

GIACINTO LIBERTINI

**Evolutionary Interpretations
of Aging,
Disease Phenomenon,
and Sex**



COPERNICAN EDITIONS

Giacinto Libertini

**Evolutionary Interpretations of Aging,
Disease Phenomenon, and Sex**

Collection of works of the Author

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INTRODUCTION

Life is amazing in its extreme variety, which is full of seemingly contradictory manifestations. This is particularly true about three categories of phenomena, all of primary importance, in particular for humankind:

1A) For many species, ours included, all individuals grow old with the passage of time, that is mortality rate increases exponentially with age [Finch, 1990; Ricklefs, 1998];

1B) On the contrary, individuals of many species do not show differences in vitality between subjects of various ages and are defined as having negligible senescence [Finch, 1990];

2A) The diseases that, with great variety of torments, afflict living beings, and our species in particular, are numberless;

2B) However, in the wild, the normal condition, which is by far the most usual, is to be healthy [Eaton et al., 1988];

3A) The ways in which living beings mate, and so recombine their genes, are amazingly various [Bell, 1982];

3B) On the other hand, many species reproduce asexually with an equally stunning variety [Bell, 1982].

Yet, these phenomena, despite their radical differences and extreme diversity of manifestations, can be studied and analyzed by a single main tool, as all have been shaped and influenced by evolution, if it is true what Dobzhansky said:

Nothing makes sense in biology except in the light of evolution [Dobzhansky, 1964]

This book expresses some arguments about their interpretation in the light of this unifying and clarifying theory.

A first question arises, however, immediate and spontaneous: what of new can one say about phenomena so well-known, discussed, and examined certainly from all angles, in evolutionary terms too?

Yet, I think that much is still to understand and deepen and, perhaps, profound changes of interpretation must be developed and, if correct, accepted.

The first theme, commonly called aging, is defined more precisely as the age-related progressive decline of every vital function, that is the progressive increase in mortality [Libertini, 1988].

A widely held belief is that this phenomenon is universal, connected to the very nature of living matter and therefore inevitable [Hayflick, 2000; Kirkwood and Austad, 2000].

However, the natural observation teaches us that many species do not show at all an age-related decline of functions [Finch, 1990] and this is difficult to explain for those who think that the phenomenon is unavoidable.

According to prevalent theories on aging, it has been also observed that a high environmental (or extrinsic) mortality should favor the accumulation of harmful mutations in advanced ages, and this has led to the prediction that a high extrinsic mortality should result in premature aging [Kirkwood and Austad, 2000]. But, the observational data show us the opposite, namely species with lower extrinsic mortality age more precociously than those with higher extrinsic mortality [Ricklefs, 1998].

These facts, and others, have given strength to the hypothesis that the age-related

decline of fitness is an active process favored by natural selection and not an inevitable degeneration insufficiently contrasted by natural selection [Libertini, 2008]. This hypothesis, which for many is a sort of provocation, implies the existence of appropriate mechanisms that determine and regulate aging, while for the opposite hypothesis they could not exist and would be totally unjustified [Libertini, 2006].

However, in the last years, a vast accumulation of scientific evidence has shown that there is a sophisticated system, called telomere-telomerase system for short, which regulates and limits the ability of cell turnover and thus ultimately determines the progressive decay of functions [Libertini, 2009a]. It was also shown as early as 1998 that the reactivation of telomerase restores all functions of a senescent cell, the ability to duplicate themselves included, proving that aging is a process reversible and controlled genetically [Bodnar et al., 1998; Counter et al., 1998; Vaziri and Benchimol, 1998; de Lange and Jaks, 1999]. In addition, a few months ago, a dramatic experiment on animals has shown that the effects of the full reactivation of telomerase stop the aging process and reverse spectacularly all its previous manifestations, even the atrophy of olfactory bulbs [Jaskelioff et al., 2011].

This new paradigm makes realistic the goal of a control of the aging process and opens up new prospects for human civilization, though not necessarily all positive [Libertini, 2009a].

The second topic, disease phenomenon, is also very important if we consider the vast numbers of cases of illness and consequential deaths that afflict humanity.

The subject is addressed by an evolutionary point of view, namely:

What is a disease in evolutionary terms? A phenomenon that is alien to evolution and absolutely not analyzable in terms of evolutionary logic or something that can and should be studied first and necessarily within that logic? [Libertini, 1983]

Modern Medicine is alien to evolutionary thinking: for the most, it does not know to frame the diseases in their evolutionary causes, neglects appropriate preventive measures based on remedies against the primary evolutionary causes, and cares only for overt diseases with great efforts and poor overall results [Libertini, 2009b].

On the contrary, if Medicine is integrated within evolutionary logic, namely if it becomes Evolutionary Medicine [Williams and Nesse, 1991; Stearns, 1999; Trevathan et al., 2008], which is not an alternative medicine but the scientific completion and therefore improvement of the Medicine, the potential effects are of extreme importance. The vast majority of cases of disease stems from violations of elementary evolutionary rules [Libertini, 2009b]. The logic of Evolutionary Medicine approach is essential, as:

One can only understand the essence of things when one knows their origin and development, Heracleitos [Minkoff, 1983]

The third topic, the evolutionary mechanisms that favor sex, unlike the previous two subjects, has no practical use and has only a theoretical importance.

Even today there is great confusion about the evolutionary significance of sex and various theories are debated to attain a shared interpretation of this important phenomenon. This was well expressed by Ridley: "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.'" [Ridley, 1993], p. 29.

The plurality of contradicting theories gives us a certainty at the outset: most of them are totally or largely wrong.

In particular, observational data show us that most of these theories predict correlations that are falsified by empirical data. Consequently, it is true that, according to the scientific method, theories clashing with the facts can no longer be considered valid hypotheses or acceptable theories, and should be mentioned only for their historical value.

By starting from this assumption, it is exposed how a simulation model based on the traditional explanation of the evolutionary advantage of sex, without any special condition, can show that in finite populations, other conditions being equal, the sex is favored by natural selection while its advantage vanishes in infinite populations. Afterwards, considering the disadvantages deriving from the search of a mate, connected to the coupling, etc., it is possible to formulate predictions about the spread of sex within the numberless species, or for the alternation between sexual and asexual phases of reproduction within the same species, and verify their confirmation in empirical data.

With this method, it is shown that the traditional theory is correct, if properly formulated in terms of finite populations, and also that the plethora of alternative theories proposed must be rejected, except for some specific and particular aspects.

Perhaps, these subjects are boring for you, my unknown Reader. Well, if you:

- do not worry about the causes for which in a few years you will have to go to a sad place from which nobody returns,
 - disdain to know how in a few decades this could change,
 - have no concern about the possibility of getting sick,
 - are not curious to learn why, in spite of the powerful and efficient modern Medicine, diseases are rampant and sufferings grow,
 - do not want to be informed about the possibilities of modifying health strategies so that this terrible trend will be reversed,
 - are not interested in understanding the logic behind men and women who reproduce in extremely complicated ways (let's admit that it is fun too), a task that could be performed by oneself without the need for copulation, as many living species do,
- so, if you do not have any of these interests, then you have the wrong book, do not go on reading these pages, and, please, do not grumble going away for the time wasted.

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SECTION I

EVOLUTION AND AGEING

Chapter II (Senescence) and Interlude (between chapters II and III) in: G. Libertini, **Evolutionary Arguments on Aging, Disease and Other Topics**, 2011, Azinet LLC (ISBN-13 978-0-9788709-2-8), English reprint of G. Libertini, **Ragionamenti Evoluzionistici**, 1983, Società Editrice Napoletana, Napoli

[For the full text both of the Italian and in the English version, and for the simulation programs, see my personal site = "http://www.r-site.org/ageing/index_e.htm"]

Chapter II - Senescence

1) Definitions

- Senescence is a general title for the group of effects that, in various phyla, lead to a decreasing expectation of life with increasing age ... In a population not subject to senescence and exposed only to random overall mortality, the decline of numbers is logarithmic, and animals die, ex hypothesis, from causes that would have killed them at any age. In a population exposed only to death from reduced resistance, due to senescence, the curve approaches a rectangular form: after a certain age, animals die from causes that would not have killed them in youth. In one case the force of mortality is constant; in the second it rises steadily with age. Thus in rats the force of mortality rises after the ninth month of life in a geometrical progression ... Real survival graphs are commonly intermediate in form between the two ideal contours. - (Comfort, A., 1979, pp. 7 and 23)

In accordance with Comfort, but with a further specification (in italics), I say:

“Senescence” is a whole series of phenomena, with causes and mechanisms to be established, which manifests itself as a progressive increase in mortality rate as the age of the living being increases. The beginning of senescence is in that period of the life when, *in natural conditions*, the increase in the mortality rate exceeds an arbitrarily established threshold value.

In Fig. II 1-1, two diagrams are shown, the first, the one on the right, concerning the curve of numerical decline of a population that ages according to Comfort's definition, the other the typical curve of a population with constant resistance to “noxae”. On the curve of the right-hand side diagram, some symbols dividing the curve into periods have been added. With respect to the human species, I show two empirical diagrams in Fig. II 1-2, of which the first illustrates the variation of the mortality rate and the second the numerical decline of a population.

I will now express three further definitions which are indispensable for an understanding of the following paragraphs.

By “mean duration of the life” (ML) I am referring to the mean duration of life of the totality of the individuals of a species - or of a population - in their natural ecological niche.

On the other hand, by the term “longevity”, I am speaking about the mean duration of life in the natural ecological niche of those individuals of a species that are not dead in the first phases of life and have escaped pathological or accidental fatal events that are damaging at any age.

Finally, the expression “maximal longevity” means the greatest observable duration of life, even in an artificial ecological condition.

* * *

As regards Comfort's definition of senescence – which I fully share, albeit with a specification -, there are some points that need to be stressed.

1) The definition is not based on morphological or physiological criteria, but only on the observation of the life table in wild conditions of a population that is homogeneous by age. The increase of the mortality rate is tautologically due to a decline in the abilities of adaptation and resistance to selective pressures, but the substrate of this decline is not specified or arbitrarily postulated in the definition. It should be mentioned that such a substrate must not necessarily be some macroscopic alteration: in natural conditions even a very slight alteration of a function x could entail a significant reduction in survival abilities.

2) The aforesaid definition, which I will describe as “gerontological”, does not necessarily coincide with the one that a morphologist or a physiologist might give. The definition of this second type, which I will call “geriatric”, could be based on evident morphological or physiological parameters, such as, for example, teeth or coat wear, which manifest themselves frequently in animals which have grown old in captivity. A geriatric definition of this type is more restrictive than the gerontological one. More formally, the senescent individuals in geriatric terms, as now defined, are a subset of those individuals that are senescent in gerontological terms. To stress this concept, I will define as “hypersenescence” those among the senescent individuals (in gerontological terms) that show evident morphological and/or physiological alterations. Depending on the seriousness of the alterations, an easy prediction is that hypersenescent individuals are rarely - or even never - observable in natural conditions (see later).

3) The definition of the term senescence, and likewise those of ML and of longevity, must be used only to refer to populations in wild conditions, something that is not stressed by Comfort. If, as is plausible, the life table depends on the conditions according to which the population lives, which also means that the beginning of senility is influenced by the conditions of life, it is clear that, if there is no reference to a unique ecological niche, the gerontological definition of senescence becomes meaningless. It must be noted that, on the contrary, the geriatric definition in itself disregards any reference to an ecological niche.

I do not think that these specifications are idle semantic disquisitions. I want to show how Comfort himself (whose definition of senescence I have accepted), makes no distinction between a “gerontological” and a “geriatric” understanding of senescence.

- ... old age is undoubtedly a relatively rare or very rare termination to the life-cycle of vertebrates studied in the field - as it is for man in societies where medical and economic conditions are bad.

... in wild voles ... and in *Peromyscus* ... senescence is never observed, judging from the state of the teeth and bones of recent and fossil animals ... tooth wear is a reliable index of age in short-tailed shrews, those over 2 years being edentulous, but age limitation by this mechanical form of senescence is more potential than actual since few survive to exhibit it. - (Comfort, A., 1979, p. 140)

In my opinion, however, the correct conclusion is: individuals that are hypersenescent - and not those that are senescent in gerontological terms - are a rarity in the natural ecological niche.

Moreover: it should be considered a prejudice – which is absent in Comfort's definition - that senescence is identified with the alterations of individuals which have grown old in captivity, reaching ages that cannot be found in natural conditions.

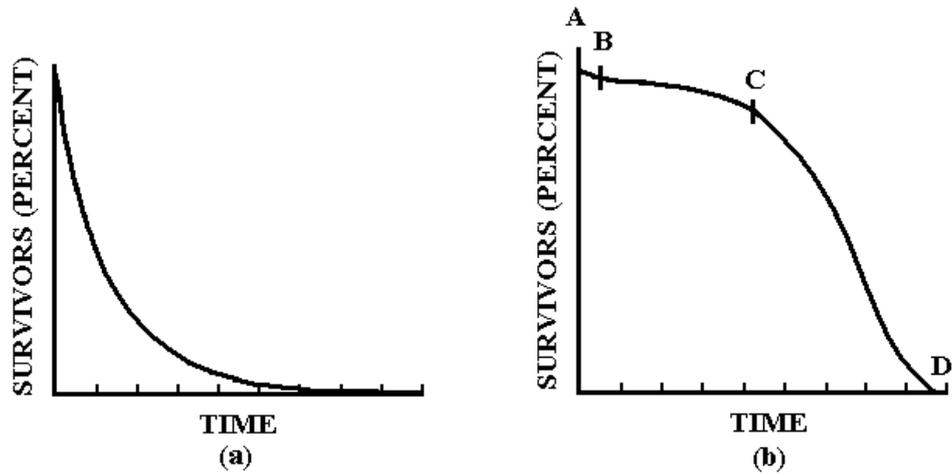


Fig. II 1-1 - Life table of a non-senescent population (a) and of a senescent population (b).

Source: Comfort, A., 1979, p. 22.

Some arbitrary symbols of delimitation have been added to the curve on the right. For this curve:

AB = first period of life with mortality higher than period BC both because the immature forms are more vulnerable to the dangers of the habitat, and because there is a loss of a certain number of genetically defective individuals;

BC = youth and adulthood with relatively constant mortality which depends on the environmental conditions;

CD = senility with a strong numerical decline in the surviving population.

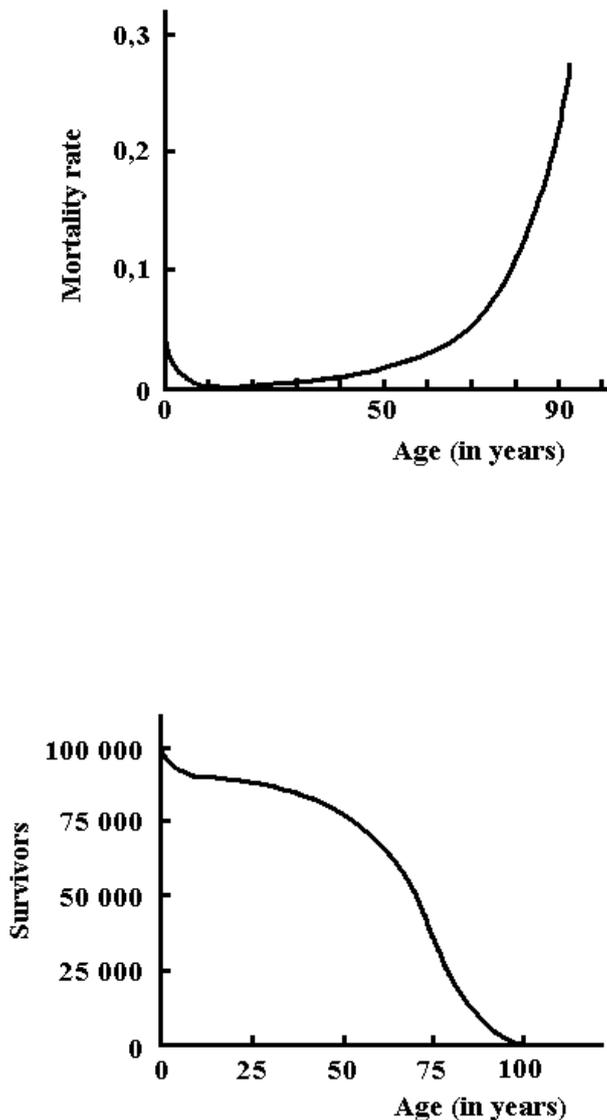


Fig. II 1-2 - Mortality and life table of a modern human population.

2) Evolutionary advantage of a lesser longevity.

From the given definitions, it can easily be deduced that greater or lesser longevity coincide with the onset of senility, the speed of which depends on the species. In this chapter, I put forward the question of *why* individuals age and therefore die a so-called “natural” death within a given time. Moreover, I wish to investigate the selective pressures that cause a greater or lesser longevity depending on the species.

It might seem strange to question the "why" of senescence for those who believe the progressive and fatal alteration of all vital functions to be *obvious*, but certainly this is an unscientific way of looking at it: discovering that something happens is not a rational reason for considering it justified. And it is also incorrect to argue that there is ongoing extensive research on those tissue and cell changes considered typical of senescence: in fact, as we must distinguish, this may explain from an evolutionary point of view *how* the organism ages and not *why*. In other words, here, I do not put forward the question of which chemical, hormonal, etc., mechanisms are implicated in the senile process, but the problem of their possible teleonomic meaning. Likewise, from an evolutionary point of view, the aforementioned research may lead to the discovery of the "how" but not of

the "why" of the unbelievable variation in longevity among the innumerable species. Among living beings, there are, in fact, organisms that live for a few days (e.g.: rotifers) and others that even seem not to age at all (e.g.: *Sequoiadendron*). The answer to the "why" of senescence, and to greater or lesser longevity, is perhaps obtainable through reasoning in evolutionary terms. First, I want to demonstrate that, between two species with different longevity, other conditions being equal, the one with the lesser longevity is advantaged.

A premise.

Remembering that the term generation (G) in Chapter I (see Fig. I 2-1) has been defined as "the time needed for there to be N deaths within a population made up of a constant number N of individuals", I wish to observe that, in a numerically constant population, ML and G coincide as values.

In fact, in a fictitious population, in which all individuals live for a period ML exactly, all N individuals born at any instant t die within, and not before nor after, the instant t + ML. Moreover, all individuals that replace the N original dead individuals, die after the instant t + ML. Therefore, as in the period t – t + ML a number N of individuals die, according to the given definition, we have:

$$G = (t + ML) - t = ML \quad (\text{II-1})$$

Moving on, then, to a real population in which the ML is a mean of unequal values because the individuals that die before reaching an age equal to the ML are perfectly balanced, by definition, by those that die after passing the ML, by repeating, with the appropriate modifications the argument expressed above, we can reach the same conclusion of a quantitative identity between G and ML in a numerically constant population. Having said that, now let us consider two species, A and B, with longevity L_a and L_b , respectively, and with $L_a < L_b$.

For now, let us also assume arbitrarily that character longevity is free from mutations that alter it and from selective pressures within each of the two species.

It is hypothesized that, for mortality in the first phases of the life and as a result of pathological and accidental causes, the ML of each of the two species is lower and proportional to their respective longevities, so $ML_a < ML_b$. Let us also assume that both species are made up of a constant number of individuals and that, therefore, $G_a = ML_a$ and $G_b = ML_b$.

Over a period of time T, we will have T / ML_a generations of A and T / ML_b generations of B. Furthermore, let us suppose that, in this period, there is a certain gradual modification of the ecological niche of the two species: selection obviously will favour the mutants that are better adapted to the new conditions of the ecological niche. But, while selection with regard to A will operate over a series of T / ML_a generations, for B it will be T / ML_b generations and, being by assumption $T / ML_a > T / ML_b$, A will be in an advantageous condition compared to B. In fact, evolution is describable as an endless diffusion within a species of mutations that somehow entail selective advantage. But it is known that a mutation, in order to reach a given frequency within a species, needs a certain number of generations, which are inversely proportional to the size of the selective advantage caused by the mutation. And, likewise, for a given selective advantage, the number of generations in the period of time considered is the critical factor in terms of the velocity with which the favourable genes are spread (see Fig. II 2-1).

As for A, over period T, there is a greater number of generations than for B, so character modification for A will be possible to a greater extent. Or, to say the same thing in another way, A will be able to acquire certain modifications of its own characters over a

shorter time period than can B. This means that A will have better possibilities than B to adapt appropriately to the subsequent, new ecological niche, which is an advantage of A over B (see Fig. II 2-2).

In other words, the shorter the ML and, consequently, longevity, which concurs to affect the ML, the greater the possibility of rapid evolution, with selective advantage over species with a greater ML, or longevity.

As a specification of the argument expressed here, I would say that the spreading velocity of a gene within a species (see definition in the model of Fig. II 2-1), is proportional to the number of generations per unit of time (NG/T).

Moreover, defining the velocity of evolution as the velocity with which a species adapts itself to the conditions of the ecological niche, I maintain that it is also proportional to the spreading velocity of a gene, and therefore to the ratio NG/T as well.

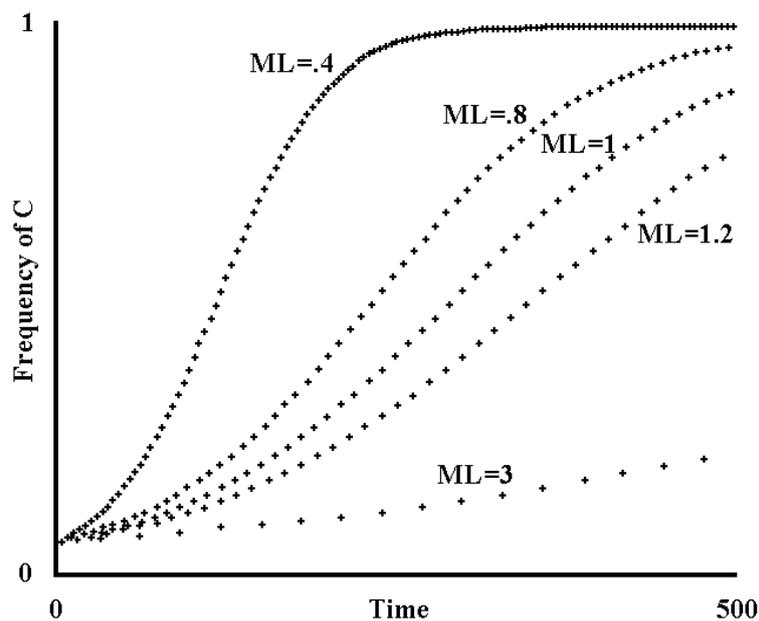


Fig. II 2-1 - Variation of the spreading velocity of a gene depending on ML variation (Theoretical model).

The expression “spreading velocity of a gene” means the inverse of the time necessary to pass from a frequency a to a frequency a' of the gene C with advantage S. The values a , a' and S are established arbitrarily, provided that $a < a'$ and $S > 0$. The formula used for the curves of the figure is the same as the iterative formula in Fig. I 2-1:

$$C_{n+1} = \frac{C_n(1+S)}{1+C_n S} \quad (\text{II-2})$$

The units of time are shown on the abscissas (10 u. from one cross to the next, with reference to the third curve, up to 500 u.). On the ordinates are the frequencies of C in 5 populations with different ML values. The populations are hypothesized as being numerically constant, so $ML = 1$ generation.

The values of C are illustrated with one cross every 10 generations. Going from top to bottom, the values of the MLs, in units of time, are:

$$ML_1 = .4 ; ML_2 = .8 ; ML_3 = 1 ; ML_4 = 1.2 ; ML_5 = 3.$$

Moreover, $S = K = .01$ and $C_0 = .05$ for all curves.

For the third curve, ML and units of time coincide. Note that the curves are morphologically equal to those in Fig. I 2-1. If we bear in mind that, in this figure, $ML = 1$ unit of time for all five curves (as for the third curve of this figure), this was obtained by varying S to an appropriate extent. In fact, for Fig. I 2-1, going from top to bottom, $S =$:

$$\frac{K}{ML_1} ; \frac{K}{ML_2} ; \frac{K}{ML_3} ; \frac{K}{ML_4} ; \frac{K}{ML_5} \quad (II-3)$$

Thus, the figure shows graphically that an increase in S or a proportional decrease in the ML, or vice versa, causes the same effects, as regards the spreading velocity of a gene. It is possible to demonstrate mathematically that this statement is roughly true for small values of S . In this demonstration, it should be noted that:

$$C_1 = \frac{C_0(1+S)}{1+C_0S}$$

$$C_2 = \frac{C_1(1+S)}{1+C_1S} = \frac{\frac{C_0(1+S)}{1+C_0S}(1+S)}{1+\frac{C_0(1+S)}{1+C_0S}S} = \frac{C_0(1+S)^2}{1+C_0S+C_0S(1+S)}$$

$$C_3 = \frac{C_2(1+S)}{1+C_2S} = \dots = \frac{C_0(1+S)^3}{1+C_0S+C_0S(1+S)+C_0S(1+S)^2}$$

.....

$$C_n = \frac{C_0(1+S)^n}{1+C_0S+C_0S(1+S)^1+C_0S(1+S)^2+\dots+C_0S(1+S)^{n-1}}$$

$$= \frac{C_0(1+S)^n}{1+C_0S((1+S)^0+(1+S)^1+(1+S)^2+\dots+(1+S)^{n-1})} \quad (II-4)$$

Using the formula of the geometric series, we obtain:

$$C_n = \frac{C_0(1+S)^n}{1-C_0S \frac{1-(1+S)^n}{1-(1+S)}} = \frac{C_0(1+S)^n}{1-C_0(1-(1+S)^n)} \quad (II-5)$$

If n is an integer, then by using Newton's binomial formula and disregarding the terms having S with an exponent greater than 1, which is justifiable as S is assumed to be small, we obtain:

$$C_n \approx \frac{C_0(1+nS)}{1-C_0(1-1-nS)} = \frac{C_0(1+nS)}{1-C_0Sn} \quad (II-6)$$

If we recall that the number of generations in a period T is inversely proportional to the ML:

$$n = \frac{T}{ML} \quad (II-7)$$

ML

By substitution, we obtain:

$$C_T = \frac{C_0(1 + S T / ML)}{1 + C_0 S T / ML} \quad (II-8)$$

that is:

$$C_1 \approx \frac{C_0(1 + S / ML)}{1 + C_0 S / ML} \quad (II-9)$$

where the coefficient of C indicates the time and not the generation and this is proof of what I wanted to show for integer values of n. If we consider that the equality is rough, by interpolation it is possible to conclude that it is valid for fractional values of n too. The exact non-iterative formula is, likewise:

$$C_T = \frac{C_0(1 + S)^{T/ML}}{1 - C_0(1 - (1 + S)^{T/ML})} \quad (II-10)$$

that is:

$$C_1 = \frac{C_0(1 + S)^{1/ML}}{1 - C_0(1 - (1 + S)^{1/ML})} \quad (II-11)$$

For the subsequent models, I will favour, where necessary, the approximate formula because it is easily compatible with other iterative formulas.

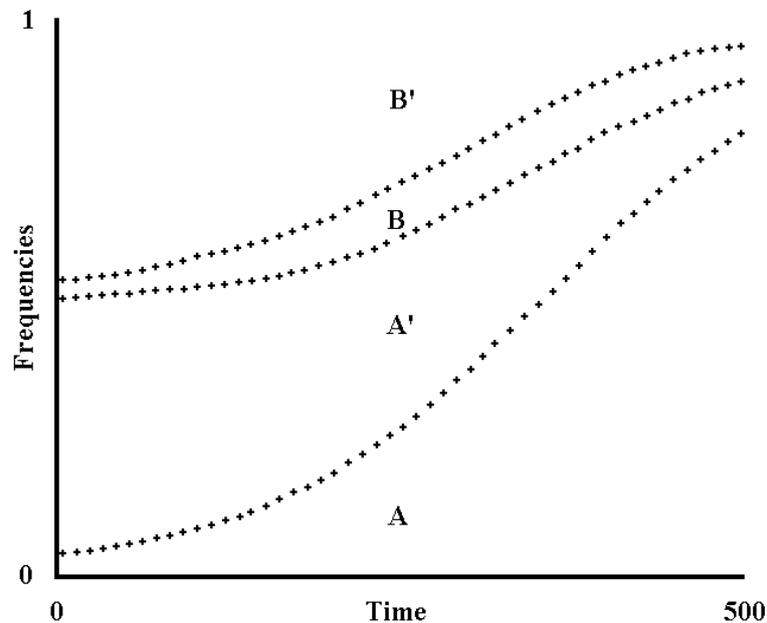


Fig. II 2-2 - Prevalence of a species over another on the basis of a different ML (Theoretical model).

Let us consider two species in competition, a and b. In species a, there are the alleles A and A' with the advantage S_a of A over A'. For species b, analogous conditions are assumed, thus defined as B, B' and S_b.

ML_a and ML_b indicate the ML of a and b, respectively.

