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Review

Aging and aging theories

G. Libertini, G. Rengo, N. Ferrara

[1] Department of Translational Medical Sciences, Federico II, University, Naples, Italy

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.

Key words: Aging, Phenoptosis, Supra-individual selection, Telomere, Subtelomere, Cell senescence, Cancer

INTRODUCTION

Definition of aging

Aging is here defined as "increasing mortality with increasing chronological age in populations in the wild", or "IMICAW" [1], a definition that is analogous to others such as "actuarial senescence" [2] and "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [3] 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 [2 4-9], for our species too [10] (Fig. 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" [3], "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" [11]), but Ricklefs highlighted that senescence reduces average life span

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Correspondence: Giacinto Libertini, Department of Translational Medical Sciences, Federico II, University, Naples, via Pansini 5, 80131 Naples, Italy - E-mail: giacinto.libertini@tin.it

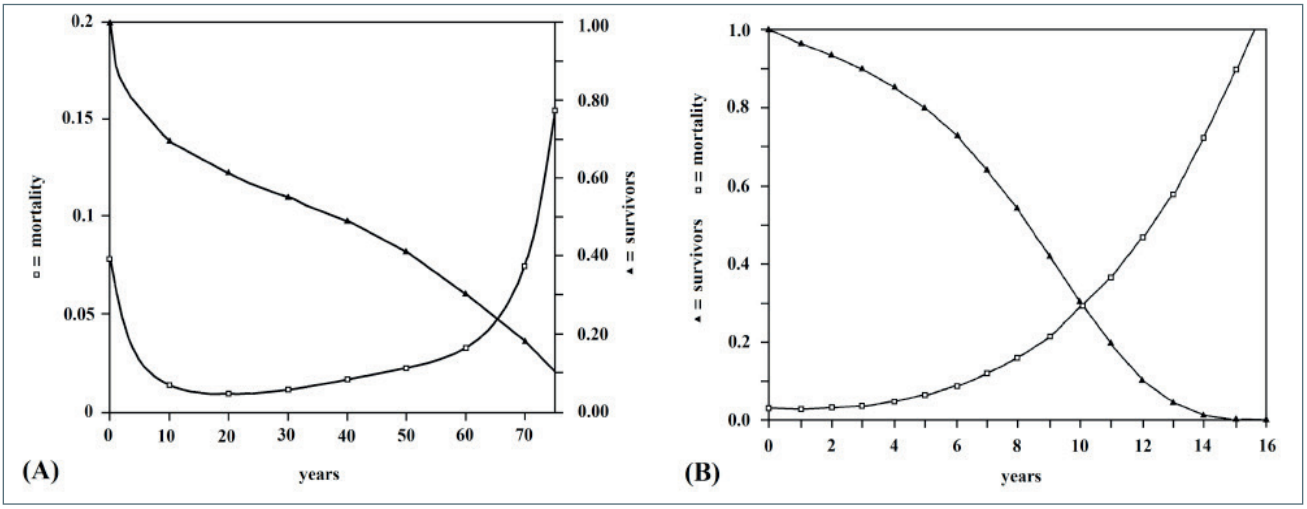

Figure 1. Some examples of aging. A: *Homo sapiens* (Ache population, data from Hill, Hurtado, 1996 [10]); B: *Pantera leo* (data from Ricklefs, 1998 [9]); for both species, observations in the wild.

up to "almost 80%" [9] and, later, a meta-analysis highlighted the evidence of aging in 175 animal species on the basis of 340 separate studies [12].

Classification of aging theories

Among the many theories that try to describe the causes of aging [13-16], 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 [17].

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 [18-23]. 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 [24-43]. 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 [44 45], 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 [46].

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 [1 47-68]. 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 [69], an expression adopted later by the same Darwin ("Natural Selection or the Survival of the Fittest" [70]), 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:

$$S \times P > 0, \quad (1)$$

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 [71-74]. 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:

$$\sum_{x=1}^{n} (S_x \times P_x \times r_x) > 0 \quad (2)$$

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 [75], 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:

$$\sum_{x=1}^{n_d} S_d + \sum_{x=1}^{n_s} S_s > S \times n, \text{ that is: } n_d \times S_d + n_s \times S_s > S \times n \quad (3)$$

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:

$$(n_{1,d} \times S_d + n_{1,s} \times S_s) + (n_{2,d} \times r_2 \times S_d + n_{2,s} \times r_2 \times S_s) \ldots + (n_{z,d} \times r_z \times S_d + n_{z,s} \times r_z \times S_s) > S \quad (4)$$

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 [74 76], 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 [77]. 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 [78-80] 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 [8]), 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" [56 57], 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" [54]).

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" [81 82].

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 [83], in particular species with no age-related mortality increase (Fig. 2).

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" [84] (e.g., rockfish, sturgeon, turtles, bivalves and possibly lobsters [84]). They have been defined species "with negligible senescence" [8]. 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 [85], but, perhaps more correctly, we should consider these species as a particular type of species with "negligible senescence".

