, such as olfactory receptor cells (ORCs), receive a signal from the external world by a single dendrite and are connected by a single axon to other neurons that process the signal [Bermingham-McDonogh and Reh 2011]. For these neurons, the connections are quite simple and stereotyped and, in principle, the turnover of these cells should not entail any particular difficulty.

2) On the contrary, each neuron in the central nervous system (CNS) is connected to a large number of other neurons. According to an estimate, in the human brain, there are 10^{11} neurons connected by 10^{14} synapses [Williams and Herrup 1988], i.e., there is an estimated average of 1,000 synapses for each neuron. Given the large number of synapses and the expected variability of the connections between neurons, the possible replacement of a neuron with a new neuron should also restore any previous connection through an unlikely mechanism that would be exceedingly complex. If brain functions are dependent on the net of synapses among neurons that have been established over time, and the connections are different for each individual neuron and, on the whole, are specific for each individual, the failure to restore the exact synapses would create unbearable harm caused by any turnover of these neurons. This would justify the absence of cell turnover for CNS neurons, with some exceptions [Horner and Gage 2000; Zhao et al. 2008], e.g. "for certain forms of brain function involving the olfactory bulb and the hippocampus, which is important for some forms of learning and memory" [Zhao et al. 2008]. This absence is likely for functions that require new connections and for which new neurons are not a problem but a necessity.

Different strategies are necessary for these two opposite neuronal cell types. For the first type, as will be expounded in more detail for the ORCs, the strategy of cell turnover can be implemented as well as it is implemented for non-neuronal cells.

For the second type of neuron, the practical impossibility of replacing the neurons without damaging brain functions and at the same time the need to ensure their cell functionality even after many years, are solved, as we will see in the following sections, by a different strategy that is very well documented for a neuronal cell type, the retinal photoreceptor cells, and is quite likely for other types of CNS neurons.

For the following discussion, endothelial cells will be used as an important example of non-neuronal cells that undergo turnover. Risk factors for cardiovascular diseases, such as age, diabetes, smoking, body mass index (i.e., overweight and obesity), and hypertension [Wilson et al. 1987], are

associated with a reduced number of endothelial progenitor cells [Hill et al. 2003], likely caused by a quicker turnover of endothelial cells: "continuous endothelial damage or dysfunction leads to an eventual depletion or exhaustion of a presumed finite supply of endothelial progenitor cells ... continuous risk-factor-induced injury may lead to eventual depletion of circulating endothelial cells" [Hill et al. 2003]. This would be analogous to what happens in patients with muscular dystrophy, where there is an exhaustion of skeletal muscle stem cells [Webster and Blau 1990; Decary et al. 2000; Seale et al. 2001] as well as in a number of age-related conditions [Tyner et al. 2002; Geiger and Van Zant 2002].

ACE inhibitors, AT1 blockers (sartans) and statins, which may be considered protective drugs for cardiovascular diseases, improve endothelial function [Su 2015]. Statin therapy accelerates reendothelialization through endothelial progenitor cells [Walter et al. 2002], and this could explain the effects of statins, and likely of other "protective drugs", on the prevention and treatment of cardiovascular diseases.

- **Olfactory receptor cells**

ORCs are specialized neurons, which are present in the upper part of the nasal cavity and allow the perception of smell. They "have a single dendrite that extends to the apical surface of the epithelium and ends in a terminal knob, which has many small cilia extending into the mucosa. A single axon projects through the basal side of the epithelium through the lamina cribrosa to terminate in the olfactory bulb. Each of the receptor neurons expresses one of a family of over 1000 olfactory receptor proteins ... The neurons are surrounded by glial-like cells, called sustentacular cells. Other cells in the epithelium contribute to the continual production of the new receptor neurons ..." [Bermingham-McDonogh and Reh 2011] (Fig. 3).

The continuous turnover of the ORCs in normal individuals is well documented [Maier et al. 2014].

"The ongoing genesis of olfactory receptor cells is common to all vertebrates (see Graziadei and Monti Graziadei, 1978, for review) and the rate of production is quite high. The production of new olfactory receptor cells is critical to the maintenance of this system, as the olfactory receptor cells only last a few months. The rate of production of new olfactory receptor cells is balanced by their loss so that a relatively stable population of these receptors is maintained." [Bermingham-McDonogh and Reh 2011]

Analyses of the healthy olfactory epithelium show that the turnover of ORCs is enabled by some slow cycling stem cells and by transit-amplifying progenitor cells (globose basal cells and horizontal basal cells, respectively) [Caggiano et al. 1994; Huard et al. 1998; Chen et al. 2004; Leung et al. 2007; Iwai et al 2008]. This two-stage modality of cell reproduction to allow cell turnover is similar to that of the epidermis and other cell types [Watt et al. 2006].

Due to their position, ORCs are highly exposed to damage by external factors but have simple connections as each cell has a single dendrite, with many small ramifications in the mucosa, and a single axon. Therefore, the turnover of the ORCs is both necessary and simple. If this turnover follows the same patterns as other cell types, one can predict an age-related slowing of cell substitution with the consequent impairment of olfactory function. Other factors, perfectly compatible with the aforesaid mechanism, which could contribute to this progressive impairment, are: (i) an increase of the proportion of ORCs with various degrees of gradual senescence; (ii) age-related slowing of cell turnover of the gliocytes that are satellites of ORCs and of olfactory bulb neurons; and (iii) age-related decay of other CNS areas that contribute to olfactory function.

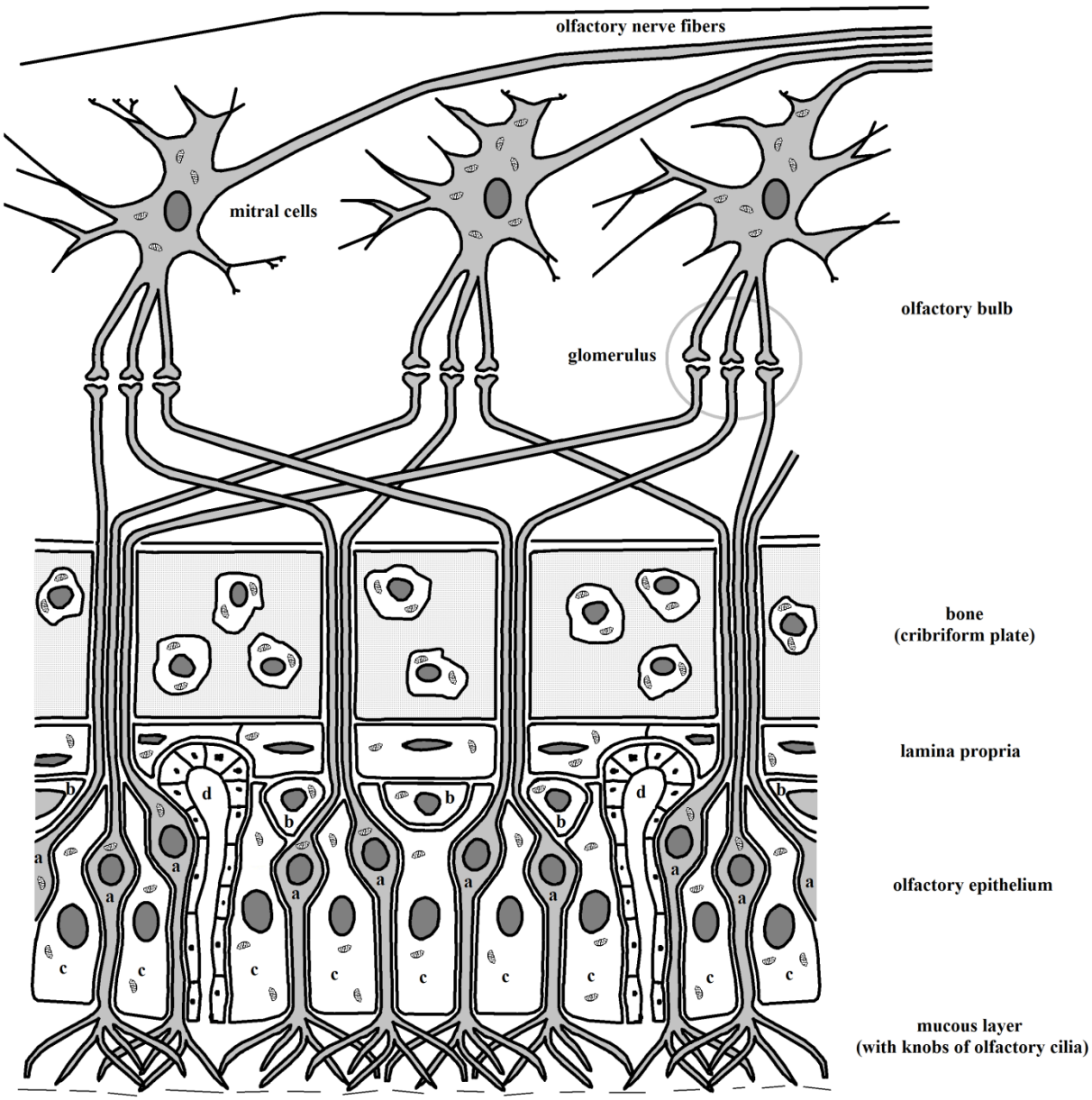


Figure 3 - Scheme of peripheral olfactory system. In the olfactory epithelium: a = olfactory receptor cells, b = regenerative basal cells; c = sustentacular or supporting cells (differentiated gliocytes); d = olfactory (Bowman's) glands.

As a matter of fact, the impairment of olfactory function is age-related [Schubert et al. 2012; Doty and Kamath 2014; Gouveri et al. 2014], and approximately 50% of 65-80 year-old individuals suffer from clear olfactory dysfunction [Doty et al. 1984; Duffy et al. 1995; Murphy et al. 2002].

If AD, PD, AMD and other diseases of the CNS are caused (as discussed below) by the failure in cell turnover of the gliocytes that are essential neuronal satellite cells, a clear relation between olfactory dysfunction and these diseases is expected. In fact: (i) olfactory dysfunction has been estimated to be as high as 90% of early-stage PD cases [Doty 2012] and 100% in AD [Duff et al. 2002]; (ii) hyposmia is a common symptom in PD dementia, in AD and in other forms of dementias [Barresi et al. 2012]; (iii) hyposmia has been reported as an early sign of PD and a precocious and constant characteristic of AD and of dementia with Lewy bodies (DLB) [Factor and Weiner 2008]; and (iv) there is a correlation between olfactory dysfunction and AMD [Kar et al. 2015].

There is a possible association between olfactory dysfunction and unhealthy lifestyles: (i) “Olfactory dysfunction is a known complication of diabetes” [Mehdizadeh et al. 2015]; (ii) there is

an association between olfactory dysfunction and type 2 diabetes or hypertension [Gouveri et al. 2014]; (iii) obesity is associated with the risk of olfactory dysfunction [Richardson et al. 2004; Patel et al. 2015]; (iv) in the rat, ethanol and tobacco smoke damage olfactory function, and this "could explain the decreased olfactory ability seen in patients who use these products" [Vent et al. 2003]; (v) "alcoholism appears to be associated with a variety of disturbances in olfactory processing" [Rupp et al. 2004]; and (vi) prevention and treatment of such diseases indicated as risk factors should also be effective for the olfactory dysfunctions. It was not possible to find studies on the specific use of drugs for such purposes. However, in a mouse model, anosmia caused by a toxic substance was successfully treated by a statin [Kim et al. 2012].

Given that ORC turnover is essential and simple for the aforesaid reasons, the turnover of other sensory neurons and consequently an age-related functional decline may also be expected. For example, in humans: (i) taste buds turnover rapidly, with an average lifespan of 8-12 days, and an age-related decline of function is documented [Feng et al. 2014]; (ii) "Reported changes include reduced total number of taste buds, reduced taste bud density in the epithelium, and reduced number of taste cells per taste bud." [Feng et al. 2014]; (iii) there is a decline of gustatory capacities in patients with Alzheimer disease [Aliani et al. 2013]. A negative correlation between age and the number of Meissner's corpuscles per mm^2 was observed [Iwasaki et al. 2003] and "there is an approximate two-thirds reduction in numbers of Pacinian and Meissner's corpuscles with age" [Griffiths 1998].

- **Retinal photoreceptors**

Retinal photoreceptors (cones and rods) are very specialized nervous cells and have complex connections with other neurons of the retina, where the initial processing of data perceived by the eye occurs. Therefore, photoreceptors belong to the aforesaid second type of neurons (as do almost all neurons) and have no turnover. However, photoreceptors depend on other cells with turnover, the cells of the so-called retinal pigment epithelium (RPE), which consist of specialized gliocytes that turnover. The heads of the photoreceptors are associated with RPE cells. Photoreceptors have particular membranes covered by photopsin molecules, which allow light reception but suffer from high oxidative damage, and each day about one-tenth of these membranes is phagocytized and metabolized by RPE cells. At the same time, photoreceptors synthesize an equal quantity of membrane so that cell structure and function remain stable. An RPE cell serves approximately 50 photoreceptors and, therefore, each day metabolizes the membranes of approximately five photoreceptors, showing an exceptional metabolic activity. RPE function is essential for the vitality of the photoreceptors [Fine et al. 2000; Jager et al. 2008] (Fig. 4).

The limits in the duplication capacities of RPE stem cells do not allow for an unlimited turnover of these cells. The age-related decline of RPE cell turnover determines the enlargement of the remaining RPE cells and the accumulation of A2E, a breakdown product derived from vitamin A, and of other damaging substances [Sparrow 2003]. The continued decline in turnover causes the formation of holes in the RPE, and the photoreceptors die at this point [Berger et al. 1999].

This decline is more precociously evident in the pivotal part of the retina, the macula, where photoreceptors are denser and A2E accumulation is most abundant [Sparrow 2003; Ablonczy et al. 2012]. For this reason, the trouble is defined as "age-related macular degeneration", although the entire retina is affected [Fine et al. 2000].

AMD may arise at precocious ages depending on the particular genetic defects affecting RPE cells or from the effects of toxic substances or metabolic stresses caused by unhealthy lifestyles. Disregarding these cases, the frequency of AMD increases exponentially with age [Rudnicka et al. 2012] and for its genesis and frequency, it should be considered a standard feature of aging.

Risk factors for cardiovascular diseases [Wilson et al. 1987] can have detrimental effects on RPE turnover that are analogous to those on endothelial progenitor cells [Hill et al. 2003], and this could justify their association with AMD [Klein et al. 2007] and between unhealthy lifestyles and AMD [Mares et al. 2011].

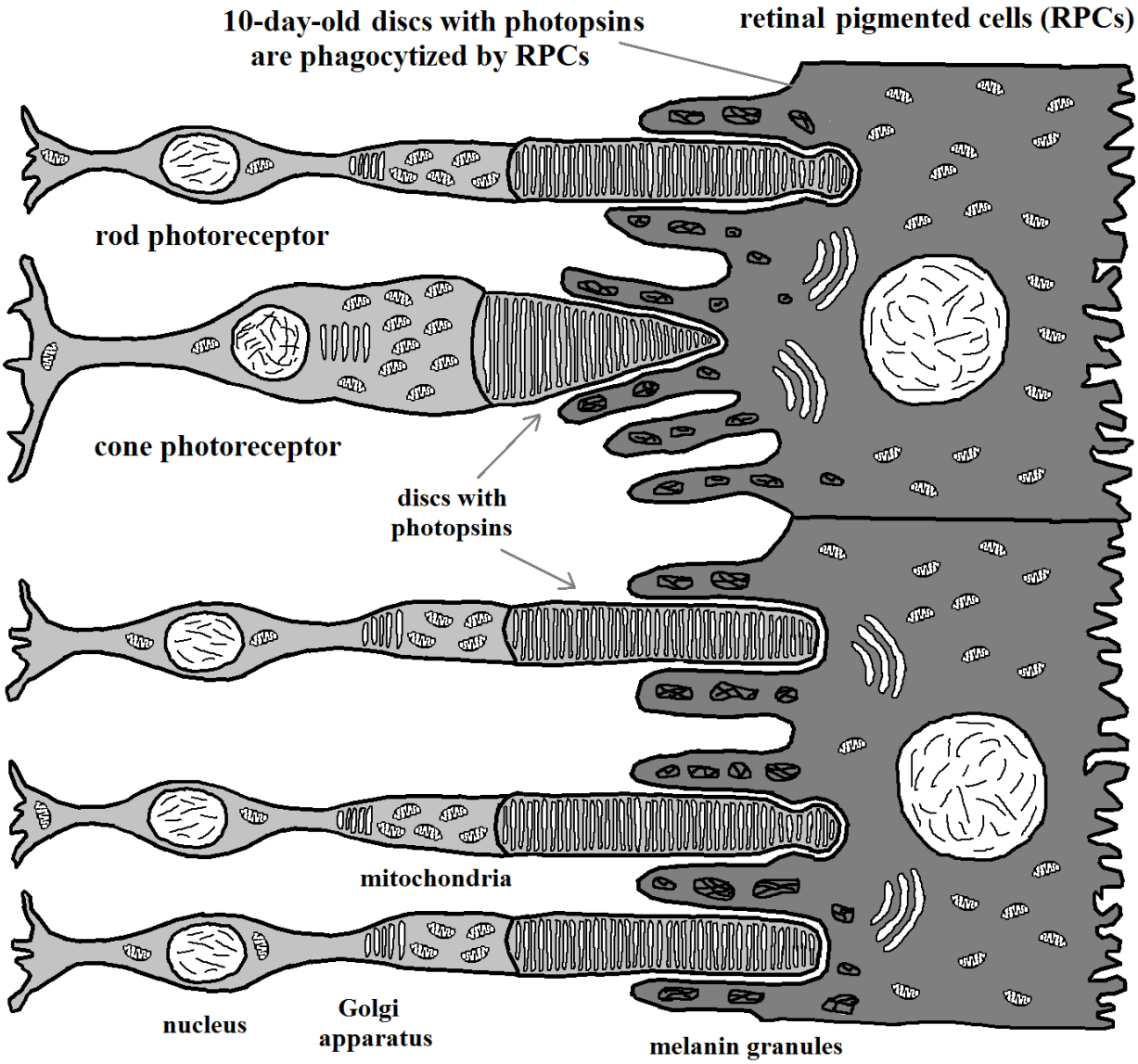


Figure 4 - A scheme of some photoreceptor cells and retinal pigmented cells.

"Smoking is the risk factor most consistently associated with AMD. Current smokers are exposed to a two to three times higher risk of AMD than non-smokers and the risk increases with intensity of smoking." [Armstrong and Mousavi 2015]

About the possible prevention or treatment of AMD by drugs, "... epidemiologic, genetic, and pathological evidence has shown AMD shares a number of risk factors with atherosclerosis, leading to the hypothesis that statins may exert protective effects in AMD. ... Evidence from currently available randomized controlled trials is insufficient to conclude that statins have a role in preventing or delaying the onset or progression of AMD." [Gehlbach et al. 2015]

As regards to alcohol, for the risk of vascular diseases: low alcohol consumption appears to have a beneficial effect [Roerecke and Rehm 2014; Gardner and Mouton 2015; de Gaetano et al. 2016]. "On the other hand, ethanol chronically consumed in large amounts acts as a toxin to the heart and vasculature." [Gardner and Mouton 2015] "Evidence consistently suggests a J-shaped relationship between alcohol consumption ... and all-cause mortality, with lower risk for moderate alcohol consumers than for abstainers or heavy drinkers." [de Gaetano et al. 2016]. For AMD: "Moderate

alcohol consumption is unlikely to increase the risk of AMD." [Armstrong and Mousavi 2015]. However, in the Beaver Dam Offspring Study, no association between a history of heavy drinking and early AMD was observed [Klein et al. 2010].

The retina, as it is constantly exposed to light and has a high oxygen content, is clearly susceptible to strong oxidative damage. However, in evident contrast with the predictions of the old paradigm, antioxidant supplements did not prevent early AMD as shown by the results of the meta-analysis of 12 studies [Chong et al. 2007].

- Brain neurons and the genesis of Alzheimer's disease

The preceding section has shown that specialized neurons without cell turnover (retina photoreceptors) depend for their function and vitality on specialized gliocytes with cell turnover (RPE cells). This suggests that other types of neurons without cell turnover (mostly CNS neurons) could also be dependent on specific gliocytes with cell turnover (Fig. 5). Therefore, the gradual senescence and cell senescence of these specialized gliocytes may cause pathologies equivalent to AMD for their genesis.

Even without considering the proposed AMD genesis, AD was hypothesized to have originated from the functional decline of particular gliocytes caused by telomere shortening [Fossel 1996, 2004]: "One function of the microglia ... is degradation of β-amyloid through insulin-degrading enzyme (IDE), a function known to falter in Alzheimer disease ..." ([Fossel 2004], p. 233).

The role of microglial cells in the degradation of β-amyloid protein is well known [Qiu et al. 1998; Vekrellis et al. 2000; Miners et al. 2008] as is the fact that this function is altered in AD [Bertram et al. 2000] with the consequent accumulation of the substance. As regards to the relation between the decline of microglial function and telomere length, a significantly reduced telomere length in circulating monocytes is associated with at least vascular dementia [von Zglinicki et al. 2000].

The hypothesis that AD is determined by the decline of microglial cells was proposed again without mentioning the association between satellite gliocyte failure and AMD [Flanary 2009] or, with stronger arguments, considering it [Libertini 2009a, 2009b].

"A cell senescence model might explain Alzheimer dementia without primary vascular involvement." ([Fossel 2004], p. 235) and it is likely that many AD cases have in part a vascular aetiology caused by age-related endothelial dysfunction [Fossel 2004]. The similarities or identities between the symptoms of a "pure" AD and those of a "pure" vascular dementia and the cases where the two conditions overlap to varying extents must not obscure an affinity that is deeper and not linked only to some identical symptoms.

If it is true that AD neuronal decay results from the decline of satellite cells and that vascular dementia is a neuronal decay caused by vascular dysfunction, there is a common pathogenetic mechanism in their origins. The vascular diseases, including those that affect the brain, are caused by endothelial dysfunction originating from the depletion of their turnover capacity marked by an insufficient number of endothelial progenitor cells [Hill et al. 2003]. Similarly, AD could originate from microglial dysfunction caused by the slowing or exhaustion of microglia cell turnover capacity. In both cases, telomere shortening in the stem cells of the endothelial cells or of the microglial cells is the primary cause.

Therefore, the risk factors for cardiovascular diseases and for AD, in general, should both accelerate telomere failure, whereas protective factors should counter these effects. Moreover, risk factors for cardiovascular diseases and for AD should coincide, and analogously protective factors for the same diseases should coincide.

In support of these claims: (i) an association between cardiovascular risk factors and AD has been shown [Vogel et al. 2006; Rosendorff et al. 2007]; (ii) cigarette smoking is a risk factor for AD [Durazzo et al. 2014]; (iii) many reviews, with some exceptions (e.g. [Li et al. 2016]), maintain a positive correlation between diabetes mellitus and the risk of developing AD [Baglietto-Vargas et al. 2016; Rani et al. 2016; Saedi et al. 2016; Vicente Miranda et al. 2016]; (iv) there is positive

relation between hypertension and AD risk [Qiu et al. 2005; Michel 2016]; (v) statins, ACE inhibitors and sartans, "protective drugs" for cardiovascular diseases, are considered effective against AD [Vogel et al. 2006; Ellul et al. 2007]; and (vi) in a recent meta-analysis, ACE inhibitors and sartans appear to reduce the risk of developing AD [Yasar et al. 2016]; (vii) "Mid-life dyslipidemia appears to play an important role in the development of AD amongst a host of other risk factors that affect vascular health. Results from observational cohorts have been mixed, though many of the highest-quality studies have found a protective effect for statins." [Wanamaker et al. 2015]

As regards to alcohol consumption "Light to moderate drinking may decrease the risk of Alzheimer's disease" [Ilomaki et al. 2015]. A study has highlighted "weak evidence" for the association between a higher risk of AD and excessive alcohol consumption while "a lower risk of AD is associated with moderate alcohol consumption" [Campdelacreu 2014].

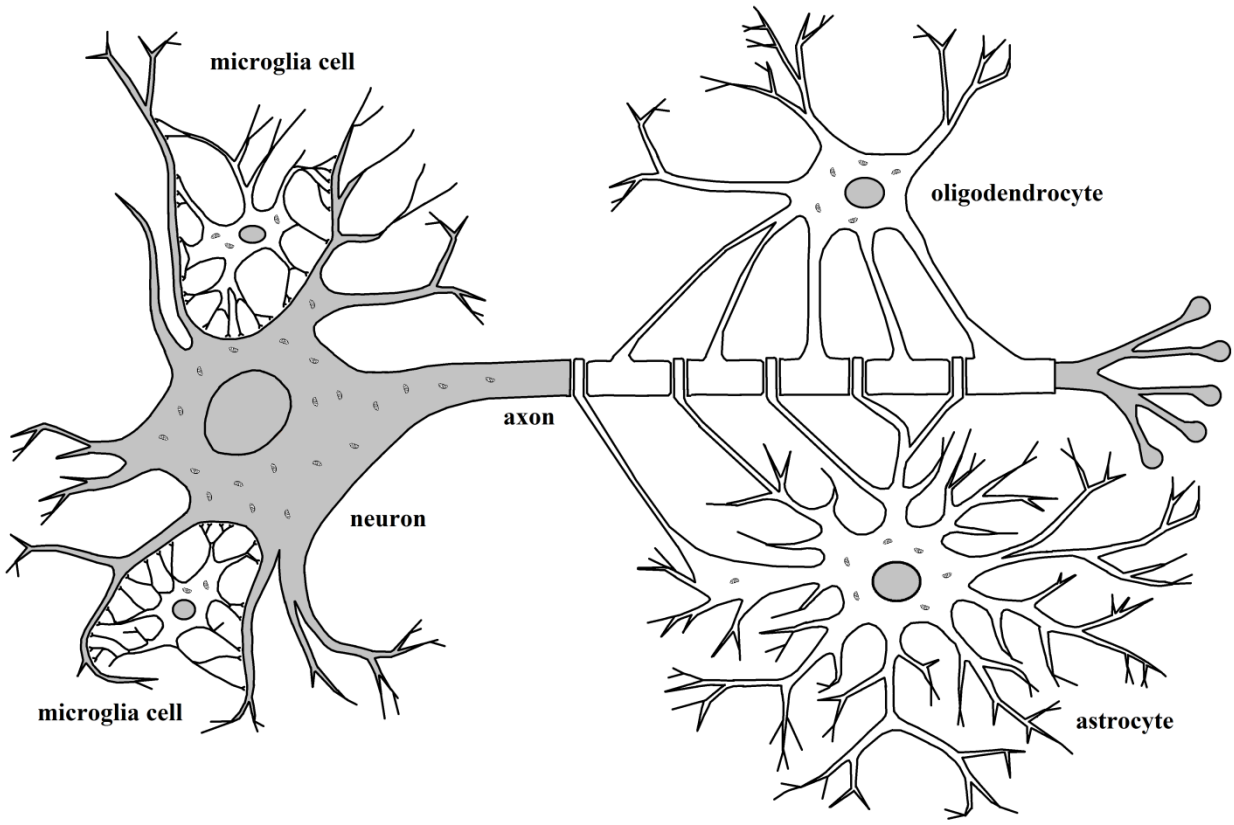


Figure 5 – A scheme of a neuron and its auxiliary gliocytes.