In the previous model (Fig. II 2-1), it was shown that a decrease in S and a proportional increase in the ML, or vice versa, cause the same effects as regards the spreading velocity of a gene. Thus, assuming, for the sake of simplicity, that ML_a = 1 unit of time and multiplying S_a by 1/ML_a = 1 and S_b by 1/ML_b, it is possible to construct curves regarding the spreading velocity of a gene, as if ML_a and ML_b were identical and equal to the unit of time. Assuming that the species are isolated from each other, but with a constant overall number of individuals, we would have:

$$\begin{aligned} A_{n+1} &= \frac{A_n(1 + S_a)}{D}; & A'_{n+1} &= \frac{A'_n}{D} \\ B_{n+1} &= \frac{B_n(1 + S_b(1/ML_b))}{D} & B'_{n+1} &= \frac{B'_n}{D} \end{aligned} \quad (\text{II-12})$$

where D indicates the sum of the numerators and maintains the sum of the frequencies constant:

$$A_y + A'_y + B_y + B'_y = 1 \quad (\text{II-13})$$

Now, if we consider that the two species are in competition with each other, and assume that, at each generation, advantage S_i is proportional to the fractions:

$$\begin{aligned} \frac{A_n}{A_n + A'_n} &= F_a & \text{for species a,} \\ \frac{B_n}{B_n + B'_n} &= F_b & \text{for species b,} \end{aligned} \quad (\text{II-14})$$

These express the degree of spreading of a favourable gene within a species; from these conditions we obtain:

$$\begin{aligned} A_{n+1} \text{ (corrected)} &= \frac{A_{n+1}(1 + S_i F_a)}{D} \\ A'_{n+1} \text{ (corrected)} &= \frac{A'_{n+1}(1 + S_i F_a)}{D} \\ B_{n+1} \text{ (corrected)} &= \frac{B_{n+1}(1 + S_i F_b)}{D} \\ B'_{n+1} \text{ (corrected)} &= \frac{B'_{n+1}(1 + S_i F_b)}{D} \end{aligned} \quad (\text{II-15})$$

where D is, as usual, the sum of the numerators.

Assuming also that gene A changes into A' with rate U_a and similarly defining U_b, we obtain, by using the same procedures for the isolated species:

$$\begin{aligned}
A_{n+1} &= \frac{A_n(1 + S_a - U_a)}{D}; & A'_{n+1} &= \frac{A'_n + U_a A_n}{D} \\
B_{n+1} &= \frac{A_n(1 + (S_b - U_b)/ML_b)}{D}; & B'_{n+1} &= \frac{B'_n + U_b B_n/ML_b}{D}
\end{aligned}
\tag{II-16}$$

and formulas identical to those above for the species in competition. Note that, if $U_a, U_b = 0$, this second group of formulas changes into the preceding one.

The curves were obtained using the second group of formulas. The time is on the abscissas (10 units from one cross to the next up to 500 units of time, equal to as many generations of a).

On the ordinates, going from bottom to top, are the frequencies:

$$A_y; \quad A_y + A'_y; \quad A_y + A'_y + B_y.$$

The assumed values are:

$$ML_b = 1.5; \quad S_a, S_b, S_1 = .01; \quad U_a, U_b = .0001; \quad A_o, B_o = .03; \quad A'_o, B'_o = .47.$$

Apart from the inequality of the ML, a and b start, therefore, with equal conditions. The curves show the prevalence of a over b as a consequence of the faster diffusion of the favourable gene within species a.

3) Evolutionary steadiness of character senescence

A character is defined as evolutionarily stable when it entails advantages greater than the possible disadvantages plus the load of disruptive mutations, so that the character is not lost.

In the reasoning of the previous paragraph, two species with different longevity were compared, with the assumption, however arbitrary, that the genes causing senescence are exempt from mutations, selective pressures and other factors that modify their frequencies within each species. The argument has shown that, between two species with different longevity, and other conditions being equal, the one with the lesser longevity is favoured; no indication has been given, however, about the steadiness of evolution within a species, of character senescence (or limited longevity, which is the same thing). I am now going to examine this fundamental question, thereby abolishing the assumption formulated in the reasoning above.

At first glance, it is difficult to justify the steadiness of the character senescence. In fact, the advantage of senescence would seem to apply over several generations and for the species *in toto*, although there are certainly a number of immediate advantages for the single organism, which is not - or is less - senescent, such as, for example: a greater ability to produce several offspring, a lesser incidence of the more vulnerable period of life, such as the period of growth, etc. (see Chapter II, par. 5 too). But it is implicit in the concept of selection that it cannot act on a future advantage or in defence of such a theoretical entity as a species.

It is necessary to prove that senescence brings about an immediate advantage at each generation for the genes causing it, and that the immediate advantages of the non - or less - senescent organism clash with such an immediate advantage. If this were not so, the genes causing the senescence would decay (see Fig. II 3-1).

I think that the answer should be looked for in the light of that which is the pivotal concept of modern sociobiology, namely the non-coincidence, in order to natural selection, of individual and genome of the same individual. I will start with the

observation - inexplicable if this concept is not considered - that, in the animal world, there are behaviours defined as “unselfish”, which are harmful for the individual but advantageous for other genetically related individuals (Wilson, E. O., 1975, Chapter V). We have, for example, the social organization of certain mammals which sometimes engender disadvantages for the single individual, but which is useful for the survival of the herd. The stronger individuals in a troop of baboons are capable of stopping a fierce animal, even at the cost of their life, to keep the herd safe. In the herds of many species, the younger and more vulnerable individuals are at the centre, while the adult animals place themselves in more dangerous positions. Moreover, the adults of some bird species are able to distance themselves from the nest, pretending to be injured in order to attract the predator’s attention to themselves, thereby saving their young at the risk of their own life. But, the more sensational examples are offered by eusocial insects where sterile - but sometimes potentially fertile – individuals, devote their energies to caring for the offspring of few other individuals (queen bee, drones, etc.). Darwin, observing these phenomena, which are, seemingly, quite in contradiction with natural selection, already hypothesized the existence of supra-individual mechanisms of selection (Darwin, C., 1859). From the study of eusocial insects alone, a rigorous sociobiological explanation originated, in evolutionary terms, of the phenomenon of “unselfishness” as an alternative to the classic explanation of group selection.

If a character defined by the gene C is harmful for individual I, in which it is present, but entails an advantage for other related individuals having a fraction F of the genes identical to those of individual I and, therefore, a probability F of having C, the spreading of the gene C is subjected to two contrasting selective pressures. If the sum of the two pressures (inclusive fitness) is positive, gene C is favoured, although it entails a disadvantage for the specific individual in which it is present. Note that, according to this logic, gene and individuals are distinct entities in order to selective process and the individual is subordinate to the gene, so much so that Wilson even phrased the aphorism thus: “the organism is only the means by which DNA is able to make other DNA” (Wilson, E. O., 1975, p. 3).

For a more formal exposition, see the model of Fig. II 3-2.

Returning to the subject of senescence, it is now necessary to evaluate the inclusive fitness of a gene C that reduces longevity. If an individual I, when it dies prematurely as a consequence of the action of C, is substituted by genetically related individuals, the advantage of the faster spreading of any gene y must be calculated to the extent that the individual substituting I is related to it, namely to the extent that it has a mean portion F of identical genes (= kinship coefficient). As the genes y that are spreading in a species are many, the overall advantage of the faster spreading of the genes should not be negligible, even if F is small. The model of Fig. II 3-3 has been constructed on these concepts. This model shows how, with minor modifications of the model of the preceding figures, it is possible to achieve a simple demonstration of the evolutionary steadiness of character senescence.

Note that, if in the model, the fraction F is assumed to be equal to 0, the formula becomes identical to that of Fig. II 3-1.

* * *

Based on what we learn from population genetics and natural observation (Wilson, E. O., 1975), namely that:

- 1) the species is often divided into many small groups (demes);
- 2) the genetic flow among the various demes is not unlimited;
- 3) if the number of individuals of a deme is not great (<100-200), genetic drift is not a negligible phenomenon;

4) interdemic selection may have its importance in the evolution;

I have worked out an alternative model, which does not exclude the other, to maintain the steadiness of senescence character. The species is hypothesized to be divided into N demes, each made up of n individuals. C is, as usual, a gene that causes reduced longevity. The frequency of gene C in a deme, a frequency on which the ML of the individuals of the deme depends, varies from one deme to another because of the genetic drift. The demes have been hypothesized to be completely isolated from each other for a certain number of generations during which gene C frequency in each deme decreases moderately because of disadvantage S' which is a result of the reduced longevity and also because the substitutions within each deme are hypothesized to be non-preferential for genetically related individuals ($F = 0$). At the same time, during the isolation period, frequency G of any favourable gene y increases to a differential extent for each deme because of the interdemic variation of C . In the period of isolation, let us assume that there is interdemic competition and selection (read: differential extinction) depending on the advantage deriving from the greater or lesser spreading of G . At the end of the isolation period, there is a phase in which all demes are merged and divided again immediately afterwards. The cycle then repeats itself once more.

This model too (Fig. II 3-4) shows that, with the appropriate values of the factors involved, the frequency of C increases.

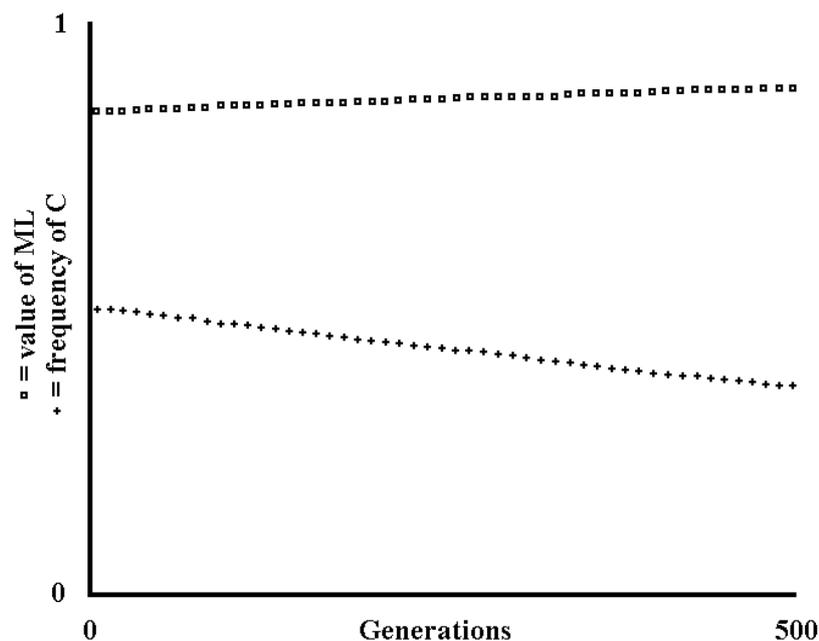


Fig. II 3-1 - Decay of the character senescence (Theoretical model).

C is a gene that brings about a more precocious senescence. The individuals with allele C' have an ML equal to 1 unit of time and those with gene C have $ML = V_c$ with $V_c < 1$. The reduced longevity results in disadvantage S' (see Chapter II, par. 5). Likewise, reduced longevity brings the advantage of a faster spreading of the favourable genes within a species, as a consequence of the faster turnover of individuals (see Chapter II, par. 2). If this advantage is in favour of any individual of the species, both individuals with gene C and those with allele C' are advantaged, so the advantage of C against C' is non-existent.

With these assumptions and using the same procedures as in the preceding models and taking the ML of the whole population at the n th generation to be:

$$ML_n = \frac{C_n V_c + C'_n 1}{1} = C_n V_c + 1 - C_n = 1 - C_n (1 - V_c) \quad (\text{II-17})$$

we have:

$$C_{n+1} = \frac{C_n (1 - S' / ML_n)}{1 - C_n S' / ML_n} \quad (\text{II-18})$$

The assumed values are:

$$C_0 = .5 ; S' = .001 ; V_c = .7.$$

In the figure, the crosses indicate the frequency of C and the squares the value of the ML. The abscissas indicate the generations (values from 0 to 500). The ordinates express both the frequency of C (values from 0 to 1), and the value of the ML (values from 0 to 1 time unit). The figure shows the decrease in frequency of C and the consequent increase of the ML.

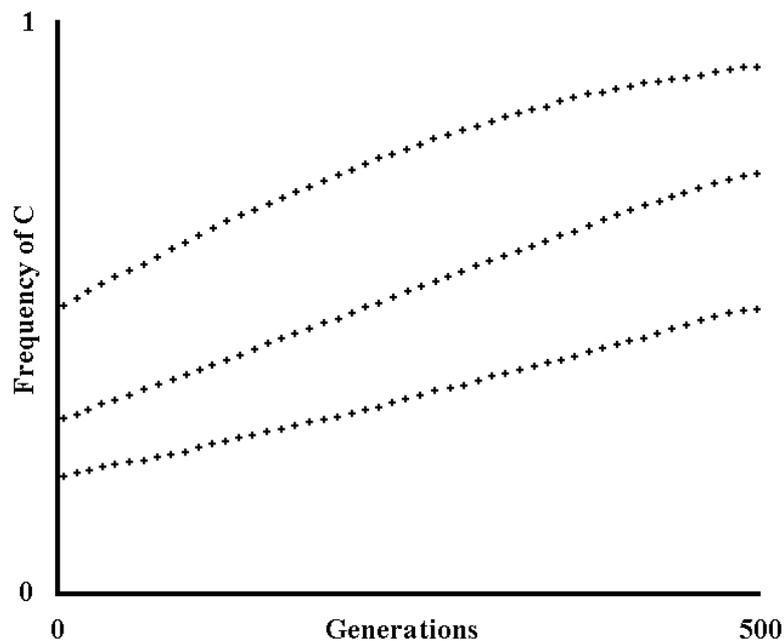


Fig. II 3-2 - Evolutionary steadiness of an “unselfish” character (Theoretical model).

C is a gene that brings disadvantage S' for the individual I in which it is present. Moreover, C brings advantage S for an individual I' having the fraction F (= kinship coefficient) of genes in common with the individual I. Gene C, which has probability F of being present in the individual I', shows, for each generation, an increase of frequency proportional to the product F S. Therefore, we have:

$$C_{n+1} = \frac{C_n (1 + F S - S')}{1 + C_n (F S - S')} \quad (\text{II-19})$$

If the advantage S is expressed towards n individuals, and to a differential extent, the product F S must be substituted with the summation:

n

$$\sum_{x=1}^n F_x S_x.$$

If we also consider a rate U of mutation of C into the allele C' , which is assumed to be inactive, it is possible, in the end, to obtain:

$$C_{n+1} = \frac{C_n (1 + \sum_{x=1}^n F_x S_x - S' - U)}{1 + C_n (\sum_{x=1}^n F_x S_x - S' - U)} \quad (\text{II-20})$$

formula used for the curves of the diagram.

Going from top to bottom, the assumed values are:

$$C_0 = .5 ; n = 1 ; S_1 = .03 ; F_1 = .5.$$

$$C_0 = .3 ; n = 2 ; S_1 = .05 ; F_1 = .125 ; S_2 = .03 ; F_2 = .25.$$

$$C_0 = .2 ; n = 3 ; S_1 = .04 ; F_1 = .25 ; S_2 = .01 ; F_2 = .125 ; S_3 = .003 ; F_3 = .5.$$

Moreover, for all curves: $S' = .01$, $U = 0$.

With the assumed values, the curves show an increase in frequency of gene C .

* * *

It should be noted that, for the sake of simplicity, an equal reproductive value for all individuals has been left out of this model (see definition in Wilson, E. O., 1975, p. 98) and all other conditions of asymmetry have been excluded. With appropriate modifications of the formulas, these factors can, however, be considered without modifying the general meaning.

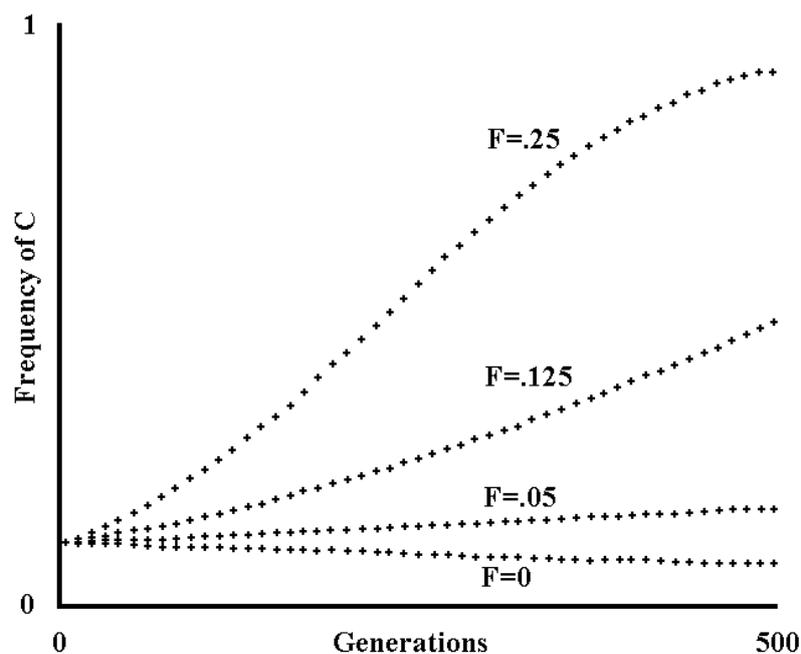


Fig. II 3-3 - Evolutionary steadiness of the character senescence (Theoretical model based on inclusive fitness).

C is a gene that brings about a more precocious senescence. The individuals with allele C' are assumed to have an ML equal to 1 unit of time, and those with gene C an ML equal to V_c and lesser than 1.

Reduced longevity results in a disadvantage S' (see Chapter II, par. 5).

It is also assumed that an individual I, when it dies, is substituted by another individual I', which has, on average, a portion F of the genes identical to those possessed by I and has, therefore, a probability F of having C (preferential substitution).

For the remaining portion (1 - F) there is, between the genes of I and I', the same likeness that there is between any two individuals of the species.

Within a species, gene y favoured by advantage S is spreading. Given that for the spreading velocity of a gene, a reduction of the ML is equivalent to a proportional increase of advantage S (see Fig. II 2-1), it is assumed, for the individuals with the lesser longevity, that:

$$S_c = \frac{S}{V_c} \quad (\text{II-21})$$

while for the individuals with normal longevity:

$$S_{C'} = \frac{S}{1} = S \quad (\text{II-22})$$

Moreover, all individuals are assumed to have a unique ML (= 1 unit of time).

The difference between the two advantages is:

$$S_c - S_{C'} = \frac{S}{V_c} - S = S (1/V_c - 1) \quad (\text{II-23})$$

This differential advantage is applied over that fraction F of genes that is identical in I and I', so, if we also consider that (see Fig. II 3-1):

$$ML_n = \frac{C_n V_c + C'_n 1}{1} = 1 - C_n (1 - V_c) \quad (\text{II-24})$$

we have:

$$C_{n+1} = \frac{C_n (1 + F S (1/V_c - 1) - S'/ML_n)}{1 + C_n (F S (1/V_c - 1) - S'/ML_n)} \quad (\text{II-25})$$

The curves of the figure were obtained assuming the following values:

$C_0 = .1$; $S = .1$; $S' = .001$; $V_c = .7$ for all curves, $F = .25$; $.125$; $.05$; 0 for the various curves, going from top to bottom.

Note that it has been assumed that $S \gg S'$ since S summarizes the advantage of the K genes y that are spreading within a species and so:

$$S = \sum_{x=1}^K S_x \quad (\text{II-26})$$

with K that is a not small number.

Note also: if we assume that $F = 0$ (non-preferential replacement), the formula changes into that of Fig. II 3-1 and the frequency of C decreases (see lower curve).

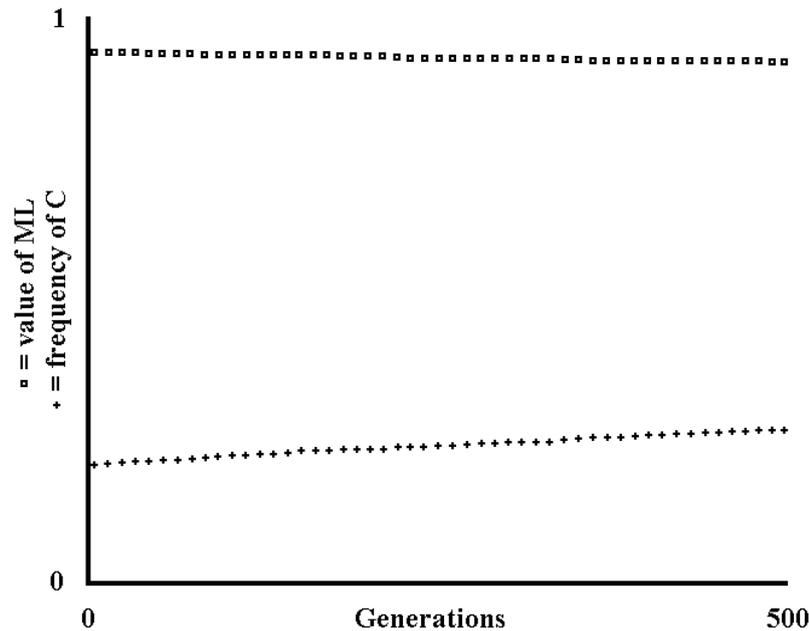


Fig. II 3-4 - Evolutionary steadiness of the character senescence (Theoretical model based on the division in demes).

Gene C, which gives rise to more precocious senescence, is present within the species. As for the preceding models, the ML of the individuals with gene C' is equal to 1 unit of time, while for those with gene C, it is equal to $V_c (< 1)$, and:

$$ML_n = 1 - C_n (1 - V_c) \quad (II-27)$$

The species is divided into N demes, each made up of a number n of individuals. Because of the genetic drift, the frequency of C in each deme is variously different from the mean value of C for the whole species. Using mathematical method and a RANDOM function, the frequency of C in each deme is calculated at each "cycle" (see definition below). For further details on this point, see the source code of the program used (s. Appendix 4).

The demes are hypothesized to be completely isolated from each other genetically for a certain number (ST) of generations (ST generations = 1 cycle). In this period, the gene C undergoes a slight decrease in frequency for the disadvantage S' , deriving from a reduced longevity and because it is assumed that the replacement of predeceased individuals is not preferential (s. Fig. II 3-1 and Fig. II 3-3). The formula used is:

$$C_{n+1} = \frac{C_n (1 - S'/ML_n)}{1 - C_n S'/ML_n} \quad (II-28)$$

In the same period, the gene G, favoured by the advantage S, is spreading within each deme with different velocities depending on the ML of the individuals of the deme. This is calculated using the formula:

$$G_{n+1} = \frac{G_n (1 + S/ML_n)}{1 + G_n S/ML_n} \quad (II-29)$$

In the model, it is also assumed that there is interdemic competition (read: differential extinction) with advantage, depending on the greater or lesser spreading of G, proportional to:

$$D_{x,nf} = \frac{D_{x,n} G_{x,nf}}{\sum_{k=1}^n D_{k,n} G_{k,nf}} \quad (\text{II-30})$$

where the terms $D_{x,n}$ and $D_{x,nf}$ indicate the fraction - with regard to the whole species - of individuals belonging to deme x at the beginning and the end, respectively, of the nth cycle and the term $G_{x,nf}$ indicates the frequency of G in deme x at the end of the nth cycle.

From this, it is possible to calculate the mean frequency of C in the whole species at the end of each cycle, and at the beginning of the next cycle (C_o):

$$C_o = \sum_{k=1}^n C_{k,nf} D_{k,nf} \quad (\text{II-31})$$

where the term $C_{k,nf}$ indicates the frequency of C in deme k at the end of the nth cycle. After the isolation period, the demes are reunified, redistributing gene C within the species. Immediately afterwards, the species is again divided into numerically equal demes and the cycle resumes again.

Note that the frequency of G is assumed to be equal to a constant ($G_o = .5$) at the beginning of each cycle. In fact, as the spreading of G means the endless spreading within a species of all genes that entail an advantage, and G therefore represents the mean of a collection of constantly renewed genes, in spreading, it is preferable to assume G to be equal, at the beginning of each cycle, to a frequency halfway between that of the lowest spreading (= 0) and that of the greatest spreading (= 1). Moreover, because G is the average of the spreading of many genes, it must be assumed that $S \gg S'$.

The figure was obtained assuming the following values:

$$N = 10 ; n = 10 ; ST = 10 ; V_c = .7 ; S' = .0001 ; S = .1 ; C_o = .2.$$

In the figure, the crosses indicate the frequency of C and the squares the ML. With the assumed values, the figure shows an increase of the frequency of C within a species. The quite limited inclination of the spreading curve of C, gives the impression that interdemic selection is secondary for the steadiness of the character senescence, with regard to the mechanisms illustrated in the model of the previous figure.

4) Other selective pressures affecting longevity

The experimental verification or the confirmation in natural observations of that which is theoretically maintained in the preceding paragraphs, comes up against the significant problem that the phenomena discussed concern a period of many generations, and thus contrasts with the limited life duration of the Experimenter or of the Naturalist. An experimental confirmation could, perhaps, be obtained using the theoretical models described so far, as well as those that will follow. Moreover, useful data could be offered by accurate comparative observations on the longevity and velocity of evolution

of species that are related and/or have a similar ecological niche, not forgetting to take into consideration the other selective pressures that contribute evolutionarily in affecting longevity.

It would, in fact, be simplistic to think that longevity is dependent only on the necessity of a greater or lesser velocity of evolution. It has to be observed that, other conditions being equal, the more long-lived species for a time likewise greater on the total of life duration are in the adult state. And the adult is usually less vulnerable to the dangers of the habitat than forms that are growing. A greater longevity has, therefore, this first advantage. This is, perhaps, particularly so in the case of trees. In fact, the development from seed to fully-grown tree, given the ruthless competition of the other plants, is a by no means short and highly problematic phase of the life cycle of trees. It is no surprise if examples of the greatest longevity are known among the trees. *Sequoiadendron* and *Pinus aristata* are species of which there are known to be millennia-old specimens which seem to not age at all. These are extreme cases and the evolutionary vulnerability caused by their non-ageing is, perhaps, indicated by the restricted nature of the zones in which they vegetate. Even among the trees, the species with limited longevity predominate.

A greater body mass should be, out of necessity, another factor influencing longevity: in such a case, the period of formation and growth of the individual will, evidently, tend to be longer and the longevity will have to increase proportionally, so that the percentage incidence of the vulnerable period of formation over the total duration of life decreases. It is probably for this reason that the whale's longevity is not low (30-50 years according to Comfort, A., 1979, and 80 years as its greatest longevity, according to the data reported by Caleb, E. F., 1977).

The extent of learning abilities is probably another important factor: the greater the learning abilities, the greater longevity must be in order for an individual to learn and benefit from the advantage consequent to the learning. If one considers that man has high learning abilities, the fact that he has the greatest longevity among the mammals would seem to be justified.

The elephant, which combines a great body mass, albeit much lower than that of the whale, with a considerable learning ability, albeit much lower than that of man, also ranks among the longer-lived mammals (40 years as longevity and 70 years as its greatest longevity, according to the data reported by Caleb, E. F., 1977).

And yet, the fact that a species is more subject to r selection or, on the contrary, to K selection (see Wilson, E. O., 1975, Chapter 4) certainly influences, evolutionarily-speaking, the longevity, in the sense that the r-selection favours those populations that are less long-lived and the opposite happens with the K-selection. (However, the conditions in which there is r- or K-selection are perhaps describable as a subset of the conditions in which a greater or lesser velocity of evolution, respectively, is necessary).

Finally, periodical climatic variations are also decisive in terms of longevity, when the ecological niche of a species is strictly dependent on the afore-mentioned variations. For a great many insects and plants, the duration of the life-cycle is, in fact, strictly dependent on seasonal or annual variations.

It remains to be explained why many species that live in conditions of high mortality by causes damaging at any age, have a great or unlimited longevity.

5) The Methuselah effect

A name that smacks of legend might be of considerable help in remembering a particular phenomenon. The somewhat longer, more technical name, might read: "the evolutionary effect of longevity increase caused by mortality increase deriving from causes damaging at any age". It is demonstrable, from a theoretical point of view, that

mortality due to the afore-mentioned causes concurs in the determination of the longevity of a species.

* * *

A certain degree of variability of the ecological niche of a species requires an adequate velocity of evolution of the species. The velocity of evolution has been said to be inversely proportional to the ML of a species. I wish to stress that the ML is, in turn, dependent on:

- 1) how fast the senile age arrives;
- 2) the mortality rates by causes damaging at any age.

In other words, both 1) and 2) contribute to limiting the ML with the advantage discussed in the preceding paragraphs of a proportionally greater spreading velocity of the genes.

Now, let us consider a species where 2) is acquiring a greater importance in ML limitation: in such a case 1), namely senescence, should come later and later if ML is to remain constant. That is, the velocity of evolution is, to an ever greater extent, an effect of the increased mortality by causes damaging at any age rather than a consequence of a limited longevity. This would be an effective explanation of the rather high longevity that is observed for many small animals, which live in conditions of high environmental mortality. Many birds of small size in captivity survive for even 15-20 years, while in the original ecological niche, the ML is much lower because very few reach the age of "natural" death.

The study of a great number of amphibians, fishes, invertebrates, etc. give analogous data (Comfort, A., 1966a and 1979).