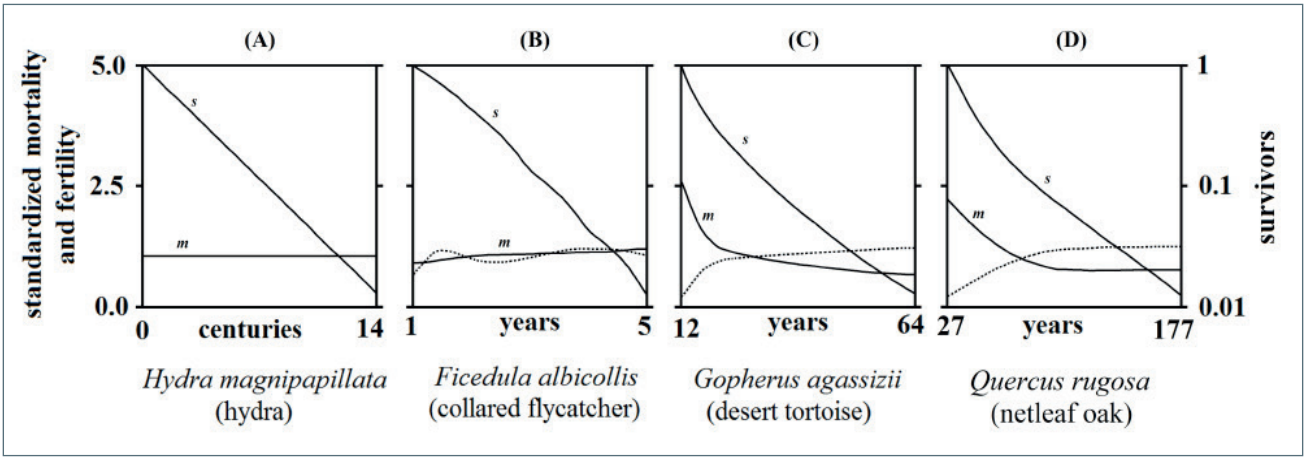

Figure 2. Some examples of life tables of non-aging species (partial and redrawn Figure 1 of Jones, Scheuerlein, Salguero-Gómez, et al., 2014 [83]). Solid lines indicate standardized mortality (*m*) and survivorship (*s*), the dotted lines the standardized fertility. (A) and (B) are cases of "negligible senescence", (C) and (D) are examples of "negative senescence". In (A), mortality and fertility lines overlap.

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 [8]). This type of phenomena, defined by Finch as "sudden senescence" [8] 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" [8].

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 [86] (Fig. 3). As this mortality increase happens only under artificial conditions, it is outside the definition of aging.

It is possible to indicate other particular cases but, for the sake of brevity, we refer to the cited work [83]. 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.

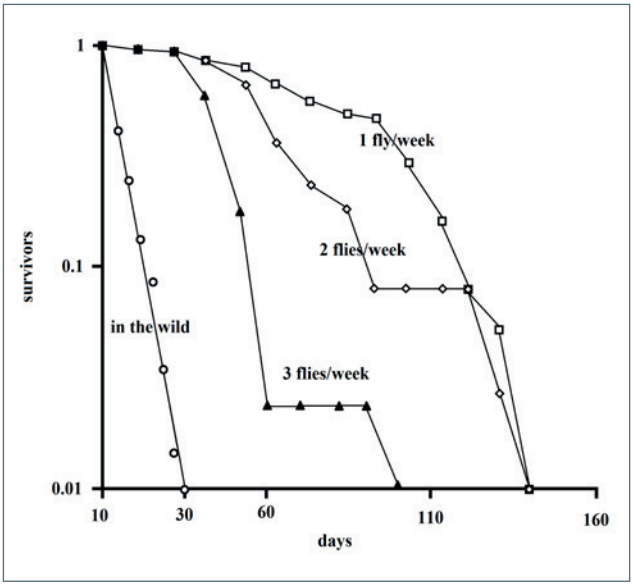

Figure 3. Survival of *Frontinella pyramitela* in the wild (circles) and in laboratory in different feeding conditions: 1 fly/week (squares); 2 flies/week (rhombs); 3 flies/week (triangles); data from Austad, 1989 [86].

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 [24 26 27 32]. A simple theoretical argument against this hypothesis has been proposed for a long time [1 51] and proposed again [16 87], 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:

$$P_e = v/s \quad (1)$$

and in the case that C is dominant:

$$P_e \approx v/s \quad (2)$$

The details of this calculation are explained elsewhere [87]. 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 $s \times Y_t$ and the equations (1) and (2) become:

$$P_e \approx v/(s \times Y_t) \quad (3)$$

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:

$$Y_{t+1} = Y_t \times (1 - \lambda) \quad (4)$$

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:

$$Y_{t+1} = Y_t \times (1 - \lambda - n \times s \times P_e) \approx Y_t \times (1 - \lambda - n \times v / Y_t) \quad (5)$$

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* [25 33], 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* [29 30], 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.