The traditional hypothesis that the decisive factor for AD pathogenesis is the damaging accumulation of substances such as β-amyloid and tau protein, has been widely contradicted by the failures of the pharmaceutical industry in the trials based on this thesis. Drugs or vaccines used with the aim to counter the formation of β-amyloid plaques have been disappointing, not for their ability to eliminate the plaques but for the purpose of obtaining positive clinical effects [Abbott 2008]. In particular, in 2008, a study showed that an experimental amyloid peptide vaccine was very effective in eliminating the plaques or in avoiding their formation, but: "Seven of the eight immunised patients who underwent post-mortem assessment, including those with virtually complete plaque removal, had severe end stage dementia before death." [Holmes et al. 2008] and the authors observed "Although immunisation with Abeta(42) resulted in clearance of amyloid plaques in patients with Alzheimer's disease, this clearance did not prevent progressive neurodegeneration" [Holmes et al. 2008]. A recent authoritative study [Sevigny et al. 2016] has used a human monoclonal antibody (aducanumab) to eliminate the amyloid-β plaques. The technique has shown

significant and dose-dependent effectiveness in eliminating the plaques, while the clinical results (for which one must observe that the study was not specifically designed) remain uncertain. After one year of treatment, one of the two clinical evaluation parameters (Mini Mental State Examination) showed positive results for the doses of antibody 3 and 10 mg/kg, but for the intermediate dose of 6 mg/kg, the result was similar to that of placebo.

The hypothesis that AD originates from the accumulation of β-amyloid (amyloid cascade hypothesis) has been strongly criticized [Herrup 2010; Mondragón-Rodríguez et al. 2010; Reitz 2012], but there is a reluctance to drop it, perhaps for the false idea that there is no plausible alternative such as the one discussed in the present work.

N-Methyl-D-aspartate receptor antagonist (e.g., memantine), acetylcholinesterase inhibitors (e.g., galantamine, donepezil, tacrine, rivastigmine), and other drugs are used to treat AD [Mendiola-Precoma et al. 2016]. However, "The only drugs available for Alzheimer's patients aim to treat symptoms ... They are marginally effective at best." [Abbott 2008]. Moreover, the treatment of AD cognitive alterations by antipsychotic drugs increased the long-term risk of mortality [Ballard et al. 2009].

- Brain neurons and the genesis of Parkinson's disease

Parkinson's disease (PD) is characterized by the accumulation of the protein α-synuclein (AS) inside CNS neurons, which forms particular inclusions known as Lewy bodies [Davie 2008; Schulz-Schaeffer 2010]. A disease that is akin to PD, classified as a Parkinson-plus syndrome, is the Dementia with Lewy bodies (DLB) [Nuytemans et al. 2010]. It has the signs of a primary parkinsonism but shows some additional features [Samii et al. 2004]. In addition, multiple system atrophy is a rare genetic disease in which AS accumulates in oligodendrocytes [Sturm and Stefanova 2014].

While AD is referred to as a tauopathy due to the accumulation of tau protein and the formation of neurofibrillary tangles, PD, DLB and multiple system atrophy, are referred to as synucleinopathies for the accumulation of AS [Galpern and Lang 2006]. Despite the difference between the accumulated substances, many symptoms and pathological manifestations are similar or identical in these neuropathies [Aarsland et al. 2009].

PD is mainly a disease of the CNS motor system because its more common and typical symptoms include disorders of movement caused by extrapyramidal motor dysfunction. However, nonmotor manifestations, such as dementia and sensory deficits are common [Barnett-Cowan et al. 2010]. In PD cases without dementia, behaviour and mood alterations (e.g., depression, anxiety, apathy, etc.) are more frequent than in the general population and are common symptoms in the cases with dementia [Jankovic 2008].

Dementia is frequent in PD patients, in particular at advanced stages, and AD patients often manifest parkinsonism [Galpern and Lang 2006]. In PD patients, the risk of dementia is two to six times that of the whole population and is related to disease duration [Caballol et al. 2007]. However, for AD patients, dementia is more precocious and senile plaques and neurofibrillary tangles are characteristic of AD, while they are uncommon in PD without dementia [Dickson 2007].

As regards to the distinction between the clinical manifestations of PD and DLB: "PD and DLB are common neurodegenerative diseases in the population over the age of 65. About 3% of the general population develops PD after the age of 65, whereas about 20% of all diagnosed dementia patients have DLB ... In both disorders movement and cognition, as well as mood and autonomic function are severely affected. Diagnosis to distinguish PD and DLB is very difficult, because of the overlap of symptoms and signs ..." [Brück et al. 2015]

In PD pathogenesis, Lewy bodies are found in the olfactory bulb, medulla oblongata and pontine tegmentum before clear symptoms appear. In the following phases, they are present in the substantia nigra, in some regions of the basal forebrain and of the midbrain, and finally in the neocortex [Davie 2008]. PD motor symptoms are interpreted as a consequence of neuronal cell

death in a particular area of the brain (the pars compacta region of the substantia nigra), which causes a reduction in dopamine secretion [Obeso et al. 2008].

In the aforesaid areas, there is neuronal degeneration and loss, for which Lewy bodies have been proposed not to be the cause of neuron death but as a protection against other factors [Obeso et al. 2010; Schulz-Schaeffer 2010]. In demented AD patients, Lewy bodies are observed in particular in cortical areas in the brain, and it has been suggested that "reduced AS clearance is involved in the generation of AS inclusions in DLB and PD" [Brück et al. 2015], but this does not clarify if Lewy bodies are the cause of neuronal degeneration or a simple consequence of the cause.

About this, some evidence must be considered:

"Glial cells are important in supporting neuronal survival, synaptic functions and local immunity ... However, glial cells might be crucial for the initiation and progression of different neurodegenerative diseases, including ASP [α-Synucleinopathies] ..." [Brück et al. 2015]

"... microglial cells contribute to the clearance of debris, dead cells and AS thereby supporting neuronal survival. But on the other hand, microglial cells can get over-activated in the course of the disease and might contribute to disease initiation and progression by enhancing neurodegeneration through elevated oxidative stress and inflammatory processes." [Brück et al. 2015]

In analogy with AMD and AD genesis, these elements suggest that the pivotal event in PD genesis is the loss of essential functions of specific gliocytes (astrocytes) mainly dedicated to axon trophism and the activation of astroglial and microglial cells could be an event caused by the consequent AS accumulation [Morales et al. 2015], while the decline of the function of these specific gliocytes would be caused by the decline of their turnover.

As regards to the associations of PD with "risk factors", there is the following evidence: (i) A study has shown that, in the middle age, high skinfold thickness is associated with PD [Abbott et al. 2002]; (ii) Metabolic syndrome (i.e., in short, an unhealthy lifestyle that causes dyslipidemia, obesity, glucose intolerance, hypertension, etc.) has been indicated as an important risk factor for PD [Zhang and Tian 2014]; (iii) Independently of other risk factors, body mass index appears related to an increased risk of PD [Hu et al. 2006], and obesity, in midlife, increases the risk of dementia [Whitmer et al. 2005]; (iv) Hyperglycemia, in aging subjects, is associated with PD [Hu et al. 2007; Tomlinson and Gardiner 2008]; (v) Type 2 diabetes is a risk factor for PD [Vicente Miranda et al. 2016]; (vi) Alcohol (light to moderate) use has been found to have an inverse association with PD [Ishihara and Brayne 2005] while alcohol use disorder appears to increase the risk of Parkinson's disease [Eriksson et al. 2013].

The risk of PD appears to be lowered by statins [Gao et al. 2012; Friedman et al. 2013; Undela et al. 2013; Sheng et al. 2016]. Captopril, an angiotensin-converting enzyme inhibitor, has been shown to protect nigrostriatal dopamine neurons in animal models for PD [Lopez-Real et al. 2005; Sonsalla et al. 2013]. A strange finding is that cigarette smoking, a risk factor for AD [Durazzo et al. 2014], AMD [Klein et al. 2007], hearing loss [Fransen et al. 2008; Chang et al 2016], and olfactory dysfunction [Vent et al. 2003], appears to lower PD risk [Li et al. 2015]. A possible explanation is that nicotine has a neuroprotective effect on dopaminergic neurons and could even be used as a medicine to contrast PD progression or symptoms [Thiriez et al. 2011; Quik et al. 2015].

Hyperhomocysteinemia (HHcy), which is considered a risk factor for endothelial dysfunction and so for vascular diseases [Woo et al. 1997], has been shown to be associated with AD and PD [Kruman et al. 2000]. However, limiting the discussion to AD, "it is still controversial if HHcy is an AD risk factor or merely a biomarker" [Zhuo et al. 2011].

- Hearing neurons

The organ of Corti is a sensory receptor inside the cochlea of the inner ear, which has hearing function. It has differentiated neurons, the auditory or hair cells, divided into two groups (inner and outer hair cells) and connected to specific neurons (spiral ganglion neurons). In a newborn, for each organ of Corti, there are approximately 35,000 neurons and 15,500 hair cells, and both of these cell types are perennial [Wong and Ryan 2015]: "... no epithelial maintenance has been described for the

hair cells of the cochlea of mammals, though hair cell addition and repair occur in lower vertebrates ...” [Maier et al. 2014] (Fig. 6).

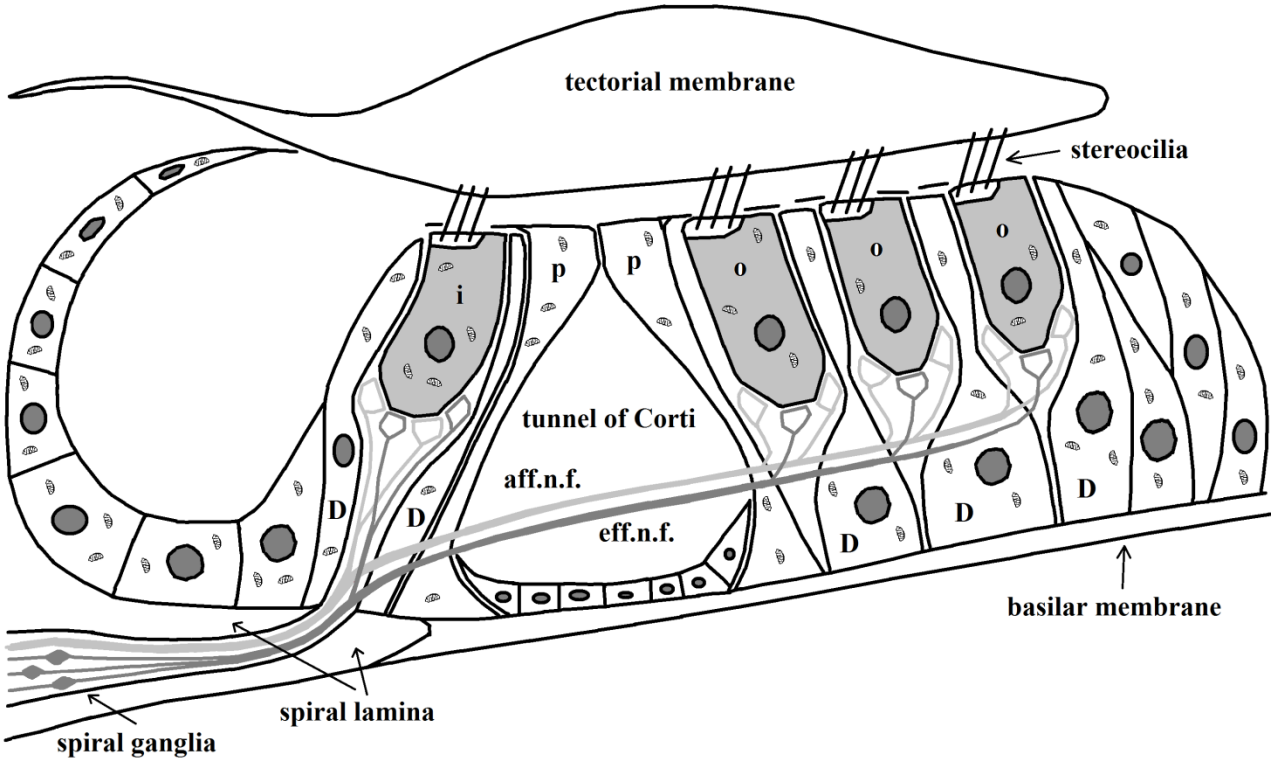


Figure 6 - Organ of Corti. Abbreviations: i = inner hair cells; o = outer hair cells; D = Deiters’ cells; p = pillar cells; aff.n.f. = afferent nerve fibres of cochlear nerve; eff.n.f. = afferent nerve fibres of cochlear nerve. Hair cells, specialized neurons that constitute the sensorineural cells of the auditive organ, are supported by Deiters’ cells, which are specialized gliocytes.

It is well known that there is an age-related progressive reduction of hearing ability [Zhan et al. 2010]. In the USA, hearing loss of more than 25 dB in speech frequency pure tone average has been reported to be 45.6, 67.6, 78.2, and 80.6% in groups aged 70-74, 75-79, 80-84, and >84 years, respectively [Lin et al. 2011b]. Noise exposure is not an indispensable cause for the development of presbycusis, as it has been observed in healthy animals that were reared in silence [Sergeyenko et al. 2013; Yan et al. 2013].

Cardiovascular and cerebrovascular diseases, diabetes, cigarette smoking have been associated with increased risk of hearing loss [Yamasoba et al. 2013]. In a study with 3,753 participants, “current smokers were 1.69 times as likely to have a hearing loss as nonsmokers” [Cruickshanks et al. 1998] and in another study with 12,935 participants “Current smoking was associated with hearing impairment in both speech-relevant frequency and high frequency across all ages.” [Chang et al. 2016]. Interestingly, “a remarkable parallelism between the risk factors for ARHI [age-related hearing impairment] and those for CVD [cardiovascular disease] with similar roles for smoking, high BMI, and regular moderate alcohol consumption.” [Fransen et al. 2008] has been observed.

Cardiovascular risk factors “adversely affect hearing acuity” [Oron et al. 2014]. Type 2 diabetes mellitus is associated with alterations in hearing [Akinpelu et al. 2014; Calvin and Watley 2015; Helzner and Contrera 2016], and hypertension has been found associated with cochlear hearing loss [Przewoźny et al. 2015]. In a highly endogamous population, “adults with DM [diabetes mellitus] and hypertension associated showed greater hearing impairment” [Bener et al. 2016].

There is an association between chronic alcohol abuse and hearing impairment [Rosenhall et al. 1993], while a protective effect of moderate alcohol consumption has been shown [Fransen et al. 2008].

Hearing impairment is common in idiopathic PD [Vitale et al. 2012], and there are 77% more cases of PD in patients with hearing loss than in those without hearing loss [Lai et al. 2014]. An association between incident dementia and hypacusia has been shown [Lin et al. 2011a].

In rats, low doses of a statin (atorvastatin) appear to prevent noise-induced hearing loss [Jahani et al. 2016]. In patients with hyperlipidaemia, tinnitus may be successfully treated by atorvastatin [Hameed et al. 2014]. In mice, another statin (pravastatin) attenuates cochlear injury caused by noise [Park et al. 2012]. In a mouse strain with accelerated aging, atorvastatin slows down the deterioration of inner ear function with age and this "suggest[s] that statins could also slow down the age-related deterioration of hearing in man" [Syka et al. 2007]. In a rat model for type 2 diabetes, losartan treatment effectively contrasts the hearing dysfunction that is typical in these animals [Meyer zum Gottesberge et al. 2015].

Conclusion

The traditional interpretation of aging, based on the tenets of the old paradigm, leads the researchers to justify the many ailments that afflict the elderly as diseases caused by various and different degenerative processes. According to the old paradigm, this implies that the disorders may well have common characteristics when there are identical or similar degenerative factors, but the similarities do not allow at all to consider them as a single disorder with various manifestations. Therefore, aging is explained as the sum and the overlapping of many different diseases and the term "aging" is only a useful term that summarizes them all without indicating a unique and distinct entity. This theoretical conception, among other things, has a practical consequence so that in the International Classification of Diseases [ICD-10 2016; ICD-9-CM 2016] a code to define aging is non-existent and, so, in the international statistics of the World Health Organization, aging is ignored as a cause of death [World Ranking Total Deaths 2014].

In complete contrast to this classical view, in the interpretation of the new paradigm, the term "aging" indicates a precise and distinct physiological mechanism, genetically determined and regulated, which manifests itself in all tissues and organs, and in the organism as a whole, as a consequence of a single, specific and well-defined mechanism. As briefly discussed above, this mechanism originates in the telomere-telomerase-subtelomere system and causes a progressive increase in the fraction of cells in replicative senescence and in various degrees of gradual senescence, with a parallel decline of cell turnover rates and of the functions of tissues and organ, which leads to a condition defined as "atrophic syndrome".

For all cell types, and then for the tissues and organs they form and for the functions resulting from them, this decline has two general modes:

(i) cells subjected to turnover (e.g., non-neuronal cells, gliocytes included, certain types of neuronal cells such as the ORCs) undergo a the direct decline;

(ii) cells not subjected to turnover (e.g., most neurons) but dependent on satellite cells that show turnover, suffer from the decline of the cells upon which they depend.

However, it is useful and necessary to add further considerations that are based on the evidence:

A) Unhealthy lifestyles, which cause diabetes, obesity, hypertension, exposure to toxic substances and other "risk factors", damage cells and accelerate their turnover causing alterations and anticipation of physiological aging;

B) By avoiding or reducing these "risk factors" and/or by using "protective drugs", such as ACE inhibitors, sartans, statins, the cellular damage and the associated disorders caused by slackened or exhausted cell turnover, are avoided or reduced. This occurs both for the decline of cells with turnover (e.g., emphysema caused by alveolocyte turnover failure, arteriosclerosis due to

endothelial cell turnover failure) and for the decline of cells without turnover but depending on cells with turnover (e.g., AMD, AD, PD, etc. caused by the decline of their trophic cells);

C) The full avoidance of the risk factors annuls the anticipation or increased probability of the aforementioned diseases but does not cancel the physiological rhythm and characteristics of aging;

D) The alterations of the characteristics of physiological, or "normal", aging are definable as diseases if "disease" is conceived as something that causes sufferings but not if "disease" is used in the meaning of alterations of a normal condition. Perhaps, a more neutral term (e.g., "trouble") should be used for the alterations that are associated with the physiological aging;

E) For the troubles of aging, if we disregard the precocious cases due to genetic anomalies, it is difficult to distinguish between the physiological forms (which we must consider as part of the physiological aging and are not prevented or modified by a healthy lifestyle and/or protective drugs) and the precocious form, which are due to some risk factor and may be undoubtedly defined as diseases.

These concepts are summarized in Figures 1, 2 and 7 and in Table 1.

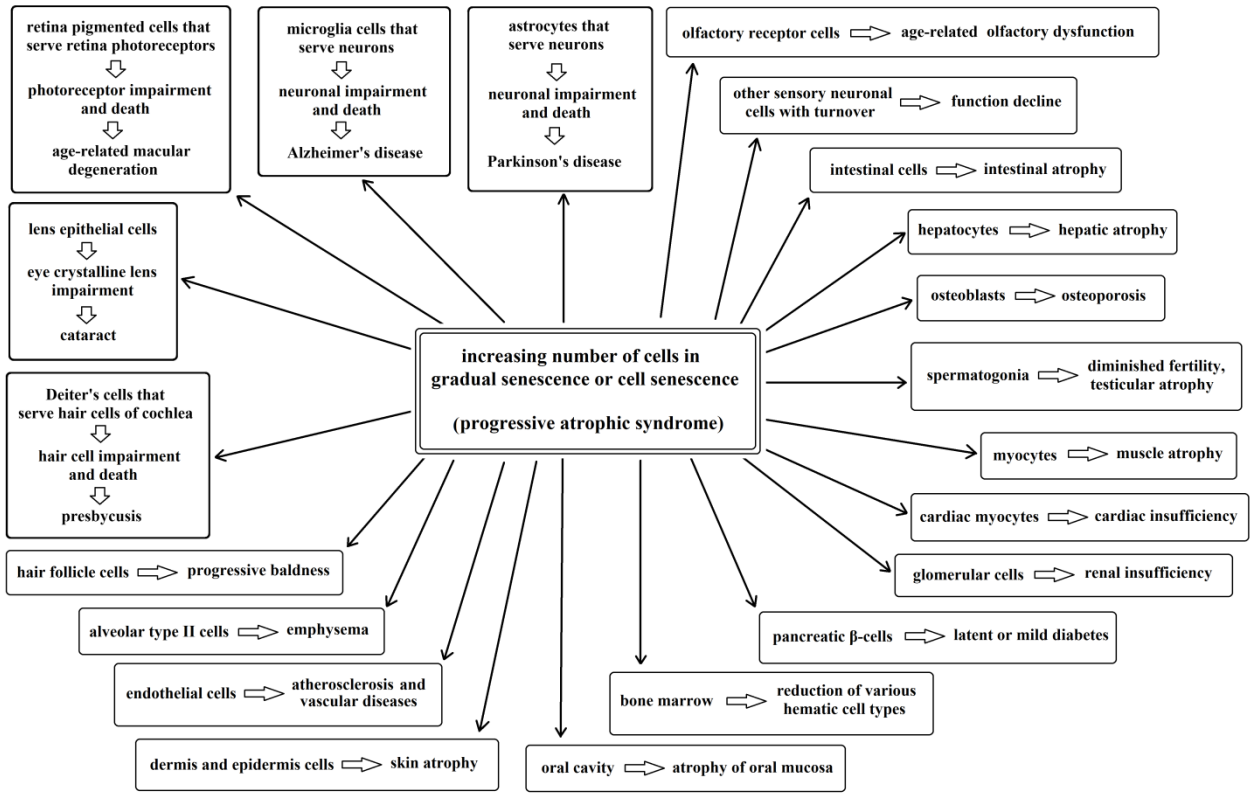


Figure 7 – A scheme of the aging process. Most cell types, including some types of neurons, are subject to turnover. Cell turnover decline causes the progressive impairment of the functions depending on these cells. Perennial neurons are compromised by turnover decline and gradual senescence of essential satellite gliocytes.

Now, some important general concepts may be expressed:

1) The telomere theory allows a unified and coherent vision of various diseases of the nervous system (e.g., AD, PD, AMD, presbycusis, age-related hyposmia), which are apparently quite distinct and without a common root;

2) The telomere theory allows a unified and consistent view of these diseases in the framework of a wide range of diseases that are not pertaining to the nervous system. Through this unitary conception, it is possible to have a rational explanation of the noteworthy commonality of factors

that increase the risks (risk factors) or reduce them (protective factors) for all the aforementioned diseases;

Table 1 – Relations between some troubles and some "risk factors" or "protective drugs".