It seems almost excessive to stress that the Methuselah effect, if it really exists, will be observable only over a sufficient number of generations; it is by no means to be understood that a variation of the mortality by causes independent of senescence significantly modifies the longevity in the space of one or few generations.

For a better expression of the Methuselah effect, see figures II 5-1 and II 5-2.

* * *

In short, if the arguments so far expounded in this chapter are correct, longevity is *increased* by:

- 1) a greater stability of the ecological niche;
- 2) an increase in the incidence of the more vulnerable period of life, such as that of initial formation and of growth;
- 3) a greater body mass;
- 4) a greater learning ability;
- 5) a prevalence of "K-selection";
- 6) an increase in mortality by causes damaging at any age;

and *decreased* by:

- 1) a lower stability of the ecological niche;
- 2) a decrease in the incidence of the more vulnerable period of life, such as that of initial formation and of growth;
- 3) a lower body mass;
- 4) a lesser learning ability;
- 5) a prevalence of "r-selection";
- 6) a decrease in mortality by causes damaging at any age.

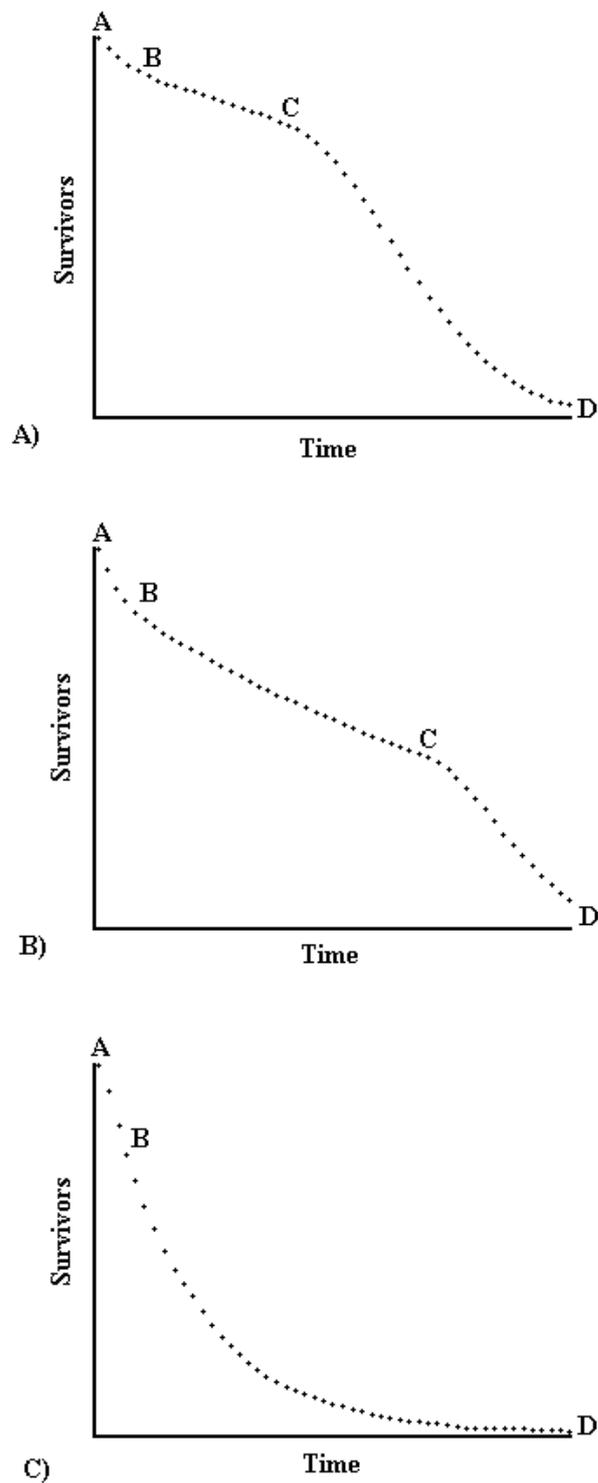


Fig. II 5-1 - Graphic illustration of the Methuselah effect.

A) Life table of an aging species. The time is on the abscissas and the percentage of the surviving individuals on the ordinates. There is an initial period AB with high mortality (see Fig. II 1-1), followed by a segment BC with almost constant mortality and which depends on the environmental conditions, and finally a segment CD with mortality that is high and increasing because of senescence. For the curve there is a calculable value z

of ML species depending on the selective pressures discussed in the preceding paragraph.

B) In this second curve, other conditions being equal, there is an increase in the inclination of the segment BC, an expression of the mortality increase by causes that are damaging at any age. This would cause a decrease of z if not compensated by the displacement of point C toward the right.

C) Limit curve: a strong increase in the inclination of BC corresponds to a displacement to infinity of point C, meaning the species becomes of unlimited longevity. Such a displacement of C could also be caused by a sufficient increase in z , as a consequence of a decreased necessity for rapid evolution of the species.

The equation that defines the curves is:

$$Y_t = Y_0 (1 - K)^t \quad (\text{II-32})$$

where: K = mortality rate; Y_t = surviving at time t .

In the first two curves K is different in the segments AB - BC - CD and, moreover, is decreasing in segment AB and increasing in segment CD.

In the third curve, K is greater in AB than in BD, and decreasing, but is constant in BD.

A program was used (see Appendix 4) to draw the curves, which are illustrative and not demonstrative. The values assumed are:

Curve A): $B = 5$; $C = 20$; $K = .01$; $I_1 = 1.1$; $I_2 = 1.1$;

Curve B): $B = 5$; $C = 35$; $K = .02$; $I_1 = 1.1$; $I_2 = 1.1$;

Curve C): $B = 5$; $C = 50$; $K = .1$; $I_1 = .01$; $I_2 = 0$.

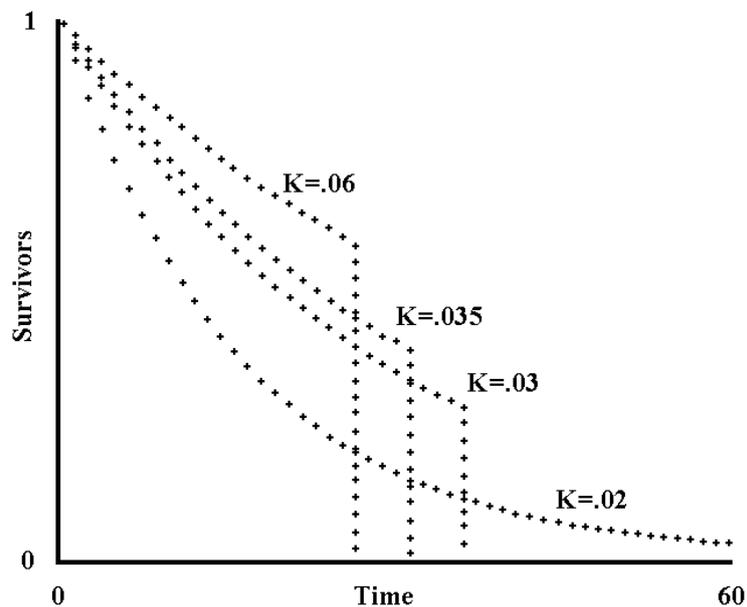


Fig. II 5-2 - Methuselah effect (Theoretical model).

In the model, the mortality rate (K) for each curve is constant from birth until an instant L , when all surviving individuals die at the same time. L is the ideal equivalent of longevity and the definition is such that will be easy to deal with mathematically. The curves are given by the formula:

$$Y_t = Y_0 (1 - K)^t \quad (\text{II-33})$$

with: $0 \leq t \leq L$.

Y_t indicates the fraction of the survivors at time t . From instant L , each curve goes down, parallel to the ordinates, until it meets the abscissas. Let us calculate the ML:

$$\begin{aligned} ML &= \frac{\int_0^L Y_0 (1-K)^t dt}{Y_0} = \int_0^L (1-K)^t dt \\ &= \left[\frac{(1-K)^t}{\text{Log}_e(1-K)} \right]_0^L = \frac{(1-K)^L - 1}{\text{Log}_e(1-K)} \end{aligned} \quad (\text{II-34})$$

Note that if $L \rightarrow \infty$, as $K < 1$, then it follows that $(1-K)^L \rightarrow 0$ and we have the equation:

$$ML = - \frac{1}{\text{Log}_e(1-K)} \quad (\text{II-35})$$

from which we have:

$$K_1 = 1 - e^{-1/ML} \quad (\text{II-36})$$

where K_1 indicates the limit value of K beyond which the equation has no meaning. If we want ML to remain constant, in spite of a variation in K , then L must also vary. So, if,

$$ML = \frac{(1-K)^L - 1}{\text{Log}_e(1-K)} = \frac{(1-K')^{L'} - 1}{\text{Log}_e(1-K')} \quad (\text{II-37})$$

by solving with regard to L (or, is the same, with regard to L'), we obtain:

$$\begin{aligned} ML \text{Log}_e(1-K) + 1 &= (1-K)^L \\ L &= \frac{\text{Log}_a(ML \text{Log}_e(1-K) + 1)}{\text{Log}_a(1-K)} \end{aligned} \quad (\text{II-38})$$

where a is any base.

This equation, to the extent that it is possible to verify, is, moreover, meaningless for values of $K > K_1$. The equations show that, when the condition of ML is constant, an increase in L corresponds to an increase in K . This is so until the value of $K = K_1$, at which point L reaches its maximal value ($= \infty$) and cannot increase further.

In the figure, four time-surviving individuals' curves are shown. The value of the ML is equal to 20 units of time. Going from bottom to top, the assumed values for K are:

.06 ; .035 ; .03 ; .02.

K_1 is obtained from the formula expounded above and is equal to: .0487705755.

6) Theories about the “how” of senescence

So far, I have investigated the “why” of senescence; that is, I have speculated on the possible teleonomic meaning that should be attributed to senescence phenomenon or to its appearance either sooner or later, depending on the species. In the light of what seems to be the conclusions of the arguments developed in the preceding paragraphs,

and within the very general limits allowed by theoretical reasonings, I now wish to consider the theories about the "how" of senescence. Several theories (see Comfort, A., 1979 and Caleb, E. F., 1977 for a review) have been put forward to explain the slow decay of the aging organisms, but disregard, in my opinion, the teleonomic question, often in a de facto manner, without any distinction between "how" and "why". I do not intend, here, to do an exposition or a history of the theories put forward so far. Likewise, I have tried to focus attention on four different ways of explaining the senescence phenomenon, reworking and interpreting freely and without mentioning, therefore, the authors that first put each concept forward, and without distinguishing between what has been already expressed by others and what is, perhaps, expressed for the first time. After this premise, I will classify the theories about the "how" of senescence in this way:

- a) Theories of senescence caused by wear;
- b) Theory of senescence caused by insufficient selection;
- c) Theory of hampered senescence;
- d) Theory of programmed senescence.

I will dedicate this paragraph to a) and b), while c) and d) will be discussed in the next paragraph.

* * *

- a) Theories of senescence caused by wear.

These theories are based on the concept of a "something" that continuously "wears out" the organs of the living being over time, progressively altering their functionality. This "something" was, at first, thought to be the simple use of the organs, but soon the untenability of this hypothesis was apparent. In fact, many organs, if not used become atrophied and, on the contrary, if used, strengthen and remain efficient for longer (e.g.: muscles). Many then tried to conceive of the "something" as being more closely related to time and independent of the use or non-use of the organs. There are, then, theories of aging caused by genetic alterations, mutations, chemical-physical alterations, stress, etc., in which the factors that cause the senescence are occasional mutations, stresses, duplication errors in division cells, progressive chemical alterations, etc. Even if the importance of one or more of these factors is a genuine factor in the genesis of the senescence, it should be noted that these theories do not put forward the question of the evolutionary usefulness or uselessness of the senescence, or of the precocity of the senescence. In the non-evolutionary terms in which they are worded, I reject them, deferring the evaluation of the importance of the empirical data, on which they are based, to the discussion about the theory of hampered senescence that, as we will see in the next paragraph, must be understood as a reformulation in evolutionary terms of the theories of senescence by wear.

* * *

- b) Theory of senescence by insufficient selection.

This theory in itself is of little importance, but it is, perhaps, useful to express it because it allows us to make an important observation. I quote a passage that expounds it:

- Today biologists tend to regard aging not a phenomenon that has evolved according to a particular function, such as A. Weismann thought, but as a phenomenon due to the accumulation of processes that selective pressure has been unable to remove at old age, when the accidental causes have reduced the individual reproductive contribution; this way, as even in species not subject to senescence there are always more young than old individuals, a point is reached where homeostasis no longer meets a sufficient selective

pressure to remain stable; on the contrary, it is possible a positive selection in favour, e.g., of a gene causing high fertility or great vigour in the first phases of the life, but disease or dysfunction in more advanced phases. - (Comfort, A., 1966b)

This theory can be criticized for various reasons:

1) It implies that a negligible percentage of the population reaches the senile age, meaning that life tables such as those allowing the definition of senescence formulated by Comfort (e.g.: see Fig. II 1-1, on the right) should not be observed. The theory in question is, perhaps, due to the observation that individuals which are clearly senescent in a geriatric sense - the same as those defined as "hypersenescent" in a gerontological sense - are rare in natural conditions. I have already stressed that such an interpretation is in intrinsic contrast with Comfort's definition: if rare individuals reach a certain age, it is probable that the increase in mortality, that is, senescence, began before, thereby implying that a considerable portion of the population has reached the senile age.

2) If we accept the existence of a gene that is favourable in young age and harmful later, it is also possible to hypothesize the existence of genes which are favourable at any age and which would be selectively advantaged over the former type of gene, in that percentage of the population reaching the age at which the former genes are harmful. Likewise, we hypothesize the existence of many harmful genes acting at various ages, with no period of life favoured or unfavoured, it is possible to prove (see Fig. II 6-2) that, in a population with non-ageing individuals, even a large number of such harmful genes would not cause a life table comparable to that of a population that ages.

3) Species with a high mortality by causes damaging at any age, as fewer individuals reach advanced ages, should have a more precocious senescence than those with low environmental mortality, which is exactly the opposite of that which is theoretically predicted by the "Methuselah effect". But:

- the greater part of small-sized Birds in the wild have constantly a high mortality, which is independent from the age: the probability of accidental death is so high to allow only to few individuals to age ... the potential life duration of the Birds is usually much greater than that of Mammals of analogous size, although the metabolism of Birds is higher and their growth period short ... Many small-sized Birds reach 15-20 years in captivity ... the slow growth of many Reptiles and Fish, not all of large size, suggests that some of these heterothermic animals age very slowly, so much that, for their mortality, diseases and accidental events have greater importance than age and decline of physical vigour in itself. Some experimental researches indicate that also in these species an aging process is noticeable. - (Comfort, A., 1966a)

I therefore consider the unreliability of this theory, according to which the senile process would be a consequence of the increasingly insufficient selective pressure caused by degenerative processes of unknown type as age advances and the number of surviving individuals decreases, to be obvious. In fact, this theory prompts us to ask the question whether, perhaps, the opposite is true, that is, that – speaking only in terms of the human species for now – the moderate incidence among senescent individuals - or rather "hypersenescent" individuals - of certain diseases caused by genetic defects is, perhaps, a consequence of the reduced selective pressure that they exert at such an age. Indeed, a disease that jeopardizes the survival of individuals that are already past their best in terms of reproductive potential and defence of their offspring, exerts a much lower selective pressure than those diseases that strike at younger ages (see Fig. II 6-1). This concept will also be discussed in Chapter III, par. 5 and in Chapter V, par. 3, 5 and 7.

* * *

Note that it would be wrong to maintain that the decline in reproductive function, as it does not allow the frequency reduction of harmful genes beyond a certain age, therefore causes an increase in mortality and, therefore, by definition, senescence. In such a case, in fact, the teleonomic question concerning senescence could be reformulated in terms of a teleonomic question about the decline in the reproductive function, all other concerns regarding greater or lesser longevity remaining unchanged.

Moreover, in Comfort's definition of senescence there is no hint at a decline in the reproductive function proportional to the increase of the mortality. It is necessary to avoid confusion between the reproductive decline in hypersenescent individuals, which is well demonstrable, and a possible reduction, which needs to be proved, in the reproductive abilities of individuals that are senescent in gerontological terms (see definition). On the other hand, the empirical confirmation of such a correlation having been accepted, it is more correct to consider the decline in reproductive abilities as a feature of senescence, rather than as an independent parameter.

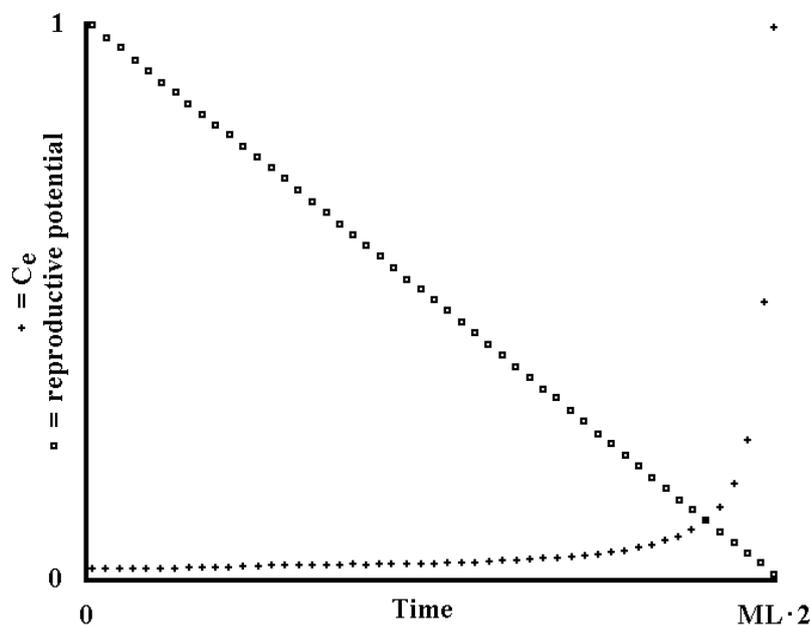


Fig. II 6-1 - Equilibrium frequencies of a gene that is harmful depending on the age of the individual when the gene expresses itself (Theoretical model).

C is a harmful gene with a C' unique allele, which does not entail damage and which changes into C at the rate of V each generation. On the contrary, the mutation frequency of C into C' is negligible.

C manifests its harmful action when the individual reaches age t. As the individual, which progresses in its vital cycle, expresses more and more of its reproductive potential and of its ability to defend its offspring, the damage S caused by C is in inverse relation to age t, in which the gene manifests its harmful action.

Therefore, we have:

$$S = S_{\max} - f(t) \tag{II-39}$$

where S_{\max} is the damage caused by C if expressed from birth and $f(t)$ is a function that must be empirically determined (but, in the present figure, it is defined arbitrarily for practical reasons).

By applying the procedures already used for other models, it is possible to obtain:

$$C_{n+1} = \frac{C_n(1-S) + VC'_n}{1-C_n S} = \frac{C_n(1-S-V) + V}{1-C_n S} \quad (\text{II-40})$$

At equilibrium, we have:

$$C_e = \frac{C_e(1-S-V) + V}{1-C_e S} \quad (\text{II-41})$$

Dividing by C_e , we obtain:

$$1 - C_e S = 1 - S - V + V/C_e$$

$$C_e^2 S - C_e(S + V) + V = 0$$

$$C_e = \frac{S + V \pm \sqrt{(S + V)^2 - 4 S V}}{2 S}$$

$$= \frac{S + V \pm \sqrt{(S - V)^2}}{2 S} = \frac{S + V \pm (S - V)}{2 S} \quad (\text{II-42})$$

Therefore, the two solutions are:

$$C_e = \frac{S + V + S - V}{2 S} = \frac{2 S}{2 S} = 1$$

$$C_e = \frac{S + V - S + V}{2 S} = \frac{2 V}{2 S} = \frac{V}{S} \quad (\text{II-43})$$

The figure has been obtained by using the second solution, but assuming $C_e = 1$ when $V/S > 1$.

Function $f(t)$ has been arbitrarily defined in this way:

$$f(t) = \frac{ML 2 - E}{ML 2} \quad (\text{II-44})$$

where ML is the mean duration of life and E indicates the age at which the gene manifests its harmful action. Thus, the formula of resolution becomes:

$$C_e = \frac{V}{S_{\max} \frac{ML 2 - E}{ML 2}} \quad (\text{II-45})$$

In the figure, the equilibrium frequencies of C are shown, with crosses, on the ordinates. On the ordinates, the fractions of reproductive potential, not yet expressed are also illustrated, with squares. The abscissas indicate the ages (E) at which the gene expresses the damage and the age to which the fraction, not yet expressed, of reproductive potential is referred. The abscissas indicate values that go from 0 to $ML 2$.

The values assumed are:

$$S_{\max} = .01 ; V = .0001.$$

The figure shows that, if C manifests itself when the greater part of reproductive potential is passed, the equilibrium frequency is high.

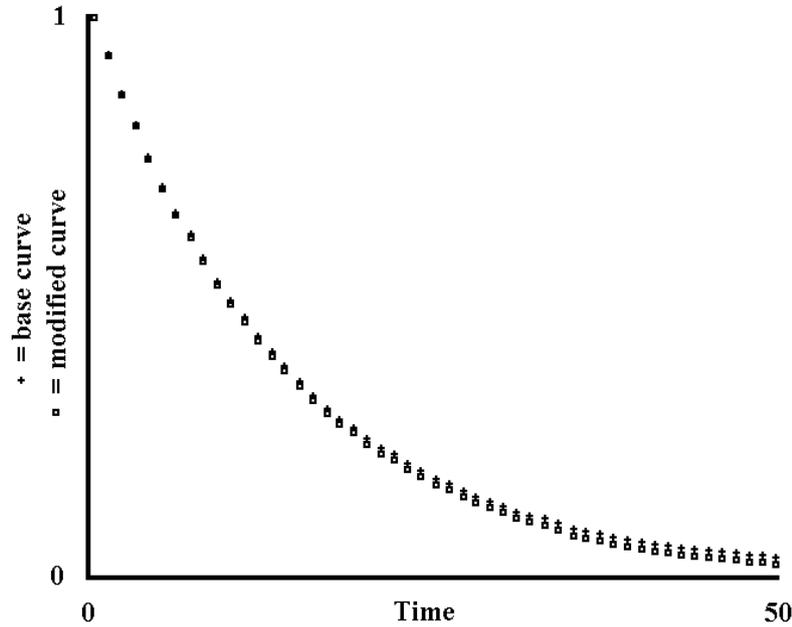


Fig. II 6-2 - Effects on a life table of a large number of genes that are harmful at various ages (Theoretical model).

There is a population made up of individuals with mortality K that is constant at any age of life and therefore not subject to senescence, according to Comfort's definition. Let us also assume that reproductive abilities do not decrease with age and, for simplicity, that the individuals are haploid. Now, I am going to consider the modifications of the life table caused by the action of numerous harmful genes that each manifest themselves exclusively at a certain age. One of these genes is C : it manifests itself at age t , causing damage S , and has no other manifestation. The only allele, C' , is inactive and changes into C with a rate of V at each generation, while C changes into C' with negligible frequency.

Using F_t to refer to the fraction of the population surviving at time t , we have:

$$C_{n+1} = \frac{C_n(1 - S F_t) + V C'_n}{1 - C_n S F_t} = \frac{C_n(1 - S F_t - V) + V}{1 - C_n S F_t} \quad (\text{II-46})$$

At equilibrium, using the mathematical procedure of the preceding figure, we have:

$$C_e = \frac{V}{S F_t} \quad (\text{II-47})$$

Thus, a fraction equal to C_e of the individuals surviving at time t , will suffer damage S , meaning that:

$$F_t(\text{corrected}) = F_t - F_t \cdot C_e S = F_t(1 - C_e S) \quad (\text{II-48})$$

Considering n genes with the same characteristics as C , it is necessary to multiply damage S by n , and so:

$$F_t (\text{corrected}) = F_t (1 - C_e S n) \quad (\text{II-49})$$

This correction having been made, the curve from time $t+1$ onwards must be properly accommodated by taking into account the individuals missing at time t .

Then, considering n genes with the same characteristics as C , but with action at time $t+1$, the same series of calculations must be carried out. Again, the same operations must be repeated for analogous genes which express themselves at times $t+2$, $t+3$, until the end of the life table.

In the figure, the base curve is expressed using crosses. With the procedure described above, and assuming $t=0$, a modified curve has been obtained, expressed using squares in the figure. The abscissas cover 50 units of time and each interval indicates 1 unit. The assumed values are:

$$K = .07 ; n = 100 ; S = .5 ; V = .00001.$$

For simplicity of calculation, constant values have been assumed for n , S and V .

The modified curve shows that a large number of harmful genes ($50 \cdot 100 = 5000$) also moves down the base curve, but does not cause any modification indicating senescence according to Comfort's definition.

7) Theories of hindered senescence and of programmed senescence

The theory of hindered senescence, which must be considered a reworking in evolutionary terms of the theories by wear, considers the living being as subjected to wear processes that it is useful to counter only in part, unless the advantage of a greater velocity of evolution is lost. According to the viewpoint of this theory, senescence is an unavoidable and universal process that the organism hinders with various and unknown mechanisms, and with varying intensity according to the species.

The theory of programmed senescence, on the other hand, considers the senile process as something that is predetermined, namely a phenomenon that needs specific genes in order to exist. According to this theory, senescence instead of being hindered, is thought to be provoked. Indeed, if the common reasoning says that the decay of any living being or thing is natural, this theory, on the contrary, rejects the truth of such a concept. The living being is an entity that auto-renews itself and is not an inanimate object: *the phenomenon, having a strangeness that needs an explanation, is the fact that such an entity ages*, that is it ceases to renew itself, and not the contrary.

I think that there are weighty arguments in support of the theory of programmed senescence:

1) Hayflick's experiments (Hayflick, L., 1961, 1965, 1966), according to which cells (embryonic fibroblasts) of man and other species are able to divide themselves a limited number of times (50 for man), are perhaps more easily interpretable if senescence is considered a pre-arranged phenomenon and, among other things, dependent on precise genetically determined limits of cell duplication capacity. Hayflick's experiments become even more interesting if one remembers that the maximum number of cell divisions varies from species to species and has a certain correlation with the longevity of the species.

2) For species with high environmental mortality, it would, perhaps, be admissible to expect that, from a certain age, reached by a very limited number of individuals, natural selection is insufficient to favour those mutations that would hinder senescence. Thus, a non-excessive longevity for the aforesaid species should, perhaps, be expected, meaning that the "Methuselah effect" would have limited possibilities for performing its action if

the theory of hindered senescence is true. This is in contrast with what we see from natural observation, as has already been stated in the previous paragraph.

3) Each species, in its embryonic and growth phases, develops in a very precise and constant manner and this certainly depends on genetic factors. Analogous precision and constancy is recognizable in senescence. This is by no means proof, but it would seem to provide evidence in favour of the hypothesis of genetic regulation of the "senescence" phenomenon.

8) Researcher and senescence

Ageing and death, in which it inevitably ends, are, perhaps, one of the aspects of reality that have influenced human thought and civilisation most.

- To a great extent human history and psychology must always have been determined and moulded by the awareness that the life-span of any individual is determinate, and that the expectation of life tends to decrease with increasing age. The Oriental could say "O King, live for ever!" in the knowledge that every personal tyranny has its term. - (Comfort, A., 1979)

The great importance of this subject urges us to evaluate that which has been written in the preceding paragraphs with the utmost attention.

There are two opposite ways of understanding the reality of the senile process.

The first is that senescence is something unavoidable due to the transitoriness of everything. As a tool or a car gradually wears out over time and is finally completely unusable, the living being, likewise, simply by living, in ways unknown, wears, ages and finally dies.

The second way of conceiving senescence, on the other hand, rejects the parallel between the unavoidability of an inanimate object wearing out and the senescence of a living being, as arbitrary and unproven. Senescence is, rather, thought of as something determined and caused by genes and has a usefulness, or teleonomic meaning, for the living being.

The conflict between these two different theses is evident and I think that the dilemma is not without possible consequences in the search for substances that hinder senescence. The Researcher, if the first hypothesis is true, is struggling against something inevitable, and his efforts are practically without hope. On the other hand, if the second thesis is true, the fight is against a very strange and little known "function", but the difficulties - which are enormous - do not leave us without the hope that we will, some day, be able to master it. To trust the latter thesis is, perhaps, only a psychological, and contestable, advantage which, in itself, adds nothing to the possible results of the research into senescence and the means to dominate it. The great importance of the psychological attitude is, on the other hand, not to be undervalued in determining the outcome of an action. In fact, among other things, to conceive of ageing as a genetically determined process certainly overcomes a deep-rooted conception according to which:

- The ageing of the organism is a condition that is so well-known and innate to our way of considering reality and our personal destiny that it is, perhaps, difficult, at first, to put it forward as object of investigation, or even of experimentation. (Prodi, G., in Favilli, 1968)

Perhaps the Researcher who decides to break with tradition will be advantaged because, once free from certain prejudices, he will be more confident in possibilities of future success. But, I think that he will also certainly run into strong opposition, be it ethical, religious, or of another type, from those who are opposed to this new conception of senescence. It is, perhaps, useful at this point, to mention two statements made by a famous scientist, warning the Researcher who chooses such a path:

- Any confusion between ideas suggested by science and science itself is to be avoided.
- Modesty befits a scientist, but not the ideas that are inside of him and that he has a duty to defend (Monod, J., 1970).