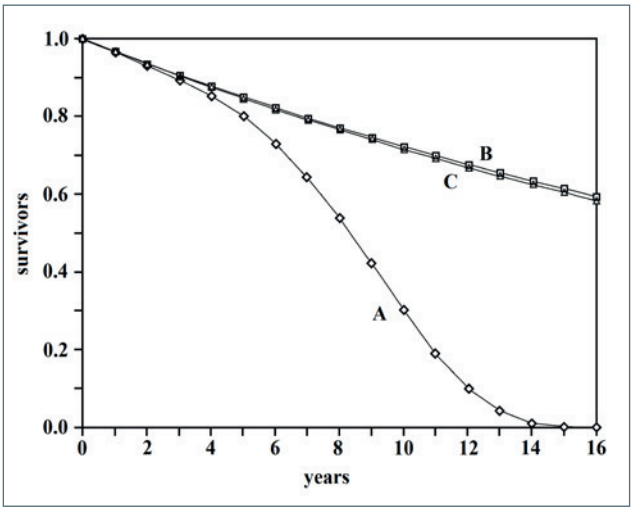

Figure 4. Hypothetical effects of a great number of t-genes on a life table. Curve A (rhombs): life table of *Panthera leo* in the wild; the death rate is described by Weibull's equation ($m_t = m_0 + \alpha t^{\beta}$) with the values $m_0 = .032$; $\alpha = .000252$; $\beta = 3$ given by Ricklefs, 1998 [9]. 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).

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 [1 51]. Subsequently, it was observed that this prediction should be valid for all theories that propose aging phenomenon as planned and favoured by natural selection [88]. 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" [68].

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" [3].
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 [9] (Fig. 5).
This plain contradiction between the empirical data and the predictions of the three classical theories was underlined by Ricklefs [9] and was subsequently deepened [88]. 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.

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 [65 89]. 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 [47 50], but a few years later, he dismissed this idea [48 50].
In 1961, a botanist proposed again the argument that senescence accelerates generation turnover and so "… in plants senescence is a catalyst for evolutionary adaptability" [49].
In 1988, after an anticipation in a non-peer reviewed book [51], a theory was proposed that explained aging as adaptive in spatially structured populations and in terms of kin selection because it accelerated evolution [1]. This hypothesis, which was later reaffirmed [52 53 55 88], starts from the following consideration.

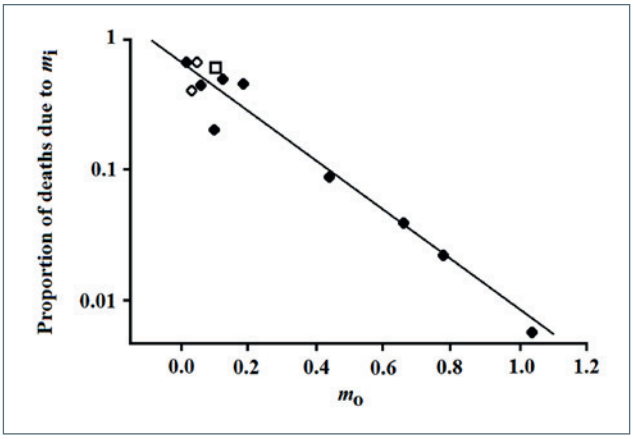

Figure 5. Inverse relationship between m_0 and the proportion of deaths due to m_i. Solid diamonds refer to bird species, open diamonds to mammal species, open square to *Homo sapiens*; ordinates are in logarithmic scale. Data for mammals and birds are from Ricklefs, 1998 [9], Table II; data for *H. sapiens* are from Hill, Hurtado, 1996 [10]. Without the data of our species, the linear regression has the following values: r = -.758708, t = -3.494043, p < 0.01 (from Libertini, 2008 [88]).

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 (Fig. 6).
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 [90]), 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:

$$r \times S \times (1/ML_C - 1) > S' \quad (6)$$

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 [63 67 68]. 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" [63]).
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 [66]. 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 [60 61]. In possible harmony with the idea that aging is adaptive and programmed, damage by mitochondrial ROS has been proposed as the essential mechanism [58 59 65]. In other papers, although a specific