Troubles	Cell turnover of the specific cells	Risk increased (+) or perhaps increased (+?) or lowered (-) or unaltered (/) by							Protective effect by:	
		Age	Diabetes	Obesity / dislipemia	Hypertension	Smoke	Alcohol moderate use	Alcohol abuse	Statins	ACE-i, sartans
Endothelial dysfunction	Yes	+[1]	+[2]	+[3]	+[4]	+[5]	-[6]	+[7]	+[8]	+[9]
Olfactory dysfunction	Yes	+[10]	+[11]	+[12]	+[13]	+[14]	[15]	+[16]	+?[17]	[18]
AMD	No	+[19]	+[20]	+[21]	+[22]	+[23]	/[24]	/[25]	+?[26]	[27]
AD	No	+[28]	+[29]	+[30]	+[31]	+[32]	-[33]	+[34]	+[35]	+[36]
PD	No	+[37]	+[38]	+[39]	+[40]	-[41]	-[42]	+[43]	+[44]	+?[45]
Hearing impairment	No	+[46]	+[47]	+[48]	+[49]	+[50]	-[51]	+[52]	+?[53]	+?[54]

Notes: 1-5) [Wilson et al. 1987; Hill et al. 2003]; 6) [Roerecke and Rehm 2014; Gardner and Mouton 2015; de Gaetano et al. 2016]; 7) [Gardner and Mouton 2015; de Gaetano et al. 2016]; 8) [Walter et al. 2002; Su 2015]; 9) [Su 2015]; 10) [Schubert et al. 2012; Doty and Kamath 2014; Gouveri et al. 2014]; 11) [Gouveri et al. 2014; Mehdizadeh et al. 2015]; 12) [Richardson et al. 2004; Patel et al. 2015]; 13) [Gouveri et al. 2014]; 14) [Vent et al. 2003]; 15) No specific study; 16) [Rupp et al. 2004]; 17) [Kim et al. 2012]; 18) No specific study; 19) [Rudnicka et al. 2012]; 20-22) [Klein et al. 2007; Mares et al. 2011]; 23) [Klein et al. 2007; Armstrong and Mousavi 2015]; 24) [Armstrong and Mousavi 2015]; 25) [Klein et al. 2010]; 26) [Gehlbach et al. 2015]; 27) No specific study; 28) [Gorelick 2004]; 29) [Rosendorff et al. 2007; Baglietto-Vargas et al. 2016; Rani et al. 2016; Saedi et al. 2016; Vicente Miranda et al. 2016]; 30) [Rosendorff et al. 2007; Wanamaker et al. 2015]; 31) [Rosendorff et al. 2007; Qiu et al. 2005; Michel 2016]; 32) [Rosendorff et al. 2007; Durazzo et al. 2014]; 33) [Campdelacreu 2014; Ilomaki et al. 2015]; 34) [Rosendorff et al. 2007; Campdelacreu 2014]; 35) [Vogel et al. 2006; Ellul et al. 2007; Wanamaker et al. 2015]; 36) [Vogel et al. 2006; Ellul et al. 2007; Yasar et al. 2016]; 37) [De Lau and Breteler 2006; Pringsheim et al. 2014]; 38) [Hu et al. 2007; Tomlinson and Gardiner 2008; Zhang and Tian 2014; Vicente Miranda et al. 2016]; 39) [Abbott et al. 2002; Hu et al. 2006; Zhang and Tian 2014]; 40) [Zhang and Tian 2014]; 41) [Li et al. 2015]; 42) [Ishihara and Brayne 2005]; 43) [Eriksson et al. 2013]; 44) [Gao et al. 2012; Friedman et al. 2013; Undela et al. 2013; Sheng et al. 2016]; 45) [Lopez-Real et al. 2005; Sonsalla et al. 2013]; 46) [Zhan et al. 2010; Lin et al. 2011b]; 47) [Akinpelu et al. 2014; Oron et al. 2014; Calvin and Watley 2015; Helzner and Contrera 2016; Bener et al. 2016]; 48) [Fransen et al. 2008; Oron et al. 2014]; 49) [Oron et al. 2014; Przewoźny et al. 2015; Bener et al. 2016]; 50) [Cruickshanks et al. 1998; Fransen et al. 2008; Oron et al. 2014; Chang et al. 2016]; 51) [Fransen et al. 2008]; 52) [Rosenhall et al. 1993]; 53) [Syka et al. 2007; Park et al. 2012; Hameed et al. 2014; Jahani et al. 2016]; 54) [Meyer zum Gottesberge et al. 2015].

3) The possible criticism against the telomere theory, and consequently against the programmed aging paradigm, caused by the evidence of tissue and organs largely composed of cells without turnover and that age, are overcome by the evidence previously expounded. Vice versa, the telomere theory and the programmed aging paradigm emerge strengthened from the overcoming of such criticism;

4) If aging is a physiological phenomenon, genetically determined and regulated, in principle it could be regulated, slackened or even cancelled. This statement, which might seem unrealistic and, for the supporters of the non-programmed aging paradigm, the declaration of something clearly utopian, i.e., impossible, on the contrary is something that has been proved: (i) since 1998, it is known that in cultivated normal cells, telomerase activation leads to longer telomeres, cancels all the manifestations of cell senescence, both the biochemical alterations and the incapacity to duplicate [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998]; (ii) telomerase reactivation in aged mice with blocked telomerase determined the clear reversal of all

the manifestations of aging, those of the nervous system included [Jaskelioff et al. 2011]; (iii) in one- and two-year-old normal mice, telomerase reactivation delayed the aging manifestations and increased lifespan [Bernardes de Jesus et al. 2012]; (iv) human skin obtained from aged fibroblasts with reactivated telomerase was not distinguishable from skin obtained from young fibroblasts [Funk et al. 2000];

5) In the search for a treatment to slacken or reverse aging, the attention should be focused on the telomere-telomerase-subtelomere system. Telomerase reactivation has been proposed as treatment for AD [Fossel 1996, 2004] and for AMD [Libertini 2009b]. As an intermediate step for the goal of a complete control of aging, the effective treatment of diseases that are strongly invalidating and expensive, such as AD, PD and AMD, by actions on telomere-telomerase-subtelomere system has been proposed [Libertini and Ferrara 2016a].

Chapter 13

Libertini G (2017c) Sex and Aging: a Comparison between Two Phenoptotic Phenomena. *Biochem (Mosc.)* 82(12), 1435-55.

Sex and aging: a comparison between two phenoptotic phenomena

Giacinto Libertini

Abstract

A phenomenon that is genetically programmed and favoured by natural selection is defined as phenoptotic when it determines death or increased risk of death, alias fitness reduction, for the individual that manifests it. Aging, here precisely defined as age-related progressive mortality increase in the wild, if programmed and favoured by natural selection, undoubtedly falls within the definition of phenoptosis. Sexual reproduction, as for the involved individuals determines fitness reduction and, in some particular species, even certain death, also falls under the definition of phenoptosis.

In this paper, aging and sex are analyzed as phenoptotic phenomena, trying to single out the similarities between them. In particular, from a theoretical standpoint, these phenomena:

- for their understanding, require an analysis that comprises both individual and supra-individual selection, because they are harmful in terms of individual selection but advantageous in particular conditions of supra-individual selection;
- determine a higher velocity and/or greater opportunities of evolution and hence a greater evolutionary potentiality (evolvability);
- are advantageous in ecological conditions of K-selection and with finite populations;
- are disadvantageous in ecological conditions of r-selection and with unlimited populations;
- are not favourable in all the ecological conditions and, so, species that reproduce asexually or species that do not age are predicted and exist.

Introduction

Since a long time, sex and aging are subjects of much debate about their justification in evolutionary terms. A minute and complete exposition and discussion of the various theories about the evolutionary primary causes of the two phenomena would require a much larger space than that possible for a review.

Therefore:

- as regards sex, reference will be made mainly to a model based on the ancient and shared theoretical firm belief that its evolutionary advantage derives from the recombination of characters. However, sex cannot be advantageous in condition of r-selection [Pianka 1970] and in numerically unlimited populations;
- as regards aging, the hypothesis proposing its advantage due to the greater speed of evolution determined by a quicker generation turnover will be exposed. However, aging may be favoured, by supra-individual selection, only in ecological conditions of K-selection [Pianka 1970] and in species divided into small demes.

Afterwards, for both phenomena, the predictions of the aforesaid hypotheses about their existence among the various species, depending on ecological conditions, will be expounded and briefly compared with the predictions of other hypotheses.

Finally, their nature as phenoptotic phenomena and a general comparison between the two phenomena will be outlined.

Sex

- The advantage of sex

The evolutionary justification of gene recombination between two individuals (defined as "mixis" or "amphimixis", or commonly as "sex"), is a much debated topic [Ghiselin 1974; Williams 1975; Maynard Smith 1978; Bell 1982; Ridley 1993; Barton and Charlesworth 1998; Agrawal 2006; Hadany and Comeron 2008; Barton 2009; Otto 2009; Hartfield and Keightley 2012; McDonald et al. 2016; Sharp and Otto 2016; Lewis-Pye and Montalbán 2017; Ho and Agrawal 2017].

The "classic" hypothesis, also known as Fisher-Muller hypothesis or, by using Bell's old eponym, "The Vicar of Bray" [Bell 1982], maintains that sex is evolutionarily advantageous because it allows a continuous rearrangement of genes (Fig. 1). This thesis was first expressed by Weismann [Weismann 1889] and by Guenther [Guenther 1906]. It was later formulated in terms of population genetics by Fisher [Fisher 1930] and Muller [Muller 1932], and afterwards, with greater mathematical formalism, by Muller [Muller 1958, 1964] and Crow and Kimura [Crow and Kimura 1965].

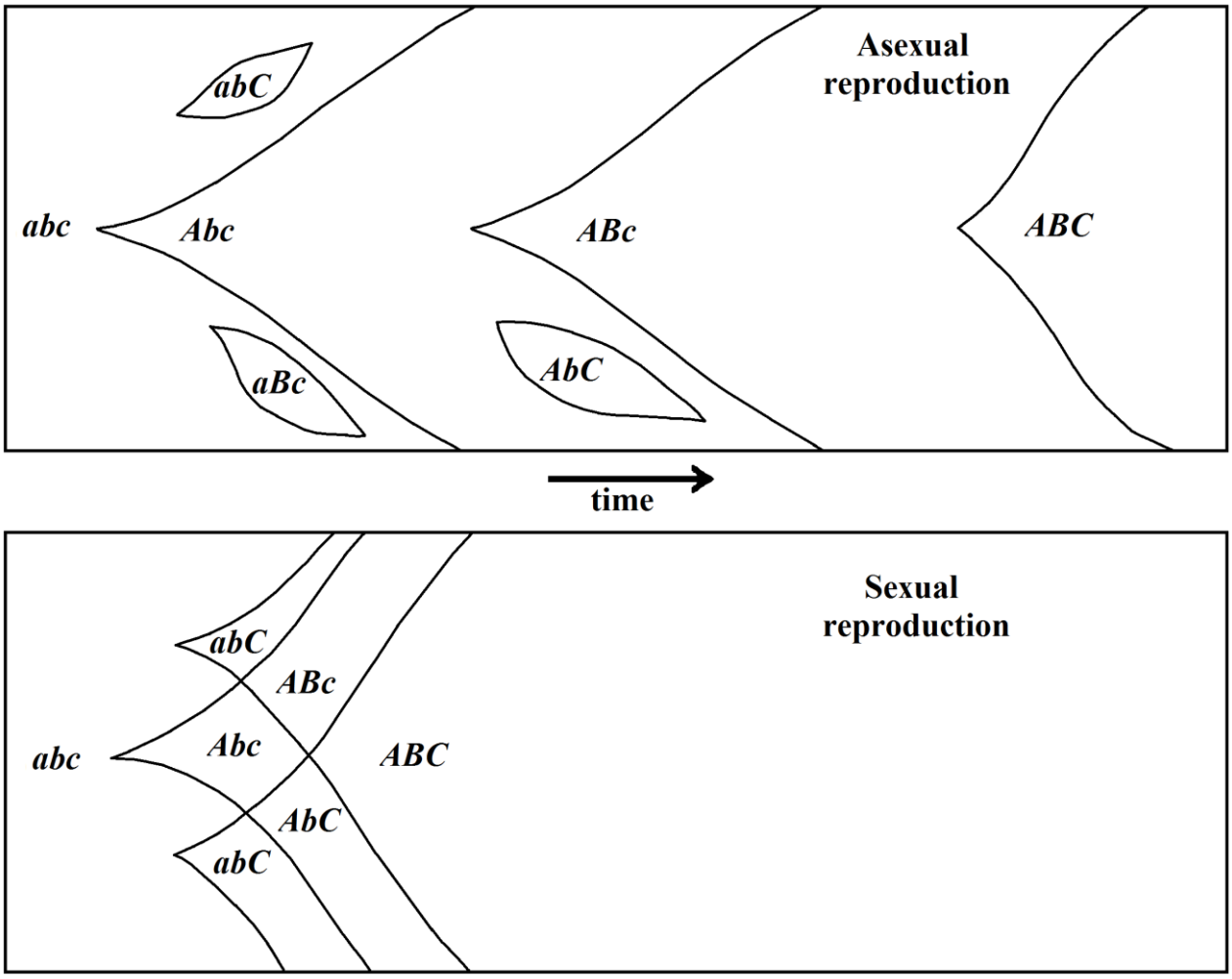


Figure 1 - For the "classic" hypothesis, sex would be evolutionarily advantageous because it allows a continuous rearrangement of genes and therefore the attainment of the best combinations earlier than with asexual reproduction [redrawn from Crow and Kimura 1965].

Maynard Smith [Maynard Smith 1968, 1978] criticised the "classic" hypothesis with the following, simple but effective, argument.

If, in an infinite population of a haploid species, there are two genes (a, b), with alleles (A, B) that have an advantage (s_A, s_B) over a and b, respectively, the combination frequencies in the next generation will be:

$$P_{n+1,ab} = P_{n,ab} / T$$
$$P_{n+1,Ab} = P_{n,Ab} (1+s_A) / T$$
$$P_{n+1,aB} = P_{n,aB} (1+s_B) / T$$
$$P_{n+1,AB} = P_{n,AB} (1+s_{AB}) / T \quad (1)$$

where:
s_{AB} = advantage of AB over ab = $[(1+s_A)(1+s_B)-1]\ k$ (2)
k = interaction between the fitnesses (epistasis);
$P_{n,xy}$ = frequency of xy combination at generation n;
T = the sum of numerators;

If, at generation n, there is no linkage disequilibrium (D), that is, if:

$$D = P_{n,ab} P_{n,AB} - P_{n,Ab} P_{n,aB} = 0 \quad (3)$$

with no epistasis, or interaction between the fitnesses (k = 1), the group of formulas (1) determines that in the next generation it will be always:

$$D = P_{n+1,ab} P_{n+1,AB} - P_{n+1,Ab} P_{n+1,aB} = 0 \quad (4)$$

with or without recombination, which can only halve linkage disequilibrium at each generation [Maynard Smith 1978]. Therefore, with these conditions sex cannot be advantageous.

With negative linkage disequilibrium ($D < 0$) sex would be advantageous, while with positive linkage disequilibrium sex would be disadvantageous.

If there is positive epistasis ($k > 1$), sex is disadvantageous because it breaks the more advantageous combination AB, and the contrary happens if there is negative epistasis ($k < 1$). However, there is no justification to assume that epistasis should be in general positive and so sex advantageous.

The same Maynard Smith tried to overcome his argument observing that it was surely valid for infinite populations but that linkage disequilibria should arise by chance in finite populations [Maynard Smith 1978], and so, in conditions of negative linkage disequilibrium, sex would be advantageous, as previously observed by Felsenstein [Felsenstein 1974].

Many scholars did not accept the counter-arguments of Maynard Smith [Crow and Kimura 1969; Williams 1975] and the doubts about the validity of the "classic" explanation of sex, here also defined as "S1" hypothesis, caused the flourishing of alternative hypotheses such as, to use the eponyms of Bell [Bell 1982]:

S2) Muller's ratchet [Muller 1964; Felsenstein 1974; Butcher 1995; Gordo and Charlesworth 2000; Keightley and Otto 2006; Gordo and Campos 2008; Wardlaw and Agrawal 2012; Hartfield and Keightley 2012] - For this theory, sex facilitates the elimination of unfavourable mutations, while, without mixis, damaging mutations would continually accumulate in a population, causing the decline of its mean fitness [Gordo and Charlesworth 2000];

S3) *Best-Man h.* [Williams 1966; Emlen 1973; Treisman 1976; Dacks and Roger 1999] - Recombination may produce individuals of extraordinarily high fitness. If these individuals have an appreciable chance of surviving in conditions where all the others die, then sexual individuals will have a greater proportion of progeny in the next generation;

S4) *Hitch-hiker h.* [Hill and Robertson 1966; Felsenstein 1974] - Stochastically generated linkage disequilibria increase the variance of fitness of any single-locus genotype and so retard the

fixation of a favourable allele. An allele increasing the rate of recombination reduces linkage disequilibria and accelerates the fixation of favourable alleles and thus, for selection, it is hitch-hiked by these favourable alleles;

S5) *Tangled Bank h.* [Ghiselin 1974; Case and Taper 1986; Burt and Bell 1987; Ridley 1993; Doncaster et al. 2000; Song et al. 2011] - Sex diversifies progeny and its advantage is greater in conditions of environmental spatial heterogeneity, that is, if there are various "ecological niches in the same small geographical area - in an environment which does not change in time" [Bell 1982]);

S6) *Red Queen h.* [Van Valen 1973; Hamilton 1975; Levin 1975; Charlesworth 1976; Glesener and Tilman 1978; Glesener 1979; Bell 1982; Bell and Maynard Smith 1987; Ridley 1993; Peters and Lively 1999, 2007; Otto and Nuismer 2004; Kouyos et al. 2007; Salathé et al. 2008; Liow et al. 2011; Brockhurst et al. 2014; Voje et al. 2015] - This popular theory maintains that sex has evolved "in response to the shifting adaptive landscape generated by the evolution of interacting species." [Otto and Nuismer 2004]; "The Red Queen Hypothesis ... suggests that the coevolutionary dynamics of host-parasite systems can generate selection for increased host recombination. ... A prerequisite for this mechanism is that host-parasite interactions generate persistent oscillations of linkage disequilibria ..." [Kouyos et al. 2007]);

S7) *Historical h.* [Williams 1975] - Sex has no general evolutionary cause and sexual/asexual condition is mainly determined by the sexuality/asexuality of the ancestors.

and, moreover, the hypotheses that:

S8) *Sex is advantageous because it slows down evolution and excessive specialization* [William 1975; Stanley 1978];

S9) *Recombination eliminates the negative linkage disequilibrium generated by synergistic epistasis* [Kondrashov 1984; Charlesworth 1990; Barton 1995; Otto and Feldman 1997];

S10) *A plurality of theories is necessary to explain the existence of sex* [West et al. 1999];

and others theories, on the whole classified by Kondrashov [Kondrashov 1993].

Well-known serious criticisms oppose many of these hypotheses [Bell 1982]. Moreover: i) various attempts to explain sex advantage in finite populations appear too complex [Kondrashov and Yampolsky 1996; Bürger 1999; Pálsson 2002; Iles et al. 2003; Barton and Otto 2005; Martin et al. 2006; Tannenbaum 2008]; and ii) sex evolutionary advantage should be investigated without hypothesizing artful and/or unduly limiting mechanisms.

However, it is possible to formulate a model that shows for sex - in terms of individual selection, as considered necessary by Felsenstein [Felsenstein 1974] - both advantage in finite populations and no advantage in infinite populations. Moreover, the model considers: i) the important observation expressed by Felsenstein: " ... those authors who have allowed finite-population effects into their models have been the ones who found an advantage to having recombination, while those whose models were completely deterministic found no consistent advantage." [Felsenstein 1974]; and ii) the suggestion that real populations are subject to genetic drift and are spatially structured [Otto and Lenormand 2002].

- The simulation model for infinite populations

Let us consider a species:

a) haploid;

b) with an infinite population;

c) with half of the individuals at generation zero having - in a specific locus - a gene R+ allowing conjugation and free recombination only with other individuals having R+, while the others have an allele R- allowing conjugation and recombination only in a fraction z of individuals. If $z > 0$ the pool of recombining individuals is constituted by all R+ individuals plus a fraction z of R- individuals. However, if $z = 0$, as in most of the following simulations, there is "no sharp distinction between individual selection and group selection", as underlined by Felsenstein and Yokoyama

[Felsenstein and Yokoyama 1976], but the selection will actually be considered only in strict terms of individual selection.

d) with R+ and R- individuals having the same ecological niche and being by no means distinguishable except for the condition expressed in c;

e) with mutation rates of R+ into R-, namely turning a sexual individual into an asexual individual, or vice versa, of zero frequency;

f) with the disadvantage for sexual individuals of finding a mate and of coupling and with any other possible disadvantage of sex, the so-called "cost of sex" included, here not considered;

g) with new alleles (A, B, C, ...) more advantageous than those prevailing in the species (a, b, c, ...), which, in the simulations, are supposed at generation zero with frequency equal to 1;

h) with independent gene transmission of any allele, i.e., the recombination fraction is assumed equal to 0.5;

i) with the mutation rate, at each generation, of an allele x into X equal to u_x and the back-mutation rate of X into x equal to w_x.

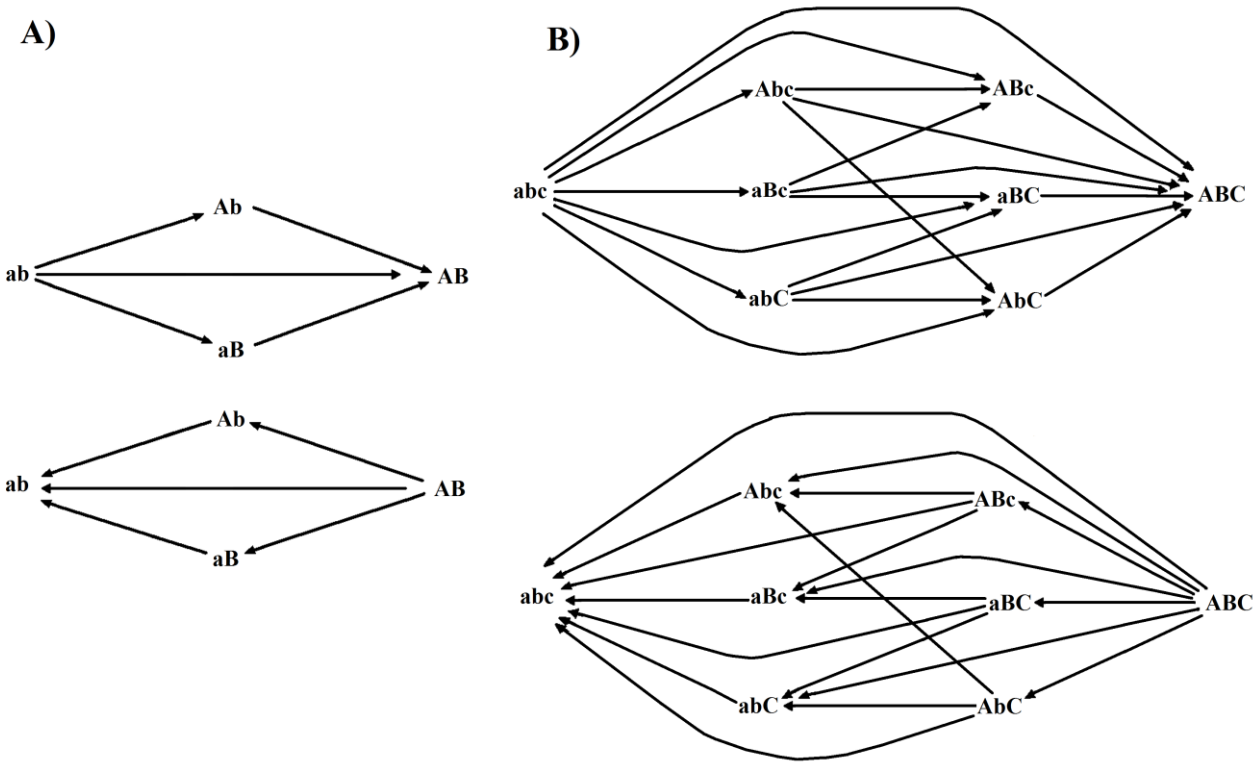


Figure 2 – A) 2G case; B) 3G case. Possible transformations of one combination into another.

The question is whether there is an advantage of sexual over asexual individuals, or vice versa, i.e., whether there is a spreading or a decay of R+.

The model is restricted to the cases of:

I) two genes (a, b) and their respective new alleles (A, B) ("2G case"), with four possible combinations (ab, Ab, aB, AB);

II) three genes (a, b ,c) and their respective new alleles (A, B, C) ("3G case"), with eight possible combinations (abc, Abc, aBc, abC, ABc, aBC, AbC, ABC).

These restrictions are not a limitation, because if sex will be proved advantageous with only 2 or 3 genes, its greater fitness will be self-evident with more genes.

For the sake of simplicity, it is hypothesized that:

$u = u_a = u_b = u_c$ (5)

$w = w_a = w_b = w_c$ (6)

$s = s_A = s_B = s_C$ (7)

With two and three genes, the possible cases of transformation by mutations from one combination into another and the probability of each transformation are indicated in Fig. 2 and Tables 1-2).

Table 1 - 2G case. Possible transformations of one combination into another and their probabilities.

From	To	Probability		From	To	Probability
aB	AB	u		aB	ab	w
Ab	AB	u		Ab	ab	w
ab	aB	$u - u^2$		AB	aB	$w - w^2$
ab	Ab	$u - u^2$		AB	Ab	$w - w^2$
ab	AB	u^2		AB	ab	w^2

Table 2 - 3G case. Possible transformations of one combination into another and their probabilities.

From	To	Probability		From	To	Probability
ABc	ABC	u		Abc	abc	w
aBC	ABC	u		aBc	abc	w
AbC	ABC	u		abC	abc	w
Abc	ABc	$u - u^2$		ABc	Abc	$w - w^2$
Abc	AbC	$u - u^2$		ABc	aBc	$w - w^2$
aBc	ABc	$u - u^2$		aBC	aBc	$w - w^2$
aBc	aBC	$u - u^2$		aBC	abC	$w - w^2$
abC	aBC	$u - u^2$		AbC	Abc	$w - w^2$
abC	AbC	$u - u^2$		AbC	abC	$w - w^2$
Abc	ABC	u^2		ABc	abc	w^2
aBc	ABC	u^2		aBC	abc	w^2
abC	ABC	u^2		AbC	abc	w^2
abc	Abc	$u - 2u^2$		ABC	ABc	$w - 2w^2$
abc	aBc	$u - 2u^2$		ABC	aBC	$w - 2w^2$
abc	abC	$u - 2u^2$		ABC	AbC	$w - 2w^2$
abc	ABc	$u^2 - u^3$		ABC	Abc	$w^2 - w^3$
abc	aBC	$u^2 - u^3$		ABC	aBc	$w^2 - w^3$
abc	AbC	$u^2 - u^3$		ABC	abC	$w^2 - w^3$
abc	ABC	u^3		ABC	abc	w^3

The fitness for individuals with two advantageous alleles (F_{XY}; XY means AB, for the 2G case; AB or BC or AC, for the 3G case) is:

$$F_{XY} = 1 + k\,[(1+s)^2 - 1] \quad (8)$$

(where $k = 1$, when there is no interaction - or epistasis - between the genes).