9) Reformulation of the four observations

In the Chapter I, par. 2, I maintained that, for the persistence of a species, it is necessary, first of all, for all the individuals to be able to survive and propagate. In the light of that which has been brought to us by sociobiology and discussed in this paragraph, I will add that the individuals may also have characters that, although disadvantageous for themselves, are, on the contrary, advantageous to a greater extent for genetically close individuals. And likewise, as the object of the selection is more precisely the gene and not the individual, although the two entities often coincide as regards selection, it is necessary to reformulate the four observations thus:

- 1) Those genes with greater overall aptitude to persistence (inclusive fitness) have the greater probabilities of persistence.
- 2) A gene that, because of changes in the ecological niche, loses its overall aptitude to persistence, tends to a zero frequency.
- 3) The genome changes from generation to generation, according to probable and not to highly improbable modifications.
- 4) The frequency of each gene, and the genome in its totality, tends to be, in any evolutionary stage, the result of the actions of all selective pressures in the ecological niche.

INTERLUDE: Built-in obsolescence

Built-in obsolescence is that characteristic of an industrial product, specifically planned and pursued, for which the product deteriorates and becomes more and more difficult to repair after a definite time, although 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 prevent the annual share of renewal of a product in a stable market from being minimal. For example, a nation in which there are 10 million motor-vehicles, with a mean duration of ten years, requires an annual production of 1 million of motor-vehicles for replacement. If the mean duration of a car increased to 20 years, annual production would fall to 0.5 million, with catastrophic consequences for profits and employment. The second advantage is the introduction of new technologies with a speed that is inversely proportional to the mean duration of the product. A product with unlimited duration would delay, or even render economically disadvantageous, the use of new and more effective technologies. The third advantage is that a productive system, organised for quick and continuous renewal, is easily adaptable to: a) unexpected market growth; b) the opening of new markets; c) conversion to the production of other items; d) transformation into a military industry, etc. On the other hand, the production of goods with very long duration, as there is a minimal annual production, is not very adaptable to the aforementioned events.

In this regard, I believe the following to be true:

Built-in obsolescence is a hidden pillar of the modern “consumer culture”. Neither manufacturers nor trade unions, nor politicians are interested in publicizing this 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 considerable efforts in the design of an industrial consumer product are, in fact, dedicated to making the product both precise and reliable up to a certain time, and then unreliable and increasingly expensive to repair thereafter.

* * *

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 appropriate modifications of the terms, the main common aim is to allow the industrial product or the living being the greatest evolution, the greatest adaptability to new conditions, the greatest competitiveness in the struggle.

It is tragic to observe that man and his machines essentially share their ultimate fate.

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

It is incredible that, in a civilisation in which built-in obsolescence is fundamental, it is not known that the living world obeys a parallel logic.

An adaptive theory of the increasing mortality with increasing chronological age in populations in the wild

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Abstract: An "increasing mortality with increasing chronological age in populations in the wild" (IMICAW) is a phenomenon shown by many species, and the greater or smaller (or non-existent) IMICAW has an adaptive value, since it reduces the "mean duration of life" (ML). As Leopold (1961) pointed out, a smaller ML brings about a greater spreading velocity, within the species, of any advantageous mutation. However, this is an argument of group selection and is, therefore, inadequate to demonstrate that within a species a C gene causing IMICAW is stable compared with a C' allele not having this effect. The problem may be solved if we consider the inclusive fitness of C with the hypothesis that the dead individuals are replaced by kin individuals. In such a case, even with low values of the coefficient of relationship (Hamilton, 1971) of the substituting individuals, C tends to be stable and favoured by the selective mechanism as compared with C'. When the preferential replacement by kin individuals does not happen and/or when the turnover of generations is swift enough, C is not favoured and hence IMICAW loses its hypothesized adaptive value. In such cases, survival curves must be of type II or III of Pianka's classification (1970). It is discussed if IMICAW might be a consequence of the action of many harmful genes that express themselves tardily in the course of life.

Links:

PubMed = <http://www.ncbi.nlm.nih.gov/pubmed/19291860>

Google Scholar: <http://scholar.google.com/scholar?q=libertini-g+adaptive&hl=en>

Personal site = http://www.r-site.org/ageing/index_e.htm

Evolutionary explanations of the “actuarial senescence in the wild” and of the “state of senility”

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Abstract: A large set of data suggests that progressive reduction of fitness and senile decay in vertebrates are in correlation with the decline of cell replication capacities. However, the limits in such capacities are hardly explained in evolutionarily terms by current gerontological theories that rule out fitness decline as something genetically determined and regulated and therefore somehow favored by natural selection.

Four theories are tested as possible explanations of the “increasing mortality with increasing chronological age in populations in the wild” (“IMICAW”[1]), alias “actuarial senescence in the wild”[2], and of the observed negative correlation between extrinsic mortality and the ratio between deaths due to intrinsic mortality and deaths due to extrinsic mortality. Only the theory attributing an adaptive value to IMICAW allows an evolutionary explanation for it and for the aforesaid inverse correlation while the other three theories (“mutation accumulation”, “antagonistic pleiotropy”, and “disposable soma” th.) even predict a positive correlation.

Afterwards, the same theories are tested as possible explanations for the “state of senility”[3], namely the deteriorated state of individuals in artificially protected conditions (captivity, civilization, etc.) at ages rarely or never observable in the wild. With the distinction between “damage resulting from intrinsic living processes”[4], alias “age changes”[5], and “age-associated diseases”[4,5], the same theory explaining IMICAW allows a rational interpretation of the first category of phenomena while another theory, the “mutation accumulation” hypothesis, gives an immediate interpretation for the second category.

The current gerontological paradigm explaining the increasing mortality with increasing chronological age as consequence of insufficient selection should be restricted to the “age-associated diseases”. For IMICAW, it should be substituted with the concept of a physiologic phenomenon genetically determined by a balance of opposite selective pressures – strictly in terms of kin selection – and, for “age changes”, with the action of the same IMICAW-causing mechanisms at ages when selection becomes ineffective.

Links:

PubMed = <http://www.ncbi.nlm.nih.gov/pubmed/16951902>

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Empirical evidence for various evolutionary hypotheses on species demonstrating increasing mortality with increasing chronological age in the wild

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Abstract: Many species show a significant increase in mortality with increasing chronological age in the wild. For this phenomenon, three possible general hypotheses are proposed, namely that: (1) it has no adaptive meaning; (2) it has an adaptive meaning; (3) the ancestry is the pivotal determinant. These hypotheses are evaluated according to their consistency with the empirical evidence. In particular, (1) the existence of many species with a constant, or almost constant, mortality rate, especially the so-called “animals with negligible senescence”; (2) the inverse correlation, observed in mammals and birds in the wild, between extrinsic mortality and the proportion of deaths due to intrinsic mortality; (3) the existence of highly sophisticated, genetically determined, and regulated mechanisms that limit and modulate cell duplication capacities and overall cell functionality. On the whole, the hypothesis of an adaptive meaning appears to be consistent with the empirical evidence, while the other two hypotheses hardly appear compatible.

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Phylogeny of age-related fitness decline function

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Organizer: Italian Society for Evolutionary Biology
(SIBE, Società Italiana di Biologia Evoluzionistica)

ABSTRACT

An age-related fitness decline in the wild is documented for many species and there is empirical evidence for an adaptive meaning of this phenomenon, which in its more advanced expression, frequent in protected conditions, is usually called 'ageing'. A theory explains this fitness decline as evolutionarily advantageous by a mechanism of kin selection that in consequence of a quicker generation turnover allows a faster spreading of advantageous mutations. According to this theory, the advantage exists in conditions of K-selection (species divided in demes, populated by kin individuals, and in saturated habitats where only the death of an individual gives space to a new individual).

A plausible mechanism of the fitness decline is the progressive slowdown of cell turnover, namely a progressive prevalence of programmed cell death (by apoptosis or other means) on cell substitution by duplication of stem cells. Limits in cell duplication and the related cell senescence (progressive decline of cell functions in relation to the number of previous cell duplications) are determined by telomere-telomerase system and its species-specific regulation. In some species, as Rockfish, telomere-telomerase regulation and mortality rate result unvaried with the age.

Telomere-telomerase system and apoptosis are ubiquitous in eukaryote species. In yeast, *Saccharomyces cerevisiae*, after 25±35 duplications telomere-telomerase system does not allow further replications and the cell dies by apoptosis, which is also triggered by: a) unsuccessful mating; b) particular stresses, as dwindling nutrients (in older cells); c) cell senescence.

Apoptosis in *S. cerevisiae*, as in all eukaryote species, is a sophisticated function that kills the cell in a well defined pattern, optimal for an useful phagocytosis of cell fragments by other cells. Apoptotic patterns in *S. cerevisiae* have been interpreted as adaptive, because useful to the survival of kin individuals. Moreover, ecological life conditions of yeast, being of K-selection type, suggest that limits in cell duplications and the related phenomenon of cell senescence are adaptive too.

These considerations induce to a phylogenetic correlation between phenomena observed in colonies of kin yeast cells and analogous phenomena in multicellular organisms, that is the formulation of a phylogenetic hypothesis of "ageing".

In particular:

- a) apoptosis in stressed cells is common to yeast and multicellular species;
- b) apoptosis as part of morphogenetic mechanisms in multicellular organisms appears to be a derived function (impossible in monocellular organisms);
- c) the same is for apoptosis as part of cell turnover;
- d) cell senescence, caused by telomere-telomerase system, is common to yeast and multicellular species;
- e) the limits in the number of cell duplications, caused by telomere-telomerase system, in yeast directly determine life span of each unicellular organism, while in multicellular

species indirectly determine life span of the whole organism with a progressive slowing down in cell turnover and the consequent fitness decline.

In shorts, “ageing” mechanisms in yeast and multicellular eukaryote species, divided by more than 600 millions of distinct evolution, are incredibly similar in their basic physiological components and selective explanations.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

Main concept:

Aging in yeast is considered adaptive while for multicellular eukaryotes this idea is excluded by current gerontological paradigm, in clear contrast with theoretical arguments and empirical evidence

An age-related fitness decline in the wild is documented for many species [1,2] (Fig. 1) and there is empirical evidence for an adaptive meaning of this phenomenon [3], which in its more advanced expressions, common in protected conditions, is usually called ‘ageing’. A theory explains this fitness decline as evolutionarily advantageous by a mechanism of kin selection that, in consequence of a quicker generation turnover, allows a faster spreading of advantageous mutations. According to this theory, the advantage exists in conditions of K-selection (species divided in demes, populated by kin individuals, and in saturated habitats where only the death of an individual gives space to a new individual) [4-6].

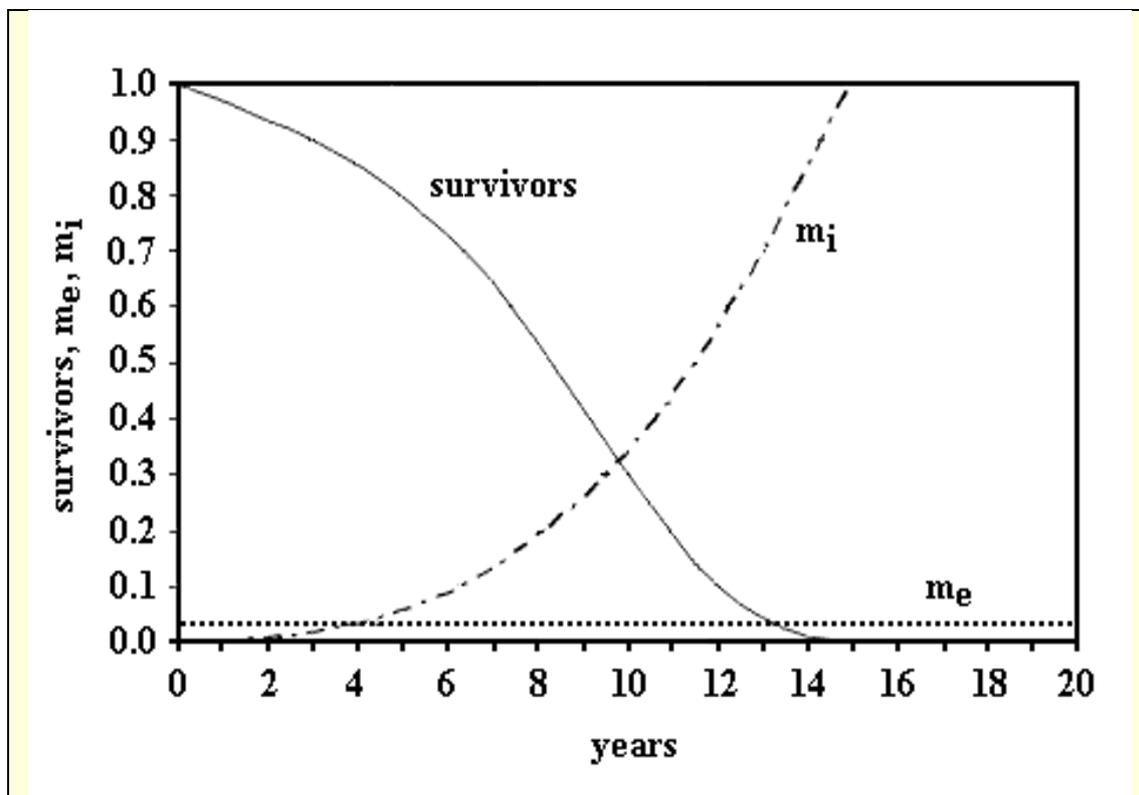


Fig. 1 – Life table of *Panthera leo*: survivors, extrinsic mortality (m_e , mortality caused by external causes, i.e., predation, accidents, infections, etc.) and intrinsic mortality (m_i , mortality caused by internal causes, i.e., aging). Data are from Ricklefs [2].

A plausible mechanism of the fitness decline is the progressive slowdown of cell turnover, namely a progressive prevalence of programmed cell death (by apoptosis or other means) on cell substitution by duplication of stem cells. Limits in cell duplication and the related cell senescence (progressive decline of cell functions in relation to the number of previous cell duplications) are determined by telomere-telomerase system and its species-specific regulation [6,7] (Fig. 2). In some species, as Rockfish and lobsters, telomere-telomerase regulation and mortality rate result unvaried with the age [8,9].

Telomere-telomerase system and apoptosis are ubiquitous in eukaryote species [10-13] (Fig. 3). In yeast, *Saccharomyces cerevisiae*, telomere-telomerase system does not allow further replications after 25 ± 35 duplications and the cell dies by apoptosis [10], which is also triggered by: a) unsuccessful mating; b) particular stresses, as dwindling nutrients in older cells; c) cell senescence [14] (Fig. 4).

Apoptosis in *S. cerevisiae*, as in all eukaryote species, is a sophisticated function that kills the cell in a well-defined pattern [15], optimal for an useful phagocytosis of cell fragments by other cells that “are able to survive longer with substances released by dying cells” [16]. Apoptotic patterns in *S. cerevisiae* have been interpreted as adaptive because useful to the survival of the clone, which is likely composed by kin individuals [13,16-20]. Moreover, ecological life conditions of yeast, being of K-selection type, suggest that limits in cell duplications and the related phenomenon of cell senescence are adaptive and explainable with the same evolutionary mechanisms proposed for multicellular species subject to K-selection [4-6].

These considerations induce to a phylogenetic correlation between phenomena observed in colonies of kin yeast cells and analogous phenomena in multicellular organisms, that is the formulation of a phylogenetic hypothesis of “ageing”.

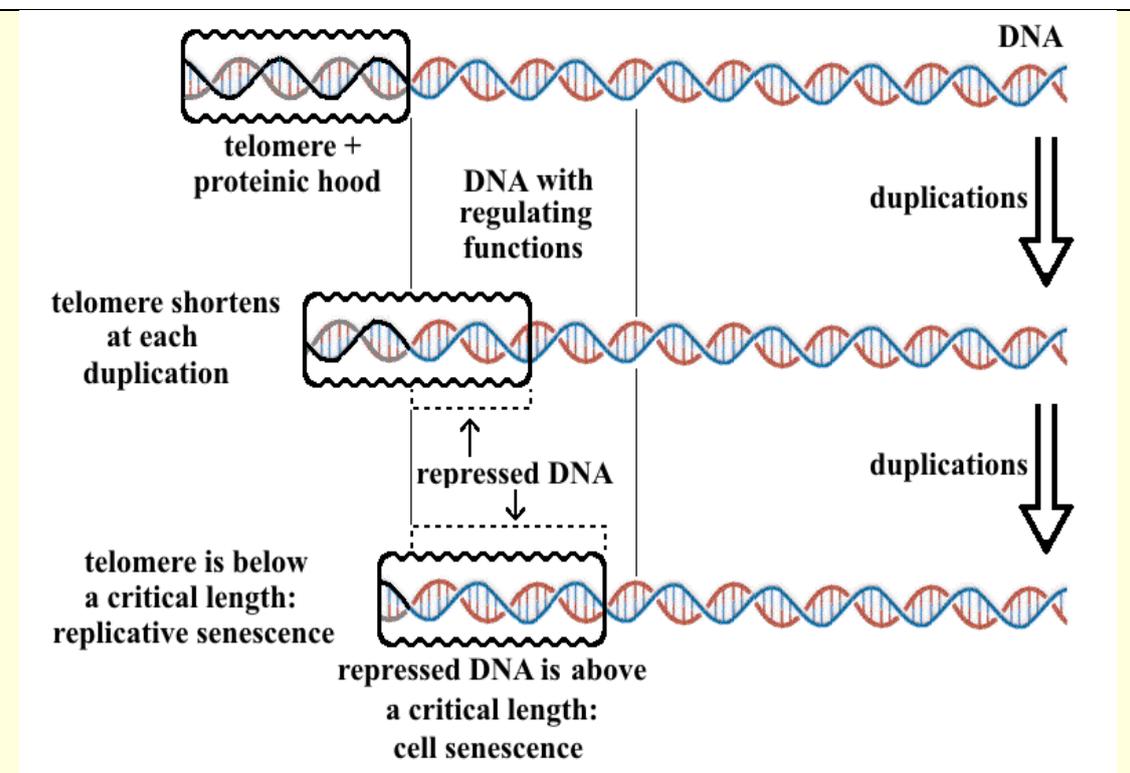


Fig. 2 – Telomere progressive shortening increases the probability of replicative senescence and impairs the expression of many genes (cell senescence). It is likely the existence near to the telomere of a tract of DNA regulating overall cell functionality: with telomere shortening the proteinc “hood” capping telomere slides down and alters this regulation [7].

In particular (Table I and Fig. 5):

a) apoptosis in yeast is triggered by starvation, damaged conditions of the cell, unsuccessful mating, etc., and in these cases it is favoured by kin selection because increases survival probability of kin cells [16]. In multicellular species, considering each individual as a clone having all cells with the same genes (coefficient of relationship “r” equal to 1) although having differentiated functions, apoptosis of less fit cells is favoured by analogous mechanisms of kin selection;

b) in multicellular organisms, apoptosis as part of morphogenetic mechanisms and of lymphocyte selection is clearly a derived function, being impossible in monocellular organisms;

c) in yeast, replicative senescence and cell senescence, caused by telomere-telomerase system, and apoptosis “limit longevity that would maintain ancient genetic variants within the population and, therefore, favor genetic conservatism” [14], which means, in other words, that these phenomena are favoured by kin selection [4-6]. In multicellular organisms, replicative senescence, cell senescence and apoptosis cause age-related limits in cell turnover with consequent age-related fitness decline [6,7] (“senile state” in its more advanced expressions [6]), and this is favoured by kin selection in conditions of K-selection [4-6].

In shorts, “ageing” mechanisms in yeast and multicellular eukaryote species, divided by about 600 millions of distinct evolution, are incredibly similar in their basic physiological components and selective explanations.

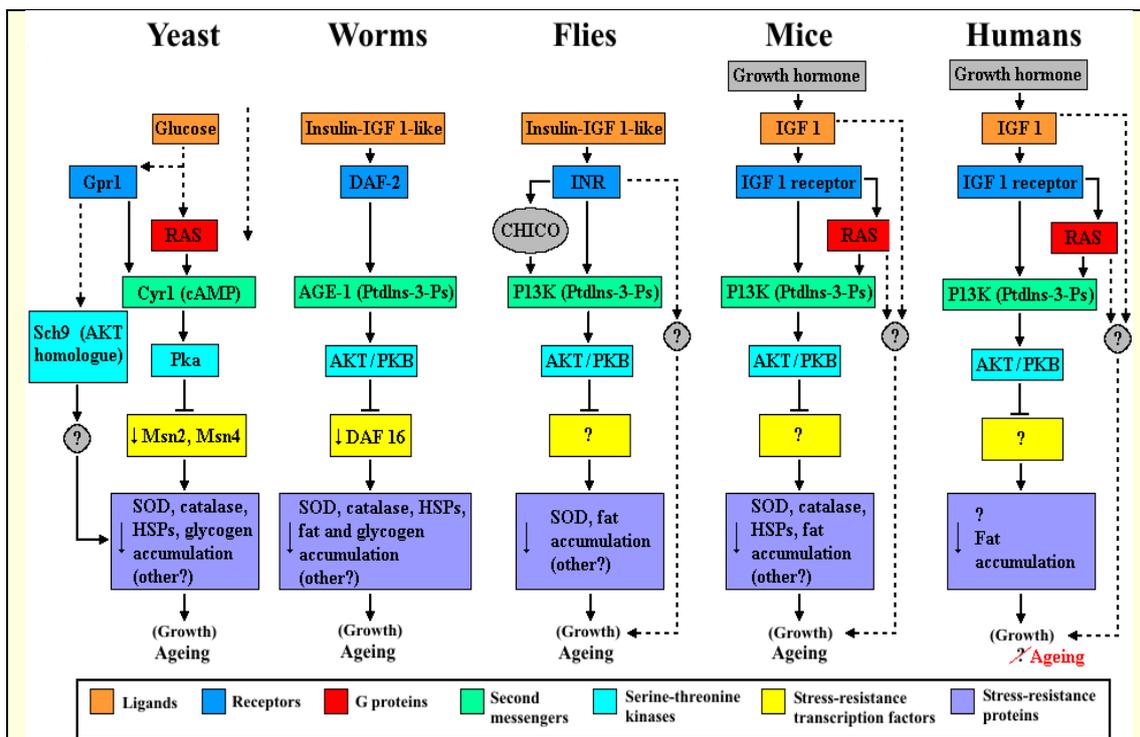


Fig. 3 – Scheme of trigger mechanisms for apoptosis in various eukaryote phyla. Figure redrawn from [13] and with a correction (in red): apoptosis is a very ancient mechanism clearly in correlation with ageing, but in the original scheme this is doubtful only for humans!

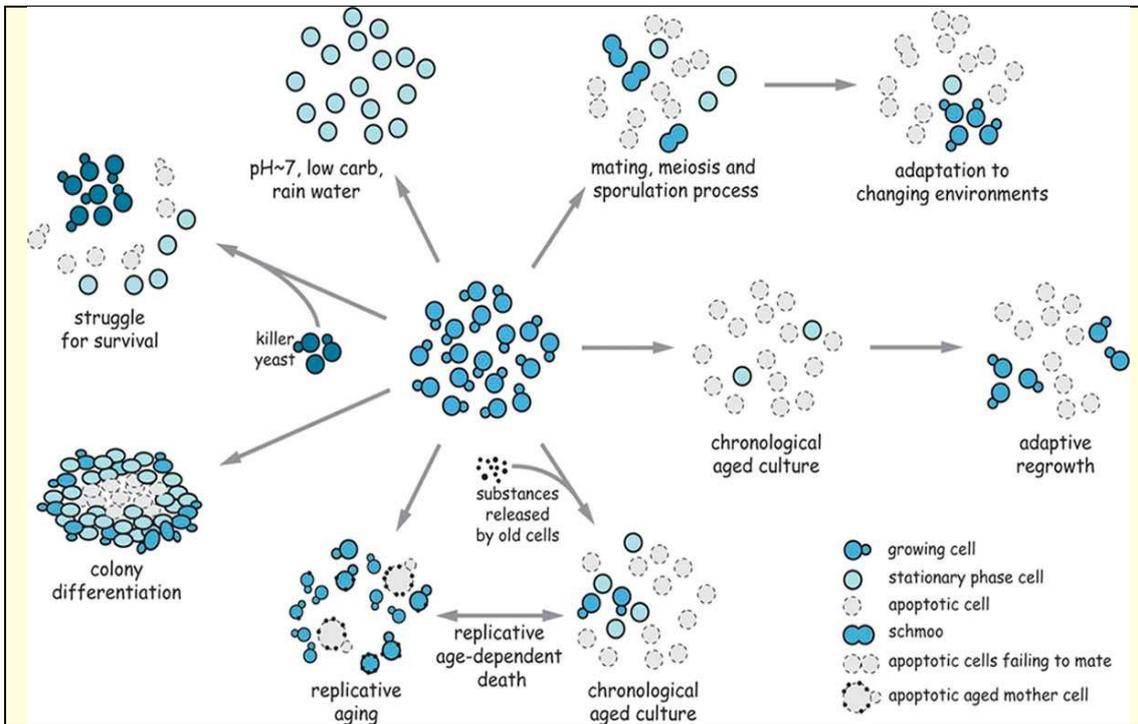


Fig. 4 – Apoptosis, that is a programmed form of death, is triggered in wild yeast by various conditions, as: a) “dwindling nutrients trigger the altruistic death of older cells”, b) “when mating is not successful”; c) replicative senescence that is genetically determined by telomere-telomerase system, for which is considered adaptive [14]. For condition c: “apoptosis coupled to chronological and replicative aging limits longevity that would maintain ancient genetic variants within the population and, therefore, favor genetic conservatism” [14]. The figure is from [14].

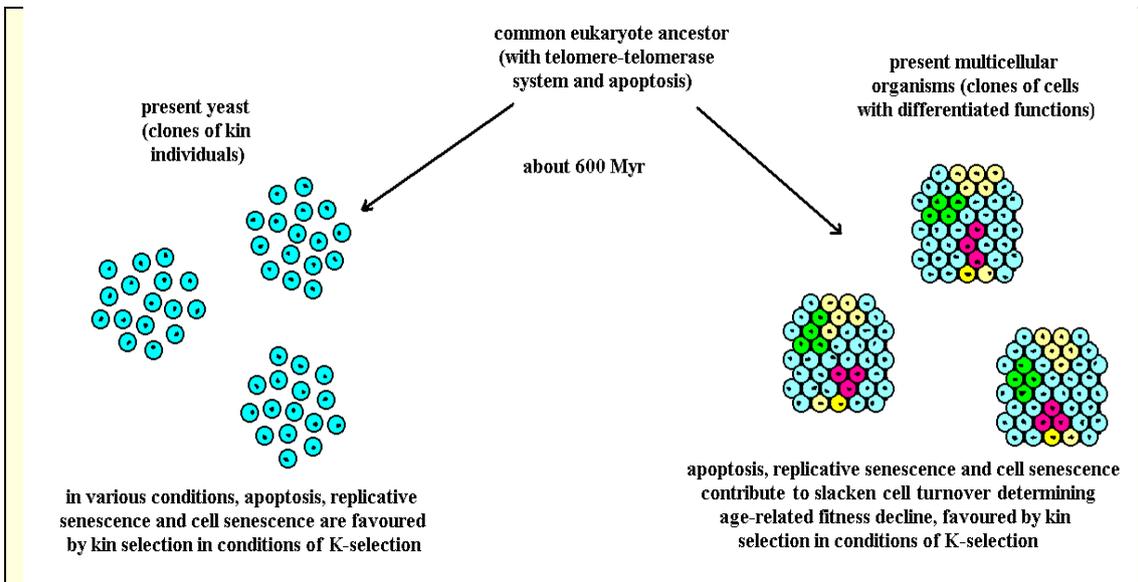


Fig. 5 – Analogous functions of apoptosis, replicative senescence and cell senescence in species separated by about 600 millions of years of distinct evolution.

Table I			
Phenomenon	Description	Function in yeast (and other monocellular eukaryotes)	Function in multicellular eukaryotes
Apoptosis	Ordinate process of cell self-destruction with modalities allowing the use of cell components by other cells	Activated when nutrients are scarce, mating is not successful and in old individuals (Note 1)	Eliminates damaged cells (Note 2); Essential for morphogenesis (Note 2); Essential to determine cell turnover whose progressive impairment contribute to age-related fitness decline (Note 1)
Cell senescence	In relation to the number of replications, progressive impairment of cell functions determined by the repression of subtelomeric DNA	Cause a quicker generation turnover (Note 1)	Contribute to slacken cell turnover determining age-related fitness decline (defined senile state in its more advanced expressions) and, therefore, a quicker generation turnover of multicellular individuals (Note 1)
Replicative senescence	In relation to the number of replications, progressive increase of the probability to lose duplication capacity		
<p>Note 1 = altruistic behaviour(s) favoured by kin selection in conditions of K-selection; Note 2 = altruistic behaviour considering the multicellular individual as a clone</p>			

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The role of telomere-telomerase system in age-related fitness decline, a tameable process

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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.