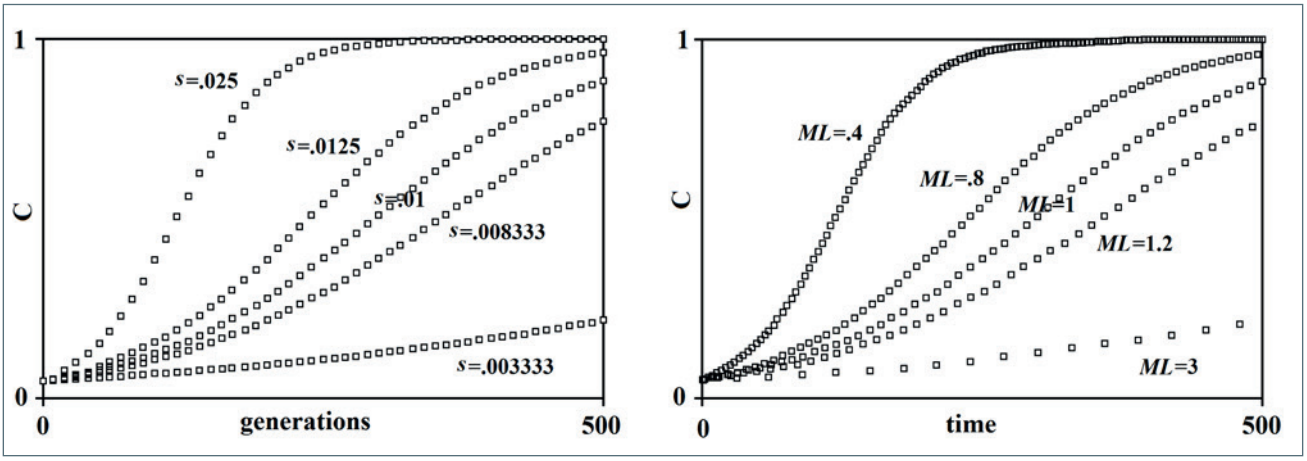

Figure 6. On the left: spreading of C according to the variation of *s* (while *ML* = 1); on the right: spreading of C according to the variation of *ML* (while *s* = 0.01); C_0 = .05. Redrawn from figures 2 and 3 in Libertini, 1988 [1], which are the same of figures I 2-1 and II 2-1 in Libertini, 1983 [51].

theory about aging is not formulated, the idea that this phenomenon is adaptive and programmed is backed with various topics [56 57 62 64 91].

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 [88].

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 I and in Figures 7A and 7B.

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

Table I. Some distinctions between old and new paradigm.

	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" (from Finch, 1990 [8])	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

Abbreviations: IMICAW: "increasing mortality with increasing chronological age in populations in the wild" (from Libertini, 1988 [1]); IMICAC: "increasing mortality with increasing chronological age in populations in captivity (i.e., under protected conditions at ages non-existing in the wild)" (from Libertini, 1988 [1]).