In the case of three advantageous alleles:

$$F_{ABC} = 1 + k^2\,[(1+s)^3 - 1] \quad (9)$$

If we indicate the frequency of combination xy in R+ individuals at the n-th generation with $P_{xy,n}$ and that in R- individuals with $P_{xy',n}$, the recombination for R+ individuals is simulated, in the 2G case, by calculating the frequencies of a, A, b, B, over the total of individuals with R+ (P_{R+}):

$$P_{a,n} = P_{ab,n} + P_{aB,n}; \ldots \quad (10)$$

and, afterwards, by using the equations:

$$P_{ab,n+1} = \tfrac{1}{2} P_{ab,n} + \tfrac{1}{2} \frac{P_{a,n}}{P_{R+,n}} \frac{P_{b,n}}{P_{R+,n}} P_{R+,n}; \ldots \quad (11)$$

The first part of the solution of each equation means that, in the recombination between individual I and another individual, in half of the cases the allele present in I does not change (see condition h). The second part means that, in the remaining 50%, the allele present in I is substituted by another allele from the other individual: the frequencies of the substituting alleles are given by the multiplication of the relative frequencies of each allele ($P_{x,n}/P_{R+,n}$), with the result multiplied for the frequency of R+ ($P_{R+,n}$).

On the contrary, for R- individuals and with $z = 0$ (see condition [c]), there is no calculation:

$$P_{ab',n+1} = P_{ab',n}; \ \ldots \quad (12)$$

In the 3G case, recombination for R+ individuals is simulated by calculating the frequencies of a, A, b, B, c, C over the total of individuals with R+:

$$P_{a,n} = P_{abc,n} + P_{aBc,n} + P_{abC,n} + P_{aBC,n}; \ldots \quad (13)$$

and, afterwards, by using the equations:

$$P_{abc,n+1} = \tfrac{1}{2} P_{abc,n} + \tfrac{1}{2} \frac{P_{a,n}}{P_{R+,n}} \frac{P_{b,n}}{P_{R+,n}} \frac{P_{c,n}}{P_{R+,n}} P_{R+,n}; \ \ldots \quad (14)$$

while for R- individuals:

$$P_{abc',n+1} = P_{abc',n}; \ \ldots \quad (15)$$

- The simulation model for finite populations

All these equations are correct in the abstract case of an infinite population, but real populations are made up of *N* individuals, with *N* a finite integer number, and are subject to random fluctuations for the number of individuals of the whole population and for each gene combination present therein.

By mutation, at each generation, an allele x may be transformed into another allele X with a probability equal to the frequency of mutation u_x. Therefore, depending on the value u_x, the frequencies of x and X at generation *n* ($P_{x,n}$ and $P_{X,n}$) are expected to pass to the frequencies $P_{x,n+1}$ and $P_{X,n+1}$ in the next generation with a difference $\Delta_x = -u_x P_{x,n}$ and $\Delta_X = +u_x P_{X,n}$ respectively.

More generally, because of mutations, advantage, recombination, genetic drift or other causes, the frequency of a combination xy is expected to pass from $P_{xy,n}$ to $P_{xy,n+1}$ in the next generation with a difference Δ_{xy} in absolute value between $P_{xy,n}$ to $P_{xy,n+1}$.

For real populations, Δ_{xy} values, multiplied by *N*, must always be integer numbers.

In the simulation model, each of these integer numbers is obtained emulating the function "rbinom" of the package R of *The R Foundation for Statistical Computing*© (http://www.r-project.org/), which generates integer random deviates.

This function is used in the program to simulate the variations of frequencies due to:

- mutations (e.g., a → A);
- back-mutations (e.g., A → a);
- advantage;
- recombination;
- genetic drift;
- diffusion of combinations among demes, when the population is not composed of a single deme (d = 1), but of several demes (d > 1) each composed of N individuals and with a mean interdemic diffusion of genes at each generation equal to f.

At each generation, the function is used several times (up to 13,000 times in 3 genes case and 100 demes).

- Results for an infinite population

With no epistasis (k = 1) and no linkage disequilibrium (D = 0), sex - as expected - is neutral with any value of u, w or s (Fig. 3).

In the figures, the value of R+ after 250 generations ($P_{R+,250}$) is 0.499997620408316 in the 2G case and 0.499998194700698 in the 3G case. The slight differences between these values and 0.5 (the frequency of R+ at generation 0) are due to the little positive linkage disequilibria caused by mutations. The frequencies of R+ and R- at generation 0 ($P_{R+,0}$; $P_{R-,0}$) have been set equal to 0.5 to give to sex and asexual individuals the same starting conditions. With any other value as well, (e.g., $P_{R+,0}$ = 0.6; $P_{R-,0}$ = 1 - $P_{R+,0}$ = 0.4), the model shows in infinite populations no significant variation from the initial frequencies of R+ and R-, as predicted by Maynard Smith [Maynard Smith 1978]. The simulations, in this and in the following figures, have been extended up to 250 generations, quite sufficient to stabilise the frequencies of combinations and R+ values.

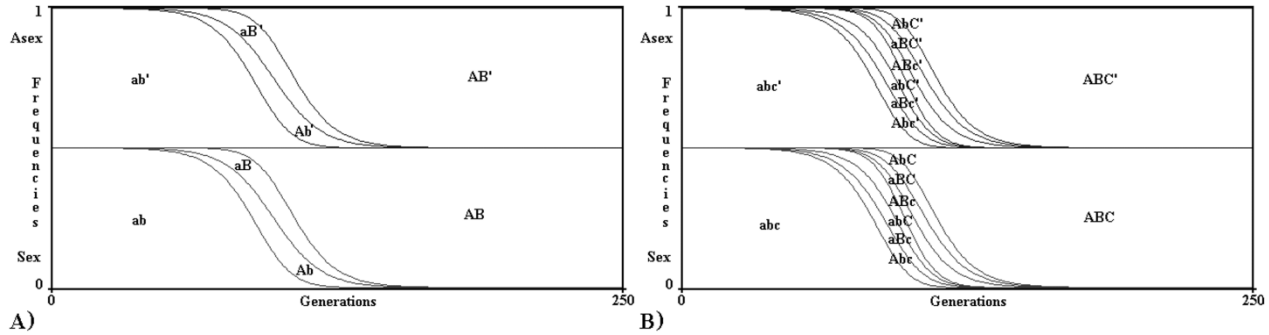


Figure 3 - A) 2G case; B) 3G case. The model shows no advantage for sex in infinite populations. The model is extremely sensible to any possible modification of the formulas shown in Table 1 or Table 2, which cause always a strong prevalence of one of the two conditions.

With any time-dependent variation of the values of s, sex is neutral too (Fig. 4), i.e., any oscillating value due to changing interactions between species cannot per se explain sex. This result needs to be remarked upon. Red Queen theory rightly underlines that biotic are likely quantitatively more important than physical factors as selective forces. From this splendid idea ("Red Queen concept"), which is undoubtedly true considering the numberless interactions between different species (predator-prey, herbivore-grass, parasite-host, host-commensal species, cooperative species, competitors for the same resources, etc.) and from the fact that in many cases these interactions cause oscillating values of the selective pressures, the theory deduces the evolutionary justification of sex [Bell 1982; Otto and Nuismer 2004; Kouyos et al. 2007].

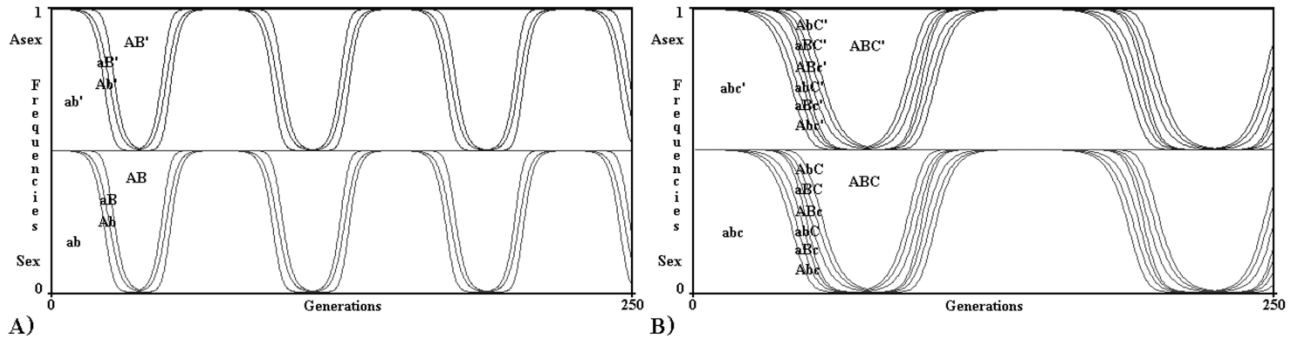


Figure 4 - A) 2G case; B) 3G case. In the simulations, *s* value oscillates from –0.1 to +0.1 every 150 generations. The model shows no advantage for sex in infinite populations if there are oscillating values of *s*.

However, the results for finite populations (see subsequent section) show that sex is advantageous, but this is in relation to the finiteness and discreteness of real populations and not to the biotic or physical character of selective pressures or to the condition of oscillating values of advantages/disadvantages. This should by no means be interpreted as a negation or diminution of the "Red Queen concept" but as a theoretical strong argument against the Red Queen hypothesis.

If k > 1 (positive epistasis), sex is disadvantageous, while, on the other hand, if k < 1 (negative epistasis), sex is advantageous. If D > 0 (positive linkage disequilibrium), sex is disadvantageous, while if D < 0, sex is advantaged (see Supplement A to this paper on the site of the journal [www.protein.bio.msu.ru/biokhimiya] and Springer site [link.springer.com/journal/10541]). However, in an unlimited population, a justification of sex as caused by prevailing conditions of negative epistasis or of negative linkage disequilibrium is unlikely.

In an infinite population, the results of the simulation model confirm the considerations obtained through analytical arguments by various authors [Felsenstein 1965; Maynard Smith 1968; Eshel and Feldman 1970; Karlin 1973].

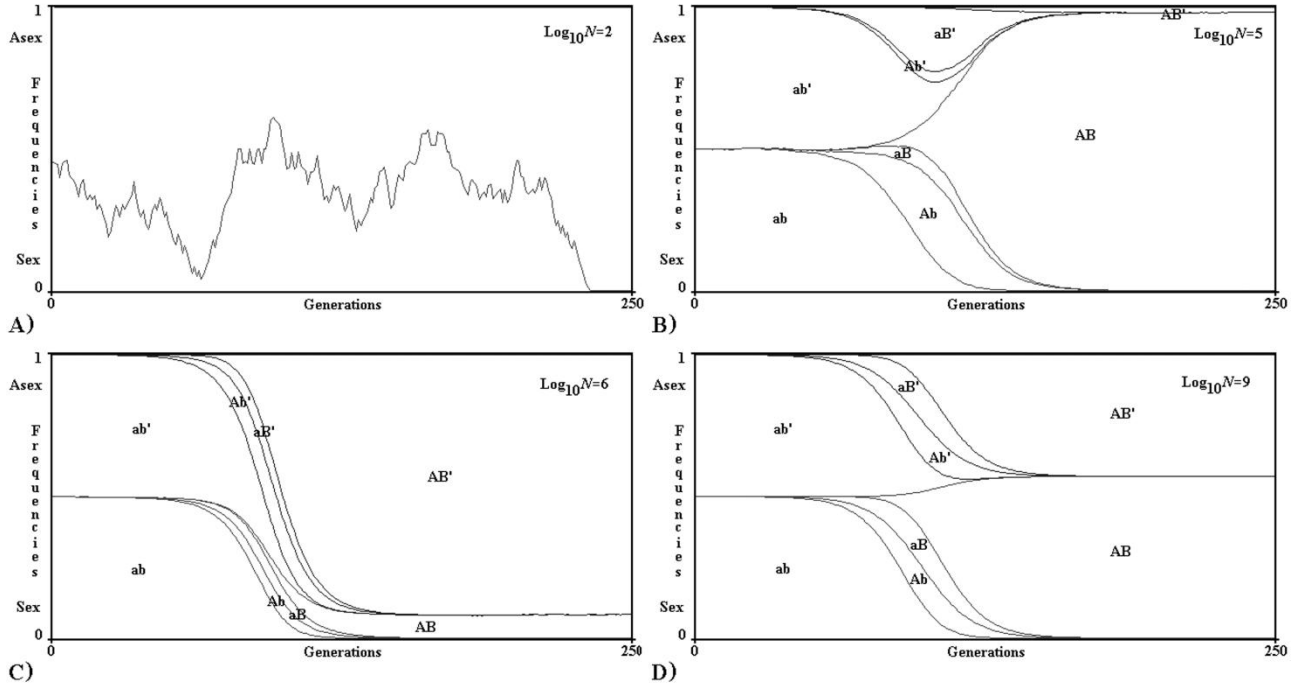


Figure 5 – 2G case, single simulations in finite populations. A) log10*N* = 2; B) log10*N* = 5; C) log10*N* = 6; D) log10*N* = 9. In the case A, only genetic drift determines the fluctuation of R+ and R- values. In the cases B, C, D, the prevalence of R+ or R- is determined by the random antecedence of mutation onset in R+ or R-.

- Results for a finite population

Examples of single simulations are illustrated in Fig. 5. With small values of N (the number of individuals), the contemporary appearance of two advantageous mutations is rare and sex cannot be favoured: prevalence of R+ or R- is determined only by genetic drift, i.e., by chance.

Figure 6 illustrates a series of simulations (1,000 for each point) with $\log_{10}N$ varying from 1 to 12 step 0.5. Mean (indicated by a square) and standard deviation (SD) are reported for each point and compared with another series of simulations (indicated by the symbol x) where R+ individuals are not allowed to recombine. Each point is marked by an asterisk if the results are significantly different ($p < 0.001$, with the t-test for two unpaired groups of data [Armitage et al. 2001]).

In the 2G case, starting from values of $\log_{10}N$ around 4, sex results generally favoured, though often it loses. With values of $\log_{10}N$ greater than 5, sex is more favoured but the advantage (difference between $P_{R+,250}$ and 0.5) becomes progressively smaller.

In the 3G case, the decline of sex advantage starts from greater values of $\log_{10}N$ and is much slower.

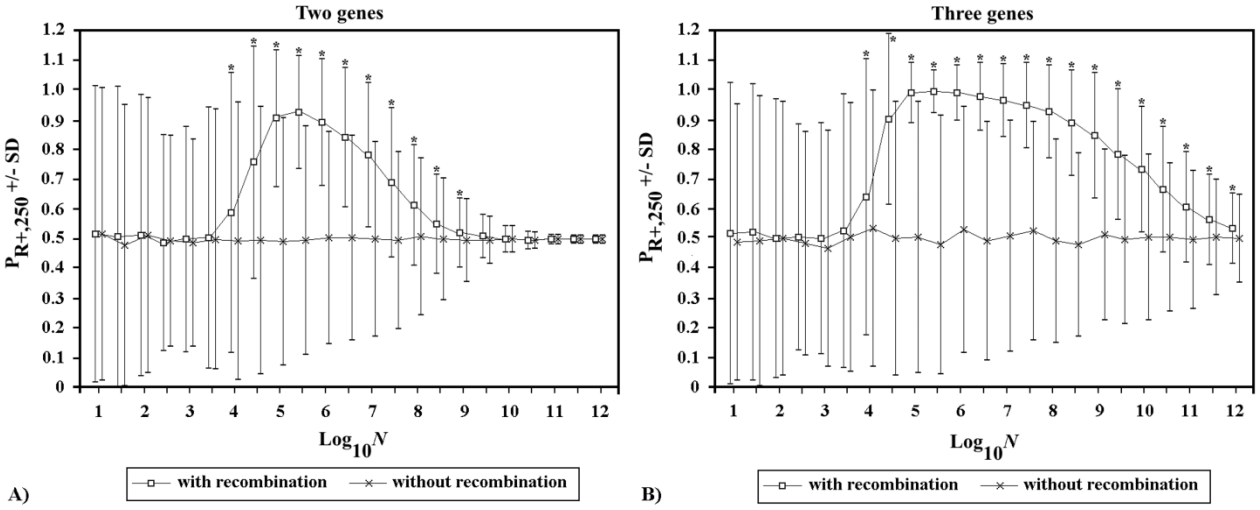


Figure 6 - Effects of recombination in finite populations. A) 2G case; B) 3G case. In these and in the following figures, if not specified otherwise: $u = w = 0.00001$; $s = 0.1$; $k = 1$; $D = 0$. Mean and standard deviation (SD) are reported for each point. For the series of simulations with recombination, an asterisk indicates a significant difference ($p < 0.001$) for each point with the corresponding point of simulations without recombination. In this and in the following figures: a) to avoid the superimposition of SD bars, the symbols of the first and of the second series have been shifted a little to the left and to the right, respectively; b) the results are always those obtained with the first runs of simulations. (Repetitions of the simulation runs for each series have given results equivalent to those of the first runs. However, these results have not substituted those of the first runs.)

In Fig. 7, the same series of simulations of the preceding figure is compared with two other simulations where, to a fraction z of R- individuals, recombination is allowed (see Supplement B to this paper on the site of the journal [www.protein.bio.msu.ru/biokhimiya] and Springer site [link.springer.com/journal/10541]) for the modifications of equations 10-15, necessary when $z > 0$). Even with small values of z the advantage of R+ over R- individuals fades.

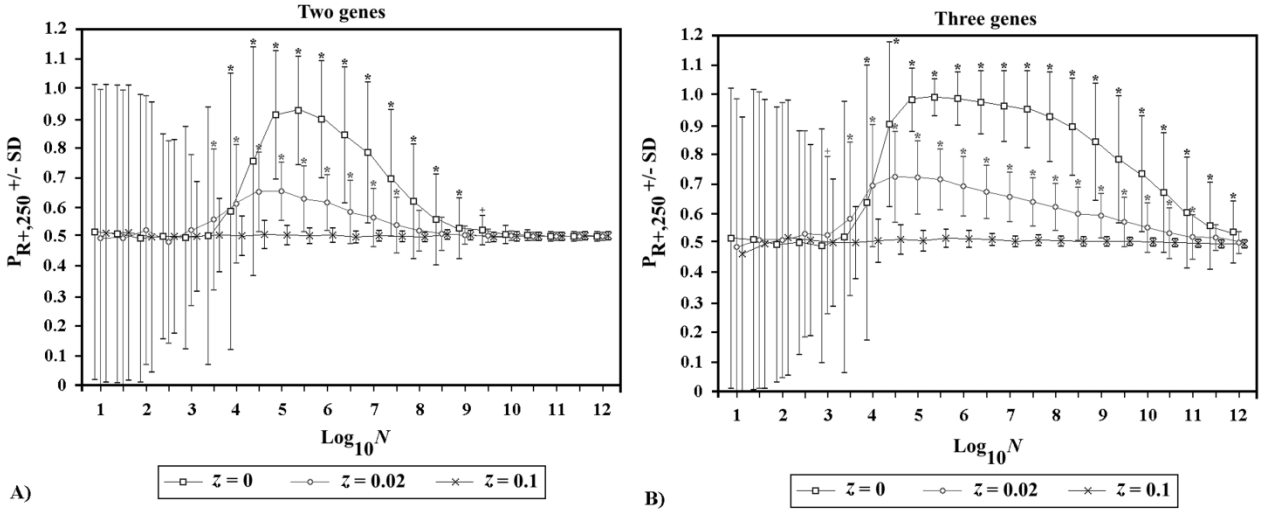


Figure 7 - Effects of the variations of *z*. A) 2G case; B) 3G case. The two series with $z = 0$ are the same as in Fig. 6 with recombination. With $z = 0.02$ the advantage for R+ individuals is greatly reduced and with $z = 0.1$ is cancelled. In these and in the following figures, an asterisk or a cross indicate a significant difference ($p < 0.001$ and $p < 0.01$, respectively) for each point versus the corresponding point of simulations without recombination in figures 6-A and 6-B.

In Fig. 8, the population (now defined as metapopulation) is divided in *d* demes, each made up of by *N* individuals, with an interdemic interchange of individuals (*f*) equal to 0.1 per generation. The results show that, for the advantage of sex, a metapopulation is equivalent to a single population of $d \cdot N$ individuals.

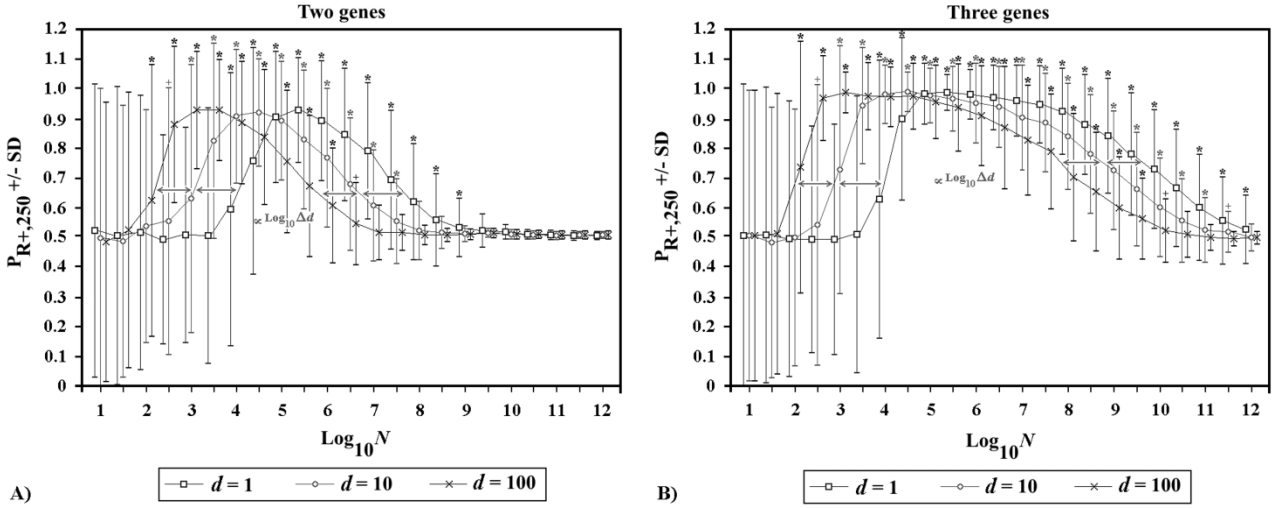


Figure 8 - Effects of the variations of *d*. A) 2G case; B) 3G case. An increase of *d* shifts both sides of the advantage curves of sex to the left in proportion to $\log_{10}\Delta d$.

- Disadvantage of sex

Sexual individuals suffer from the disadvantages deriving from the search of a mate and related to the coupling. "Amphimicts [i.e., individuals of sexual species] have one ... handicap: they must be able to find a mate, and this may be an expensive, risky and time consuming process." [Bell 1982, p. 357]). Moreover, courtship and copulation take up more precious time and involve further risks for life, which may be considered a form of optional phenoptosis [Libertini 2012a].

In some species, death during or after mating is obligatory, in particular by the so-called sexual cannibalism:

- it “occurs in various arthropod taxa” [Zuk 2016], in particular among insects, arachnids and amphipods [Polis 1981]; e.g., the males of *Araneus*, *Argiope*, *Crytophria*, species of arachnids, are killed by the female during mating [Foelix 1982];

- there are cases of sexual cannibalism in copepods and gastropods [Bilde et al. 2006].

The phenomenon, which is clearly a form of obligatory phenoptosis [Libertini 2012a] associated to mating, is considered adaptive [Zuk 2016], e.g., because “Sexual cannibalism had direct and positive effects on female fitness, as sexually cannibalistic females exhibited increased fecundity irrespective of their size, condition and foraging rate. Male consumption was almost complete and represented a relevant food intake to females. We interpret sexual cannibalism as a strategic foraging decision ...” [Fernández-Montraveta et al. 2014]

Here, these handicaps will be defined as “disadvantage of sex” (DS). Their existence allows the definition of sex as a form of phenoptosis, which is obligatory in some particular cases and optional in most cases, according to the definition of phenoptosis proposed elsewhere [Libertini 2012a].

It is important to consider the DS because it hinders the spread of sex: clearly, if it is greater than the advantage of sex, mixis cannot be favoured by natural selection.

The DS will be critically greater in severely disturbed habitats and in conditions of r-selection. As a matter of fact, in severely disturbed habitats the search for a mate is too expensive and risky. Likewise, in conditions of r-selection (and in phases of exponential growth of population) the crucial factor is reproduction swiftness, and sex, a “time consuming process” [Bell 1982], is highly disadvantageous.

Given these considerations, Gerritson’s opinion that DS is greater in conditions of low population density [Gerritson 1980] and, so: “reproduction following long-distance dispersal should be parthenogenetic” [Bell 1982, p. 357], perhaps cannot be shared, because there is no severely disturbed habitat. On the contrary, as a matter of fact: “parthenogenetics insects .... very often live in small patches of high local population density” [Bell 1982, p. 357], which is a condition of r-selection.

A comparison between the predictions of various hypotheses about the evolutionary causes of sex and the empirical data is proposed and discussed in the next section.

But, first of all, it is necessary to mention a very popular topic that is a source of misunderstandings and erroneous conclusions.

The undisputable fact that “a copy of a given gene is certain to be present in any asexual egg, but has only a 50 per cent chance of occurring in any given sexual ovum” [Bell 1982], has been described as “cost of sex” [Maynard Smith 1971] or “cost of meiosis” [Williams 1975] or “twofold selective advantage” of parthenogenesis [Maynard Smith 1978]. This creates an aura of doubt about the advantage of sex that should overcome this enormous cost (50% at each generation!) to justify its existence in evolutionary terms [Lehtonen et al. 2012].