Links:

Nova Science Publishers = https://www.novapublishers.com/catalog/advanced_search_result.php?keywords=libertini+telomeres

Personal site = http://www.r-site.org/ageing/index_e.htm

Are *C. elegans* and *D. melanogaster* valid animal models for studies on aging?

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Belozersky Institute of Physico-Chemical Biology,
Moscow State University, Moscow

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Organizer: Italian Society for Evolutionary Biology
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Abstract

C. elegans and *D. melanogaster* are common animal models for studies on aging, but there are strong arguments against the validity of these models for this type of studies:

I) Many bird and mammal species - our species included - show an increment of mortality with increasing chronological age in natural conditions. This phenomenon ("A" phenomenon) is well documented and, being existent in the wild, is influenced by natural selection. On the contrary, animals as *C. elegans* and *D. melanogaster* show in natural conditions a constant mortality rate, but, in artificial protected conditions, they display an age-related mortality increment starting from ages not existing in the wild. In fact, in natural conditions: 1) the longevity of *C. elegans* is reduced up to 10 fold compared with standard laboratory culture conditions and few individuals of this species remain fertile in the wild after 10 days; 2) *D. melanogaster* has a reported adult life span in the wild of 10-12 days. Therefore, the mortality increment for these two species ("B" phenomenon), being a laboratory artefact, cannot be influenced by selection. "A" and "B" phenomena are radically different in their possible evolutionary determinants and so the results of experiments on "B" phenomenon are not automatically applicable to "A" phenomenon.

II) *C. elegans* and *D. melanogaster* (and in general the adult insects) are composed by cells with no turnover, while birds and mammals have cells and tissues with turnover. If, as it seems likely, the slowdown and later the stopping of cell turnover, and the correlated cell senescence, are pivotal elements in the age-related fitness decline of birds and mammals, it is rather doubtful to use experiments on animals with no cell turnover to explain the fitness decline in animals with cell turnover.

III) Animals as *C. elegans* and *D. melanogaster* have life cycles thoroughly different from those of bird and mammal species. Studies on aging that use these animal models implicitly assume that their adult stages are equivalent to the postnatal stages of birds and mammals for the extension of their results to these species. But this assumption is not proved and seems quite doubtful.

The appropriateness of *C. elegans* and *D. melanogaster* as animal models for aging is a problem that cannot be neglected in aging studies. Unfortunately, in renowned texts and very influential journals, the issue is not considered and it is frequent that experiments

modifying – in laboratory conditions and at ages non-existent in the wild - the modifications of *C. elegans* and *D. melanogaster* life tables are presented as meaningful advances in the understanding of human aging!

Links:

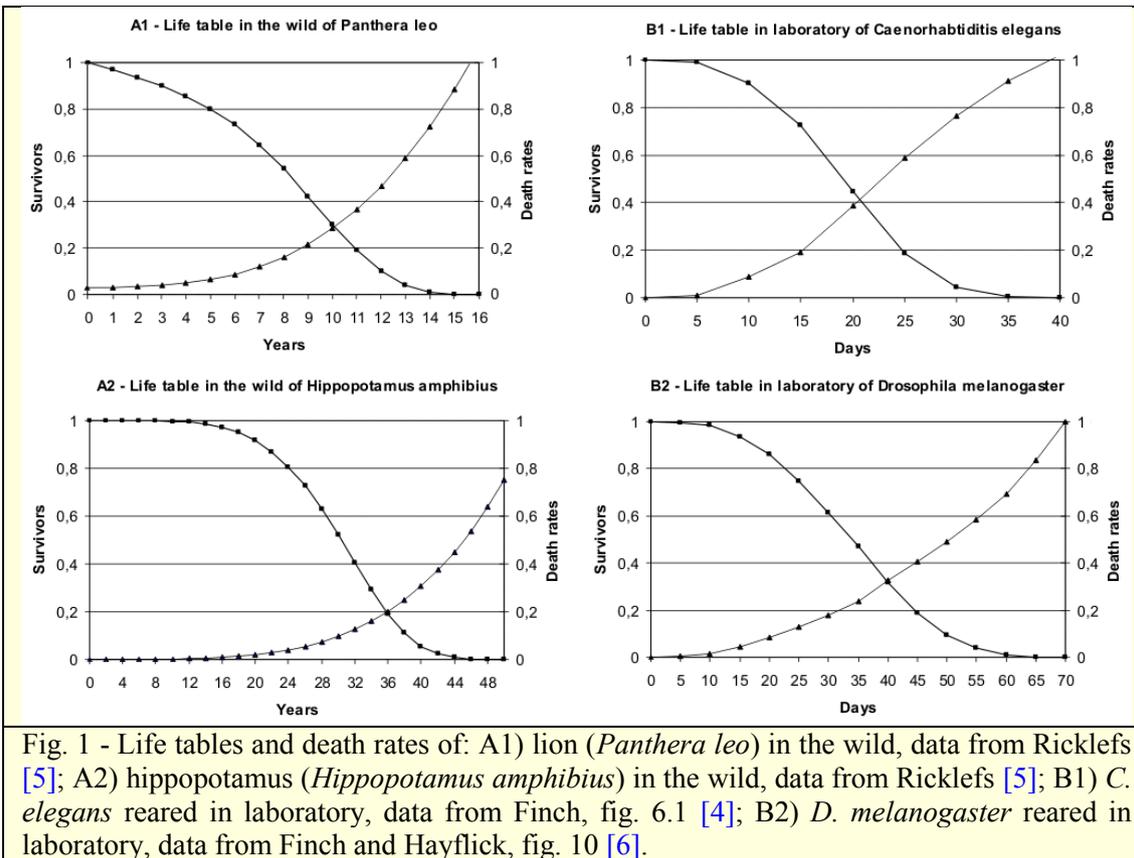
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Main concept:

***C. elegans* and *D. melanogaster* are common animal models for studies on “aging”.**

There are strong arguments against the reliability of these models for an effective explanation of the age-related mortality increase observable in natural conditions for other species.

In a discussion about aging, two precise definitions are a necessary premise because: “... 'ageing' is used with so many different meanings in so many different contexts that it is sometimes highly confusing when used without proper qualification.” [1] Many bird and mammal species - *H. sapiens* included - show an “increment of mortality with increasing chronological age in the wild” (IMICAW [2]), alias “actuarial senescence in the wild” [3]. This phenomenon is well documented [4,5] and is illustrated in Fig. 1 (A1 and A2). Other animals show in natural conditions a constant mortality rate, but, in artificial protected conditions, they display an age-related mortality increment starting from ages not existing in the wild. This “increment of mortality with increasing chronological age in captivity” (IMICAC [2]) is documented for well-known species as the worm *Caenorhabditis elegans* [4] and the fly *Drosophila melanogaster* [6]. In fact, the longevity of *C. elegans* “under more natural conditions is reduced up to 10 fold compared with standard laboratory culture conditions” [7] and few individuals of this species remain fertile in the wild after 10 days [8]. *D. melanogaster* has a reported adult life span in the wild of 10-12 days [4]. For both these animals, the age-related increasing mortality described in Fig. 1 (B1 and B2) starts at ages non-existent in the wild and, so, it is only a laboratory artefact.



As *C. elegans* and *D. melanogaster* are easily available in laboratory, many studies on "aging" have used these two species as animal models [9,10]. But:

- 1) is IMICAC a phenomenon that can be compared to IMICAW?
- 2) are these two species reliable animal models for studies on IMICAW?

Most likely, the answer is negative for three main reasons:

I) By definition, IMICAW exists in the wild and therefore is influenced by natural selection. On the contrary, by definition, IMICAC is non-existent in the wild and therefore cannot be influenced by natural selection. This means, in principle, that IMICAW, and not IMICAC, could be modeled by natural selection and that the two phenomena are radically different in their evolutionary determinants and mechanisms. This argument could be contested with the assumptions that IMICAW does not exist in the wild and/or is not determined or influenced by natural selection [11] but these prejudice are contradicted by natural observations [4,5] and theoretical arguments [2,12].

II) *C. elegans* and *D. melanogaster* (and in general the adult insects) are composed by cells with no turnover [4,13], while lion, hippopotamus and man (and, in general, birds and mammals) - species that show the IMICAW phenomenon - have cells and tissues with turnover (Fig. 2). If, as it seems likely, the slowdown and later the stopping of cell turnover, and the correlated cell senescence, are pivotal elements in the fitness decline of animals as lion, hippopotamus and our species [14,15], it is rather doubtful to use experiments on animals with no cell turnover to explain the fitness decline in animals with cell turnover.

III) Animals as *C. elegans* and *D. melanogaster* have life cycles thoroughly different from those of bird and mammal species (for *C. elegans*, see Fig. 3). Studies on "aging" that use these animal models implicitly assume that their adult stages are

equivalent to the postnatal stages of birds / mammals for the extension of their results to bird / mammal species. But this assumption is not proved and seems quite doubtful.

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 [9], and it is frequent that, in very influential journals, experiments modifying – in laboratory conditions and at ages non-existent in the wild - the life table of our dear worm or of our beloved fly are presented as meaningful advances in the understanding of human aging [10,16,17]!

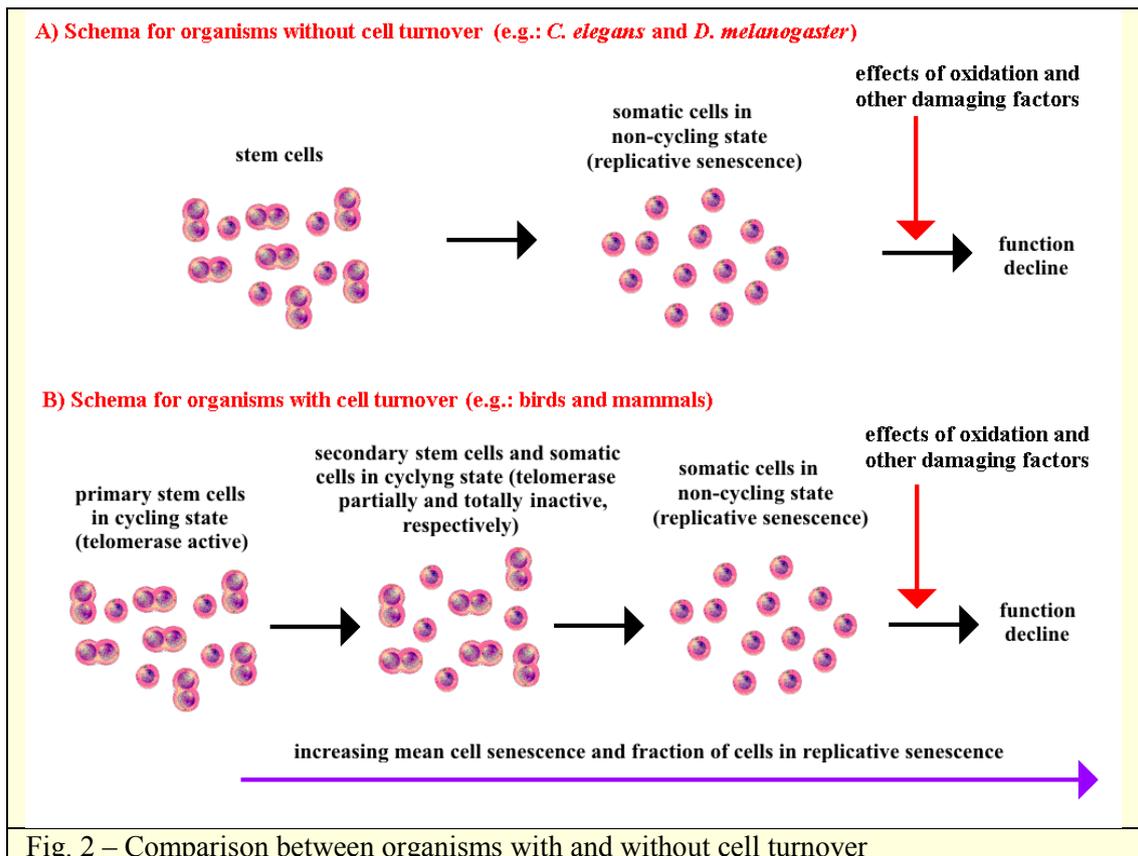
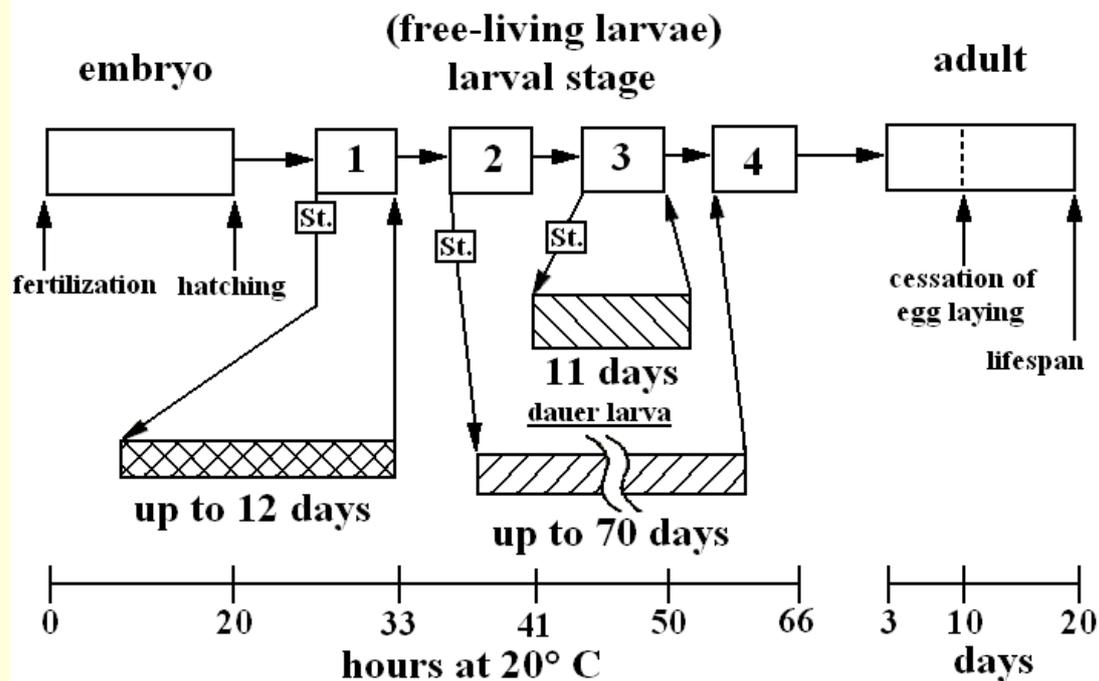


Fig. 2 – Comparison between organisms with and without cell turnover

A) Stages of *C. elegans* life cycle



B) Stages of birds / mammals life cycle

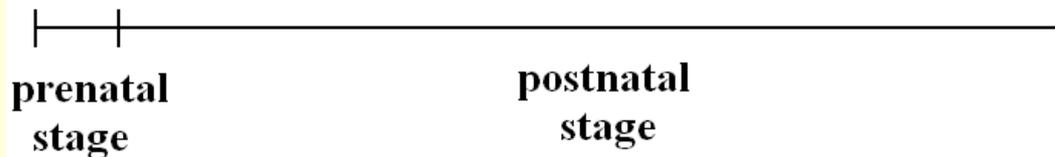


Fig. 3 – A) Stages of *C. elegans* life cycle (redrawn from [4]). The lifespan reported in the figure is in laboratory conditions, while it is reduced up to 10 fold in the wild [7]; B) Stages of bird / mammal life cycle. For studies on “aging”, the equivalence between adult stage of *C. elegans* and postnatal stage of birds / mammals is not at all a self-evident assumption.

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Comparison between two paradigms about aging

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Abstract

According to the current prevailing interpretations, the age-related fitness decline shown by many species in natural conditions, commonly defined as "aging", is an effect of:

- 1) the age-related decline of natural selection (mutation accumulation hypothesis);
- 2) a balance between possible advantages at a younger age and the disadvantages of fitness decline (antagonistic pleiotropy hypothesis);
- 3) limited "resources" - not better defined - which are used preferentially for reproduction and not for soma maintenance (disposable soma hypothesis).

This interpretation ("first paradigm") is challenged by a different paradigm ("second paradigm") that explains aging as an adaptive phenomenon and, in short, maintains:

- 1) Age-related decline of natural selection cannot explain age-related fitness decline;
- 2) There is no evidence for antagonistic pleiotropic genes or for limited "resources" causing age-related fitness decline;
- 3) The first paradigm predicts a direct relation between environmental mortality and the proportion of deaths caused by aging. The second paradigm predicts the opposite. Observational data falsify the prediction of the first paradigm and confirm that of the second.

4) The limitations in cell turnover determined by telomere-telomerase system are a plausible mechanism underlying senescence. This is hardly explainable by the first paradigm. On the contrary, this is compatible with the second paradigm and, in fact, the adaptive hypothesis predicts and requires the existence of specific mechanisms causing the fitness decline.

5) For the first paradigm aging is only a common term for many age-related different diseases: aging as a distinct entity does not exist and, in principle, cannot be mastered. On the contrary, for the second paradigm, all manifestations of aging have common mechanisms: aging is a distinct entity and, in principle, can be mastered.

The coexistence of the two paradigms or the formulation of intermediate hypotheses appears impossible. Therefore, a choice based on scientific data is indispensable.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

Definitions:

IMICAW = Increasing Mortality with Increasing Chronological Age in the Wild [1], alias “actuarial senescence in the wild” [2]

IMICAC = Increasing Mortality with Increasing Chronological Age in Captivity [1]

“aging” or “senescence” = imprecise term that includes both IMICAW and IMICAC [1,3]

“state of senility” = the deteriorated state of individuals in artificially protected conditions with low mortality (captivity, civilization, etc.) at ages rarely or never observable in the wild, namely the state of individuals with age-related reduced fitness to wild conditions smaller than an arbitrarily established value [4,5]

m_e = extrinsic mortality (= mortality due to environmental factors) [6]

m_i = intrinsic mortality (= mortality due to intrinsic factor, i.e. senescence) [6]

P_s = proportion of deaths due to intrinsic mortality [6]

t-gene = a harmful gene that acts only from age t [1]

(1st paradigm)

MAH = Mutation Accumulation Hypothesis [7-11]

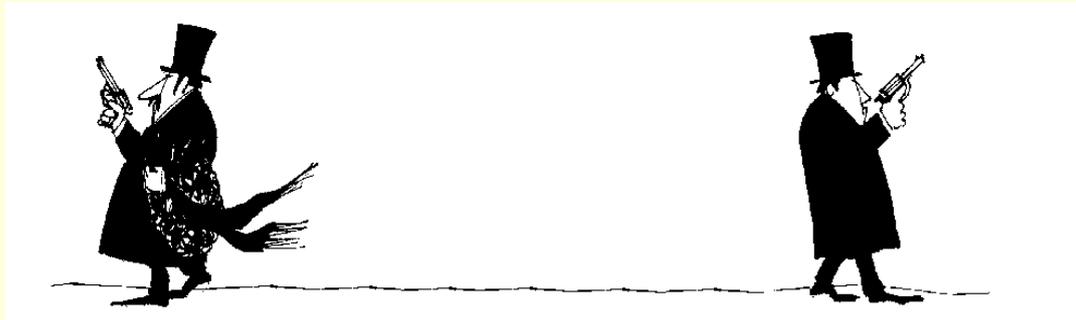
AP H = Antagonistic Pleiotropy Hypothesis [4,12]

DSH = Disposable Soma Hypothesis [13,14]

+ Wear and tear hypotheses, Stochastic hypothesis, etc.

(2nd paradigm)

AAH = Adaptive Aging (or, more precisely, IMICAW) Hypothesis [1,5,15-18]



The world is too small for both of them

	First paradigm (Aging is not adaptive) [4,7-14,19]	Second paradigm (IMICAW / aging is adaptive) [1,5,15-18]
I	The evident alterations of “aging” or “senescence”, in the meaning of “state of senility” [4], are totally or almost incompatible with the survival in the wild.	In the study of evolutionary mechanisms it is illogical to have as principal object an artificial condition, the “state of senility”, totally or almost absent in the wild and, hence, not or little influenced by natural selection.
II	So, “senescence”, in the meaning of the “state of senility”, being nearly absent in the wild, is not	IMICAW, a documented reality [6,20] (Fig. 1), is influenced by natural selection and is, consequently, a proper object for

	object of efficacious selection.	the analysis of evolutionary mechanisms.
III	Moreover, “any hypothetical ‘accelerating aging 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 selection advantage.” [19]	An IMICAW-causing gene reduces the individual fitness, but this does not mean inevitably that the inclusive fitness of the same gene is negative. Only when a gene has no effect on the fitness of other individuals where the gene is present, the evaluation of its inclusive fitness may be disregarded. In the particular case of an IMICAW-causing gene it has been shown that such a gene may, in certain conditions, have a positive inclusive fitness [1,5].
IV	Therefore, “senescence” is the outcome of insufficient selection (against harmful genes for MAH, antagonistic pleiotropic genes for APH, physiological, biochemical or environmental constraints for DSH, etc.).	Therefore, IMICAW is not the result of insufficient selection, but of a balance between positive and negative selecting factors.
V	From this, it can be deduced that the less efficacious is the remaining selection, in particular when m_e is greater, the more rapid must be the onset of the “senescence”. With m_e at its greatest values, P_s should be at the highest. But, this prediction is falsified by data from natural observation [6] (Fig. 2) with no sound justification for this contradiction.	From this, it can be deduced that in the case of a weaker favorable selection, e.g. as when m_e is high, an IMICAW-causing gene is less selectively favored and, therefore, P_s is reduced. With m_e at its greatest values, P_s should be zero [1,5]. These apparently paradoxical predictions, contrary to those of the first paradigm, are confirmed by data from natural observation [6] (Fig. 2).
VI	There is no explanation for the existence of non-IMICAW species and, indeed, they should not exist for MAH, APH, DSH, etc. (except when there is no separation between soma and germ line [22]).	The existence of non-IMICAW species is predicted in well-defined and common conditions [1,5].
VII	In short, “senescence”, in the meaning of “state of senility”, is the result of insufficient selection pro a greater longevity and against noxious agents.	“Age changes” in their initial expression coincide with the greatest IMICAW alterations observable in the wild, while in their advanced manifestation are the artificial (by reduction of m_e) utmost and

		unnatural amplification of IMICAW alterations [5]. The “state of senility” is the “age changes” plus the effects of t-genes insufficiently eliminated by natural selection in the wild because of the tardiness of their manifestation (“age-associated diseases”) plus damages by wrong lifestyles [5] (category 2 in Masoro’s classification [23]).
VIII	So, to contrast “senescence”, identifying the damaging factors (harmful genes, pleiotropic genes, physiological alterations such as oxidant factors, etc.) is an indispensable prerequisite.	Since IMICAW is determined by genes that are in part favored by natural selection, it is necessary to investigate the physiological mechanisms that reduce the fitness in the wild and that cause the “age changes” in conditions of low mortality. The existence of these mechanisms is the main prediction of AAH. For the “age-associated diseases”, plainly interpreted as the outcome of t-genes effects, it is appropriate to act as for other diseases caused by genetic alterations [1,5].
IX	The life limiting mechanisms caused by the limits in cell duplication capacities determined by telomere-telomerase system are not predicted by the first paradigm and are hardly compatible with it [24].	Life limiting mechanisms as the limits in cell duplication capacities determined by telomere-telomerase system are entirely compatible with the second paradigm and essential for its validity [24].
X	The concept of IMICAC is absent and so there is no distinction between IMICAW and IMICAC and no specific care in experimental data evaluation.	The concept of IMICAC is well defined, with a clear distinction between IMICAW and IMICAC, and the necessity of considering this distinction in experimental data evaluation is strongly underlined [1,5].
	Aging is only a common term for many age-related different diseases: aging as a distinct entity does not exist and, in principle, cannot be mastered	All manifestations of IMICAW, usually defined aging in their more advanced expressions, have common mechanisms: aging is a distinct entity and, in principle, can be mastered

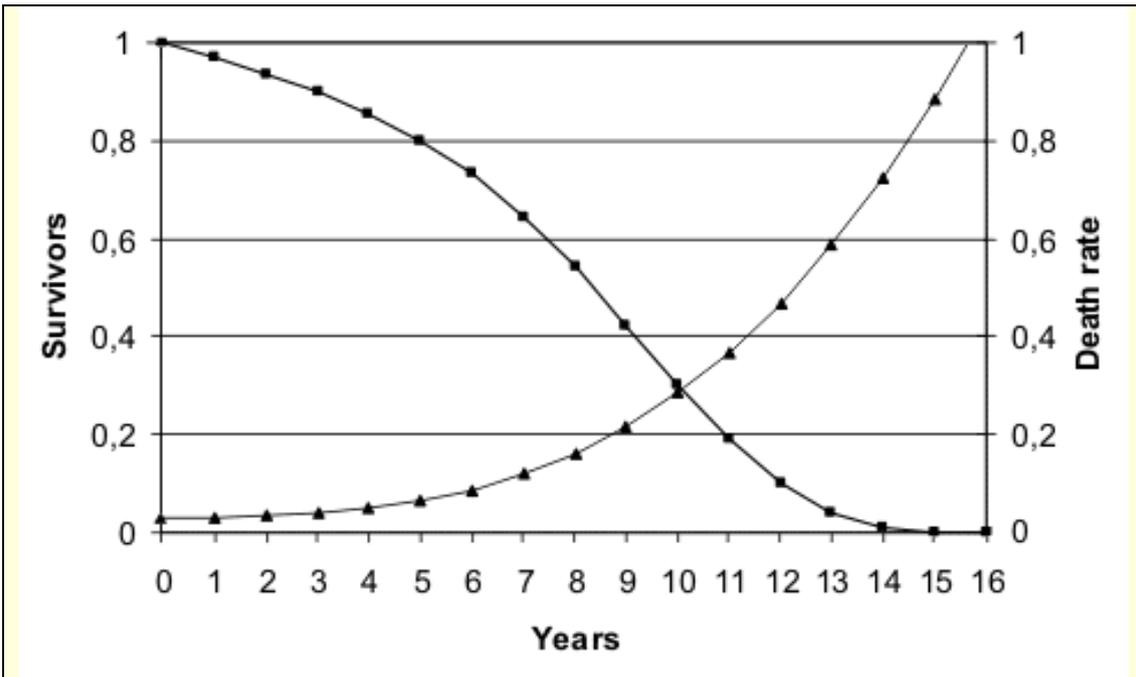


Fig. 1 – Life table of *Panthera leo* in natural conditions (Data from [6]). Fitness decline is well documented in the wild for many species [6,20].

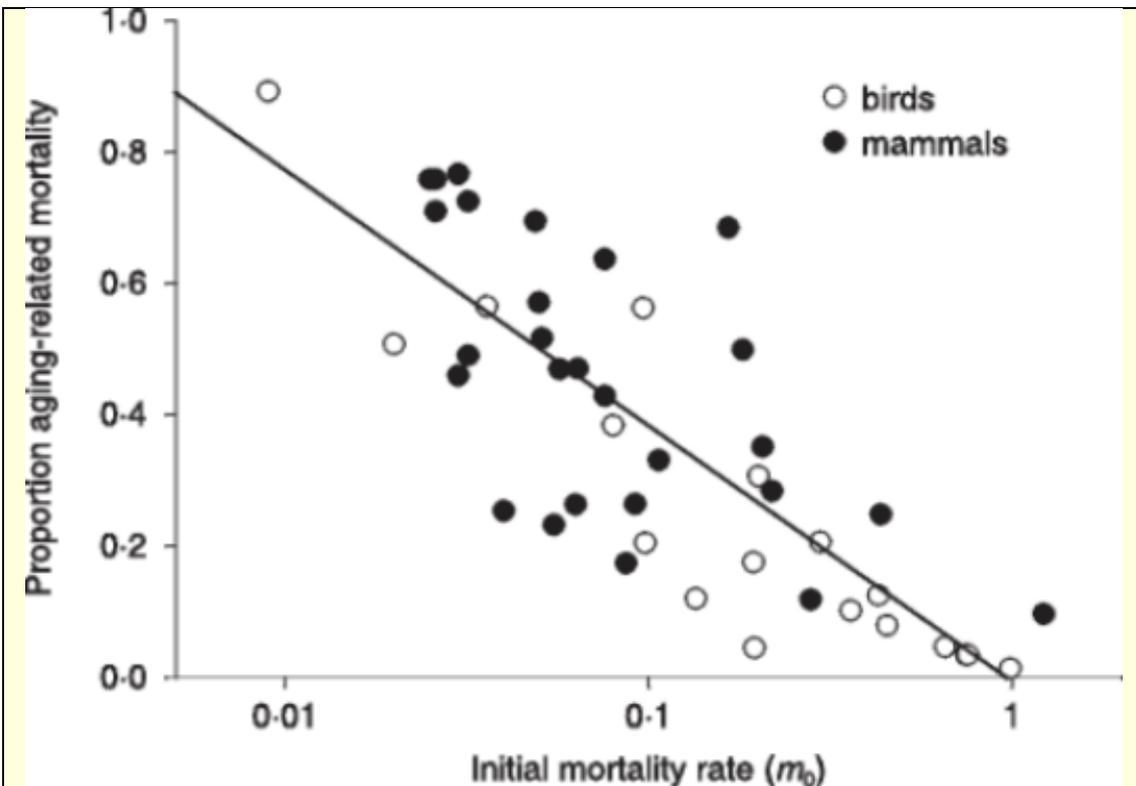


Fig. 2 – Inverse relation between extrinsic mortality and the proportion of deaths due to intrinsic mortality (Figure from [21]).