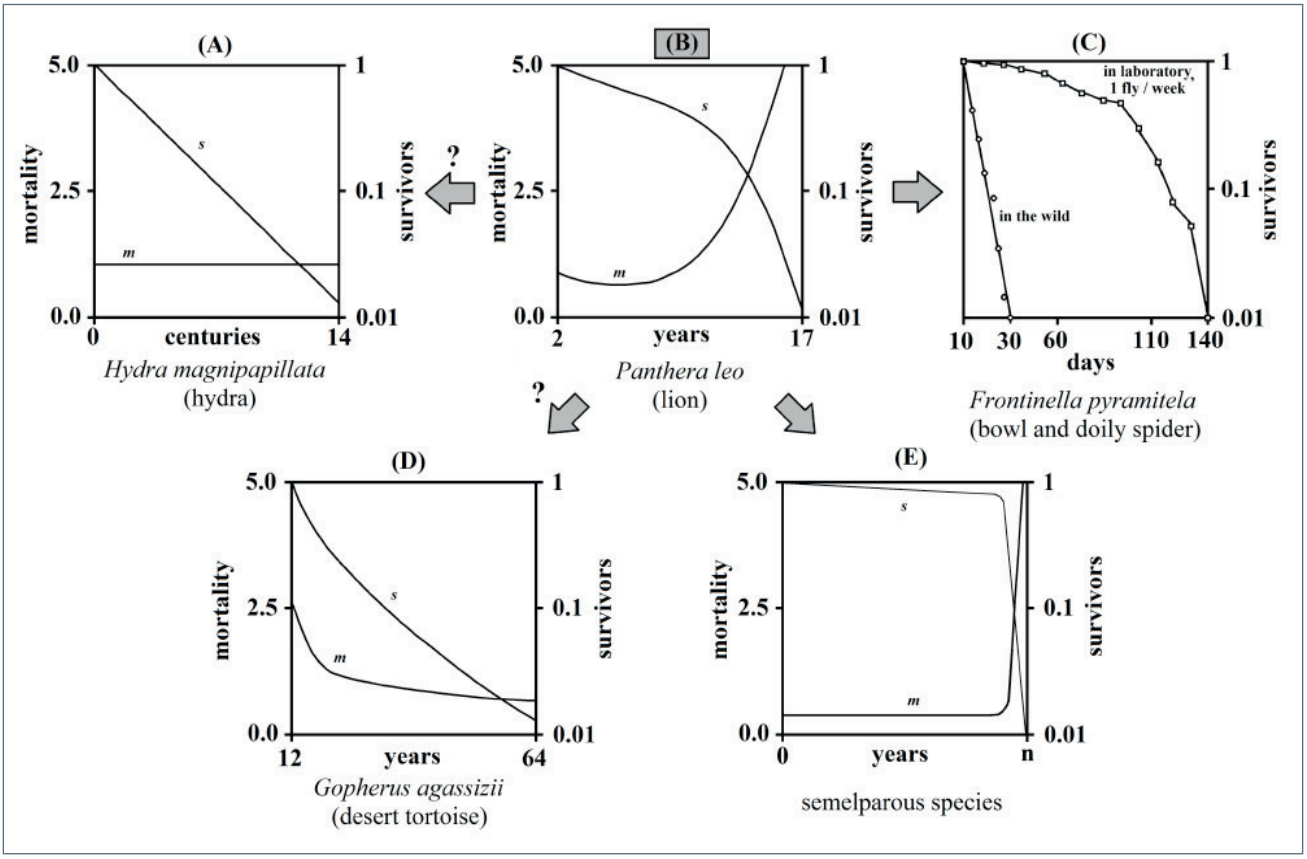

Figure 7A. For the old paradigm, the primary condition is (B) and the other conditions are derived, although (A) and (D) are difficult to explain. (A), (B) and (D) are from Figure 1 of Jones, Scheuerlein, Salguero-Gómez, 2014 [83], partial and redrawn, only mortality (*m*) and survivorship (*s*) are indicated; (C) has been drawn by using data from Austad, 1989 [86]; (E) is an ideal life table of a semelparous species as reported in Finch, 1990 [8].

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 [16]. 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 [92]. It is ubiquitous in the eukaryotic world [64] and is certainly very old phylogenetically: it is observed, with some differences, even in

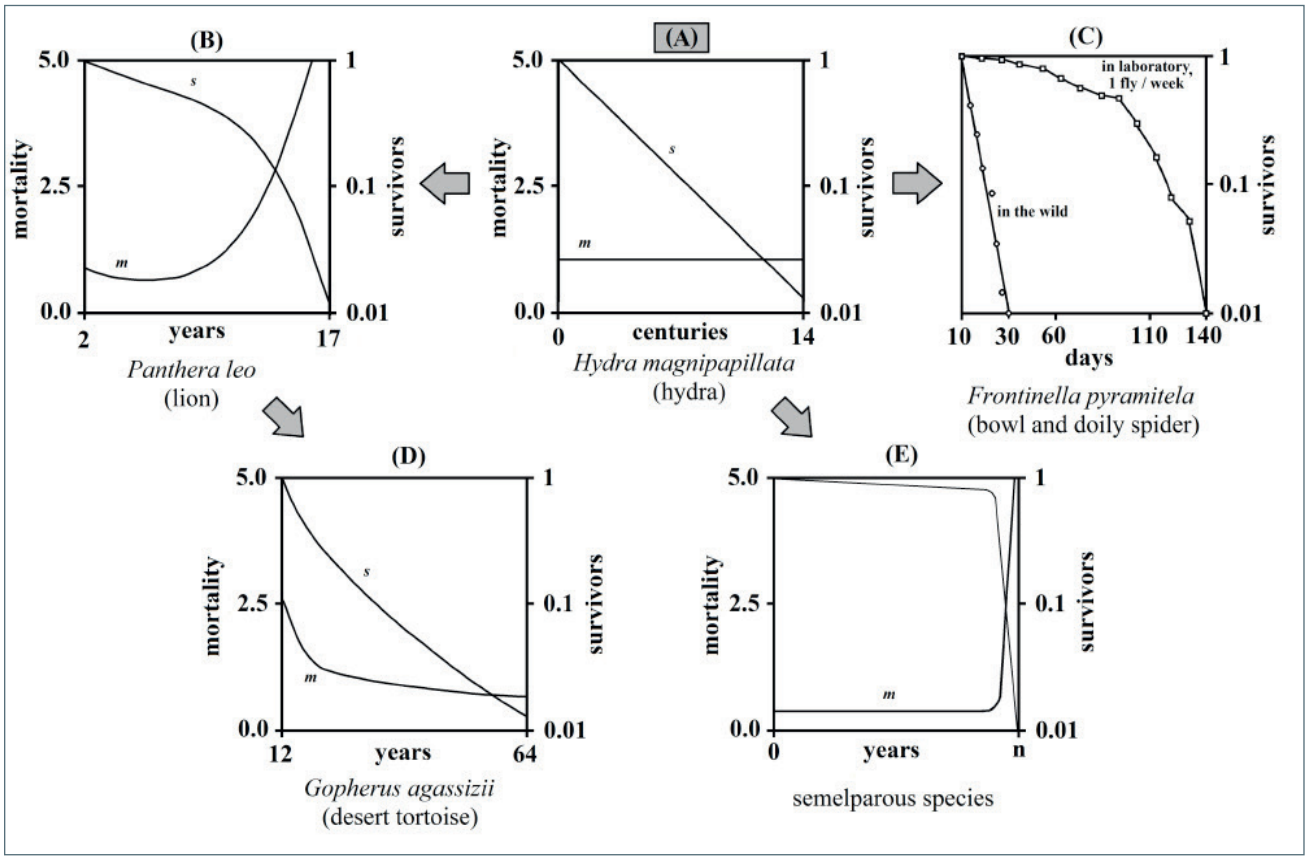

Figure 7B. For the new paradigm, the primary condition is (A) and the other conditions are derived.

unicellular species such as yeast [93]; furthermore, there are similar and phylogenetically related phenomena, defined as "proapoptosis", in prokaryotes [94 95]. Apoptosis is clearly different from necrosis, as it follows an ordered sequence, does not damage other cells and does not trigger an inflammatory response [96]. Apoptosis shows itself in many healthy tissues and organs [97-109] and is essential to ensure cell turnover [110-113], although it has other important functions (e.g.: removal of cells that are injured or infected [114 115], lymphocyte selection [116 117], morphogenetic mechanisms [118], wound healing [119] 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 [120].