However, in keeping with this idea, for a man who produces every day millions of spermatozoa and only every few years is successful in having from his partner a child with half of his genes, and similarly for the many species of animals and plants that spread a huge number of eggs, spermatozoa and seeds while only few of them are fertilized, or fertilize, we should speak of a “billion-fold selective cost of sex”. This type of reasoning would make absurd to find for sex an evolutionary advantage of the same weight that could justify its existence.

More correctly, the reasoning should compare the quantity of resources invested with the number of successful offspring obtained.

It is easy to show that in the particular, very simple, case of isogamous species (i.e., species with gametes of equal size), the “cost of sex” does not exist.

Indeed, in the case of an isogamous species, if m is the optimal size of a zygote, the production of a single asexual zygote of m size has a cost proportional to m. This cost is equal to the cost of two sexual gametes of size $m/2$ that, when coupled with two other gametes of the same size obtains the optimal size m for two zygotes. In both cases, a gene has the same probability of being present in a zygote (1 in the first case; $0.5 \cdot 2 = 1$ in the second case).

This is perfectly true if the relation between zygote size and viability is linear. In fact, if we symbolize survival with *s*, the relationship between zygote size and its viability can be expressed as $m = A\ s^B$, where A and B are constants and, in the model of some Authors [Parker et al. 1972; Bell 1978; Charlesworth 1978], in isogamous species, with B = 1 (linear relation between zygote size and viability) the overall cost of sex is zero, with B < 1 the cost of sex is > 0, and with B > 1 the cost of sex is < 0 [Bell 1982]. This means that for isogamous species, in particular conditions only (B < 1), there is a cost of sex, and in other conditions (B>=1) the cost of sex is an advantage or is inexistent.

So, for isogamous species the cost of sex is inexistent, although in some particular cases (when $B \neq 1$) an advantage (!) or a disadvantage could exist.

However, for most species there are many differences between female and male individuals and between their roles and investment of resources in the reproduction, which must be considered as extreme forms of anisogamy.

Well, if in the evolution from isogamy to these types of anisogamy there are certainly advantages/disadvantages caused by the second condition, they are related to anisogamy and not to sex in itself. In any case, the critical factor is not simplistically the number of DNA copies invested by each parent, but the ratio between the amount of resources invested by the two parents and the number of successful offspring.

- A comparative and experimental critique of the theories (Empirical evidence for the prediction of various theories)

As a theory is sound or unsound according to whether predictions are confirmed or falsified by empirical data, it is necessary to verify whether predictions of the "classic" hypothesis and of other hypotheses about sex evolutionary advantage are confirmed or falsified by data from natural observation.

In Table 3, the predictions of the "classic" hypothesis and those of three other theories – S3) Best-Man, S5) Tangled Bank, and S6) Red Queen - regarding the expected trends of the distribution in nature of sex and of some related phenomena are compared with evidence from natural observation.

Table 3 - Comparison between the predictions of four hypotheses on the evolutionary cause of sex and data from natural observation (Expected trends of prevalence of sex/asexual forms). Page numbers refer to Bell's book [Bell 1982].
Abbreviations: S = sexual; A = asexual; DS = disadvantage of sex; T. = thelytoky (a rare type of parthenogenesis in which females are produced from unfertilized eggs).

	PREDICTIONS OF				
	S3) Best-Man hypothesis	S5) Tangled-Bank hypothesis	S6) Red Queen hypothesis	S1) Classic hypothesis (Fisher-Muller)	Data from natural observation
PART 1: INTERSPECIFIC COMPARISON					
Correlation with different habitats (pp. 359-365)					
Freshwater, Higher latitudes, Severely disturbed environments, r-selection, Ecological periphery of a species range, Novel habitats, Recently glaciated areas, Xeric environments	S (p. 359, 364)	A (p. 359, 364)	A[1]	A[2]	A (p. 359)
Ocean, Lower latitudes, Constant environments, K-selection, Ecological centre of a species range, Ancient habitats, Unglaciated areas, Non-xeric environments	A (p. 359, 364)	S (p. 359, 364)	S[3]	S[4]	S (p. 359)
Other conditions (pp. 378-383 and 364)					
Parasitism	The same as observed	The same as observed in	The same as observed	The same as observed	S whenever possible. T.

	in nature (p. 378-383)	nature but T. is expected not rare (p. 378-383)	in nature (p. 378-383)	in nature[5]	extremely rare, more common in free-living form (p. 378-383)
Very small size of soma	S (p. 364)	-	A[1]	A[2]	A (p. 364)
Large size of soma	A p. 364)	-	S[3]	S[4]	S (p. 364)
Recombination (pp. 411-436)					
Correlation between achiasmy and Ocean, Lower latitudes, constant environment, K-selection, etc.	Expected negative (p. 433)	Expected positive (p. 433)	Expected positive (p. 433)	Not expected[6]	Not found (p. 411-35)
Correlation between chromosome number and sexual reproduction	Expected negative (p. 433)	Expected positive (p. 433)	Expected positive (p. 433)	Not expected[6]	Not found (p. 411-35)
Correlation between crossing over frequency and sexual reproduction	Expected negative (p. 433)	Expected positive (p. 433)	Expected positive (p. 433)	Not expected[6]	Not found (p. 411-35)
PART 2: INTRASPECIFIC COMPARISON					
Intermittent sexuality (pp. 365-370)					
During growing season (exponential growth of population)	A (p. 365)	- (p. 367)	-	A[2]	A (p. 368)
Before climatic changes	S (p. 365-366)	- (p. 367)	-	-	Not related (p. 367)
At times of minimal population density	S (p. 368)	A (p. 367)	A[1]	A[2]	A (p. 367)
At times of high population density	A (p. 368)	S (p. 367)	S	S[4]	S (p. 367)
Elicitation of sex in laboratory (pp. 370-371)					
Signals of a change in environment	Sex elicited (p. 370)	-	-	-	Not related (p. 370-371)
Crowding and starvation in constant conditions	-	Sex elicited (p. 371)	Sex elicited[3]	Sex elicited[4]	Sex elicited (p. 370-371)
Dispersal and dormancy (pp. 371-777)					
Actively dispersing stage	S (p. 371)	S (with some reservation) (p. 371)	-	S[7]	S (p. 373)
Dormant stage	S (for most Best-Man models) (p. 377)	S/A (p. 377)	-	S[7] (A if the change of environment conditions is abrupt[8])	S/A (p. 371-377)

Note:
*[1] Because of a smaller interspecific competition;
*[2] Because of a greater DS;
*[3] Because of a greater interspecific competition;
*[4] Because of a smaller DS;
*[5] As DS is likely to be small in parasitic phase and great in free-living phase;
*[6] As there is no likely related DS difference;
*[7] There is no particular reason to suppose a greater DS;
*[8] With an abrupt change of environment conditions a greater DS is likely;

		Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	Classic hypothesis
Totals:	Differences	12	4	3	0
	No prediction	1	5	5	2
	Concordances	3	7	8	14

This paragraph has the same name as chapter 4 of Bell's book [Bell 1982] and has in common with it aims, methods and predictions for the Best-Man, Tangled-Bank and Red Queen hypotheses, and references to data from natural observation. The predictions for the aforesaid hypotheses are identical to those expounded by Bell, but in some cases, in the absence of Bell's opinion, a prediction, explained in an appropriate note, has been proposed.

The predictions of the "classic" hypothesis may be formulated by using one simple criterion: as the theory and the simulation model maintain and show that sex - disregarding DS - is always advantageous except in small and isolated populations, sex is predicted to be always favoured except for the above-mentioned populations and when DS is important (severely disturbed environments, r-selection, phases of exponential growth of population, etc.). Moreover, because DS does not exist as regards recombination, no correlation between certain phenomena of recombination (achiasmy, chromosome number, and crossing over frequency) and amphimixis or parthenogenesis is expected.

In various cases, the predictions of the "classic" hypothesis and those of other hypotheses coincide, although the motivations are different (e.g., predictions of the "classic" hypothesis and those of the Red Queen for "Correlation with different habitats").

The noteworthy result appears to be a strong correspondence between predictions of the "classic" hypothesis and data from natural observation.

The utter failure of the Best-Man hypothesis is remarkable and Bell's negative opinion on this theory must be shared. In Table 3, it is a plain example of a hypothesis in almost constant contradiction with data from natural observation.

For the Tangled-Bank and the Red Queen hypotheses, there are the wrong predictions of correlation between certain phenomena of recombination and amphimixis/parthenogenesis ("Amphimixis is to parthenogenesis as high rates of recombination are to low; the correlates of low levels of recombination will therefore be the same as the correlates of parthenogenesis." [Bell 1982]), a significant contradiction described and underlined by Bell in ch. 5.2 [Bell 1982]. On the other hand, since for such phenomena as achiasmy, frequency of crossing over and number of chromosomes intrinsically DS does not exist, a correlation between these phenomena and parthenogenesis is not predicted by the "classic" hypothesis, in accordance with data from natural observation [Bell 1982].

Moreover, for the Tangled-Bank hypothesis, the prediction for parasitism is not completely adequate, as Bell underlines [Bell 1982].

As regards other theories that are not considered in the table:

S2) Muller's Ratchet hypothesis - It could justify sex only for small populations as "Muller's ratchet operates only in small or asexual populations ... harmful mutations are unlikely to become fixed in sexual populations unless the effective population size is very small." [Keightley and Otto 2006]. Therefore, this theory, which is not contradicted by the results reported in this paper, could integrate the "classic" theory;

S4) Hitch-hiker hypothesis - An R+ gene could be described as a gene that is advantageous because it hitchhikes favourable genes that are better spread by its action. The hitch-hiker hypothesis could, therefore, be defined as a different and indirect way of expounding the "classic" theory;

S7) Historical hypothesis - This theory, which does not justify sex existence, is refuted by the evidence that sexual or asexual reproduction is influenced by many conditions. However, if it is considered not a theory explaining sex but as an inertial factor restraining an easy passage from sexual to asexual reproduction, or vice versa, it should deserve a certain amount of attention;

S8-S10) These hypotheses make no prediction.

Williams proclaimed [Williams 1975]: "... the unlikelihood of anyone ever finding a sufficiently powerful advantage in sexual reproduction with broadly applicable models that use only such general properties as mutation rates, population sizes, selection coefficients, etc." (p. 14), and

Ridley wrote [Ridley 1993]: “I asked John Maynard Smith, one of the first people to pose the question ‘Why sex?’, whether he still thought some new explanation was needed. ‘No. We have the answers. We cannot agree on them, that is all.’ ” (p. 29).

Perhaps, now Williams’ unlikelihood is a likelihood and the uncertainty of Maynard Smith has been solved. By theoretical arguments, the advantage of sex is rationally explained by the “classic” theory in terms of individual selection and using only the “general properties ...” that Williams considered necessary [Williams 1975]. Moreover, if we consider the DS, it is possible to formulate predictions about the trends of the diffusion of sexuality in nature that are confirmed by evidence from natural observation.

For small populations, Muller’s Ratchet hypothesis, if confirmed, could reinforce and integrate the “classic” theory. Historical hypothesis deserves attention as an inertial factor in the prediction of trends of diffusion of sex and related phenomena.

The correct concept that biotic factors - which should often determine oscillating *s* values - are quantitatively more important than physical factors as selective forces in determining evolution (Red Queen concept), the pivotal idea at the roots of the Red Queen theory, is not at all against the “classic” theory, although it is insufficient in itself to explain sex, and should be considered an argument that reinforces the “classic” hypothesis.

Somehow, the “pluralist approach to sex and recombination” [West et al. 1999] seem to be the correct solution, but with this specification: “classic” theory is the trunk with the main branches while some of the other theories complete the tree.

Aging

As regards aging, here precisely defined as “increasing mortality with increasing chronological age in populations in the wild” [Libertini 1988], there are many theories [Comfort 1979; Medvedev 1990; Weinert and Timiras 2003; Libertini 2015a], which can be classified in two broad groups based on opposed paradigms:

- the first (“old paradigm”) interprets aging as the cumulative and progressive effect of many damaging factor that are insufficiently opposed by natural selection;
- the second (“new paradigm”) explains aging as a physiological function determined by natural selection in terms of supra-individual selection, although opposed by individual selection.

Within the old paradigm, there are the following hypotheses:

O1) *Cessation of somatic growth h.* - For this old theory, when the growth shows no limit, as in many lower vertebrates, the organism is always young and there is no aging. On the contrary, for species with a fixed growth, aging begins when the formation of new tissues ends [Minot 1907; Carrel and Ebeling 1921a; Brody 1924; Bidder 1932; Lansing 1948, 1951].

O2) *Damage accumulation h.* - This is a vast group of old and new theories that explain aging as caused by the accumulation of various types of damage (“wear and tear”, deteriorations in cell colloids, degeneration of nervous/endocrine/vascular/connective/other specific organs or tissues, toxic metabolites, damaging substances produced by gut bacteria, cosmic rays, etc. [Comfort 1979],

chemical damage due to DNA transcription errors [Weinert and Timiras 2003], oxidative effects by free radicals on the whole body [Harman 1972; Croteau and Bohr 1997; Beckman and Ames 1998; Oliveira et al. 2010], on the DNA [Bohr and Anson 1995; Weinert and Timiras 2003], or on the mitochondria [Miquel et al. 1980; Trifunovic et al. 2004; Balaban et al. 2005; Sanz and Stefanatos 2008]).

O3) *Mutation accumulation h.* - Aging is caused by many noxious genes insufficiently removed by natural selection as they manifest their actions late in life [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987; Partridge and Barton 1993].

O4) *Antagonistic pleiotropy h.* - Hypothetical pleiotropic genes, which are advantageous in the younger ages and disadvantageous in the older ages, are insufficiently eliminated by natural selection and so cause a progressive fitness decline [Williams 1957; Rose 1991].

O5) *Disposable soma h.* - It is assumed that the organism has limited - although not well defined - resources. Therefore, natural selection must divide them between the necessities of reproductive capacity and those of the maintenance systems, and the maintenance at older ages is sacrificed [Kirkwood 1977; Kirkwood and Holliday 1979].

O6) *Quasi-programmed aging h.* [Blagosklonny 2006] - This theory is a variation of the preceding hypothesis: "nature blindly selects for short-term benefits of robust developmental growth ... aging is a wasteful and aimless continuation of developmental growth" [Blagosklonny 2013].

O7) *Historical h.* - In analogy with the historical hypothesis for sex, a species could be more or less precociously aging, or even non-aging, if it belongs to a group of species, or to a phylum, where there is the same condition [De Magalhães and Toussaint 2002].

Within the new paradigm:

N1) *Aging as phenoptotic phenomenon* - Aging appears to be under genetic determination and modulation and so must be somehow adaptive [Skulachev 1997; Bredesen 2004; Mitteldorf 2004; Longo et al. 2005]. Aging is a particular type of a well-known group of phenomena [Finch 1990], for which the neologism "phenoptosis" was coined [Skulachev 1997], which defines the cases when an individual sacrifices itself, or its siblings, obligatorily or potentially, by mechanisms that are favoured by natural selection at a supra-individual level [Skulachev 1997, 1999a; Libertini 2012a]. However, this is a pivotal observation about aging, but not a specific hypothesis about the causes of aging.

N2) *Aging is useful because it frees space for the next generation* – Wallace, the co-author of the first paper on evolution by natural selection, proposed that aging was favoured by selection because predeceasing individuals do not compete with their offspring [Wallace 1865-1870; Skulachev and Longo 2005]. Likewise, Weissmann suggested, without any evidence, that aging was favoured by evolution as the death of old individuals frees space for the next generation and this was useful to accelerate evolution [Weismann 1889], but later he repudiated this idea [Weismann 1892; Kirkwood and Cremer 1982].

N3) *Aging as increasing evolvability factor h.* - Following Weismann's suggestion, aging has been considered as favoured by natural selection because it increases the speed and so the potentiality of evolution, or evolvability [Goldsmith 2004, 2008a].

N4) *Red Queen h. for aging* - A proposed explanation for aging is that it would limit the spread of diseases caused by other living beings, by analogy with Red Queen hypothesis of sex [Mitteldorf and Pepper 2009], which, however, although very popular, appears to be untenable as shown by the simulation model and the evidence of the preceding section.

N5) *Aging as caused by mitochondrial oxidative substances h.* - Mitochondrial ROS are interpreted as the pivotal cause of aging explained as a phenomenon favoured by natural selection [Skulachev and Longo 2005; Skulachev 1999b, 2001].

N6) *"Chronomeres" and "printomeres" h.* - Hypothetical "chronomeres" and "printomeres" have been proposed as pivotal parts of programmed aging mechanisms [Olovnikov 2003, 2015]),

N7) *Aging as accelerating factor of evolution h.* - In 1961, Leopold, a botanist, posed again Weismann's suggestion that aging accelerates generation turnover and so favor evolution: "… in plants senescence is a catalyst for evolutionary adaptability" [Leopold 1961]. In 1983 and later, starting from the observation that an increase of the advantage (*S*) of a gene or an equivalent reduction of the mean duration of life (*ML*) have the same effect on the spreading of a gene within a species (Figure 9), the advantage of aging in spatially structured populations and in terms of kin selection was proposed [Libertini 1983, 1988, 2006, 2008, 2009a, 2013]. An analogous idea of aging advantage in spatially structured populations was later proposed by using more complex

models [Travis 2004; Martins 2011; Mitteldorf & Martins 2014]. However, the first model is much simpler and easily understandable. It maintains that a gene C that determines a shorter *ML* is favoured when:

$$\sum_{x=1}^{n} [r\, S_x\, (1/ML_C - 1)] > S' \qquad (16)$$

where: ML_C = shorter *ML* of the individuals with C (while the individuals with the neutral allele C' have $ML_{C'} = 1$); S_x = advantage of an x allele that is spreading within the species; m = number of the alleles that are spreading within the species; r = coefficient of relationship between the individual that dies by action of C and the substituting individual; S' = advantage of the greater *ML* of the individuals with the neutral allele C'.

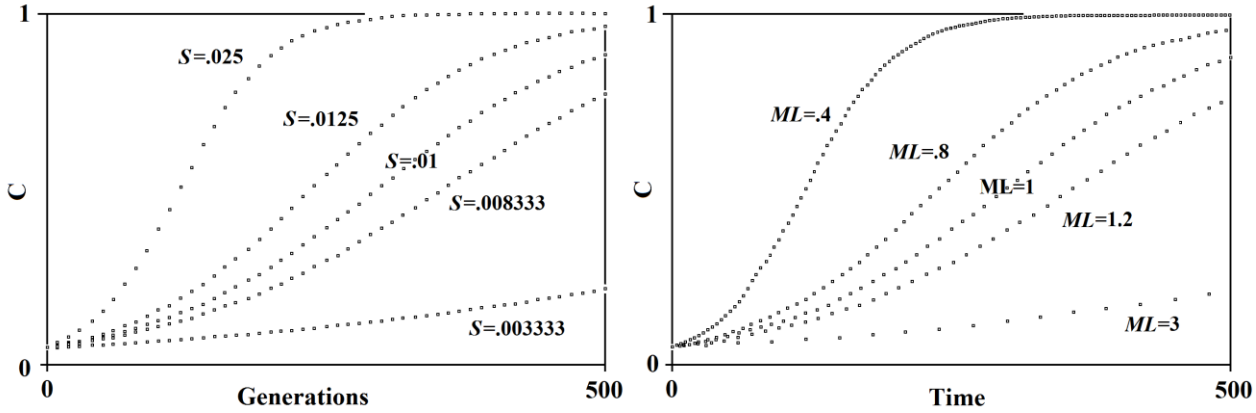


Figure 9 - On the left: spreading of the gene C according to the variation of *S*, if *ML* is supposed constant; on the right: spreading of the gene C according to the variation of *ML*, is *S* is supposed constant. The simulation programs for Figures 9 and 10 are available in the Supplementary materials (see Supplement A to this paper on the site of the journal [www.protein.bio.msu.ru/biokhimiya] and Springer site [link.springer.com/journal/10541]).

If the m alleles that are spreading within the species have a mean advantage S_m, the formula (16) becomes:

$$m\, r\, S_m\, (1/ML_C - 1) > S', \qquad (17)$$

which has been used for Figure 10.

Moreover, if C is not a favourable gene ($S > 0$) but a harmful gene ($S < 0$), the same mechanism causes a quicker elimination of C.

It is necessary to highlight that formulas (16) and (17) are equivalent to formula (10) used in the original work [Libertini 1988]:

$$r\, S\, (1/V_C - 1) > S', \quad \text{i.e.,}\ r\, S\, (1/ML_C - 1) > S',$$

where it was "hypothesized S » S', since S sums up the advantages of the m genes that are spreading within the species." By disregarding this essential specification, now included in the formulas, the model has been wrongly considered as unlikely [Kowald and Kirkwood 2016].

It is possible to highlight some common predictions for the hypotheses of the new paradigm: i) possible existence of non-aging species; ii) in the comparison among different species, inverse relationship between extrinsic mortality and *ML* reduction caused by senescence (Ricklefs' P_s, or

proportion of senescent deaths [Ricklefs 1998]); and iii) existence of specific, genetically determined and modulated mechanisms that cause aging [Libertini 2008].

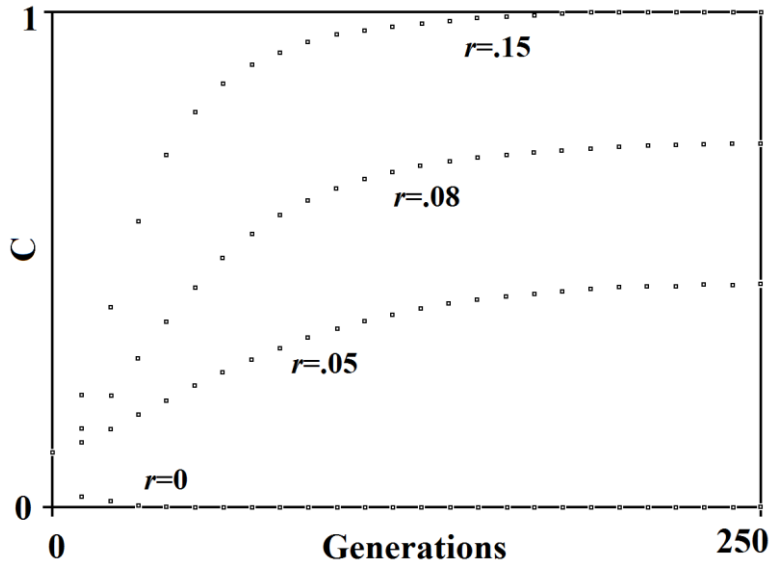


Figure 10 – Spreading or elimination of a gene C that determines a reduced *ML* (ML_C = .7). The starting value of C is .1. Moreover: S_m = .01; *m* = 600; *S'* = .2. The values of *r* are indicated near each curve.

The arguments and the evidence that are in support of or against the two paradigms have been discussed in another work [Libertini 2008] and for brevity will not be repeated here. However, considering it and adding the evaluation of the *Historical hypothesis* (O7), it is easily possible to organize Table 4 that shows the correspondences or non-correspondences of the predictions of various hypotheses (O1-O7, N7). The other hypotheses are not considered in the table for the following motives:

- N1 (*Aging as phenoptotic phenomenon*) is the main concept of the new paradigm but is not a specific hypothesis;
- N2 (*Aging is useful because it frees space for the next generation*) may be considered an old and not well defined formulation of N7;
- N3 (*Aging as increasing evolvability factor h.*): If aging accelerates generation turnover, this certainly increases the speed and the potentiality of evolution, i.e., the evolvability. However, the proposed effect of aging should not be interpreted as the cause of it. For each function, it is correct to search its meaning (teleonomy), but it is wrong to attribute a finalistic aim to this meaning (teleology). In other words, N3 should be considered a very important comment on the effects of aging and not a hypothesis about the evolutionary cause of aging.
- N4 (*Red Queen h. for aging*), N5 (*Aging as caused by mitochondrial oxidative substances h.*), and N6 (*"Chronomeres" and "printomeres" h.*) do not appear to give specific predictions that may be tested.

The table shows that the predictions of N7 appear to be in perfect concordance with empirical data and theoretical arguments, while all the other hypotheses are clearly falsified. Only the old *Cessation of somatic growth h*. (O1) and the *Historical h.* (O7) show a partial match with empirical data and appear even less unacceptable that the three theories (O2, O3 and O4) currently presented as the solid scientific explanation of aging.

Table 4 - Correspondences or non-correspondences between empirical data or theoretical arguments and the prediction of various hypotheses.
Abbreviations: O1 = *Cessation of somatic growth h.*; O2 = *Damage accumulation h.*; O3 = *Mutation accumulation h.*; O4 = *Antagonistic pleiotropy h.*; O5 = *Disposable soma h.*; O6 = *Quasi-programmed aging h.*; O7 = *Historical h*; N7 = *Aging as accelerating factor of evolution h.*;
N = not explained or predicted by the hypothesis or in contrast with its predictions; - = irrelevant for accepting/rejecting the hypothesis; Y = predicted by the hypothesis or compatible with it.

Theories:	O1	O2	O3	O4	O5	O6	O7	N7
1) Absence of aging in many species	Y	N/-	N/-	N/-	N/-	N/-	Y	Y
2) Great variation of aging rates among aging species	Y	N/-	N/-	N/-	N/-	N/-	Y	Y
3) Effects of caloric restriction on lifespan	-	-	-	-	N	-	-	Y
4) Damage of aging for the senescing individual but its advantage in terms of supra-individual selection	-	N	N	N	N	N	-	Y
5) Existence of age-related fitness decline under natural conditions	Y	N	N	N	N	N	Y	Y
6) In the comparison among different species, *ML* reduction caused by deaths due to intrinsic mortality (P_s) inversely proportional to extrinsic mortality	N	N	N	N	N	N	N	Y
7) Impossibility of explaining age-related fitness decline as a consequence of genes that are harmful at a certain age	-	-	N	-	-	-	-	Y
8) Age-related progressive decline of cell turnover capacities	N	N	N	N	N	N	N	Y
9) Gradual cell senescence	N	N	N	N	N	N	N	Y
10) Cell senescence (replicative senescence + gradual cell senescence to the highest degree)	N	N	N	N	N	N	N	Y

Totals:									
	N	4	6	7	6	7	6	4	0
	N/-	0	2	2	2	2	2	0	0
	-	3	2	1	2	1	2	3	0
	Y	3	0	0	0	0	0	3	10

Comparison between sex and aging

In the comparison between sex and aging, despite the huge differences between the two phenomena, it is possible to highlight some interesting similarities.