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Arguments against telomere-telomerase system as general defence against cancer

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Abstract

Telomere-telomerase system, which is genetically determined and regulated, causes cell senescence and limits cell replication capacity. The existence of life-limiting mechanisms is specifically predicted by adaptive aging theories while the contrary is true for non-adaptive aging theories. Therefore, for this second type of theories, it is essential an adaptive function to justify the existence of these life-limiting mechanisms. A popular interpretation is that they are a general defence against cancer, but there are strong arguments against this hypothesis:

1) It does not justify: a) the existence of animals that show in the wild no observable increase in age-specific mortality rate, cancer mortality included; b) the great differences of duplication limits and of cell overall functionality decay from species to species, unless cancer risk is postulated as varying from species to species in direct correlation with the limits imposed to cell duplication capacities and to cell overall functionality by the genetic modulation of telomere-telomerase system;

2) Shortened telomeres increase vulnerability to cancer as a consequence of dysfunctional telomere-induced instability;

3) The decline of duplication capacities and of overall cell functionality weakens immune system efficiency, which is inversely related to cancer incidence;

4) The role of the telomere in chromosomal stability argues that telomerase protects against carcinogenesis.

5) In yeast, a eukaryotic species, replicative senescence and cell senescence, although not caused by telomere shortening but by another mechanism related to the number of duplications, cannot be a consequence of an impossible cancer risk. Moreover, these phenomena and others strictly associated observed in yeast have been interpreted as adaptive.

6) *Dyskeratosis congenita*, an inherited human disease, is characterized by an altered telomerase. Problems tend to occur in tissues in which cells multiply rapidly and there is a higher rate of cancer that can likewise be explained by the lack of telomerase, which results in unstable chromosomes.

But: 7) in rodents, telomerase activity is not related to maximum lifespan while is inversely related to body mass and this has been interpreted as a fact in support of the defensive role against cancer risk of telomere-telomerase system, as a greater body mass presumably increases cancer risk.

In short, with the important exception of point (7), which stimulates further data and discussions, there are not specific arguments and experimental tests in support of the hypothesis that telomere-telomerase age-related limiting actions on cell turnover is a general defence against cancer. Therefore, telomere-telomerase age-related limits on

cell turnover are hardly justifiable as a defence against cancer risk and, lacking other plausible explanations, only the adaptive hypotheses of age-related fitness decline appear a rational cause for their existence.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

Main concept:

**Telomere-telomerase age-related limits on cell turnover require an evolutionary justification.
For adaptive aging theory, these limitations are essential life span limiting mechanisms.
For non-adaptive aging theory, they are a general defence against cancer, but the strong arguments against this interpretation should be falsified**

Telomere-telomerase system, which is genetically determined and regulated, causes cell senescence and limits cell replication capacity [1] and these phenomena are a plausible general mechanism of senescence [1,2].

Non-adaptive aging theories do not predict the existence of mechanisms genetically determined and regulated that cause age-related mortality increment, i.e. fitness decline. Mechanisms of this type could be compatible with non-adaptive aging theories only if an adaptive function is a plausible and exhaustive evolutionary justification for their actions.

On the contrary, adaptive aging theories predict and require the existence of mechanisms genetically determined and regulated causing age-related mortality increment.

Therefore, in absence of an alternative explanation, the existence of telomere-telomerase system with its effects on cell turnover is a strong argument against non-adaptive aging hypotheses and in support of adaptive aging hypotheses [3].

Non-adaptive hypotheses try to explain replicative senescence and cell senescence as a general defence against malignant neoplasia [4,5], that is a terrible evolutionary trade-off between ageing and defence against cancer [6].

There are strong arguments against this interpretation:

1) It does not justify the existence of animals that 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” [7], alias animals with “negligible senescence” [8] (Fig. 1-2), and the great differences of duplication limits and of cell overall 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 cell overall functionality by the genetic modulation of telomere-telomerase system. But, old lobsters and rainbow trouts, animals with negligible senescence, have, in the wild, the same levels of telomerase activity as young individuals [9,10] 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 [3]. For these animals, telomerase action involves no evident oncogenic risk and, therefore, telomerase hypothesized oncogenic effect could be explained and documented in its possible existence only for other animals.

2) Shortened telomeres increase vulnerability to cancer because of dysfunctional telomere-induced instability [11,12] (Fig. 3);



COURTESY JOSEPH VAINA

Fig. 1 – January 2009: A giant lobster named George, estimated to be 140 years old, escaped a dinner-table fate and was released into the Atlantic Ocean after a New York seafood restaurant granted him his freedom (<http://www.cnn.com/2009/US/01/10/maine.lobster.liberated/>).

3) The decline of duplication capacities and of overall cell functionality weakens immune system efficiency [1], which has, for a long time, been known to be inversely related to cancer incidence [13];

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 (Oshimura et al., 2000); expression is late and permissive, not causal (Seger et al., 2002).” [1];

5) In yeast, a eukaryotic species, replicative senescence and cell senescence, although not caused by telomere shortening but by another unknown mechanism related to the number of duplications (likely, the accumulation of extrachromosomal ribosomal DNA circles - ERCs -, which block subtelomeric DNA) [14], is a well-documented phenomenon [15-17], and, being yeast unicellular, cannot be a consequence of an impossible cancer risk. (Moreover, these phenomena and others strictly associated [18,19] observed in yeast have been interpreted as adaptive [20-25] and are consistent with the explanation that they determine a greater evolution rate and are favoured in conditions of K-selection [2,26].)

6) *Dyskeratosis congenita* (DC), an inherited human disease [27], is characterized by an altered telomerase [28]. “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.” [29]. DC patients present defects in these tissues [29]

and also suffer from a higher rate of cancer that can likewise be explained by the lack of telomerase, which results in unstable chromosomes [30,31].

But: 7) In rodents, telomerase activity is not related to maximum lifespan while is inversely related to body mass [32]. This has been interpreted as a fact in support of the defensive role against cancer risk of telomere-telomerase system, as a greater body mass presumably increases cancer risk [32].

In short, with the important exception of point (7), which stimulates further data and discussions, there are not specific arguments and experimental tests in support of the hypothesis that telomere-telomerase age-related limiting actions on cell turnover is a general defence against cancer. Therefore, telomere-telomerase age-related limits on cell turnover are hardly justifiable as a defence against cancer risk and, lacking other plausible explanations, only the adaptive hypotheses of age-related fitness decline appear a rational cause for their existence.

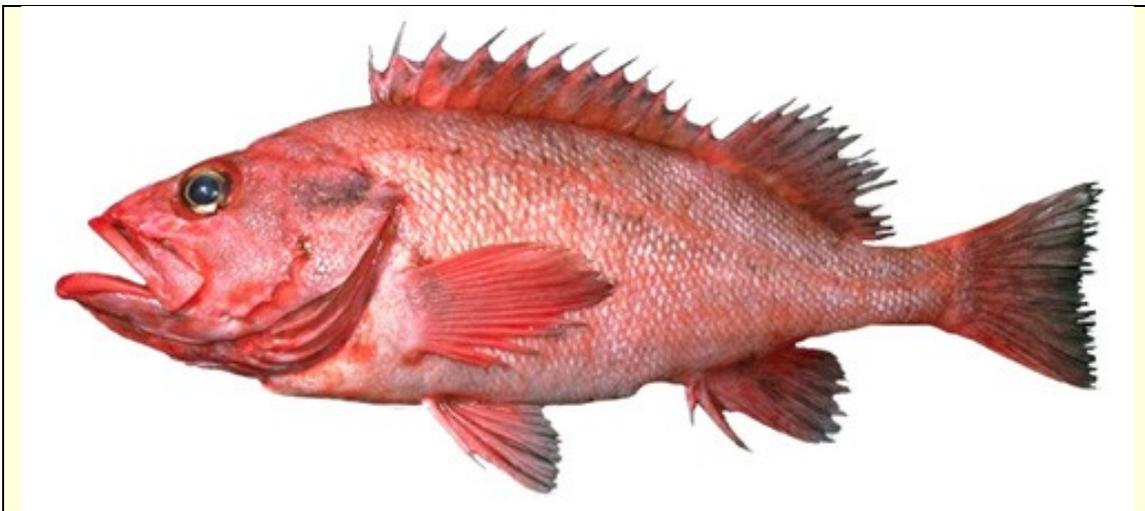
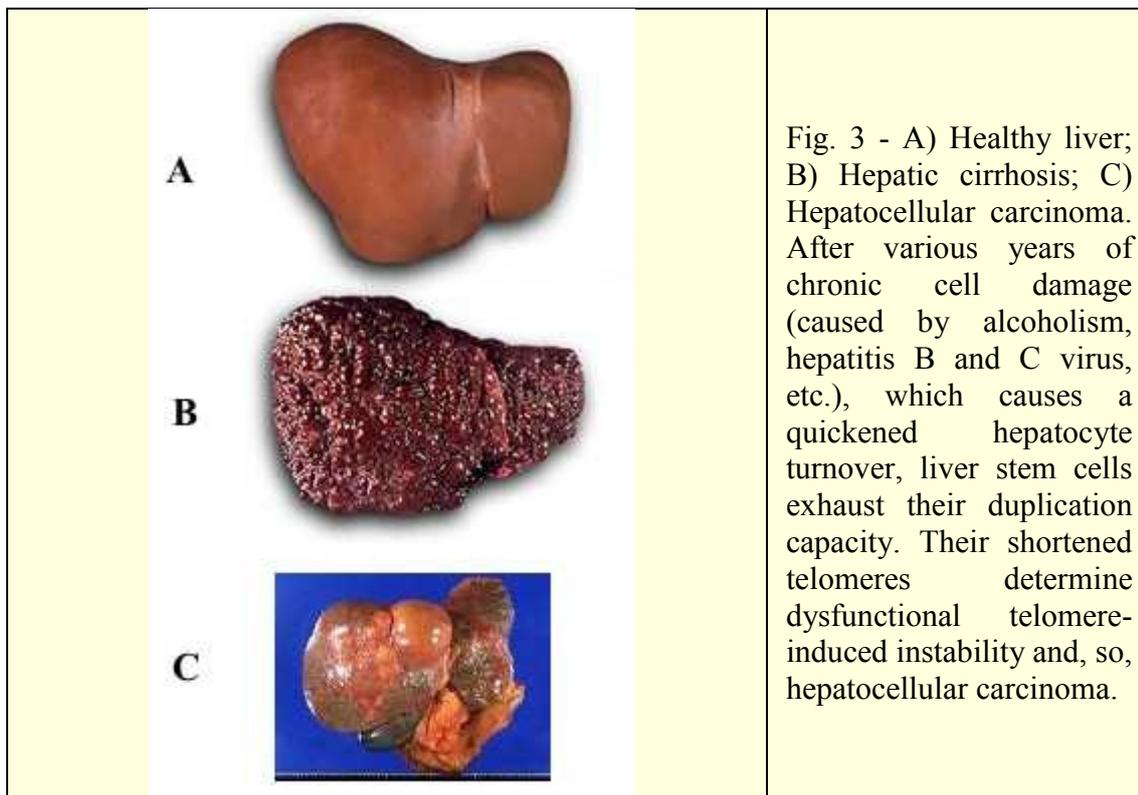


Fig. 2 – Rougheye rockfish (*Sebastes aleutianus*) are probably among the longest-lived marine fishes on Earth, living as old as 205 years. For Yelloweye rockfish (*Sebastes ruberrimus*, living as old as 118 years), commercially caught off Sitka, Alaska, "16% of the fish going to people's dinner tables were 50 years of age or older, with several over 100 years old!" (from the site <http://www.agelessanimals.org>)



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A proposal: Project "*Homo sapiens liberatus II*"!

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Abstract

Ageing is commonly 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, in analogy with diseases showing the same alterations, and the cures allow often an increase in survival time in conditions of low quality of life.

But, it is indispensable to acquire the awareness that ageing is something other than a disease and that it needs specific measures. It is possible to conceive an ambitious project for the solution to the problem, with a possible honorific name indicating the inspiration of Prof. Skulachev's great design.

In the first phase, some preliminary targets will be pursued, that is a better understanding of: a) telomere-telomerase system; b) apoptosis phenomenon; c) cell turnover of all tissues and its effect on the function of the organs in each age of the life; d) morphogenetic mechanism, in particular for dentition. Another important goal of the same phase is the development of genetic techniques for: a) the effective and precise insertion of a genetic sequence in a point of the genome not causing dangerous alterations; b) the effective and precise substitution of a genetic sequence with another sequence.

In the second phase, with experiments on animals, genetic sequences will be inserted or substituted with the aim of modifying the modulation of telomere-telomerase system for increasing longevity. The same techniques will be applied for the treatment of severe genetic diseases and for age-related severe diseases such as Age-Related Macular Degeneration and Alzheimer's disease. In the same phase, on animals, experiments will be performed with the same techniques to obtain multiple dentitions and other experiments for testing possible drugs with increasing longevity qualities.

In the third phase, on man, first experiments of gene therapy (not on germinal cells) will be performed and possible drugs with increasing longevity qualities will be tested, with the verification of the results and progressive widening of the experiments.

In the fourth phase, the project plans possible experimentation and application of gene therapy on human germinal cells and applications on a large scale of safe and tested techniques and drugs.

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

However, to go on the moon or to live one thousand years must not be foolish attempts to compete with the Infinite, but just other ways to contemplate It.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

Main concept:

**Aging may be mastered, but it is necessary to consider it a function and not a muddled array of diseases:
a paradigm change is an essential preliminary!**

The idea of this project was stimulated by T. Goldsmith's encouragement and approval and was published (without a name defining it) as an operating proposal in a recent work [1].

For the details and the premises, the reading of the entire work is necessary.

Prof. V. Skulachev's strong example has prompted the possible name.

Introduction

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 of 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 and that needs specific measures. It is possible to conceive an ambitious project for the solution to the problem in four steps:

Step 1 (Duration: at least a decade)

Parallel pursuit of various targets

- a) Widening of the studies on 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 each organ;
- d) The same for the morphogenesis of each organ, 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 without causing dangerous alterations (Fig. 1-3);
- f) Development of genetic techniques for the effective and precise substitution of a genetic sequence with another sequence (Fig. 1-3);
- g) Research of possible safe drugs to modify telomere-telomerase actions and/or cell turnover (or other) so that longevity is increased.

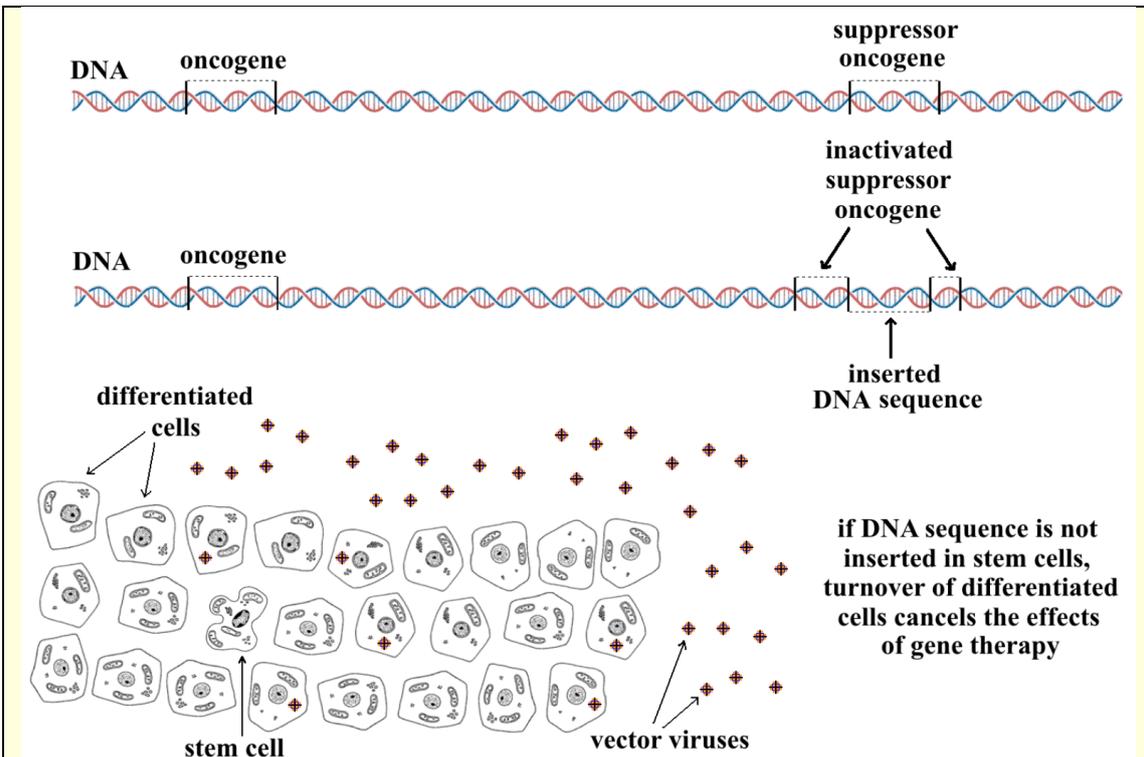


Fig. 1 - Current gene therapy. DNA sequence is inserted in a random position by a vector virus. If an insertion inactivates a suppressor oncogene, this may cause a cancer. The type of vector virus and/or limits in the dose of viruses inoculated may cause the transformation only of differentiated cells 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.

Step 2 (Duration: at least a decade)

Parallel pursuit of various targets

- Experiments on animals of insertion of genetic sequences to modify the modulation of telomere-telomerase system for increasing longevity;
- The same with techniques of genetic substitution;
- First applications of the above-mentioned techniques on man for the treatment of severe genetic diseases;
- First applications of the above-mentioned techniques on man for the treatment of age-related severe diseases such as **Age-related Macular Degeneration** and **Alzheimer's disease** (Fig. 4);
- As with (a) and (b) to obtain multiple dentitions;
- Experiments on animals of possible drugs with increasing longevity qualities.

Step 3 (Duration: at least two decades)

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

Step 4 (Duration: indeterminate)

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

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

Means: 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 (Fig. 5A-B).

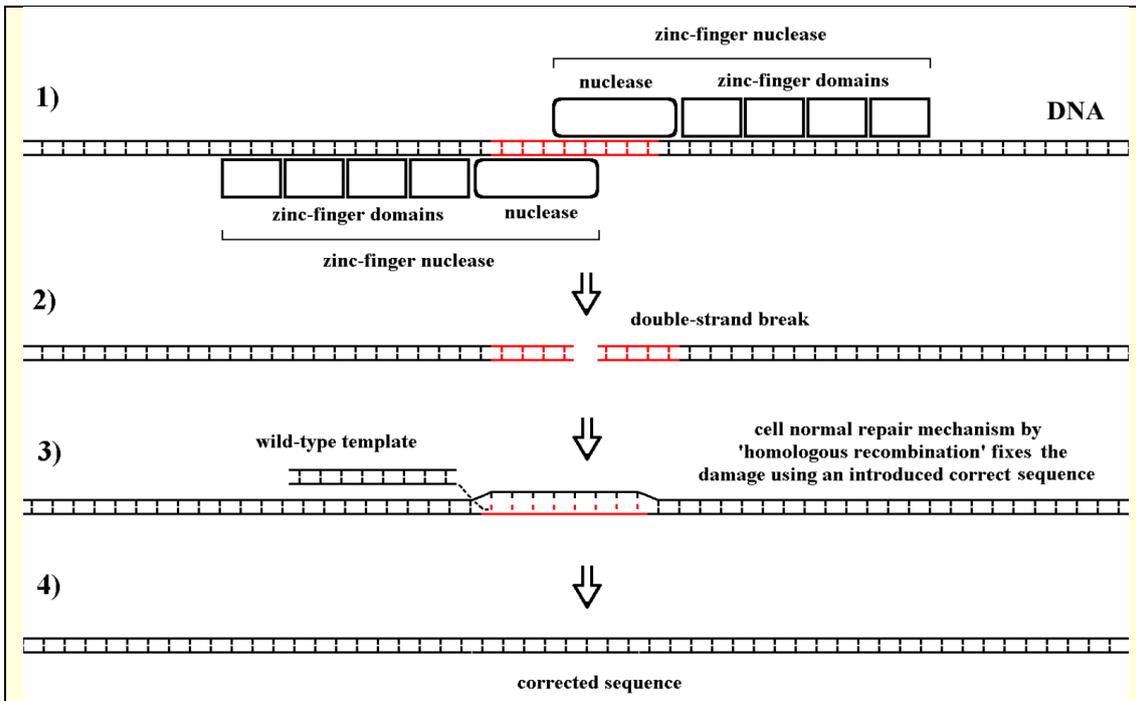


Fig. 2 - The corrected gene is inserted in substitution of the altered gene. With the use of two zinc-finger nucleases, composed of zinc-finger domains (each specific for a particular three-base DNA sequence) and a nuclease (a Type IIS restriction enzyme), it is possible to break DNA double-strand in a precise point with the successive correction by normal cell DNA-repair system by using an introduced DNA corrected sequence [2]. This method appears very promising [3].

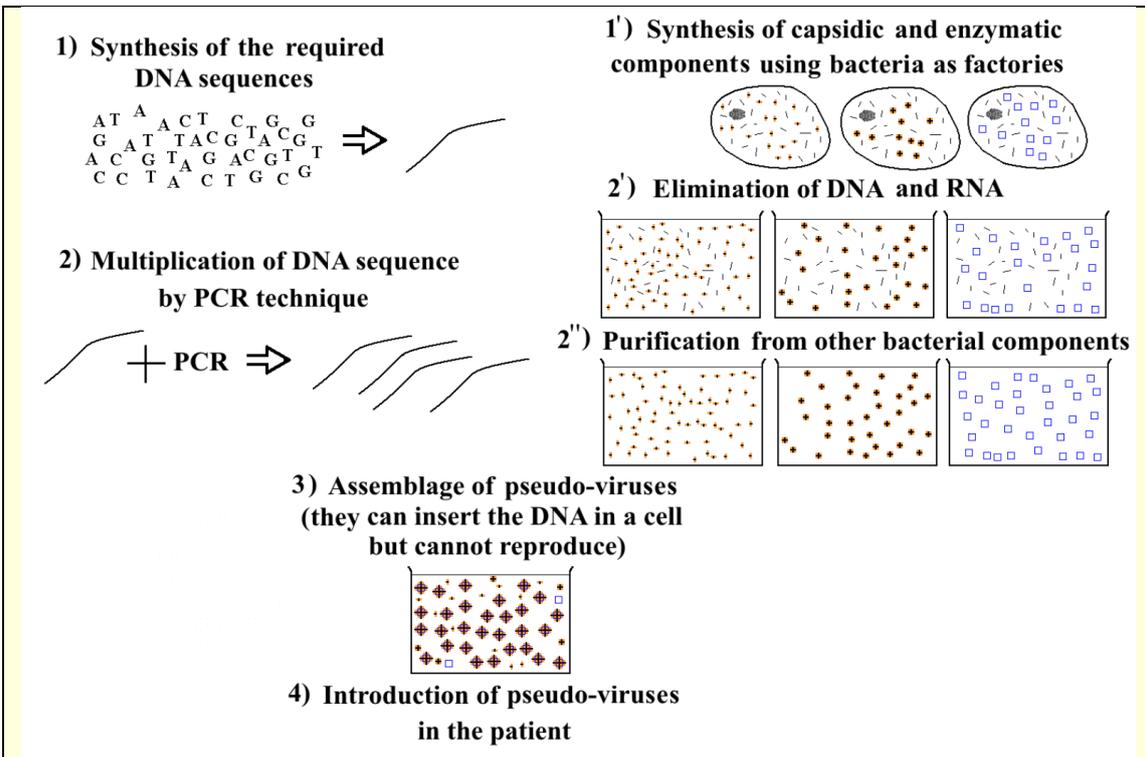


Fig. 3 - Creation of gene vectors (hypothetical scheme). The required DNA sequences (for the specific zinc-finger nucleases, for the gene to be modified, etc.) are created starting from defective viral sequences and from single nucleotides and multiplied by using PCR technique. Capsidic components and 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.



Fig. 4 – The defeat of Alzheimer's disease by telomerase activation in neuron satellite glyocytes will be a plausible preliminary goal to master ageing.



To go on the moon or to live one thousand years must not be foolish attempts to compete with the Infinite, but just other ways to contemplate It.

Fig. 5A-B - Moon landing was the aim of an ambitious and complex project that was successful because based on solid scientific grounds and strongly supported both economically and intellectually.

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Oxidative damage and aging

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Abstract: The risk of cardiovascular diseases is positively related to hypercholesterolemia, hypertension, diabetes, smoking, age, etc. and lowered by preventive lifestyle measures and by anti-hypertensive, hypoglycaemic and anti-lipidemic drugs.

The common interpretation is that modifiable risk factors increase oxidative damage while preventive lifestyle measures and lowering-risk-factors drugs reduce this harm. Moreover, aging, interpreted as the consequence of cumulative oxidative damage, is necessarily the cause of age-related cardiovascular increasing risks and is not modifiable with preventive measures and drugs.

Statins, ace-ACE-inhibitors and sartans (“protective drugs”) are known to be effective in reducing the cardiovascular risk even without acting on risk factors, namely with a direct action on atherogenesis, but this is compatible with the above-said general interpretation.

These ideas are challenged by the observation that the number of circulating endothelial progenitor cells (EPC) is negatively related to the cardiovascular risk and to the increasing age and that the intake of protective drugs is associated with higher values of EPC.

A likely deduction is that: 1) Excessive stress (oxidative or of other types) increases the apoptotic rate of endothelial cells (which show continuous cell turnover ensured by EPC) and quickens their turnover, so lowering renewal capacities and reducing EPC count; 2) Older endothelial cells, which suffer by cell senescence, increase the probability of atherosclerosis; 3) In old individuals, with or without excessive stress, EPC are reduced because of EPC stem cell exhaustion by telomere shortening: diseases derived from compromised blood circulation are a common end to the life of healthy old individuals with no particular risk factor.

In short, oxidative damage is important in the atherogenic process and in aging, but the key actor is the progressive failure of cell turnover caused by cell duplication limits, which are determined by the genetic regulation of telomere-telomerase system.

The scheme proposed for endothelial cells and atherogenesis is likely valid for other organs and tissues and for the whole organism.

This stimulates a general view where: I) Organism shows a continuous renewal of its cells; II) Aging is the consequence of the progressive slackening of this turnover and can be described as the progressive atrophy of each tissue and organ; III) Many diseases are the effect of the acceleration of the physiologic turnover of some cell types and the consequent exhaustion of their renewal capacities; IV) Many risk factors and many drugs contrasting these factors act by increasing or reducing, respectively, the turnover acceleration.

However, a well-founded objection needs a sound justification: some cells or tissues (as muscle and heart myocytes, eye crystalline lens, photoreceptor cells and neurons of

central nervous system) appear to have no turnover and so should not be included in this scheme, thus greatly weakening it.

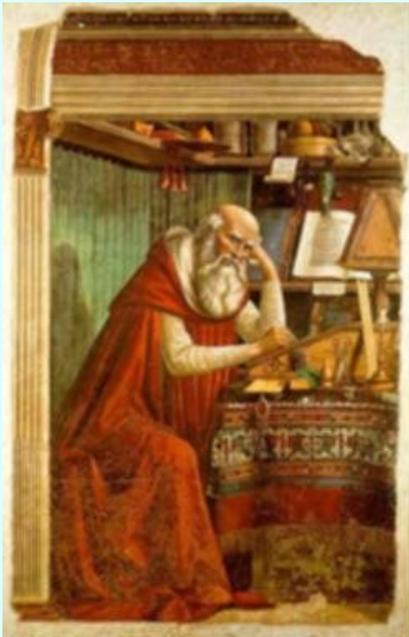
But: A) Muscle and heart myocytes are cells with turnover; B) The functionality of crystalline lens depends on lens epithelial cells that show turnover; C) Photoreceptor cells, particularly exposed to oxidative damage, and neurons of central nervous system, which have high metabolic activity, both depend from specialized types of gliocytes that show turnover. Turnover decline of these cells is a likely cause of age-related macular degeneration (ARMD) and of Alzheimer disease (AD), respectively; D) Smoking, diabetes, and obesity are risk factors for these diseases while "protective drugs" lower the risk.

Cures for ARMD and for AD that try to contrast ARMD and AD by lowering the oxidative damage or reducing the accumulation of metabolic substances result ineffective. A rational cure should contrast the decline of gliocyte turnover by the activation of telomerase, a possibility that is well documented *in vitro* by important experiments.