Cell turnover varies greatly in its rhythms depending on organ and cell type [121]. At one extreme we have the cells of colon mucosa that are replaced in 3-6 days [122], at the other extreme "the heart is replaced roughly every 4.5 years" [123] and the "bone has a turnover time of about ten years in humans" [122].

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 [48 50]. 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 [124 125]. Many years later, breaking this inveterate prejudice, it was demonstrated, *in vitro*, that the duplication capabilities were limited [126 127]. Later, it was shown that this limitation (Hayflik's limit) was also evident *in vivo* [128] and for many cell types [129-131]. The duplication capacities were shown to be inversely correlated with age [132] and, in the comparison between species, directly correlated with longevity [133]. In 1975, it was shown that something in the nucleus was the cause of the limit [134].

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 [135 136]. 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 [137]. In subsequent years, the telomere was shown, in a protozoan, to be a simple repeated sequence of nucleotides (TTGGGG) [138]. The same sequence with minimal variation (TTAGGG) was present in our species and in mammals [139] and in many other species that are phylogenetically distant [140]. 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 [141]. It was later shown that: telomerase is repressed by specific regulatory proteins [142]; telomere length shows, in many cell types, an age-related progressive shortening [143]; in individuals of animal species studied in the wild there is association between life expectancy and telomere length [144-146]; inactivated telomerase and/or short telomeres increase the probability of apoptosis [147-151].

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 [151]. This repressing effect, which has been known for some time as the "telomere position effect" [152], defined as "gradual senescence" too [75], alters also the functioning of genes placed "over long distances" in the DNA molecule [153] 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 [151].

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" [154], (ii) "A common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences" [155]. 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" [156], which involves the inability of the cell to duplicate further (replicative senescence) as well as maximal alterations of gradual senescence.

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 [97 157]. This was later explained by Blackburn [158]: 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 [159] and among hamsters, mice and men [160]; (ii) two *Mus* strains with different telomere lengths exhibit the same aging rhythms and equivalent longevity [151], (iii) similarly, for cloned animals derived from somatic cells, i.e., with shortened telomeres, and non-cloned individuals [151]. 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 [75 151] (Fig. 8).

These phenomena ("gradual senescence" and "cell senescence", which includes "gradual senescence" to its maximum degree) are completely reversed in vitro by the activation of telomerase [161-165]. As "cell senescence" may be completely and quickly triggered or, on the contrary, cancelled, it has also been defined as "on/off senescence" [16 75 166].

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 [167].

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 [168], (ii) in one- and two-year-old normal mice, increased lifespan and delayed all aging manifestations [169].

Germ-line cells duplicate without limits and no transformation into senescent cells or manifestation of gradual senescence. On the contrary, these phenomena happen for somatic cells but are completely reversed by telomerase activation. The differences between germ-line