Both phenomena are not favoured by natural selection in populations numerically unlimited. Similarly, in conditions where there is high mortality and the need for rapid reproduction, mostly definable as conditions of r-selection, the disadvantages that are inherent to each of the two phenomena are greater.

Therefore, species that reproduce asexually and species that do not age are predicted and exist.

As regards sex, there are species that may alternate sexual and asexual phases and the second alternative is preferred in r-selection conditions. For aging, there are not species with similar alternation of phases, but in particular conditions, e.g. caloric restriction and other conditions, individuals of aging species appear to slow down the aging process [Calabrese and Baldwin 1998; Masoro 2003, 2005, 2007; Calabrese 2005; Ribarič 2012, Lee and Min 2013], a phenomenon that is

incompatible with the theories that interpret aging as due to the action of damaging factors more or less effectively opposed.

Both phenomena, in order to be understood in their evolutionary dynamics, require an analysis in terms of supra-individual selection.

Both phenomena increase the evolutionary potentiality of a species, i.e., the evolvability, however by two different mechanisms: sex accelerates the achievement of the best genetic combinations, aging accelerates the spreading of favourable genes and the elimination of harmful genes. In any case, the greater evolvability must not be seen as the factor that determines the evolution of the two phenomena: a greater evolvability is the effect and not the cause of the mechanisms, favoured by supra-individual selection, which at each generation favours the genes determining sex or aging.

As regards aging and sex as phenoptotic phenomena:

- Aging is a type of obligatory phenoptosis. It is a particular type of phenoptosis with a slow and progressive fitness decline and so defined as "slow phenoptosis" [Skulachev 2002b].

- In general, sex does not imply obligatory death, but reduces the fitness as a consequence of the dangers caused by partner search, courtship, mating, etc. Most of this fitness reduction is connected not so much to the sex itself but to sexual differentiation. In some cases, sexual differentiation involves the sacrifice of one of the partners at the time of mating, or after, or at the time of reproduction. Therefore, in general sex may be classified as optional phenoptosis although there are cases of obligatory phenoptosis.

The analogies and differences between sex and aging are summarized in Table 5.

Table 5 - Analogies and differences between sex and aging.

Sex	Aging
It is favoured by natural selection as, in numerically finite populations, it allows the easier achievement of advantageous genetic combinations.	It is favoured by natural selection as, in numerically finite populations separated into demes, it allows the faster spreading of favourable genes and the faster elimination of harmful genes.
It is not favoured in numerically unlimited populations.	It is not favoured in numerically unlimited populations.
It is favoured in condition of K-selection.	It is favoured in condition of K-selection.
For species that may alternate sexual and asexual phases, r-selection conditions favour the asexual stages.	Under certain adverse conditions (e.g., caloric restriction), it is possible to observe a reduction of aging rate.
By comparing similar species, some of which with sexual reproduction and others with asexual reproduction, asexual ones are favoured in r-selection conditions.	By comparing similar species, a greater environmental mortality favours a delay of aging or non-aging species.
It increases the so-called evolvability.	It increases the so-called evolvability.
Its advantage is evident only in terms of supra-individual selection.	Its advantage is evident only in terms of supra-individual selection.
It involves disadvantages that imply an increase in mortality and so it is classifiable as optional phenoptosis. However, in some cases connected with the sexual differentiation, there is obligatory phenoptosis.	It involves a progressive increase in mortality and therefore is a phenomenon classifiable as obligatory phenoptosis, subtype slow [Skulachev 2002b; Libertini 2012a].
Many species do not show sex or have alternative forms of amphimixis [Bell 1982].	Aging is not universal. Indeed, in an overall assessment of all living species, aging, although common in vertebrates, is shown only by a minority of the living species [Jones et al. 2014; Libertini et al. 2017].

Conclusion

Both sex and aging are sometimes described as biological mysteries as regards their evolutionary justification.

However, if we analyze both phenomena in terms of supra-individual selection, as absolutely necessary for any phenoptotic phenomenon, the advantages and disadvantages of the two phenomena appear clear and without any aura of mystery. The analysis of their advantages (in particular, in terms of supra-individual selection), together with the analysis of their disadvantages (in particular, at the individual level), enables predictions about the conditions under which each of the two phenomena is favoured or opposed by natural selection. These predictions, in the general evaluations of this review, appear to have full confirmation by the evidence.

Another element that derives from the analysis in terms of supra-individual selection is that the cases of individual sacrifice, i.e., the cases of phenoptosis, also in its various forms for sex or aging (obligatory and slow, optional, obligatory and rapid, etc.), appear normal phenomena and not anomalies that require always explanations as strange exceptions.

Chapter 14

Libertini G, Rengo G, and Ferrara N (2017) Aging and aging theories. *Journal of Gerontology and Geriatrics*, 65, 59-77.

Aging and Aging Theories

Giacinto Libertini, Giuseppe Rengo, Nicola Ferrara

Abstract

Several theories have sought to explain aging, here precisely defined as "increasing mortality with increasing chronological age in populations in the wild". They all fall within one of two opposite and incompatible paradigms. For the first ("old paradigm"), aging is the result of degenerative phenomena that natural selection cannot counteract completely, due to insufficient strength or opposing selective pressures. For the second ("new paradigm"), aging is favoured by natural selection in terms of supra-individual selection: it belongs to a broader category of phenomena, on the whole defined as "phenoptosis", which are explicable only in terms of supra-individual selection. For the new paradigm, aging is a specific function that is genetically determined and regulated, with its own physiology, pathology and phylogeny. This paper describes the theoretical arguments and the empirical evidence that support or are in contrast with each of the two paradigms. Subsequently, on the basis of an imposing and authoritative amount of research, aging mechanisms at the cellular and organismal levels are described. The clear existence of such mechanisms is indispensable proof to support the new paradigm and is in complete and unsolvable contrast with the old paradigm.

Introduction

- Definition of aging

Aging is here defined as "increasing mortality with increasing chronological age in populations in the wild", or "IMICAW" [Libertini 1988], a definition that is analogous to others such as "actuarial senescence" [Holmes and Austad 1995] and "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [Kirkwood and Austad 2000] with the essential difference that these do not have the condition "in the wild".

It is essential that this condition is present and explicit because its absence may lead to false conclusions. In fact, let us consider a species that shows no mortality increase in the wild, but under protected conditions, e.g., in captivity, may reach ages, which are non-existent in nature, where there is evidence of an age-related increasing mortality (e.g., see below the case of the spider *F. pyramitela*). For the first definition, this species does not age; for the other two definitions, the species may be considered as subject to aging. However, a death rate increase that is not present in the wild and is shown, only under protected conditions, at ages which are non-existent in the wild cannot be subject to natural selection. So, its causes cannot be an explanation for the increase in mortality shown by other species under natural conditions.

It is also important to have full awareness that aging, as described in the first definition, exists and that this is well documented from a long time [Deevey 1947; Laws and Parker 1968; Spinage 1970, 1972; Finch 1990; Holmes and Austad 1995; Ricklefs 1998], for our species too [Hill and Hurtado 1996] (Figure 1). The existence of the phenomenon has been minimized and deemed insignificant ("there is scant evidence that senescence contributes significantly to mortality in the wild" [Kirkwood and Austad 2000], "senescence-associated increases in age-related mortality . . . even where they are observed, they contribute only to a relatively small fraction of deaths within the population" [Kirkwood and Melov 2011]), but Ricklefs highlighted that senescence reduces average

life span up to “almost 80%” [Ricklefs 1998] and, later, a meta-analysis highlighted the evidence of aging in 175 animal species on the basis of 340 separate studies [Nussey et al. 2013].

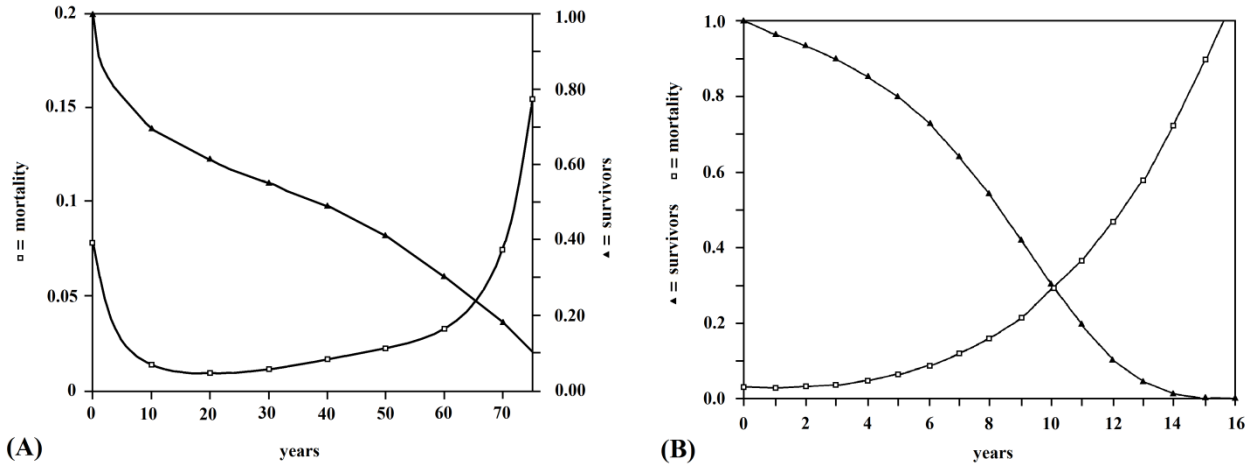


Figure 1 - Some examples of aging. A: *Homo sapiens* (Ache population, data from [Hill and Hurtado 1996]); B: *Pantera leo* (data from [Ricklefs 1998]); for both species, observations in the wild.

- Classification of aging theories

Among the many theories that try to describe the causes of aging [Comfort 1979; Medvedev 1990; Weinert and Timiras 2003; Libertini 2015a], a first possible distinction is between non-evolutionary and evolutionary theories. The theories of the first group are formulated without any consideration of the natural selection as possible factor that somehow affects aging. Within this group there are almost all of the oldest hypotheses, including those explaining aging as a result of progressive wear and tear. In the second group, there are theories that in various ways try to reconcile their explanations of aging with the mechanisms of natural selection.

A more interesting distinction is between two different and opposing interpretations:

1) aging is a non-programmed phenomenon; it is a set of degenerative phenomena that natural selection cannot contrast completely due to insufficient strength or opposing selective pressures;

2) aging is a programmed phenomenon; it is caused by mechanisms genetically determined and programmed that, despite being harmful to the individual, are in some way advantageous in terms of supra-individual natural selection.

As the contrast between the two interpretations is strong and complete and does not appear solvable by some form of compromise, the two interpretations have the value of opposite paradigms, in the sense of the term defined by Kuhn [Kuhn 1962].

All non-evolutionary theories, and a large part of the evolutionary theories, refer to the first interpretation, defined as “old paradigm”. It includes a significant number of hypotheses according to which aging is caused by the progressive accumulation of damage of various types and the consequent fitness impairment. In the older theories, the phenomenon is conceived without any consideration of the evolutionary mechanisms, i.e., with the implicit assumption that natural selection is irrelevant for this phenomenon [Minot 1907; Carrell and Ebeling 1921a; Brody 1924; Bidder 1932; Lansing 1948, 1951]. Some less old theories consider natural selection and propose that the damaging mechanisms are poorly contrasted by selection, (i) as few individuals survive at older ages; (ii) for the constraints imposed by genes with pleiotropic effects; (iii) for the limits caused by other physiological needs [Medawar 1952; Williams 1957; Hamilton 1966; Edney and Gill 1968; Harman 1972; Kirkwood 1977; Kirkwood and Holliday 1979; Miquel et al. 1980; Mueller 1987; Rose 1991; Partridge and Barton 1993; Bohr and Anson 1995; Croteau and Bohr 1997; Beckman and Ames 1998; Trifunovic et al. 2004; Balaban et al. 2005; Blagosklonny 2006,

2013; Sanz and Stefanatos 2008; Oliveira et al. 2010]. For all the hypotheses of the old paradigm, aging: (i) is not favoured by natural selection; and so (ii) cannot have specific mechanisms genetically determined and regulated that determine it. Furthermore, as aging is seen as a set of degenerative processes, the term "aging" must be considered as a useful word to summarize the overall effects of heterogeneous phenomena: aging as a distinct entity does not exist. According to this paradigm, which is currently dominant: (i) in the present International Classification of Diseases [ICD-9-CM 2016; ICD-10 2016], there is no code for aging; (ii) aging as a distinct cause of death is excluded and, for the international official statistics of the World Health Organization, aging as a distinct cause of death is left out [World Ranking Total Deaths 2014].

Only some of the evolutionary aging theories refer to the second interpretation, defined as the "new paradigm". They interpret aging as a physiological phenomenon, determined and regulated by specific genetically programmed mechanisms, which are favoured by natural selection as advantageous in terms of supra-individual selection despite the disadvantages caused by them on the individuals [Weismann 1889; Leopold 1961; Kirkwood and Cremer 1982; Libertini 1983, 1988, 2006, 2009a, 2012a, 2013; Skulachev 1997, 1999a, 1999b, 2001; Goldsmith 2004, 2008a; Mitteldorf 2004; Travis 2004; Longo et al. 2005; Skulachev and Longo 2005; Mitteldorf and Pepper 2009; Martins 2011; Mitteldorf and Martins 2014]. It is intrinsic to this conception that the aging mechanisms must have (i) a physiology; (ii) a pathology; and (iii) a phylogeny.

- Some basic concepts

Some essential premises are necessary for the subsequent discussion.

A) Subjects of aging theories

It is essential to make a distinction about the specific topics of aging theories. In fact, a first subject is the explanation of the "why" of aging in evolutionary terms and another subject is the "how" of aging. For the theories that attempt to explain aging without considering evolutionary mechanisms, this distinction does not exist, and the "why" and the "how" are the same thing. Even for some of the theories that try to take into account the mechanisms of evolution but attribute aging to an insufficient selection against damaging factors, the distinction between the "why" and the "how" is weak or non-existent. On the contrary, for other evolutionary theories the discussion about the "why" is clearly distinct from the discussion about the "how".

B) Various descriptions of natural selection

In its most famous and popular simplification, natural selection is "the survival of the fittest" of Spencer [Spencer 1864], an expression adopted later by the same Darwin ("Natural Selection or the Survival of the Fittest" [Darwin 1869]), i.e., in modern terms, the preferential spreading of the genes of individuals who are fittest to survive and reproduce.

This may be expressed by a simple formula that tells us the condition for which a gene (C) is favoured by natural selection:

$$S \cdot P > 0, \quad (1)$$

where: S = advantage caused by the expression of C; P = reproductive value of the individual at the age when C is expressed.

In a more general conception, natural selection operates in terms of kin selection [Hamilton 1964, 1970; Trivers 1971; Wilson 1975]. It is necessary to consider the inclusive fitness of a gene (C) whose action has effects not only on the individuals I_1, where C exists, but also in individuals I_2, I_3, . . . I_n, which are related with I_1 and for which there is a probability that C is in the genome equal to the coefficient of kinship (r) between I_x and I_1. Therefore, C will be favoured by natural selection when:

$$\sum_{x=1}^{n} (S_x \cdot P_x \cdot r_x) > 0 \quad (2)$$

Clearly, when n=1, as r_1=1, formula (2) becomes formula (1), and so individual selection is only a particular case of kin selection.

Now, as already discussed in another paper [Libertini 2015b], if we consider a species,

- subdivided into monoclonal demes and subjected to catastrophic events that cause a disadvantage *S* for every individual;
- in which, by action of a gene (C), among the n individuals with C, some (n_d) sacrifice themselves and die (S_d = -1) while the survivors (n_s) have an advantage S_s;
- for the sake of simplicity, the reproductive value is assumed to be constant at any age (P_x=1),

by considering that in a monoclonal deme r_x=1, the formula (2) becomes:

$$\sum_{x=1}^{n_d} S_d + \sum_{x=1}^{n_s} S_s > S \cdot n, \text{ that is: } n_d \cdot S_d + n_s \cdot S_s > S \cdot n \quad (3)$$

Moreover, if we suppose that in the deme there are several clones (1, 2, ..., z) and C exists in all the individuals of the first clone, the probability that C is in the individuals of a clone x is equal to the coefficient of kinship between the individuals of clone x and those of clone 1 (r_x), and C will be favoured by natural selection if:

$$(n_{1,d} \cdot S_d + n_{1,s} \cdot S_s) + (n_{2,d} \cdot r_2 \cdot S_d + n_{2,s} \cdot r_2 \cdot S_s) \ldots . + (n_{z,d} \cdot r_z \cdot S_d + n_{z,s} \cdot r_z \cdot S_s) > S \quad (4)$$

where, in a clone x: $n_{x,d}$ = the individuals that sacrifice themselves; $n_{x,s}$ = the survivors.

By considering these particular conditions, and certainly other possible cases, the inclusive fitness formula is transformed into equations that describe how C could be favoured in terms that are definable as group selection.

As a further significant example, the social organization (eusociality) of haplodiploid species such as ants, bees and wasps was described for many years as a result of mechanisms of kin selection [Wilson 1971, 1975], but later, together with the eusociality of other non-haplodiploid species such as termites, bathyergid mole rats, etc., "the standard natural selection theory in the context of precise models of population structure", which includes "multilevel selection", was considered a better and more fruitful explanation [Nowak et al. 2010]. Also in this case natural selection is always the same phenomenon but is studied in different conditions and through different mathematical models.

This shows that individual selection, kin selection and at least certain types of group selection are always natural selection but under different conditions or with a different descriptive approach. Moreover, this means that some old arguments against group selection as a possible valid form of natural selection [Maynard Smith 1964, 1976; Williams 1966] should be reconsidered. The key concept is that if we exclude individual selection, all the other descriptions of natural selection can be described by the comprehensive term "supra-individual selection": the substantial difference between these two categories of natural selection is that individual selection cannot justify a gene that is detrimental to the individual, while, in contrast, supra-individual selection may favour, under particular conditions, genes that are harmful or even fatal for the individual.

C) The concept of "phenoptosis"

Apart from the cases of eusociality, these theoretical considerations have a sure confirmation in a wide range of phenomena in which an individual sacrifices himself, or a closely related individual, through the direct or indirect effect of genes favoured by natural selection, in terms of supra-individual selection. These phenomena, although very common and well known for a long time (see the chapters: "Rapid Senescence and Sudden Death" and "Gradual Senescence with Definite Lifespan" in Finch's 1990 textbook [Finch 1990]), until a few years ago did not have a general term that defined them. Skulachev proposed this needed definition at the end of the nineties: "Phenoptosis [is] the programmed death of an individual" [Skulachev 1997, 1999a], and afterwards this concept has been extended to the sacrifice of related individuals ("Phenoptosis is the death of an individual caused by its own actions or by actions of close relatives ... and not caused primarily by accidents or diseases or external factors, which is determined, regulated or influenced by genes favoured by natural selection." [Libertini 2012a]).

Aging, seen as an event that is favoured and determined by natural selection, falls into the category of phenoptotic phenomena and was indeed defined by the same Skulachev as "slow phenoptosis" [Skulachev 2002b, 2010].

D) Non-universality of aging

A widespread belief is that aging, as before precisely defined (age-related mortality increase in the wild), is a phenomenon shown by all living species with few exceptions. In contrast, the natural observation shows us that aging is shown only by a small number of species, ours included, although these species are among those most familiar to us. A recent work has shown among the numberless species an incredible variety of life tables or age patterns of mortality [Jones et al. 2014], in particular species with no age-related mortality increase (Figure 2).

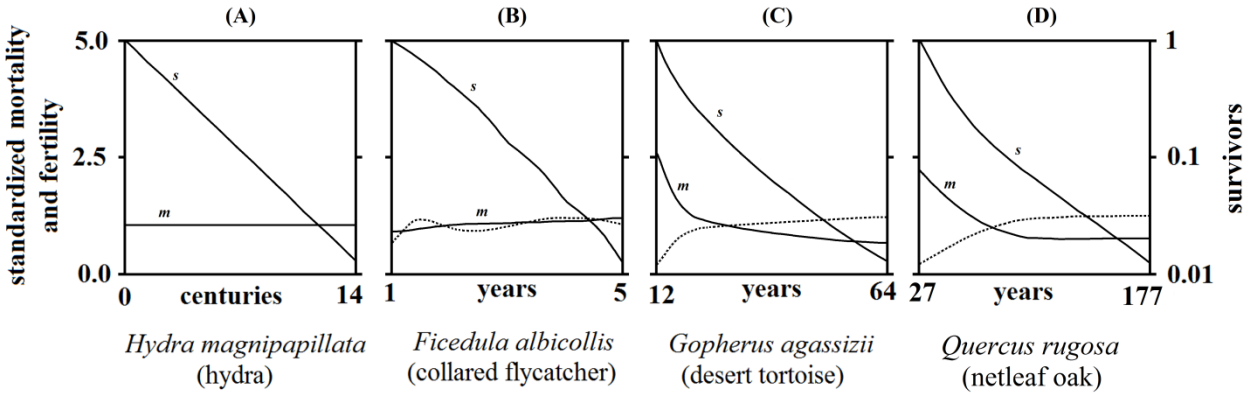


Figure 2 - Some examples of life tables of non-aging species (partial and redrawn Figure 1 of [Jones et al. 2014]). Solid lines indicate standardized mortality (*m*) and survivorship (*s*), the dotted lines the standardized fertility. (A) and (B) are cases of "negligible senescence", (C) and (D) are examples of "negative senescence". In (A), mortality and fertility lines overlap.

In fact, some species show "no observable increase in age-specific mortality rate or decrease in reproduction rate after sexual maturity; and . . . no observable age-related decline in physiological capacity or disease resistance" [Finch and Austad 2001] (e.g., rockfish, sturgeon, turtles, bivalves and possibly lobsters [Finch and Austad 2001]). They have been defined species "with negligible senescence" [Finch 1990]. Indeed, individuals of these species do not grow old but this is difficult to admit for some current theories (see below): the aforesaid expression is a prudent way of saying that they could also grow old but the pace is so slow as to be undetectable.

In particular species, there is even an age-related decrease in mortality. These are species whose death rate would be constant at all ages except that the age-related increase in body size causes less vulnerability to predation and then reduces mortality. The definition "negative senescence" has been coined for them [Vaupel et al. 2004], but, perhaps more correctly, we should consider these species as a particular type of species with "negligible senescence".

Other species do not age, but, at the time of reproduction, their individuals suddenly undergo rapid degenerative processes that cause imminent death (e.g., many Anguilliformes and Salmoniformes, some rodents and dasyurid marsupials, many plants, in particular monocarpic angiosperms [Finch 1990]). This type of phenomena, defined by Finch as "sudden senescence" [Finch 1990] is quite distinct by aging as before defined.

Many species are congenitally incapable of being able to live more than a short time. "Aphagy from defective mouthparts or digestive organs is very common during the adult phases of insects (Weismann, 1889b; Metchnikoff, 1915; Norris, 1934; Brues, 1946; Wigglesworth, 1972; Dunlap-

Pianka et al., 1977) and is *the* limiting factor in the adult lifespan of many short-lived species." [Finch 1990].

Other species, including many insects and spiders, in the wild have high mortality and show no age-related increase in mortality during their short lives (e.g., under natural conditions, the lifespan of *Frontinella pyramitela* ("bowl and doily" spider) is less than 30 days and shows no age-related increase in mortality). However, under laboratory conditions, at ages that are non-existent in the wilds, this spider shows an age-related increase in mortality that is strongly conditioned by the amounts of available food [Austad 1989] (Figure 3). As this mortality increase happens only under artificial conditions, it is outside the definition of aging.

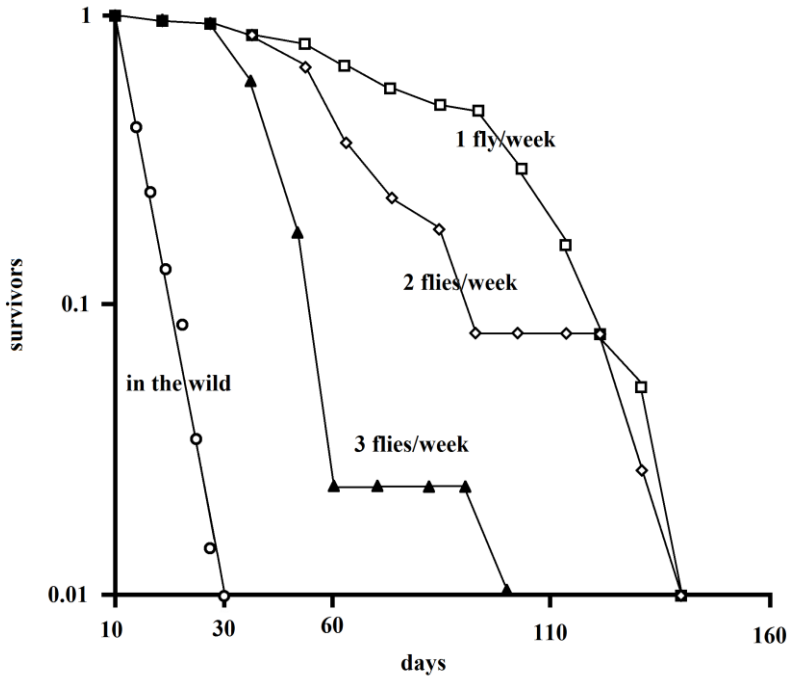


Figure 3 - Survival of *Frontinella pyramitela* in the wild (circles) and in laboratory in different feeding conditions: 1 fly/week (squares); 2 flies/week (rhombs); 3 flies/week (triangles); data from [Austad 1989].