This type of cures for ARMD and AD would be very important *per se*, but would be as much important in a more general perspective: ARMD and AD are the pivotal expression of aging for the nervous system and the control of these diseases would be an important step in the control of aging.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm



Oxidative Damage and Aging

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www.programmed-aging.org

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Framingham Heart Study [1] and, afterwards, many other studies documented [2] that the risk of coronary heart disease is positively related to:

[Modifiable risk factors]
Hypercholesterolemia
Low HDL cholesterol level
Hypertension
Glucose intolerance (Diabetes)
Cigarette smoking

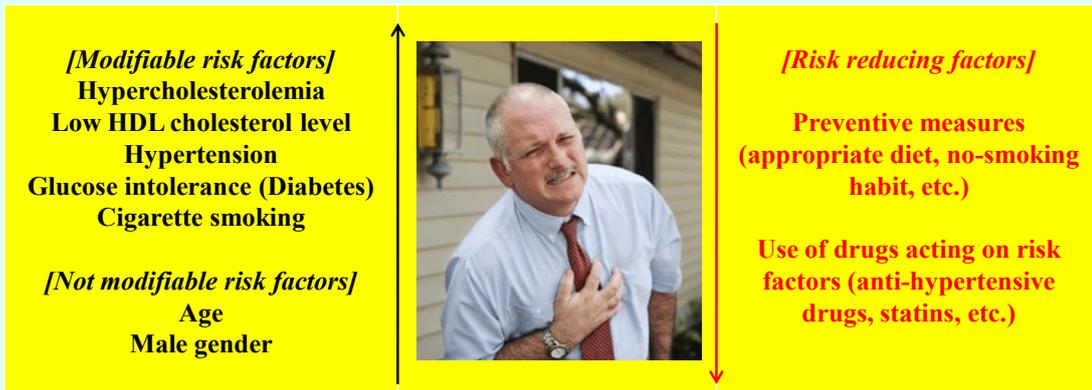
[Not modifiable risk factors]
Age
Male gender



[1] Wilson PW et al. (1987) Coronary risk prediction in adults (the Framingham Heart Study) *Am J Cardiol.* 59, 91G-94G.

[2] Wayne R et al. (eds) (1998) *Hurst's The Heart, Arteries and Veins* - 9th edit. McGraw-Hill, New York.

Moreover, the risk of coronary heart disease was lowered by [1]:



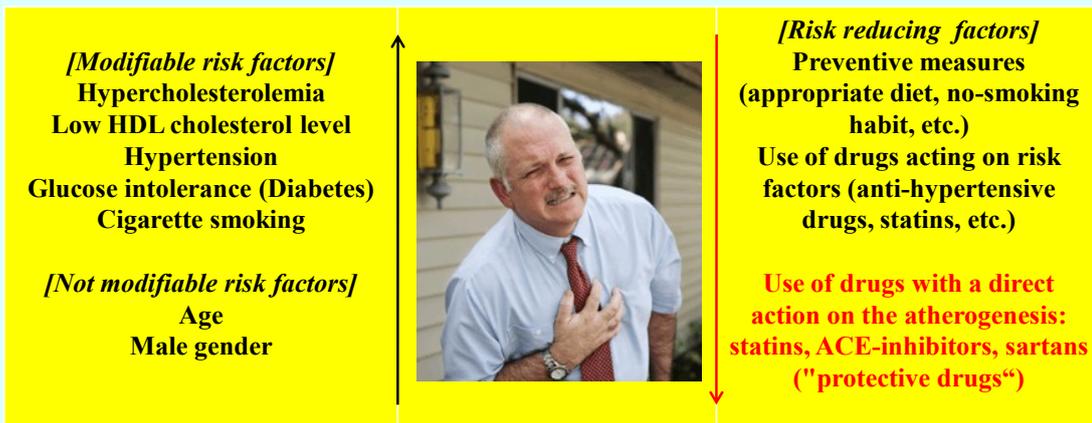
The general interpretation of these data seemed pacific and easy:

- 1) Modifiable risk factors increase oxidative damage (or cause other damages) while preventive measures and drugs avoid or reduce these harms.
- 2) Aging, as a consequence of cumulative oxidative damage (and/or of other damages), was necessarily the cause of age-related cardiovascular increasing risks, not reducible with preventive measures and drugs.

[1] Wayne R et al. (eds) (1998) *Hurst's The Heart, Arteries and Veins* - 9th edit. McGraw-Hill, New York.

Later, statins [1], ACE-inhibitors and sartans [2] (“protective drugs”), were shown to be effective in reducing the risk even without acting on risk factors, namely with a direct action on atherogenesis.

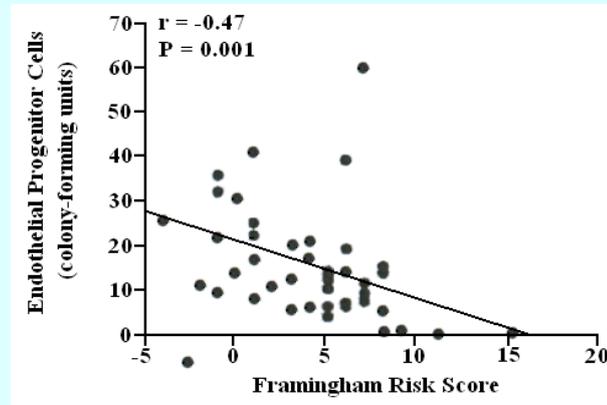
These new data were compatible with the above-said general interpretation.



- [1] Davidson MH (2007) Overview of prevention and treatment of atherosclerosis with lipid-altering therapy for pharmacy directors. *Am. J. Manag. Care* 13, S260-9.
[2] Weir MR (2007) Effects of renin-angiotensin system inhibition on end-organ protection: can we do better? *Clin. Ther.* 29, 1803-24.

But, this peaceful picture was challenged by the results of Hill et al. [1] and of other Authors that have confirmed and widened them (e.g.: [2]):

They showed that the number of circulating Endothelial Progenitor Cells (EPC) is significantly negatively related to the Framingham Risk Score.



- [1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.*. 348, 593-600.
- [2] Werner N et al. (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* 353, 999-1007.

Moreover:

"the levels of circulating EPC were a better predictor of vascular reactivity than was the presence or absence of conventional risk factors. In addition, EPC from subjects at high risk for cardiovascular events had higher rates of *in vitro* senescence than cells from subjects at low risk." [1]

The age-related decline of EPC, hinted by Hill et al. (P=.07) was confirmed by other studies (e.g.: [2]; P=0.013).

Statins, ACE-inhibitors and sartans are associated with higher values of EPC [2].

- [1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.*. 348, 593-600.
- [2] Xiao Q, et al. (2007) Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis—results from a large population-based study. *PLoS One.* 2: e975.

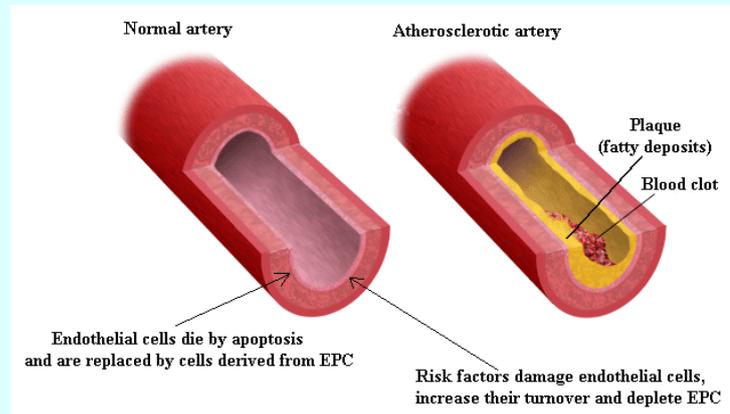
Interpretation of these data [1]

Endothelial cells manifest a continuous turnover assured by EPC, which derive from primary stem cells of bone marrow.

Excessive stress (oxidative or of other types) increases apoptosis rate of endothelial cells and quickens their turnover and this is manifested by the reduction of EPC count.

Older endothelial cells, which suffer by cell senescence, increase the probability of atherosclerosis:

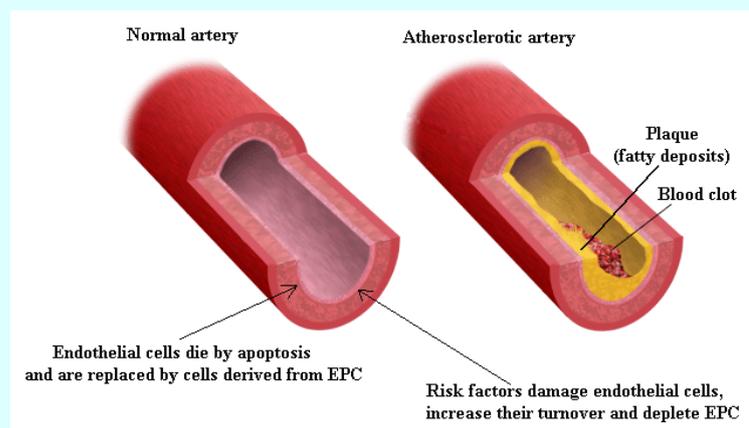
cell senescence -> endothelial dysfunction -> inflammation, plaques, blood clot, etc. ...



[1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593-600.

... In old individuals, with or without excessive stress, EPC are reduced because of EPC stem cell exhaustion by telomere shortening: diseases derived from compromised blood circulation are a common end to the life of healthy old individuals with no particular risk factor [1].

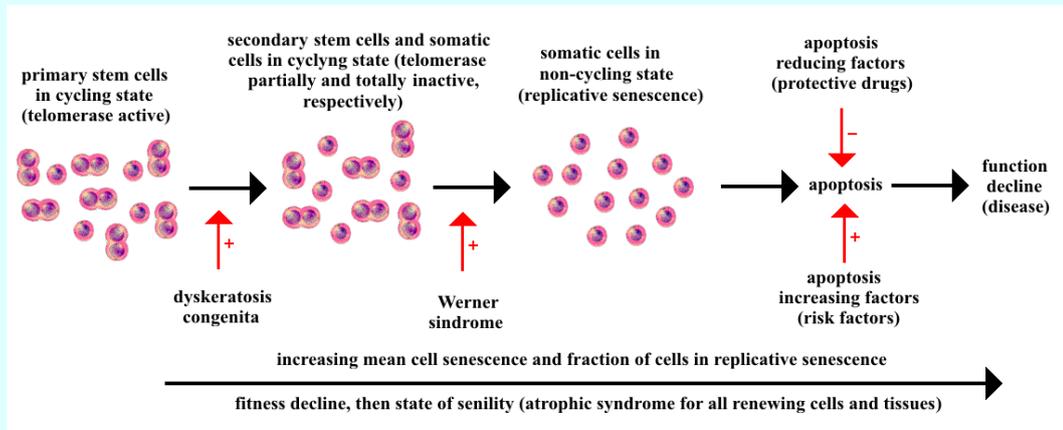
Some genetic diseases (as *Dyskeratosis congenita* and Werner syndrome) increase apoptosis rate and cell turnover, so accelerating atherogenesis [2].



[1] Tallis, RC, Fillit, HM & Brocklehurst, JC (eds) (1998) *Brocklehurst's Textbook of Geriatric Medicine and Gerontology* (5th ed.) Churchill Livingstone, New York.

[2] Marciniak, R & Guarente, L (2001) Human genetics. Testing telomerase. *Nature*, 413, 370-2.

These concepts may be generalized in the following scheme (concepts from [1]; figure from [2]):



[1] Marciniak R & Guarente L (2001) Human genetics. Testing telomerase. *Nature*, 413, 370-2.
 [2] Libertini G (2009) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Science Publishers Inc., New York.

An important concept:

Oxidative damage (+ other damaging factors) are important in atherogenic process and in aging, but the key actor is the **progressive failure of cell turnover caused by cell duplication limits**, which are determined by the genetic regulation of telomere-telomerase system.

The scheme proposed for endothelial cells and atherogenesis is likely valid for other organs and tissues and for the whole organism.

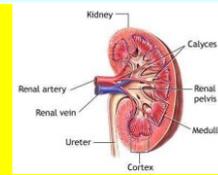
E.g.:

Apoptosis is well documented, in healthy organisms, for glomerular cells [1], alveolocytes type II [2], pancreatic β cells [3, 4], etc.

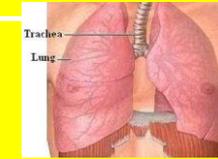
This means that these cells have turnover, and so ...

[1] Cardani R & Zavarella T (2000) Age-related cell proliferation and apoptosis in the kidney of male Fischer 344 rats with observations on a spontaneous tubular cell adenoma. *Toxicol. Pathol.* 28, 802-806.
 [2] Sutherland LM et al. (2001) Alveolar type II cell apoptosis. *Comp. Biochem. Physiol.* 129A, 267-285.
 [3] Bonner-Weir S (2000) Islet growth and development in the adult. *J. Mol. Endocrinol.* 24, 297-302.
 [4] Cerasi E et al. (2000) [Type 2 diabetes and beta cell apoptosis] [Article in French]. *Diabetes Metab.* 26, 13-6.

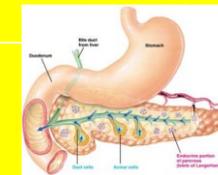
- for glomerular cells: microalbuminuria, a marker of renal damage and also a good marker of atherogenesis, is corrected by "protective drugs" [1]



- for alveolocytes type II: the decline in lung function in smokers is reduced by statins, which are among the "protective drugs" [2]



- for pancreatic β -cells: diabetes in the case of a wrong diet. The risk of diabetes is reduced by "protective drugs" [3, 4]



[1] Weir MR (2007) Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol.* 2, 581-90.
[2] Alexeeff SE et al. (2007) Statin use reduces decline in lung function: VA Normative Aging Study. *Amer. J. Respir. Crit. Care Medic.* 176, 742-7.
[3] McCall KL et al. (2006) Effect of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers on the rate of new-onset diabetes mellitus: a review and pooled analysis. *Pharmacotherapy* 26, 1297-306.
[4] Ostergren J (2007) Renin-angiotensin-system blockade in the prevention of diabetes. *Diabetes Res. Clin. Pract.* 78, S13-21.

This means a general view where [1-4]:

- the organism is in continuous renewal (turnover) of its cells;
- aging is the consequence of the progressive slackening of this turnover;
- many diseases are the effect of the acceleration of the physiologic turnover of some cell types and the consequent exhaustion of renewal capacities;
- many risk factors and many drugs contrasting these factors act by increasing or reducing, respectively, this turnover acceleration.

Aging can be described as the progressive atrophy of each tissue and organ

[1] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.
[2] Libertini G (2006) Evolutionary explanations of the "actuarial senescence in the wild" and of the "state of senility". *The Scientific World JOURNAL* 6, 1086-108 DOI 10.1100/tsw.2006.209.
[3] Libertini G (2009a) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Science Publishers Inc., New York.
[4] Libertini G (2009b) The Role of Telomere-Telomerase System in Age-Related Fitness Decline, a Tameable Process, in *Telomeres: Function, Shortening and Lengthening*, Nova Sc. Publ., New York.

The **atrophic syndrome** of a tissue or organ is characterized by [1]:

- a) reduced cell duplication capacity and slackened cell turnover (replicative senescence);
- b) reduced number of cells (atrophy);
- c) possible 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 (cell senescence);
- 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 [2].



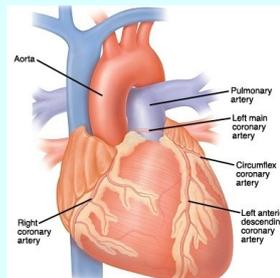
[1] Libertini G (2006) Evolutionary explanations of the “actuarial senescence in the wild” and of the “state of senility”. *The Scientific World JOURNAL* 6, 1086-108 DOI 10.1100/tsw.2006.209.
 [2] DePinho RA (2000) The age of cancer. *Nature* 408, 248-54.

This view stimulates an immediate objection:
There are cells or tissues that have no turnover and so cannot be included in this scheme, thus greatly weakening it:

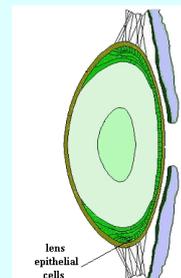
1) Muscular tissue



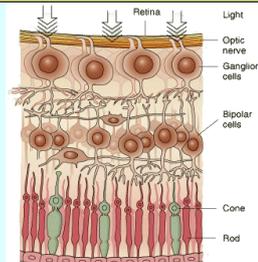
2) Heart muscle tissue



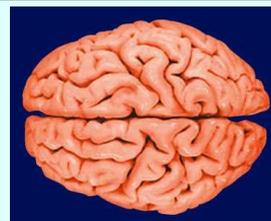
3) Eye crystalline lens



4) Photoreceptors of retina



5) Neurons of the Central Nervous System



But ...

1) Muscular tissue



Myocytes are cells with turnover!

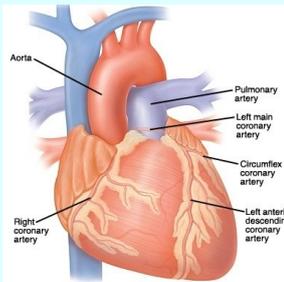
Stem cells from muscles of old rodents divide in culture less than cells from muscles of young rodents [1];

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 [2];

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 [3].

- [1] Schultz E & Lipton BH (1982) Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech. Age. Dev.* 20, 377-83.
[2] Carlson BM & Faulkner JA (1989) Muscle transplantation between young and old rats: age of host determines recovery. *Am. J. Physiol.* 256, C1262-6.
[3] Adams V et al. (2001) Apoptosis in skeletal muscle. *Front. Biosci.* 6, D1-D11.

2) Heart muscular tissue



Heart myocytes are cells with turnover!

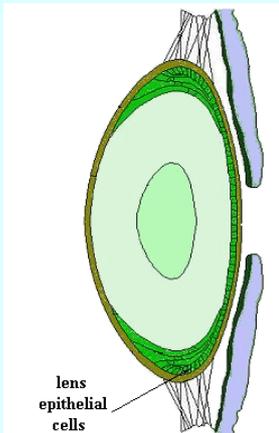
“It remains a general belief that the number of myocytes in the heart is defined at birth and these cells persist throughout life ... But myocytes do not live indefinitely – they have a limited lifespan in humans and rodents. Cell loss and myocyte proliferation are part and parcel of normal homeostasis ...” [1]

“Age-associated left ventricular hypertrophy is caused by an increase in the volume but not in the number of cardiac myocytes.” [2]

“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.**” [2]: This causes atrial fibrillation and “protective drugs”, as ACE-inhibitors, sartans and statins, are effective in the prevention of it [3, 4].

- [1] Anversa P & Nadal-Ginard B (2002) Myocyte renewal and ventricular remodelling. *Nature* 415, 240-3.
[2] Aronow WS (1998) Effects of Aging on the Heart. In *Brocklehurst's Textbook of Geriatric Medicine and Gerontology*.
[3] Jibrini MB et al. (2008) Prevention of atrial fibrillation by way of abrogation of the renin-angiotensin system: a systematic review and meta-analysis. *Am. J. Ther.* 15, 36-43.
[4] Fauchier L et al. (2008) Antiarrhythmic effect of statin therapy and atrial fibrillation a meta-analysis of randomized controlled trials. *J. Am. Coll. Cardiol.* 51, 828-35.

3) Eye crystalline lens



The crystalline lens has no cell in its core, but its functionality depends on lens epithelial cells that show turnover [1].

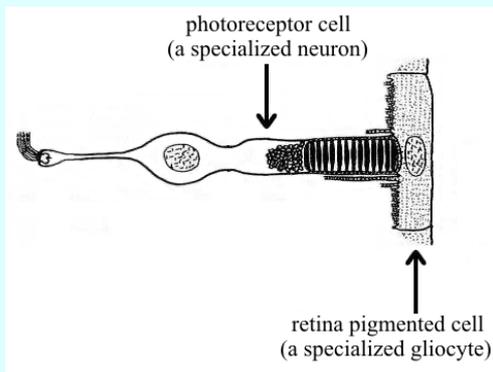
“Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells” [2], which is consistent with age-related reduction in growth potential for lens epithelial cells reported for normal human subjects [1].

Smoke and diabetes are risk factors for cataract [3].

Statins lower the risk of cataract [4]. This has been attributed to “putative antioxidant properties” [4] but could be the consequence of effects on lens epithelial cells analogous to those on endothelial cells [5].

- [1] Tassin J et al. (1979) Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. *Exp. Cell Res.* 123, 388-92.
- [2] Martin GM & Oshima J. (2000) Lessons from human progeroid syndromes. *Nature* 408, 263-6.
- [3] Delcourt C et al. (2000) Risk factors for cortical, nuclear, and posterior subcapsular cataracts: the POLA study. *Pathologies Oculaires Liées à l'Age. Am J Epidemiol.* 151, 497-504.
- [4] Klein BE et al. (2006) Statin use and incident nuclear cataract. *JAMA* 295, 2752-8.
- [5] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593-600.

4) Retinal nervous cells



Photoreceptor cells (cones and rods) are highly differentiated nervous cells with no turnover, but metabolically depending on other cells with turnover, retina pigmented cells (RPC), which are highly differentiated gliocytes.

The top of a photoreceptor cell leans on a RPC.

Each day, every RPC phagocytizes about 10% of the membranes with photopsin molecules of about 50 photoreceptor cells and, so, each day a cell of RPC metabolizes photopsin molecules of about 5 cones or rods, demonstrating a very high metabolic activity.

Without the macrophagic activity of RPC, photoreceptor cells cannot survive.

4) Retinal nervous cells - continued



Effects of ARMD on vision

With the age-related decline of RPC turnover, in RPC cells there is accumulation of damaging substances as A2E (a vitamin A-derived breakdown product) [1].

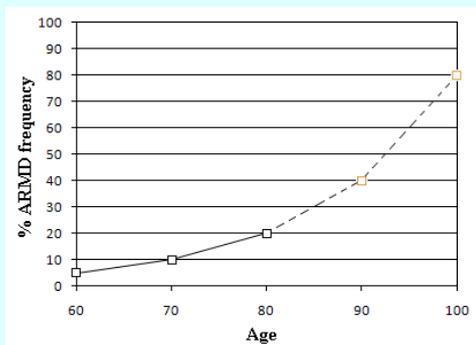
The death of RPCs by action of these substances causes holes in RPC layer and the deficiency of their function kills the photoreceptors not served.

This is above all manifested in the functionality of the more sensitive part of the retina, the macula - where the accumulation of A2E is more abundant [1] -, from which the name "age-related retina macular degeneration" (ARMD) [2]. ...

[1] Sparrow JR (2003) Therapy for macular degeneration: Insights from acne. *Proc Natl Acad Sci USA* 100, 4353-4.

[2] Fine SL et al. (2000) Age-related macular degeneration. *N. Engl. J. Med.* 342, 483-92.

4) Retinal nervous cells - continued



ARMD affects 5%, 10% and 20% of subjects 60, 70 and 80 years old, respectively [1], and it is likely that a large proportion of older individuals suffer from ARMD.

Smoking, diabetes, and obesity are risk factors for ARMD [2].

"The retina, with its high oxygen content and constant exposure to light, is particularly susceptible to oxidative damage" [3].

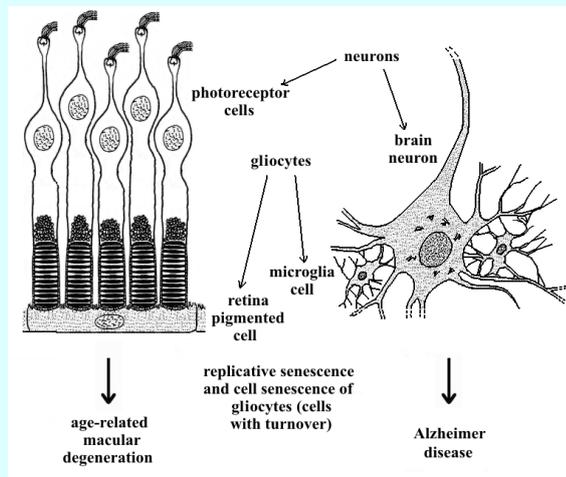
But the meta-analysis of 12 studies did not show that antioxidant supplements prevented early ARMD [3].

[1] Berger JW et al. (1999) *Age-related macular degeneration*, Mosby (USA).

[2] Klein R et al. (2007) Cardiovascular disease, its risk factors and treatment, and age-related macular degeneration: Women's Health Initiative Sight Exam ancillary study. *Am. J. Ophthalmol.* 143, 473-83.

[3] Chong EW et al. (2007). Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 335, 755.

5) Neurons of the Central Nervous System



As photoreceptor cells (specialized types of neuron with no turnover) depend on other cells (a specialized type of gliocytes with turnover), other types of neurons - as those of the Central Nervous System - depend on other types of gliocytes.

If this is true, replicative senescence and cell senescence of these gliocytes should cause pathologies similar to ARMD. ...

5) Nervous cells of the Central Nervous System - continued

The hypothesis that Alzheimer Disease (AD) is caused by replicative senescence and cell senescence of microglia cells has been proposed [1-3].

Microglia cells degrade β -amyloid protein [4, 5] and this function is known to be altered in AD [6] with the consequent noxious accumulation of the protein. ...

- [1] Fossel MB (1996) *Reversing Human Aging*. William Morrow and Company, New York.
- [2] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.
- [3] Libertini G (2009) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Sc. Publ., New York.
- [4] Qiu WQ et al. (1998) Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem.* 273, 32730-8.
- [5] Vekrellis K et al. (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci.* 20, 1657-65.
- [6] Bertram L et al. (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science*, 290, 2302-3.

5) Nervous cells of the Central Nervous System - continued

Telomeres have been shown to be significantly shorter in patients with probable AD than in apparently healthy control subjects [1].

AD could have, at least partially, a vascular aetiology due to age-related endothelial dysfunction [2] but "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." [2]

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 decline (atrophy) of cells with turnover.

Moreover, AD frequency, as ARMD, affects 1.5% of USA and Europe population at age 65 and 30% at 80 [3] and a centenarian has a high probability of suffering from it. ...

[1] von Zglinicki T et al. (2000) Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab. Invest.* 80, 1739-47.

[2] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.

[3] Gorelick PB (2004) Risk factors for vascular dementia and Alzheimer disease. *Stroke* 35, 2620-2.

5) Nervous cells of the Central Nervous System - continued

There is association between Alzheimer disease and cardiovascular risk factors [1, 2].

"Protective drugs" as statins, ACE-inhibitors and sartans, are effective against Alzheimer disease [1, 3].

[1] Vogel T et al. (2006) [Risk factors for Alzheimer: towards prevention?] [Article in French] *Presse Med.* 35, 1309-16.

[2] Rosendorff C et al. (2007) Cardiovascular risk factors for Alzheimer's disease. *Am J Geriatr Cardiol.* 16, 143-149.

[3] Ellul J et al. (2007) The effects of commonly prescribed drugs in patients with Alzheimer's disease on the rate of deterioration. *J. Neurol. Neurosurg. Psychiatry* 78, 233-9.



Possible cures for ARMD and for AD

1) Cures that are rational but effective within obvious limits:

- Reduction or avoidance of modifiable risk factors;
- Use of "protective drugs" against the effects of modifiable risk factors.

LIMITS: ineffective against age-related increasing risk of ARMD and AD (age is a non-modifiable risk factor and is not contrasted by "protective drugs"!))

2) Cures that are in accordance with the view that ARMD and AD are caused by the accumulation of damaging substances:

- For ARMD, dietary antioxidants: FAILURE shown in the meta-analysis of 12 studies [1];
- For AD, drugs against the formation of β -amyloid peptide: FAILURE [2];
- For AD, vaccine against β -amyloid peptide: "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" [2] ...

[1] Chong EW et al. (2007). Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 335, 755.

[2] Abbott A (2008) The plaque plan. *Nature* 456, 161-4.

Possible cures for ARMD and for AD - continued

3) Cures that treat cognitive alterations:

- For AD, cholinesterase inhibitors (donepezil, galantamine, rivastigmine) and NMDA receptor antagonist (memantine): "They are marginally effective at best" [1]
- For AD, antipsychotic drugs: Increase of long-term risk of mortality [2]

4) Cures that treat the key mechanism of ARMD and, likely, of AD, that is the turnover progressive failure of EPC and neuron-satellite microglia, respectively:

It is well-known from 1998 that with the activation of telomerase, telomeres result elongated and cells acquire unlimited duplication capacities [3-6]. ...

[1] Abbott A (2008) The plaque plan. *Nature* 456, 161-4.

[2] Ballard C et al. (2009). "The dementia antipsychotic withdrawal trial (DART-AD): long-term follow-up of a randomised placebo-controlled trial". *Lancet Neurology* 8, 151-7.

[3] Bodnar AG et al. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349-52.

[4] Counter CM et al. (1998) Dissociation among *in vitro* telomerase activity, telomere maintenance, and cellular immortalization. *Proc. Natl. Acad. Sci. USA* 95, 14723-8.

[5] Vaziri H (1998) Extension of life span in normal human cells by telomerase activation: a revolution in cultural senescence. *J. Anti-Aging Med.* 1, 125-30.