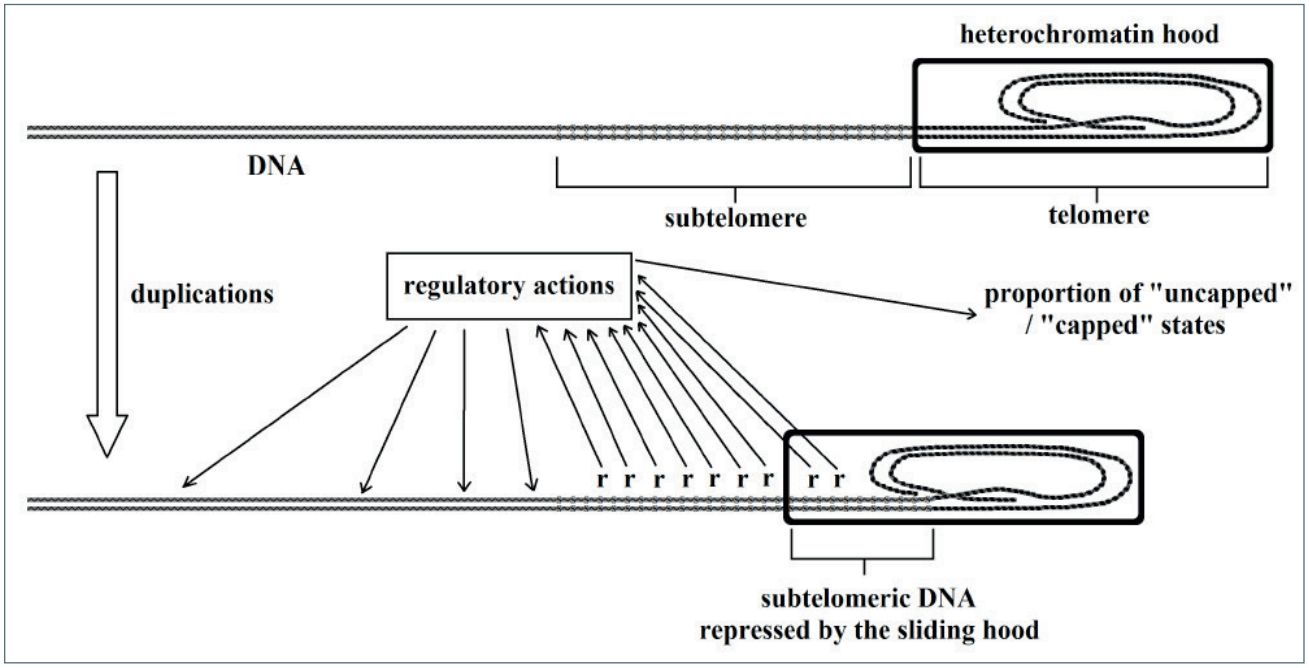

Figure 8. Sliding of the heterochromatin hood over the subtelomere represses an increasing portion of the subtelomere, which probably has repeated regulatory ("r") sequences. This alters gene expression in near and distant parts of the DNA and, moreover, increases the proportion of telomere "uncapped" phase that is vulnerable to the triggering of cell senescence.

and somatic cells and the reversibility of gradual and on/off senescence are hardly explainable by the hypothesis that gradual and on/off 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 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 [53]. 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 senescence or in cell senescence;
g) increasing cancer risk due to dysfunctional telomere-induced instability [170].

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 [171] 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 [87 166]. Figures 9 and 10 are schemes of these concepts.

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 [172-174]. So, aging would be an evolutionary necessity to contrast cancer [175], a hypothesis that could be compatible with some theories of the old paradigm (antagonistic pleiotropy theory [25 33], disposable soma theory [29 30]). However, this hypothesis is contrasted by strong arguments [55 87 176], e.g.: (i) telomere shortening increases the probability of cancer [170 177 178], (ii) gradual

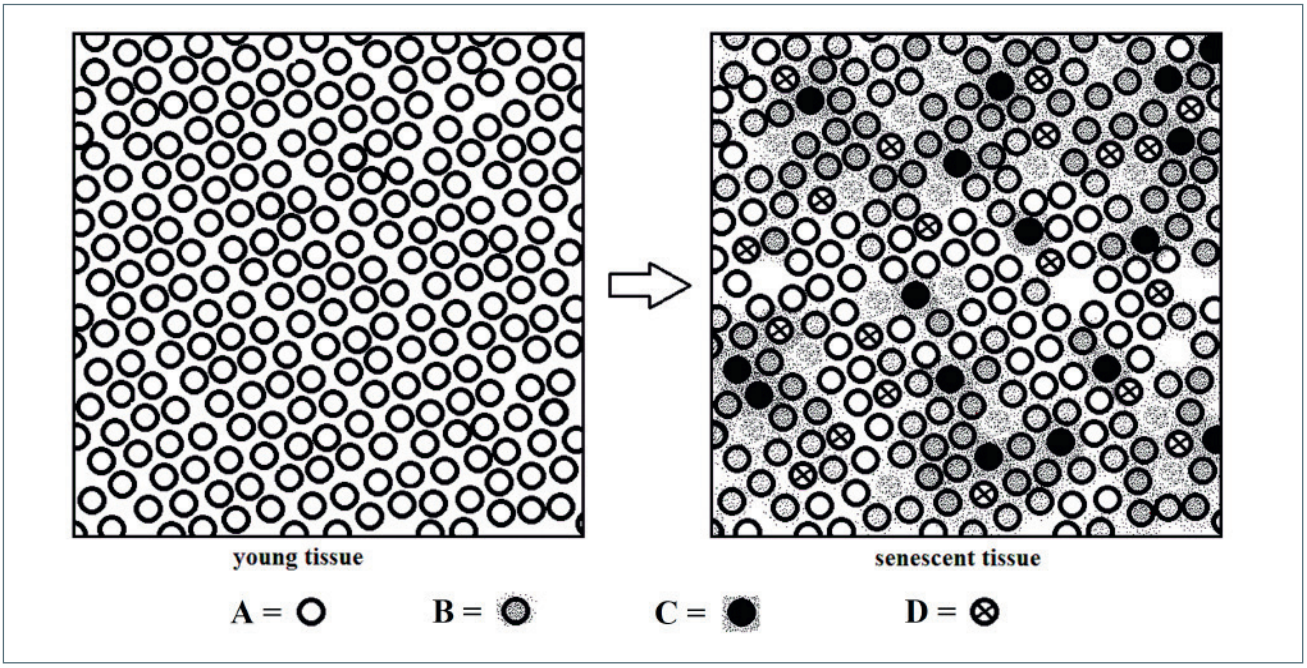

Figure 9. Scheme of the transformation of a young tissue into an old tissue. A: normal cell; B e C = cells in "gradual" and "on/off" senescence with alterations of the surrounding milieu; D = nonspecific substituting cells.