It is possible to indicate other particular cases but, for the sake of brevity, we refer to the cited work [Jones et al. 2014]. However, a consideration is necessary and due. If we weigh the enormous number of species that do not age, and consider that aging occurs in a minority of species, we must agree as a matter of fact that aging is not an inevitable and almost universal condition but, on the contrary, a peculiar condition of a limited number of species.

The "why" of aging

- Non-programmed aging theories

The "classical" evolutionary theories that try to explain aging are three and are all within the old paradigm. The first, *mutation accumulation hypothesis*, explains aging as the combined effect of many harmful genes that act later in life and are insufficiently removed by natural selection [Medawar 1952; Hamilton 1966; Edney and Gill 1968; Mueller 1987]. A simple theoretical argument against this hypothesis has been proposed for a long time [Libertini 1983, 1988] and proposed again [Libertini 2009b, 2015a], but no one has attempted to invalidate it.

In short, if we have a gene (C) that is harmful and causes a disadvantage *s*, with a neutral allele (C') and a mutation frequency from C' to C equal to *v*, it is possible to obtain the equilibrium frequency between mutations C' -> C and their elimination by natural selection. From this equilibrium frequency we calculate the frequency of the phenotypic expression of the gene (P_e) both in the case that C is recessive:

$P_e = v/s$ (1)

and in the case that C is dominant:

$P_e \approx v/s$ (2).

The details of this calculation are explained elsewhere [Libertini 2009b].

Now, let us hypothesize genes that are harmful, by a value *s*, at time t and with no effect on preceding ages. As these genes ("t-genes") are harmful only for the survivors at time t (Y_t), natural selection contrast them in function of $s \cdot Y_t$ and the equations (1) and (2) become:

$P_e \approx v/(s \cdot Y_t)$ (3).

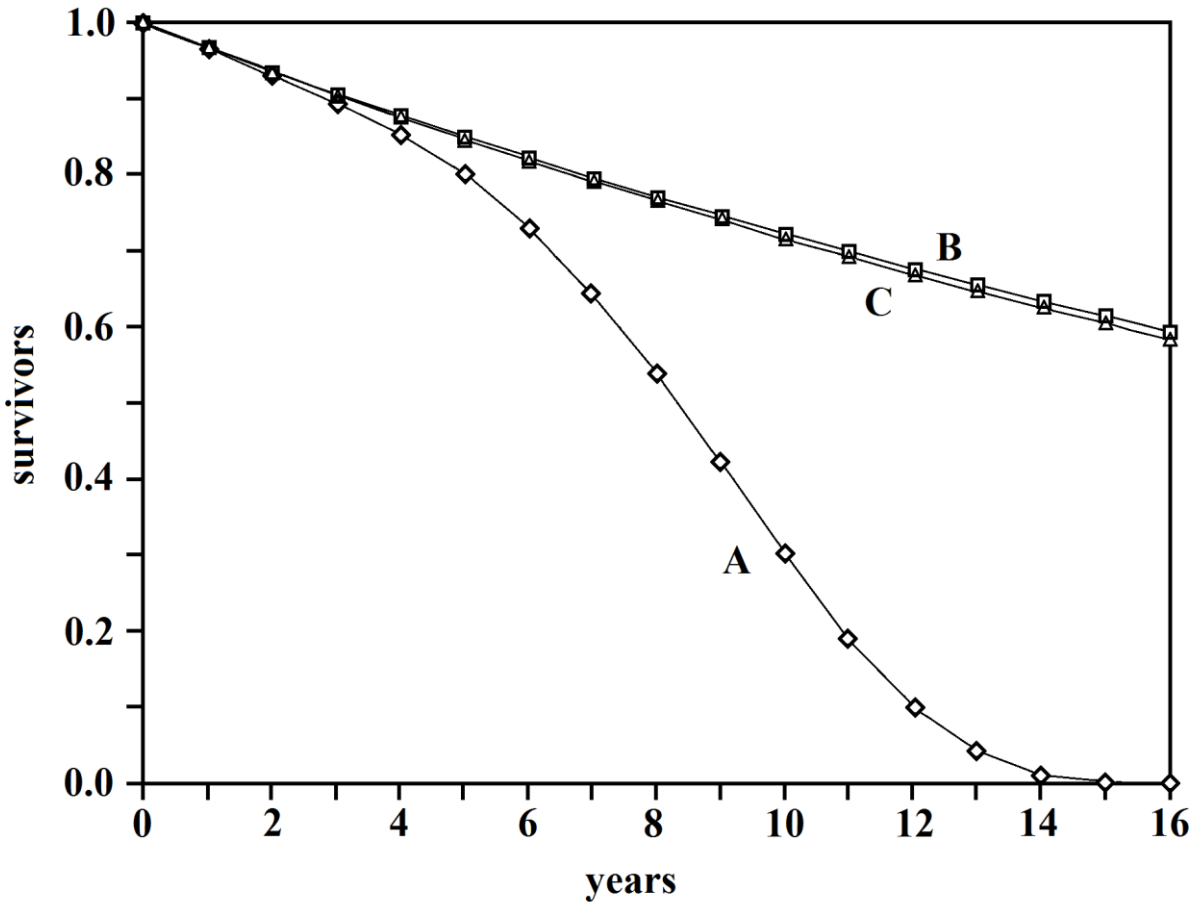


Figure 4 - Hypothetical effects of a great number of t-genes on a life table. Curve A (rhombs): life table of *Panthera leo* in the wild; the death rate is described by Weibull's equation ($m_t=m_0+\alpha t^{\beta}$) with the values m_0=.032; α=.000252; β=3 given by Ricklefs [Ricklefs 1998]. Curve B (squares): a life table with constant mortality equal to m_0 of *Panthera leo*. Curve C (triangles): the curve B plus the effects of many t-genes (n=1000; *v*=.000001).

In a population with a death rate (λ) that is constant at any age, namely, a non-aging population, the life table is obtained from the simple equation:

$Y_{t+1} = Y_t \cdot (1 - \lambda)$ (4).

By supposing n t-genes that act at time t, as many t-genes that act at t+1, and so on, and that, for the sake of simplicity, the harm caused by each of these has always the value *s*, the survivors at t+1 will be:

$$Y_{t+1} = Y_t \cdot (1 - \lambda - n \cdot s \cdot P_e) \approx Y_t \cdot (1 - \lambda - n \cdot v / Y_t) \quad (5).$$

This equation (5) is independent from the value of *s* and, as the value of *v* is small, the decrease in Y from t to t+1 will be notable only with small values of Y_t.

Curve C in Figure 4 shows the effects of a great number of t-genes (n=1000) on a life table with a constant death rate (curve B). Curve C is completely different from that of a real population (curve A), which, in the first ages, has the same mortality as the other two curves, but afterwards shows a progressive age-related increase in mortality.

The second of the "classical" theories, the *antagonistic pleiotropy hypothesis* [Williams 1957; Rose 1991], postulates the existence of many genes that are harmful at older ages but advantageous at earlier ages. Therefore, natural selection contrasts them only in part, and organisms grow old.

The third theory, the *disposable soma hypothesis* [Kirkwood 1977; Kirkwood and Holliday 1979], postulates the existence of mechanisms that are useful and advantageous at the young or adult stage but harmful at later ages. The body must economize resources, which are not well defined by the theory, and so natural selection, by these mechanisms, operates a compromise in the allocation of resources, which must be divided between reproduction or other physiological needs and the preservation of soma integrity that would allow for greater longevity.

These two theories are not vulnerable to the theoretical argument presented earlier. However, all the three classical hypotheses, together with those that explain aging as caused by the accumulation of harmful effects, do not explain the huge variability of aging rates in the comparison among species and do not justify in any way the existence of species in which the death rate is constant at any age. Perhaps *ad hoc* hypotheses could try to explain: (i) why the mechanisms proposed act to varying degrees depending on the species; (ii) why they do not act at all in some species. However, a theory cannot be considered plausible if it is built on postulates and *ad hoc* assumptions.

There is also another strong argument against any hypothesis of aging interpreted as non-programmed phenomenon.

In the formulation of the first theory that hypothesized aging as planned and favoured by natural selection, it was proposed that the supra-individual advantage of aging originated from the reduction of the mean duration of life (ML). It followed from this that, in case of major extrinsic or environmental mortality, the hypothesized advantage caused by ML reduction was lower and therefore the proportion of deaths due to aging could be reduced. Therefore, in a paradoxical way, the theory stated that extrinsic mortality and ML reduction caused by aging had an inverse relationship [Libertini 1983, 1988]. Subsequently, it was observed that this prediction should be valid for all theories that propose aging phenomenon as planned and favoured by natural selection [Libertini 2008]. In particular: "... senescent mortality tends to complement background mortality. Both contribute to the population turnover rate, and thus to evolvability ... [the] relationship between background death rate and evolved senescence is characteristic of adaptive theories of aging. A high background death rate leads to a longer evolved life span. This contrasts with classical theories, in which a high background death rate leads to a shorter evolved life span." [Mitteldorf and Martins 2014].

The three classic hypotheses, and, implicitly, also the non-evolutionary theories of aging, formulate the opposite prediction. According to these hypotheses, since aging is countered, though insufficiently, by natural selection, the increase in extrinsic mortality weakens natural selection, and therefore aging should be accelerated. So, a direct relationship between mortality and extrinsic aging rates is predicted: "The principal determinant in the evolution of longevity is predicted to be the level of extrinsic mortality. If this level is high, life expectancy in the wild is short, the force of selection attenuates fast, deleterious gene effects accumulate at earlier ages, and there is little selection for a high level of somatic maintenance. Consequently, the organism is predicted to be

short lived even when studied in a protected environment. Conversely, if the level of extrinsic mortality is low, selection is predicted to postpone deleterious gene effects and to direct greater investment in building and maintaining a durable soma." [Kirkwood and Austad 2000].

However, in 1998, Ricklefs' data on populations studied in the wild showed that the inverse relationship predicted by the hypothesis of aging as a programmed phenomenon was true [Ricklefs 1998] (Figure 5).

This plain contradiction between the empirical data and the predictions of the three classical theories was underlined by Ricklefs [Ricklefs 1998] and was subsequently deepened [Libertini 2008]. However, for this contradiction, there remains no satisfactory explanation that might be compatible with the aforementioned classical theories and with non-adaptive theories of aging.

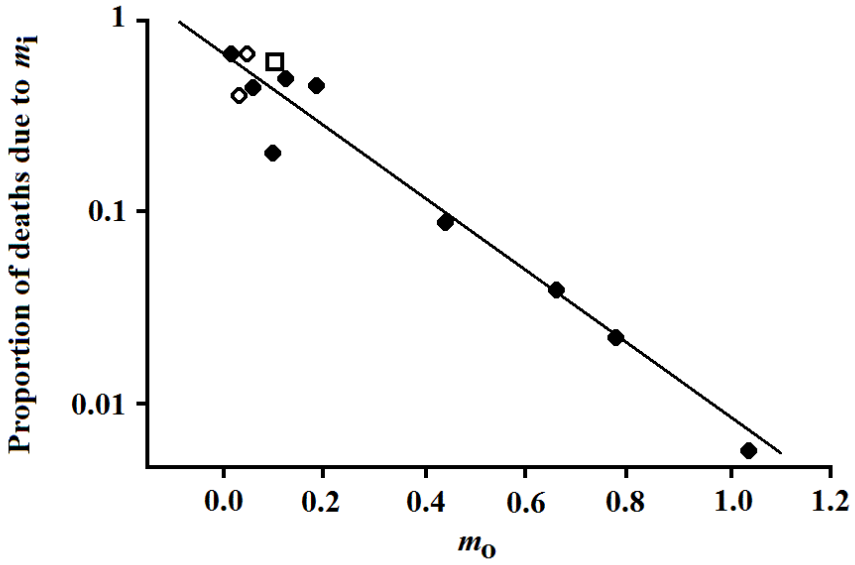


Figure 5 - Inverse relationship between m_0 and the proportion of deaths due to m_i. Solid diamonds refer to bird species, open diamonds to mammal species, open square to *Homo sapiens*; ordinates are in logarithmic scale. Data for mammals and birds are from [Ricklefs 1998], table 2; data for *H. sapiens* are from [Hill and Hurtado 1996]. Without the data of our species, the linear regression has the following values: r = -.758708, t = -3.494043, $p < 0.01$ [Libertini 2008].

- Programmed aging theories

Alfred Russel Wallace, who co-authored the first paper on the theory of evolution through natural selection with Charles Darwin, was also the first who, in 1865-1870, proposed that aging was programmed because individuals who die as a consequence of aging do not compete with their offspring [Wallace 1865-1870; Skulachev and Longo 2005]. Likewise, August Weissmann, in 1889, hinted that aging was somehow favoured by natural selection because the death of old individuals frees space for the younger generations and so for the spread of new genes [Weismann 1889; Kirkwood and Cremer 1982], but a few years later, he dismissed this idea [Weismann 1892, Kirkwood and Cremer 1982].

In 1961, a botanist proposed again the argument that senescence accelerates generation turnover and so "... in plants senescence is a catalyst for evolutionary adaptability" [Leopold 1961].

In 1988, after an anticipation in a non-peer reviewed book [Libertini 1983], a theory was proposed that explained aging as adaptive in spatially structured populations and in terms of kin selection because it accelerated evolution [Libertini 1988]. This hypothesis, which was later reaffirmed [Libertini 2006, 2008, 2009a, 2013], starts from the following consideration.

The spread within a species of a favourable gene (C) with an advantage *s*, is a function of both *s* and the speed of generation turnover, which is inversely proportional to the mean duration of life (*ML*) of the individuals. If *s* is multiplied for x or if *ML* is divided by x, we will have exactly the same effect on the spreading of C (Figure 6).

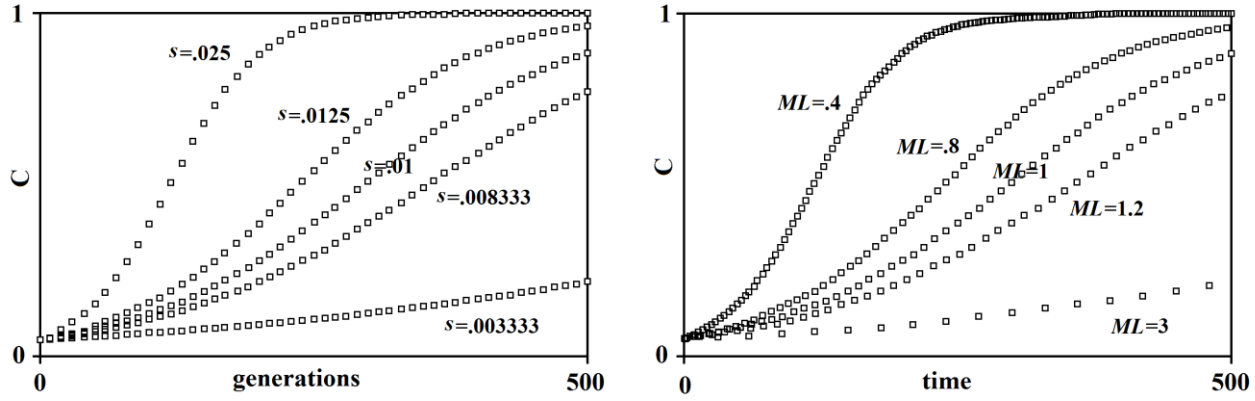


Figure 6 - On the left: spreading of C according to the variation of *s* (while *ML* = 1); on the right: spreading of C according to the variation of *ML* (while *s* = 0.01); C_0=.05. Redrawn from figures 2 and 3 in [Libertini 1988], which are the same of figures I 2-1 and II 2-1 in [Libertini 1983].

So, a shorter ML has the great advantage of a higher spreading diffusion for all favourable genes (and also a quicker elimination of all unfavourable alleles), but also entails the disadvantages that result from the shorter ML (which are increased by a greater body mass and a greater duration of the physical and neurological maturation periods). However, it was noted that, in populations divided into small groups of related to each other individuals and in condition of demographic saturation (i.e., k-selection [Pianka 1970]), the advantage would overcome the disadvantages and a hypothetical gene (C) determining a reduced *ML* (ML_C < 1) would be favoured by selection against a neutral allele C' (with $ML_{C'}$ = 1) if:

$$r \cdot S \cdot (1/ML_C - 1) > S' \quad (6)$$

where: *r* = coefficient of relationship among the individuals of the group; *S* = summation of the advantages of all the favourable genes that are spreading; *S'* = summation of the disadvantages for the individual caused by a reduced *ML*.

In the following years, some theories also proposed that aging was favoured by natural selection in spatially structured populations [Travis 2004; Martins 2011; Mitteldorf and Martins 2014]. In fact, these new contributions proposed again the same advantage for aging that resulted from a faster gene spreading but by using more sophisticated models of population genetics.

However, the first and the new theories predicted that in the case of populations not divided into groups, or those with unlimited dispersal, the aging genes were not favoured by natural selection (e.g.: "In a freely mixing population with global dispersal, evolution selects for individuals with ever-increasing life span." [Travis 2004])

Another theory, in 2009, explained aging as a defence against the spread of infective diseases, analogous to the Red Queen hypothesis on the advantages of sexual reproduction [Mitteldorf and Pepper 2009]. Later, following Weismann's insight, it was highlighted that aging increases evolvability, i.e., the speed of evolution, and so it is favoured by natural selection [Goldsmith 2004, 2008a]. In possible harmony with the idea that aging is adaptive and programmed, damage by mitochondrial ROS has been proposed as the essential mechanism [Skulachev 1999b, 2001; Skulachev and Longo 2005]. In other papers, although a specific theory about aging is not formulated, the idea that this phenomenon is adaptive and programmed is backed with various topics [Skulachev 1997, 1999a; Mitteldorf 2004; Bredesen 2004; Longo et al. 2005].

Despite the substantial differences among the various hypotheses about aging interpreted as an adaptive and programmed phenomenon, in 2008, some possible common predictions were highlighted: (i) the existence of non-aging species; (ii) among different species, an inverse relationship between the proportion of senescent deaths and extrinsic mortality; (iii) the existence of genetically determined and regulated mechanisms for aging. Moreover, it was highlighted that: the point (i) was difficult or impossible to explain by many non-programmed aging theories; and the points (ii) and (iii) were incompatible with them [Libertini 2008].

Regarding the various life table types, it is possible to highlight some general distinctions between old and new paradigm hypotheses, which are summarized in Table 1 and in Figures 7A and 7B.

Table I - Some distinctions between old and new paradigm.

Abbreviations: IMICAW = "increasing mortality with increasing chronological age in populations in the wild" [Libertini 1988]; IMICAC = "increasing mortality with increasing chronological age in populations in captivity (i.e., under protected conditions at ages non-existing in the wild)" [Libertini 1988].

	Species that ...	for the old paradigm ...	for the new paradigm ...
1	show IMICAW;	**this is the primary or most primitive condition;**	this is a particular evolved condition that is favoured only under particular ecological conditions;
2	do not show IMICAW or, prudentially, are defined as "with negligible senescence" [Finch 1990];	these are exceptions that must be explained;	**this is the primary or most primitive condition, not exceptions that must be explained;**
3	do not show IMICAW and, in certain periods of the life, even show a decreasing mortality;	these are exceptions that must be explained;	this is a variant of the primary condition, determined by particular causes (e.g., an increment in body mass that reduces predation);
4	do not show IMICAW, show very high mortality, very short life spans and IMICAC;	these are not exceptions because show IMICAC (which is not distinguished from aging);	these are non-aging species and IMICAC cannot have an evolutionary meaning because cannot be determined by natural selection;
5	do not show IMICAW, but in a certain phase, e.g. in reproduction, show a sudden death;	this is a particular type of aging and the absence of IMICAW is disregarded;	these are not aging species and their death is a form of phenoptosis, i.e. an adapted condition;

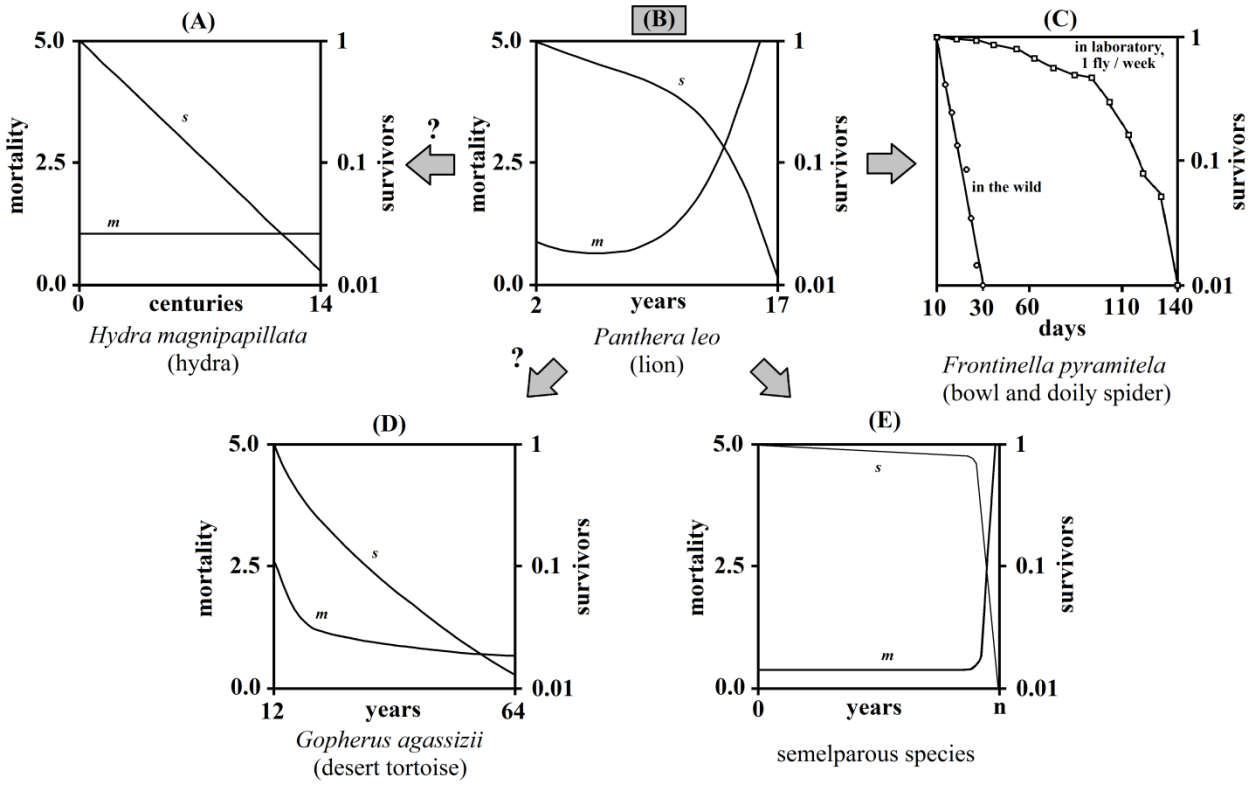


Figure 7A - For the old paradigm, the primary condition is (B) and the other conditions are derived, although (A) and (D) are difficult to explain. (A), (B) and (D) are from Figure 1 of [Jones et al. 2014], partial and redrawn, only mortality (*m*) and survivorship (*s*) are indicated; (C) has been drawn by using data from [Austad 1989]; (E) is an ideal life table of a semelparous species as reported in [Finch 1990].

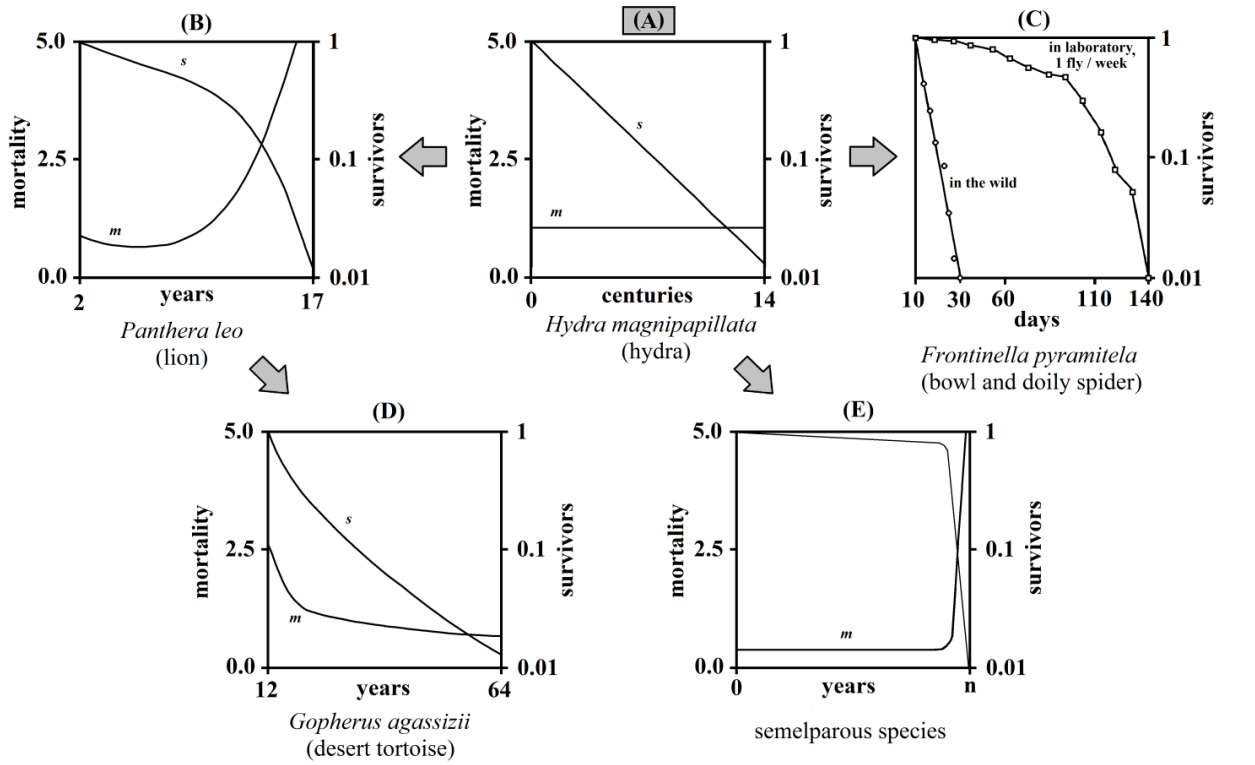


Figure 7B - For the new paradigm, the primary condition is (A) and the other conditions are derived.

The “how” of aging

For the new paradigm, as aging is considered an adaptive phenomenon, it is predictable and indeed imperative that aging is genetically programmed and regulated by specific mechanisms. On the contrary, for the old paradigm, as aging is a considered a consequence of degenerative processes insufficiently countered by natural selection, the aforesaid mechanisms simply cannot exist and, so, are indeed in utter contradiction with the paradigm. Also, for the old paradigm, the various degenerative mechanisms proposed as causes of aging represent a description of the “how” of aging.

Beyond the general issues exposed in the previous section, the existence or non-existence of genetically programmed and regulated specific mechanisms that determine aging is a fundamental and definitive evidence to settle the alternative between the old and new paradigm [Libertini 2015a]. This section is an overview of aging mechanisms as they are shown by the evidence and highlights that they are necessarily determined and regulated by genes. This description is the result of decades of work by researchers who often were, and are, not supporters or even aware of the new paradigm. On the contrary, these researchers were sometimes influenced, more or less consciously, by the tenets of the old paradigm. As we will see, the new paradigm allows for the interpretation of the experimental results within a consistent and understandable framework, while, for the old paradigm many results appear inexplicable and difficult or impossible to harmonize in a general and consistent theory.

- Cell turnover: Programmed Cell Death

In vertebrate species, organisms show a continuous renewal of their cells. Disregarding the cases in which cells die as a result of accidental events, cells usually die through the action of genetically determined and regulated mechanisms that are defined in general as “programmed cell death” (PCD). For example, epidermis cells are transformed by keratinization, die and then become detached; mucosal cells that line the intestine continually come off; erythroblasts transform themselves into erythrocytes and are subsequently removed by macrophages.

Apoptosis is a type of PCD described only in quite recent times that affects healthy tissues previously considered to lack cell turnover [Kerr et al. 1972]. It is ubiquitous in the eukaryotic world [Longo et al. 2005] and is certainly very old phylogenetically: it is observed, with some differences, even in unicellular species such as yeast [Herker et al. 2004]; furthermore, there are similar and phylogenetically related phenomena, defined as “proapoptosis”, in prokaryotes [Hochman 1997; Koonin and Aravind 2002]. Apoptosis is clearly different from necrosis, as it follows an ordered sequence, does not damage other cells and does not trigger an inflammatory response [Erwig and Henson 2008]. Apoptosis shows itself in many healthy tissues and organs [Pontèn et al. 1983; Benedetti et al. 1988; Dremier et al. 1994; Finegood et al. 1995; Migheli et al. 1997; Prins and O'Rahilly 1997; Spelsberg et al. 1999; Cardani and Zavanella 2000; Harada et al. 2000; Héraud et al. 2000; Pollack and Leeuwenburgh 2001; Sutherland et al. 2001; Xia et al. 2001] and is essential to ensure cell turnover [Wyllie et al. 1980; Lynch et al. 1986; Israels and Israels 1999; Medh and Thompson 2000], although it has other important functions (e.g.: removal of cells that are injured or infected [Tesfaigzi 2006; White 2006], lymphocyte selection [Cohen 1993; Opferman 2008], morphogenetic mechanisms [Nijhawan et al. 2000], wound healing [Greenhalgh 1998], etc.).

Cell turnover is a massive phenomenon: an estimate for our species is that about 50 to 70 billion cells are eliminated each day by PCD events (580,000 to 810,000 cells per second), i.e., in one year, a mass equal to that of the entire weight of the body [Reed 1999].

Cell turnover varies greatly in its rhythms depending on organ and cell type [Richardson et al. 2014]. At one extreme we have the cells of colon mucosa that are replaced in 3-6 days [Alberts et al. 2013], at the other extreme “the heart is replaced roughly every 4.5 years” [Anversa et al. 2006] and the “bone has a turnover time of about ten years in humans” [Alberts et al. 2013].

- Cell turnover: Cell replication and its limits

To compensate for cells eliminated by PCD, cell turnover clearly requires cell replication that, however, is restrained by known mechanisms.

In the late nineteenth century, August Weissmann proposed, without deepening the idea, that the limits to cell replication were an explanation for aging [Weismann 1892; Kirkwood and Cremer 1982]. For many years, his insight was considered unsustainable because it was wrongly believed, with the authoritative endorsement of a Nobel prize, that somatic cells of an organism were capable of unlimited replication [Carrel 1912; Carrel and Ebeling 1921b]. Many years later, breaking this inveterate prejudice, it was demonstrated, *in vitro*, that the duplication capabilities were limited [Hayflick and Moorhead 1961; Hayflick 1965]. Later, it was shown that this limitation (Hayflik's limit) was also evident *in vivo* [Schneider and Mitsui 1976] and for many cell types [Rheinwald and Green 1975; Bierman 1978; Tassin et al. 1979]. The duplication capacities were shown to be inversely correlated with age [Martin et al. 1970] and, in the comparison between species, directly correlated with longevity [Röhme 1981]. In 1975, it was shown that something in the nucleus was the cause of the limit [Wright and Hayflick 1975].

However, it was observed that the linear DNA of eukaryotes was duplicated only partially by the DNA polymerase. During each replication, a small part of one end of the DNA molecule (telomere) is not replicated [Olovnikov 1971; Watson 1972]. As an unlimited shortening was not compatible with the functionality of the cell, it was predicted the existence of an enzyme that had to restore the unduplicated part [Olovnikov 1973]. In subsequent years, the telomere was shown, in a protozoan, to be a simple repeated sequence of nucleotides (TTGGGG) [Blackburn and Gall 1978]. The same sequence with minimal variation (TTAGGG) was present in our species and in mammals [Moyzis et al. 1988] and in many other species that are phylogenetically distant [Blackburn 1991]. In 1985, we identified an enzyme (telomerase) that confirmed Olovnikov's prediction because it added the sequence of non-duplicated nucleotides. This explained the capacity of certain cells, such as stem cells and germ-line cells, to reproduce many or unlimited times [Greider and Blackburn 1985]. It was later shown that: telomerase is repressed by specific regulatory proteins [van Steensel and de Lange 1997]; telomere length shows, in many cell types, an age-related progressive shortening [Takubo et al. 2010]; in individuals of animal species studied in the wild there is association between life expectancy and telomere length [Haussmann et al. 2005; Pauliny et al. 2006; Bize et al. 2009]; inactivated telomerase and/or short telomeres increase the probability of apoptosis [Ozen et al. 1998; Holt et al. 1999; Seimiya et al. 1999; Ren et al. 2001; Fossel 2004].

- Subtelomere-Telomere-Telomerase system

The telomere is covered by a heterochromatin hood. In cells in which telomerase is inactive, or partially active, as the telomere shortens, the hood slides over the part of the DNA molecule that is adjacent to the telomere (subtelomere) and causes progressive transcriptional silencing of the subtelomere and alters the functions regulated by subtelomere [Fossel 2004]. This repressing effect, which has been known for some time as the "telomere position effect" [Gottschling et al. 1990], defined as "gradual cell senescence" too [Libertini 2015b], alters also the functioning of genes placed "over long distances" in the DNA molecule [Robin et al. 2014] and causes many alterations of cell functions, cellular secretions included (e.g., elastin, collagen, etc.), which cause modifications of the intercellular matrix, damages to other cells and inflammation [Fossel 2004].

The hypothesis that the subtelomere has a regulatory function is supported by evidence: (i) the subtelomere has an "unusual structure: patchworks of blocks that are duplicated" [Mefford and Trask 2002]; (ii) "A common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences." [Torres et al. 2011]. These repeated sequences are likely to have regulatory functions and are suppressed one after the other by the sliding of the telomere hood.

When the telomere shortens to a critical point, this inevitably triggers a chain of events, called "cell senescence" and defined as a "fundamental cellular program" [Ben-Porath and Weinberg

2005], which involves the inability of the cell to duplicate further (replicative senescence) as well as the alterations of gradual cell senescence in the highest degree.

However, in the culture of cells with equal numbers of previous duplications, there was a progressive reduction of the average capacity of duplication, or growth potential, and not a contemporary collapse in replication capacity of all cells after a certain number of duplications [Pontèn et al. 1983; Jones et al. 1985]. This was later explained by Blackburn [Blackburn 2000]: the telomere, which is covered by the aforesaid hood, oscillates between "uncapped" and "capped" conditions. In the first state, there is vulnerability to the transition to replicative senescence, i.e., activation of the cell senescence program. Furthermore, the duration of the "uncapped" state is proportional to the reduction in telomere length, but, even when the telomere is minimally reduced, there is a small uncapped phase and so a small probability that replicative senescence will be triggered.

All this could suggest that the critical element is the "absolute" length of the telomere and that therefore the initial telomere length (i.e., that in the first cell of an organism) is the factor that determines the number of possible duplications and consequently potential longevity. However, the evidence shows: (i) no correlation between telomere length and longevity among different species of rodents [Gorbunova et al. 2008] and among hamsters, mice and men [Slijepcevic and Hande 1999]; (ii) two *Mus* strains with different telomere lengths exhibit the same aging rhythms and equivalent longevity [Fossel 2004]; (iii) similarly, for cloned animals derived from somatic cells, i.e., with shortened telomeres, and non-cloned individuals [Fossel 2004]. In fact, the key factor is not the initial "absolute" length of the telomere but rather the progressive inhibition of the subtelomere, which is a function of "relative" telomere shortening and not of its initial "absolute" length [Fossel 2004; Libertini 2015b] (Figure 8).

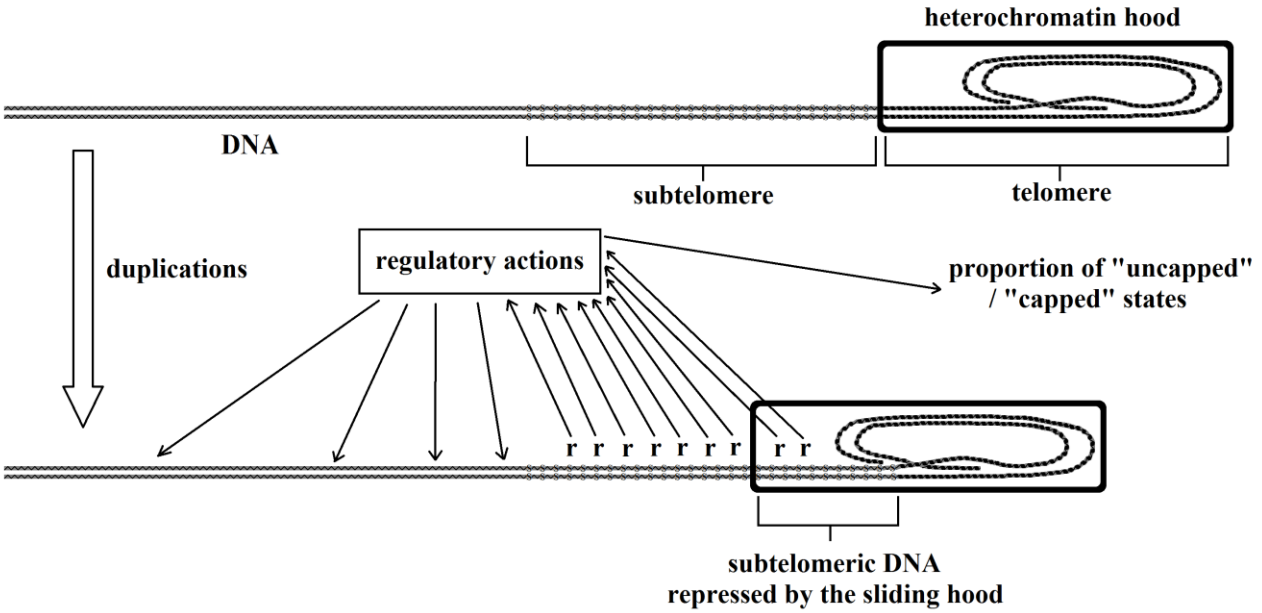


Figure 8 - Sliding of the heterochromatin hood over the subtelomere represses an increasing portion of the subtelomere, which probably has repeated regulatory ("r") sequences. This alters gene expression in near and distant parts of the DNA and, moreover, increases the proportion of telomere "uncapped" phase that is vulnerable to the triggering of cell senescence.

These phenomena ("gradual cell senescence" and "cell senescence", which includes "gradual cell senescence" in the highest degree) are completely reversed in vitro by the activation of telomerase [Bodnar et al. 1998; Counter et al. 1998; Vaziri 1998; Vaziri and Benchimol 1998; de

Lange and Jacks 1999]. As “cell senescence” may be completely and quickly triggered or, on the contrary, cancelled, it has also been defined as “on/off senescence” [Libertini 2014a, 2015a, 2015b].

Notably, aged fibroblasts in which telomerase was reactivated in vitro were used to form human skin that could not be distinguished from skin reconstituted from young fibroblasts [Funk et al. 2000].

In vivo, telomerase reactivation: (i) in aged mice with blocked telomerase, showed a clear reversal of all aging manifestations, even those of the nervous system [Jaskelioff et al. 2011]; (ii) in one- and two-year-old normal mice, increased lifespan and delayed all aging manifestations [Bernardes de Jesus et al. 2012].

Germ-line cells duplicate without limits and no transformation into senescent cells or manifestation of gradual cell senescence. On the contrary, these phenomena happen for somatic cells but are completely reversed by telomerase activation. The differences between germ-line and somatic cells and the reversibility of gradual cell senescence and cell senescence are hardly explainable by the hypothesis that gradual cell senescence and cell senescence are caused by damaging factors, while it is perfectly compatible with the thesis that they are programmed phenomena. This is in clear support of the new paradigm and in clear contrast with the old paradigm.

- Effects on the whole organism

The gradual increase in the number of cells that show cell senescence or gradual cell senescence, the slowing of cell turnover, and the resulting alterations in other cells, cause an “atrophic syndrome” in each organ, tissue and apparatus, already described elsewhere [Libertini 2009a]. It is characterized by:

a) reduced number of functional cells;
b) hypertrophy of the remaining functional cells;
c) partial substitution of the lost cells with nonspecific cells;
d) reduced mean cell duplication capacity;
e) slower cell turnover;
f) increasing number of cells in gradual cell senescence or in cell senescence;
g) increasing cancer risk due to dysfunctional telomere-induced instability [DePinho 2000].

Regarding the cell types without turnover (e.g., most neuron types, crystalline lens fibre cells), they are dependent from cells with turnover and so suffer from the consequences of turnover decline in these cells. This topic has been developed in a recent paper [Libertini and Ferrara 2016a] and for brevity will not be repeated.

Through the effects of harmful substances and unhealthy lifestyles, the aging process is accelerated, and, on the contrary, “protective drugs” and healthy lifestyles contrast this acceleration. These topics and a comprehensive description of the aging process for various organs and tissues have been concisely expounded upon elsewhere [Libertini 2009b, 2014a]. Figures 9 and 10 are schemes of these concepts.

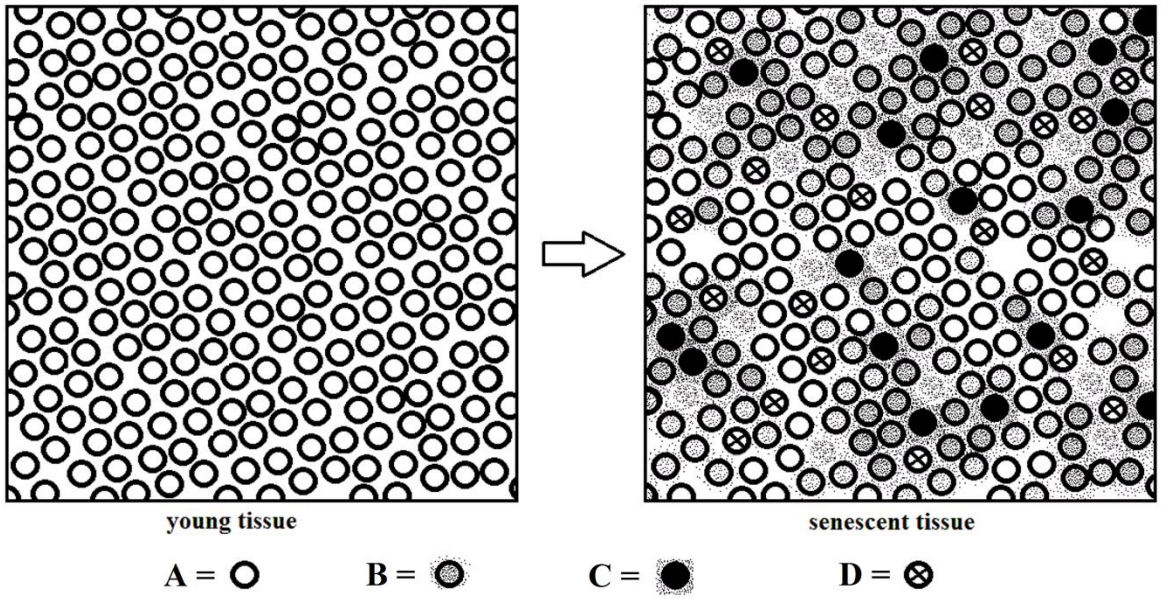


Figure 9 - Scheme of the transformation of a young tissue into an old tissue. A: normal cell; B e C = cells in "gradual" cell senescence and cell senescence with alterations of the surrounding milieu; D = nonspecific substituting cells.

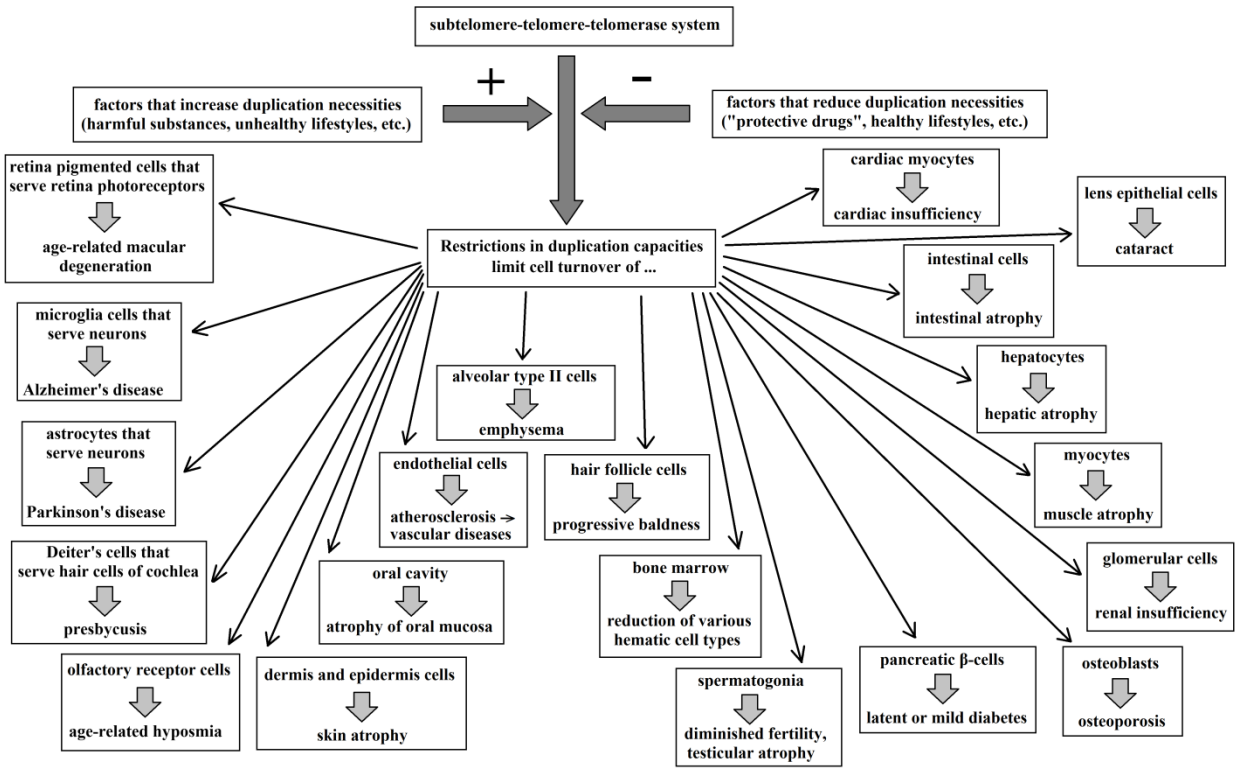


Figure 10 - Scheme of aging mechanisms at organismal level.

- Aging and cancer

The subtelomere-telomere-telomerase system is the key part of the mechanisms required by the new paradigm to explain aging. At the same time, these mechanisms are utterly incompatible with the old paradigm if there is no alternative evolutionary motivation for their existence. The only (old) explanation proposed is that they are a defence against cancer because replicative senescence would

pose an obstacle to neoplastic proliferation [Campisi 1997; Wright and Shay 2005; Rodier and Campisi 2011]. So, aging would be an evolutionary necessity to contrast cancer [Campisi 2000], a hypothesis that could be compatible with some theories of the old paradigm (antagonistic pleiotropy theory [Williams 1957; Rose 1991], disposable soma theory [Kirkwood 1977; Kirkwood and Holliday 1979]). However, this hypothesis is contrasted by strong arguments [Libertini 2009b, 2013; Mitteldorf 2013], e.g.: (i) telomere shortening increases the probability of cancer [DePinho 2000; Artandi 2002; Artandi and DePinho 2010]; (ii) gradual cell senescence and cell senescence weakens immune system efficiency [Fossel 2004] and so increases vulnerability to cancer [Rosen 1985]; (iii) old individual "animals with negligible senescence" [Finch 1990] have the same telomerase activity as young individuals [Klapper, Heidorn et al. 1998; Klapper, Kühne et al. 1998] without any increased cancer vulnerability as proven by their constant mortality; (iv) in humans, there is relationship between cancer risk and short telomeres [Wu et al. 2003; Wright and Shay 2005; Ma et al. 2011]; (v) increased expression of telomerase in normal mice increases lifespan and does not cause cancer [Bernardes de Jesus et al. 2012]; (vi) "If cellular senescence is designed to cut off cancerous cell lines, why would senescent cells remain alive and toxic? . . . from the perspective of the cancer theory, the poisoning of the body must be regarded as an unexplained evolutionary error." [Mitteldorf 2013]; (vii) in humans studied in the wild, cancer was a possible cause of death only for few older individuals (> 70 years), while most of the deaths were a consequence of the decreasing fitness caused by aging [Hill and Hurtado 1996]. It is unjustifiable that a hypothetical defence against rare events, which happen at later ages, kills many younger individuals [Libertini 2013].

A recent attempt to explain some of these contradictions within the fence of the old paradigm [Rodier and Campisi 2011] has been considered insufficient and biased [Mitteldorf 2013].

- Pathology of aging

This is a subject concisely discussed in other works [Libertini 2009b, 2014a] and, for brevity, cannot be expounded upon here. In general, it is necessary to distinguish between rare diseases originated by genetic alterations (e.g., Werner syndrome [Martin and Oshima 2000], dyskeratosis congenita [Marciniak and Guarente 2001]) and frequent or very frequent diseases caused by risk factors resulting from unhealthy lifestyles that accelerate and alter physiological aging. It is important to note the possibility of a distinction between the physiology and pathology of aging in accordance with the predictions of the new paradigm.

- Phylogenesis of aging

The phylogenesis of aging has been debated in a recent paper [Libertini 2015b] and, for brevity, only a single fact will be highlighted. In yeast (*S. cerevisiae*), telomerase is always active and mother-line cells manifest aging alterations due to increasing subtelomere inhibition caused by the progressive accumulation of particular molecules (ERCs). In daughter-line cells, this does not happen but, in *tlc1Δ* mutants in which telomerase is deficient, the telomere is shortened with each cell duplication and the subtelomere is inhibited by the progressive sliding of the cap on it [Lesur and Campbell 2004], similarly to what occurs in mammals.

Conclusion

Among numberless types of phenoptosis, which are all considered adaptive [Finch 1990; Libertini 2012a], it is odd that aging, also defined as "slow phenoptosis" [Skulachev 2002b, 2010], is the only one still considered by many as non-adaptive. In 1977, Hayflick wrote: ". . . if normal animal cells do indeed have only a limited capacity for division in cell culture, then manifestations of aging might very well have an intracellular basis." [Hayflick 1977]. As these limits for cell division was later shown to be genetically determined and regulated, this statement could be considered a wise anticipation of the new paradigm.

However, twenty-five years later, an authoritative “position statement”, written by the same Hayflick and two other leaders in aging sciences and endorsed by about 50 known worldwide scientists, stated: “No genetic instructions are required to age animals”, “... longevity determination is under genetic control only indirectly”, “... aging is a product of evolutionary neglect, not evolutionary intent” [Olshansky et al. 2002].

The concepts of this “position statement”, which is a comprehensive expression of the old paradigm, appear to be strongly contradicted by the arguments and the evidence presented in this review. The same arguments and facts appear to be in accordance with 1977 Hayflick's insight and entirely compatible with the new paradigm.

Therefore, a paradigm shift should be considered necessary and unavoidable.

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