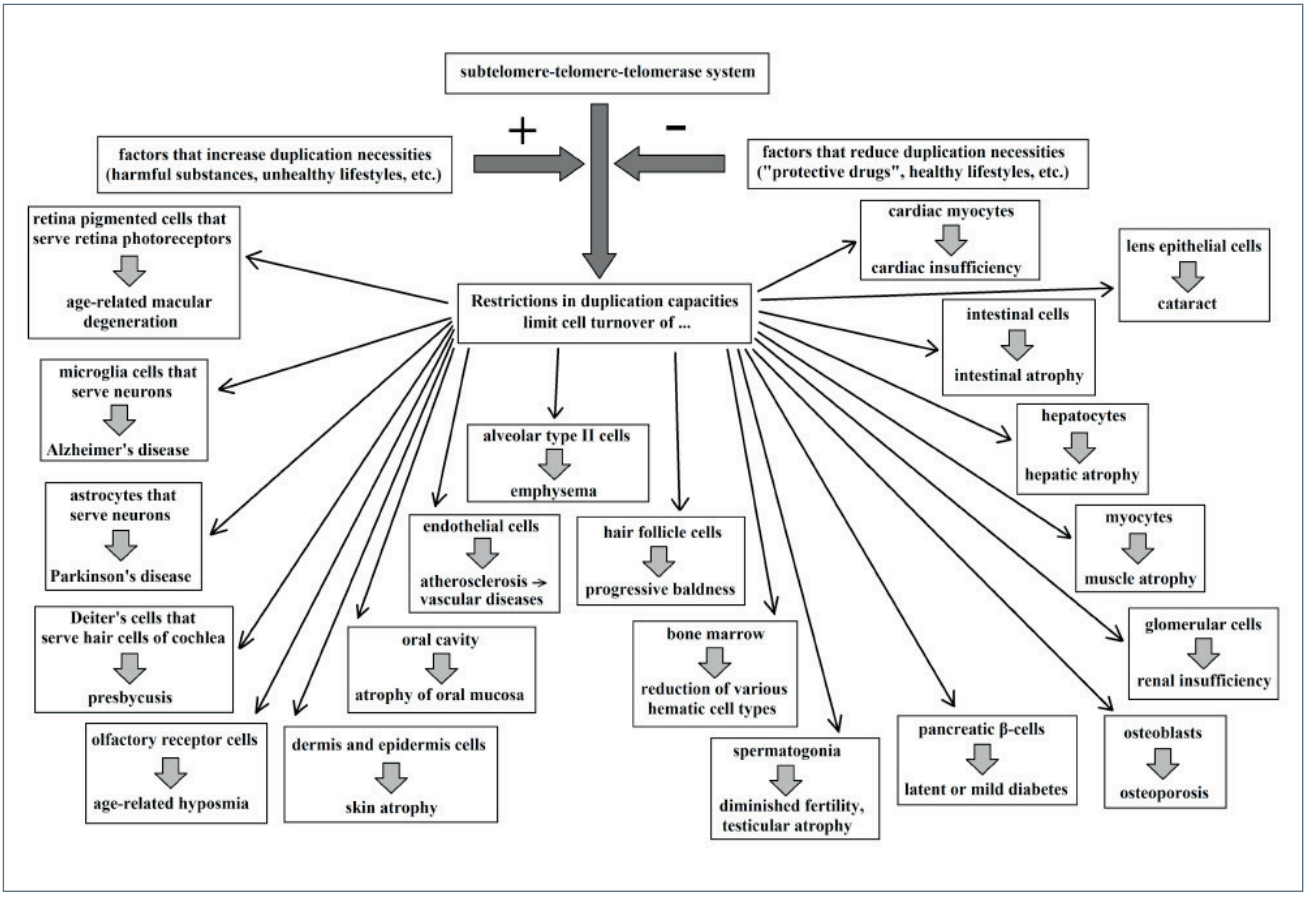

Figure 10. Scheme of aging mechanisms at organismal level.

and on/off senescence weakens immune system efficiency [151] and so increases vulnerability to cancer [179], (iii) old individual "animals with negligible senescence" [8] have the same telomerase activity as young individuals [180 181] without any increased cancer vulnerability as proven by their constant mortality, (iv) in humans, there is relationship between cancer risk and short telomeres [173 182 183], (v) increased expression of telomerase in normal mice increases lifespan and does not cause cancer [169], (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" [176], (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 [10]. It is unjustifiable that a hypothetical defence against rare events, which happen at later ages, kills many younger individuals [55].
A recent attempt to explain some of these contradictions within the fence of the old paradigm [174] has been considered insufficient and biased [176].

Pathology of aging

This is a subject concisely discussed in other works [87 166] 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 [184], dyskeratosis congenita [185]) 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 [75] 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 [186], similarly to what occurs in mammals.

CONCLUSIONS

Among numberless types of phenoptosis, which are all considered adaptive [8 54], it is odd that aging, also defined as "slow phenoptosis" [81 82], 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" [187]. 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" [188].
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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