[6] Vaziri H & Benchimol S (1998) Reconstitution of telomerase activity in normal cells leads to elongation of telomeres and extended replicative life span. *Cur. Biol.* 8, 279-82.

Possible cures for ARMD and for AD - continued

Moreover, in the first experiment, a very important study by Bodnar et al. (which Google Scholar reports has been cited 2,771 times):

"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 -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." [1]

[1] Bodnar AG et al. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349-52.

Conclusion

Well, this is an extraordinary coincidence: it is not necessary to demonstrate that RPC can be rejuvenated by action of telomerase.

It is rational to hint that by action of telomerase it could be possible to reactivate the turnover of RPC and, so, to cure the key mechanism of ARMD.

Furthermore, it is rational to hint that by action of telomerase it could be possible to reactivate the turnover of neuron-satellite microglia and, so, to cure the key mechanism of AD.

Conclusion - continued

ARMD and AD are terrible diseases and the cure of them by the correction of their key mechanism is very important *per se*.

But this type of cure is important in a more general perspective.

ARMD and AD are the pivotal expression of aging for the nervous system.

This type of cures could be:

- the first step in the control of aging,
- the demonstration that aging is a tameable process,
- the proof that the ambitious goal of an *Homo sapiens liberatus* is a real aim and not utopia.

This presentation is on my personal pages too:

www.r-site.org/ageing.

**Please, write your possible questions (now or when you will prefer).
Any question will have a written and public answer**

(e-mail: giacinto.libertini@tin.it)

**Thanks
for your attention**

Phylogeny of age-related fitness decline in the wild and of related phenomena

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ABSTRACT

Apoptosis, the telomere-telomerase system, cell senescence and replicative senescence, a characteristic of cell senescence, are ubiquitous in eukaryotic species. Moreover, in some eubacterial species, "proapoptosis", a type of cell suicide, is determined by molecules homologous to apoptotic proteins, suggesting a common phylogenetic origin. The sophisticated mechanisms and regulators underlying these phenomena are genetically determined.

A common feature is that they are always harmful for the individual cell or for the multicellular organism or for the single cell in a multicellular organism in which they act. However, they are probably advantageous for kin cells or individuals.

In particular, in some eukaryotic species, a significant effect is that they may cause, in natural conditions, an age-related fitness decline, which is also referred to as "aging", an imprecise term.

Here I suggest that their evolutionary meanings lie in kin selection, and the analogies between their action in monocellular and multicellular eukaryotes are underlined.

A phylogenetic reconstruction based on their adaptive meanings is proposed.

Keywords: IMICAW, IMICAC, aging, telomere, telomerase, apoptosis, proapoptosis

Preliminary remarks

Some preliminary considerations are indispensable to avoid misunderstandings.

The phenomenon of an "increasing mortality with increasing chronological age in populations in the wild" ("IMICAW" [Libertini, 1988]), alias "actuarial senescence in the wild" [Holmes and Austad, 1995], alias "age-related fitness decline in the wild", is a real and well documented phenomenon [Deevey, 1947; Laws and Parker, 1968; Spina, 1970, 1972; Finch, 1990; Holmes and Austad, 1995; Ricklefs, 1998].

By definition, according to its presence in wild conditions, IMICAW phenomenon is subject to natural selection and should not be mixed up with the "increasing mortality with increasing chronological age in captivity" ("IMICAC" [Libertini 1988]), which is found in laboratory conditions at ages not existing in the wild for species that in natural conditions do not show IMICAW phenomenon. By definition, according to its absence in wild conditions, IMICAC is not subject to natural selection. In particular, the "fitness" in "age-related fitness decline in the wild" definition is unsuitable to the artificial conditions defined in IMICAC concept.

This paper regards only IMICAW, alias age-related fitness decline, and related phenomena and not IMICAC phenomenon. This remark is important as in current scientific literature and in the prevailing ideas about age-related fitness decline both phenomena are confused in a single imprecise term, namely "aging" (or "senescence").

The concepts and the results referred to "aging" in its imprecise meaning but, in fact, to IMICAC phenomenon (e.g., the numberless papers regarding the survival in laboratory conditions and at ages not existing in the wild of *C. elegans* and *D. melanogaster*) will

not be considered in this paper, not for inaccuracy or for the sake of brevity but as not regarding the topic.

Moreover, in this paper, the term "aging" will be used only making reference to current ideas where a precise meaning is not defined.

INTRODUCTION

If species separated by different evolutionary histories of hundreds of millions of years show equal or similar features that are clearly of common phylogenetic origin, it is necessary to inquire about an equality or analogy of functions explaining their evolutionary persistence and similarity.

Phenomena such as apoptosis, the telomere-telomerase system, cell senescence and replicative senescence (that is, in relation to the number of cell replications, in a single cell: abrupt decline of cell functions and block of duplication capacities; in a cell culture: overall progressive decline of cell functions and of duplication capacities), which will be discussed in the next section, exist in yeast, a monocellular eukaryote, and in multicellular eukaryotic species with a divergent evolutionary history at least from the beginning of Cambrian period, about 600 millions of years ago [Minkoff, 1983].

Moreover, "proapoptosis" [Hochman, 1997], a form of eubacterial cell suicide with mechanisms clearly related to eukaryotic apoptosis [Koonin and Aravind, 2002], indicates a much older evolutionary persistence and similarity.

The present paper expounds the main common features of these phenomena, trying to explain their general evolutionary meanings and phylogenetic relations.

EMPIRICAL EVIDENCE

A) LIMITS IN DUPLICATION CAPACITIES

A-1) In multicellular eukaryotes

Normal eukaryotic non-germ cells of multicellular organisms with limited lifespan can, in general, duplicate themselves only a limited number of times both *in vitro* [Hayflick, 1965; Hayflick and Moorhead, 1961] and *in vivo* [Schneider and Mitsui, 1976]. This phenomenon (Hayflick limit), well documented for many types of cells [Rheinwald and Green, 1975; Bierman, 1978; Tassin et al., 1979], shows an inverse relation with the ages of donors of origin [Martin et al., 1970] and, with exceptions that will be discussed later, a rough direct correlation with the life span of the species from which cells are derived [Röhme, 1981].

The main cause of the phenomenon, for many years known to be caused by something acting in the nucleus [Wright and Hayflick, 1975], was suggested to be a result of the incomplete action of DNA polymerase, which at each duplication leaves out a part of the terminal portion of DNA, the telomere [Watson, 1972]. This incomplete replication leads to a progressive shortening of the DNA molecule with a related increase in duplication impairment [Olovnikov, 1973].

Telomeres are highly conserved repetitive sequences of DNA (e.g., TTGGGG in a protozoan [Blackburn and Gall, 1978], TTAGGG in mammals [Moyzis et al., 1988] and many other species [Blackburn, 1991]). Telomeres shorten with every duplication event [Harley et al., 1990], but an enzyme, telomerase, can elongate telomeres at each replication, thereby compensating for the incomplete action of DNA polymerase. The action of telomerase explains why some cells, such as those of germ line, have unlimited duplication capacities [Greider and Blackburn, 1985]. With telomerase deactivation, telomeres shorten at each duplication and, in a cell culture or in a tissue, overall duplication capacity is reduced [Yu et al., 1990]. On the other hand, telomerase

activation elongates telomeres and cells become capable of numberless duplications [Bodnar et al., 1998; Counter et al., 1998; de Lange and Jacks, 1999; Vaziri, 1998; Vaziri and Benchimol, 1998]. Moreover, active telomerase was demonstrated in immortal human cell lines [Morin, 1989], while in other cells it was proven to be repressed by regulatory proteins [van Steensel and de Lange, 1997].

In a cell culture, the final incapability of a cell to duplicate (replicative senescence) was shown not to be an abrupt event for all the cells at the same time, but a progressive reduction of cell culture growth potential that depended on the reduction of telomere length [Jones et al., 1985; Pontèn et al., 1983].

According to Blackburn's model [Blackburn, 2000], particular protective nucleoproteins cap telomeres, which oscillate between capped and uncapped conditions: the duration of the first state directly correlates with telomere length while the other state is vulnerable to the passage to "noncycling state" or final stage of replicative senescence (fig. 1).

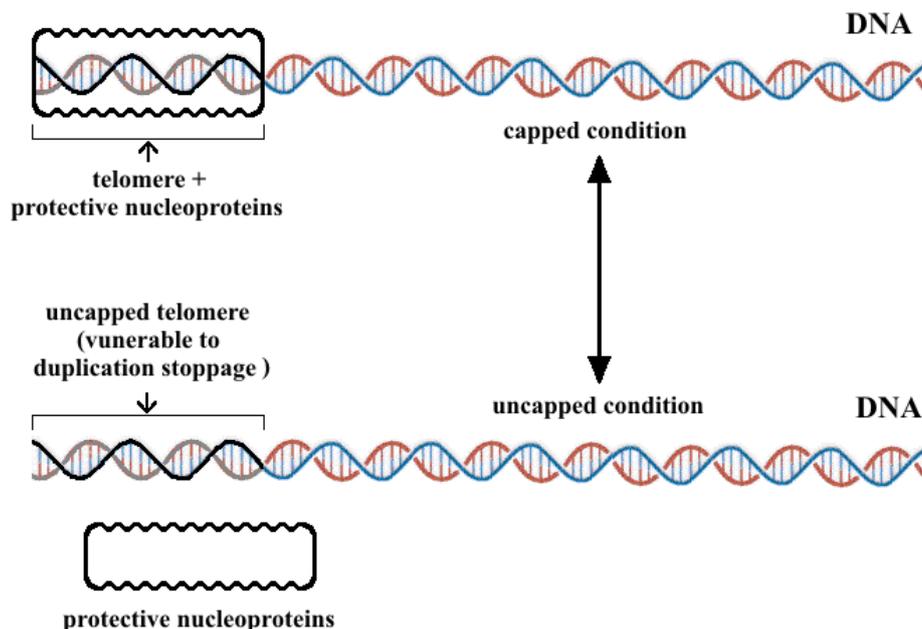


Figure 1 – Telomere oscillates between capped and uncapped conditions. The probability of uncapped condition increases at each duplication in relation to telomere shortening. Uncapped telomere acts as a broken end that can cause an end-to-end joining and a block of cell duplications.

A population of cells with telomeres at their maximum length, but inactivated telomerase, shows a progressive decline in replication capacities. Even cells with telomerase activated and so telomeres constantly at maximum length, should show a small percentage of cells passing to noncycling state at each division. Moreover, it has been proposed that stem cells, unlike germ cells, have levels of telomerase activity that are only partially able to stabilise telomere length [Holt et al., 1996] and for this reason they cannot indefinitely replace the apoptotic elements for cell populations in renewal [Fossil, 2004].

The absolute length of telomeres does not enable one to predict a species life span. Species, such as the mouse and the hamster have long telomeres [Slijepcevic and Hande, 1999], yet they age more precociously than species such as man, which have shorter telomeres. Moreover, in rodents, telomerase activity is not related to maximum lifespan [Gorbunova et al., 2008]. However, Blackburn's hypothesis does not postulate for different species a fixed ratio between telomere length or telomerase activity and the stability of telomere-capping nucleoproteins complex: it is easy to suppose that the

stability of the complex and, in general, the modulation of telomere-telomerase system is different from species to species. What is likely important is the species-specific critical telomere length and the relative rather than absolute telomere shortening [Fossel, 2004].

In relation to the mean number of duplications in cell culture or in a tissue, there is an increasing probability of cell senescence, a "fundamental cellular program" [Ben-Porath and Weinberg, 2005], which is characterized by an altered expression of many genes usually active in the cell, compromising cell overall functionality, and by replicative senescence. A senescent cell has deleterious consequences on the extracellular matrix as well as other cells that are physically near or physiologically interdependent. Cell senescence, and replicative senescence that is one of its characteristic, certainly derive somehow from the relative shortening of telomere (Fossel's "cell senescence limited model") [Fossel, 2004].

About the mechanism underlying cell senescence:

"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].

These statements are largely based on experiments in yeast, but possible deductions for monocellular eukaryotes must consider the invariability of telomere length with duplications in these organisms (see next paragraph).

On the other hand, the likelihood that a mechanism of this type is true for multicellular eukaryotes is widely discussed by Fossel (see pages 45-56 in Fossel, 2004; a plausible scheme is illustrated in fig. 2).

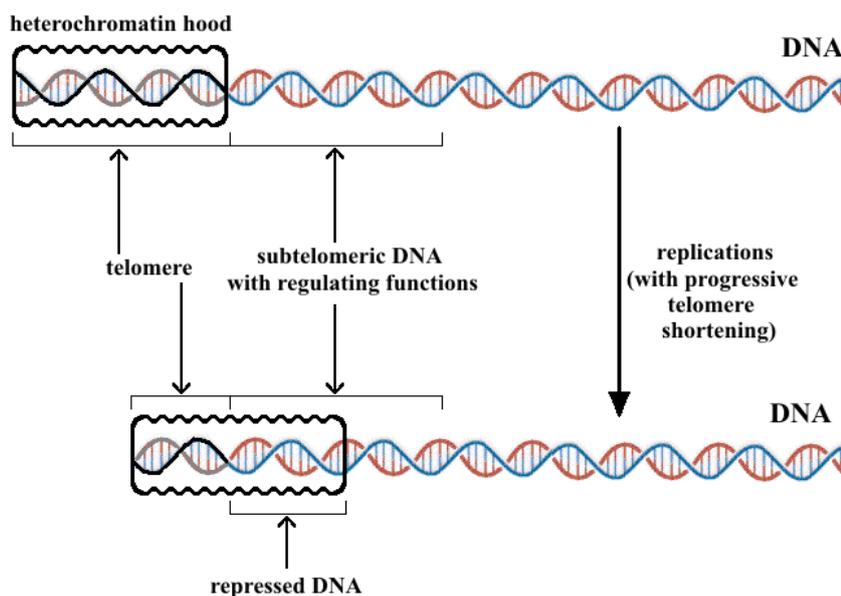


Figure 2 – The expression of many genes is impaired in relation with telomere progressive shortening. As likely hypothesis, a subtelomeric DNA tract regulates overall cell functionality and its action is impaired by the progressive sliding of the heterochromatin ‘hood’ caused by telomere shortening [Fossel, 2004].

Heterochromatin ‘hood’ [Fossel, 2004] and capping nucleoproteins [Blackburn, 2000] are most likely the same thing because: 1) they are supposed in the same part of the chromosome; 2) telomerase activation and the consequent telomere lengthening cause the reversal both of manifestations of cell senescence and of replicative senescence [Bodnar et al., 1998; Counter et al., 1998; de Lange and Jacks, 1999].

For germ line cells and for donor somatic cells that originate a cloned animal, the resetting of telomere clock is indispensable [Fossel, 2004]. The starting length of telomere must be established as with each subsequent shortening of the telomere, the probability of cell senescence and replicative senescence will increase. The absolute value of “telomere length is irrelevant” [Fossel, 2004]: two *Mus* strains with different telomere length (10 and 20 kb, respectively) show the same life span and an equal timing of cell senescence; analogously the same is true for donor animals and for cloned animals derived from cells with shortened telomeres [Fossel, 2004]. An appropriate shaping of the heterochromatin hood depending on telomere length could explain the equal timing of cell senescence and replicative senescence in spite of different telomere lengths (fig. 3).

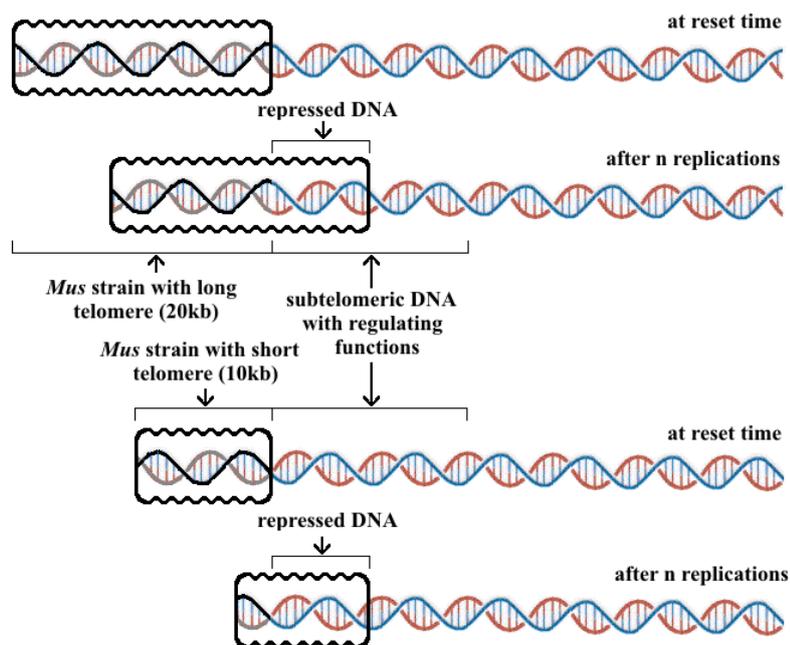


Figure 3 – In the reset of telomere clock, the heterochromatin hood is shaped proportionally to telomere length and does not vary for all the cell life. Telomere shortening in relation to the number of duplications causes the sliding of heterochromatin hood over subtelomeric DNA that regulates both overall cell functionality and telomere capped / uncapped condition equilibrium. The progressive repression of subtelomeric DNA increases the degree of cell senescence and the probability of replicative senescence. This hypothetical model could explain the large irrelevance of initial telomere length for the consequences of its subsequent shortening [Fossel, 2004].

Mice and other animals have a shorter life span, despite a baseline telomerase activity in most somatic cells [Prowse and Greider, 1995] and much longer telomeres than our species [Slijepcevic and Hande, 1999]. (But, in mice microglia cells, 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 knockout (mTR^{-/-}) mice, which have telomerase genetically inactivated, only after four [Herrera et al., 1999] to six [Blasco et al., 1997] generations, with very shortened telomeres, fertility and viability are jeopardized, although organs with high cell turnover show dysfunctions in early generations [Herrera et al., 1999; Lee et al., 1998]. The model of fig. 3, as expounded in fig. 4, could explain this apparently paradoxical phenomenon.

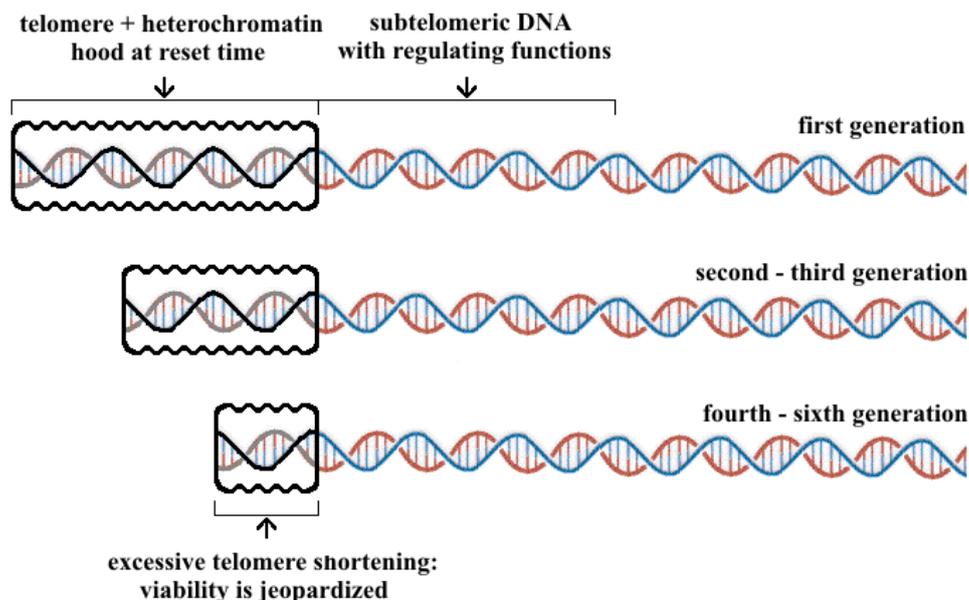


Figure 4 – For the model of fig. 3, the length of heterochromatin hood in knockout mice, defined in the reset phase, is proportional to telomere length. Subsequent sliding of the heterochromatin hood over subtelomeric DNA and the consequent genetic repression is independent from the length of the hood. With an excessive telomere shortening, the mechanism is compromised and cell viability is lost. The short life span of mice and other species with long telomeres is explained by a species-specific low degree of telomere + heterochromatin hood complex stability.

Subtelomeric DNA appears to have both a pivotal importance for overall cell functionality and a position vulnerable to inactivation by telomere shortening itself. Excluding the possibility of an absurd evolutionary illogicality, this coincidence can be explained only as something favoured by natural selection to determine cell senescence and replicative senescence. A possible scenario for the evolution of the telomere-cell senescence system is proposed below.

A-2) In a monocellular eukaryote

Yeast (*Saccharomyces cerevisiae*), a well studied eukaryotic monocellular species, reproduces by asymmetric division between mother and daughter cells. The mother lineage can reproduce a limited number of times only, specifically between 25 and 35 generations in about 3 days [Jazwinski, 1993].

Both in mother and daughter yeast cells, telomere length does not decrease with duplications [D’Mello and Jazwinski, 1991; Smeal et al., 1996]. “Budding [= daughter] yeast cells express telomerase and divide indefinitely.” [Maringele and Lydall, 2004]

In mother cells of wild-type yeast, extrachromosomal ribosomal DNA circles (ERCs) accumulate in proportion 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].

ERCs, or some other unknown factor, interfere with gene expression and mutants such as *dna2-1*, which show abnormalities in the replication of DNA and therefore increased rates of ERCs accumulation, suffer by precocious alterations of gene expression. Specifically, transcriptome of older (18-generation-old) individuals of wild-type yeast are similar to those of young (8-generation-old) individuals of *dna2-1* mutants [Lesur and Campbell, 2004].

Telomerase-deficient mutants (*tlc1Δ* mutants) show, both in mother and daughter cells, telomere shortening. Additionally, older individuals of daughter cell lineages, which have no ERCs accumulation, show an overall expression of genes (transcriptome) similar to that of older individuals of wild-type yeast, and of young individuals of *dna2-1* mutants [Lesur and Campbell, 2004]. It is possible that in telomerase-deficient yeast mutants, as in cells of multicellular eukaryotes, telomere shortening causes the sliding of a telomere heterochromatin hood that interferes with a critical part of subtelomeric DNA, while in wild-type yeast subtelomeric DNA is somehow repressed by ERCs, or by some other unknown factor (fig. 5).

In old yeast cells, besides the replicative senescence, there are increasing metabolic alterations [Lesur and Campbell, 2004], which can be defined as cell senescence.

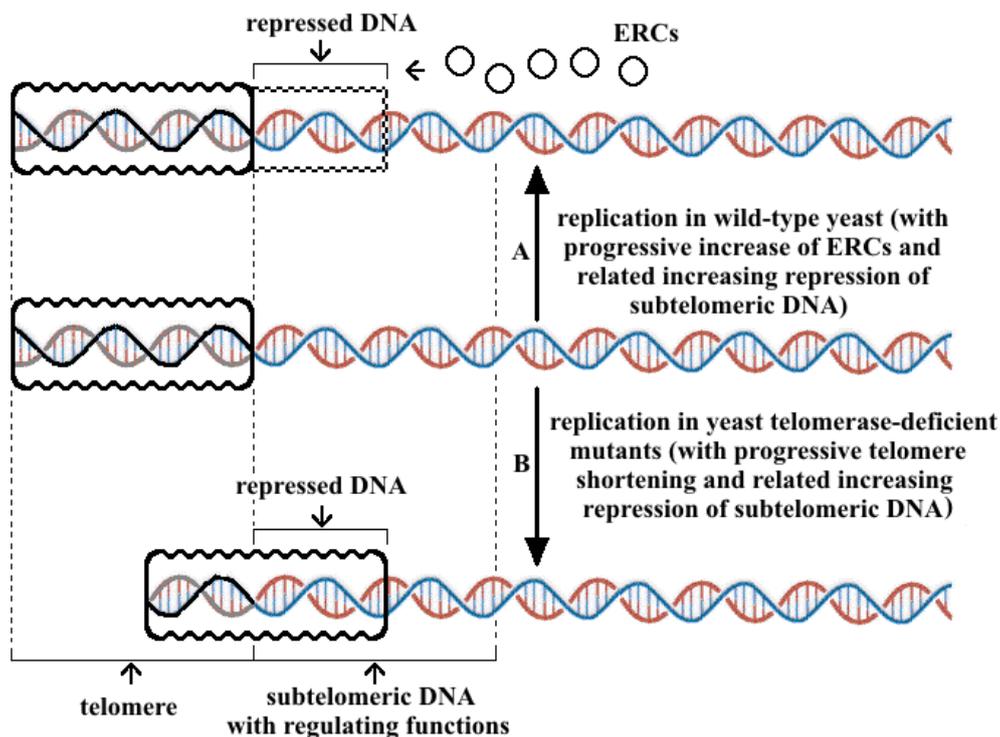


Figure 5 – A) In wild-type yeast, ERCs, or some other unknown factor increasing at each duplication, interfere with subtelomeric DNA, e.g. adding something to the telomere heterochromatin hood. This process is accelerated in *dna2-1* mutants; B) In telomerase-deficient mutants, in daughter cell line without ERCs accumulation, telomere shortening cause a sliding of telomere heterochromatin hood with subtelomeric DNA repression similar to that of case A.

B) APOPTOSIS

In contrast with necrosis, which is the cell death caused by acute cellular injury, apoptosis is an ordered form of cell self-destruction, which is ubiquitous in eukaryotic species [Longo et al., 2005].

Apoptosis was characterised and clearly differentiated from necrosis for the first time during observations of normal liver hepatocytes [Kerr et al., 1972]. It is described as a definite series of biochemical events leading to specific morphological changes (blebbing, loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, etc.).

B-1) In multicellular eukaryotes

Programmed cell death by apoptosis, selectively triggered for some cells in specific times, is essential for morphogenetic mechanisms (e.g., embryo neural development [Nijhawan et al., 2000], wound healing [Greenhalgh, 1998]), lymphocyte selection [Cohen, 1993; Opferman, 2008], cell turnover in healthy adult organs [Israels and Israels, 1999; Lynch et al., 1986; Medh and Thompson, 2000; Wyllie et al., 1980] (as documented for many tissues and organs [Libertini, 2006]), removal of damaged or infected cells [Tesfaigzi, 2006; White, 2006], etc.

Apoptotic cellular debris does not damage other cells because phagocytes remove such cell fragments in an orderly manner without eliciting an inflammatory response [Erwig and Henson, 2008].

Inactivated telomerase and short telomeres increase the probability of apoptosis [Fossel, 2004; Ozen et al., 1998; Holt et al. 1999; Seimiya et al., 1999; Ren et al., 2001].

B-2) In a monocellular eukaryote

In yeast, a phenomenon closely resembling apoptosis of multicellular eukaryotes was described quite recently [Madeo et al., 1997]. It was soon evident that the overexpression of human Bcl-2, an apoptosis inhibiting factor, in yeast delays processes leading to the phenomenon [Longo et al., 1997], while the overexpression of an apoptosis inducing factor in mammals (BAX) could elicit it [Ligr et al., 1998].

A growing body of evidence has documented similarities between this phenomenon in yeast and apoptosis in multicellular eukaryotes, to the extent that both deserve the same name. This data thus suggest that the two phenomena share a common phylogenetic origin [Kaeberlein et al., 2007; Longo et al., 2005; Madeo et al., 1999]: "... since the first description of apoptosis in a yeast (*Saccharomyces cerevisiae*) strain carrying a CDC48 mutation (Madeo et al., 1997), several yeast orthologues of crucial mammalian apoptotic proteins have been discovered (Madeo et al., 2002; Fahrenkrog et al., 2004; Wissing et al., 2004; Qiu et al., 2005; Li et al., 2006; Walter et al., 2006), and conserved proteasomal, mitochondrial, and histone-regulated apoptotic pathways have been delineated (Fig. 1; Manon et al., 1997; Ligr et al., 2001; Ludovico et al., 2002; Fannjiang et al., 2004; Ahn et al., 2005a; Gourlay and Ayscough, 2005; Pozniakovskiy et al., 2005)." [Büttner et al., 2006]

In yeast, there is an increasing vulnerability to apoptosis and replicative senescence, when the number of duplications increases, together with the metabolic alterations of cell senescence [Büttner et al., 2006; Fabrizio and Longo, 2008; Herker et al., 2004; Laun et al., 2001]. The age-related death rate increments in yeast follow exponential dynamics [Laun et al., 2007], as they also do for multicellular organism [Ricklefs, 1998].

Apoptosis is also triggered or favoured by: a) unsuccessful mating [Büttner et al., 2006]; b) dwindling nutrients [Granot et al., 2003]; c) chemical alterations [Madeo et al., 1999]; and d) killer toxins secreted by competing yeast tribes [Büttner et al., 2006].

When a yeast individual dies by apoptosis, cellular fragments do not damage other cells and are usefully phagocytised by other cells, which, consequently, “are able to survive longer with substances released by dying cells” [Herker et al., 2004].

A schematic comparison between apoptosis in yeast and multicellular eukaryotes is illustrated in fig. 6.

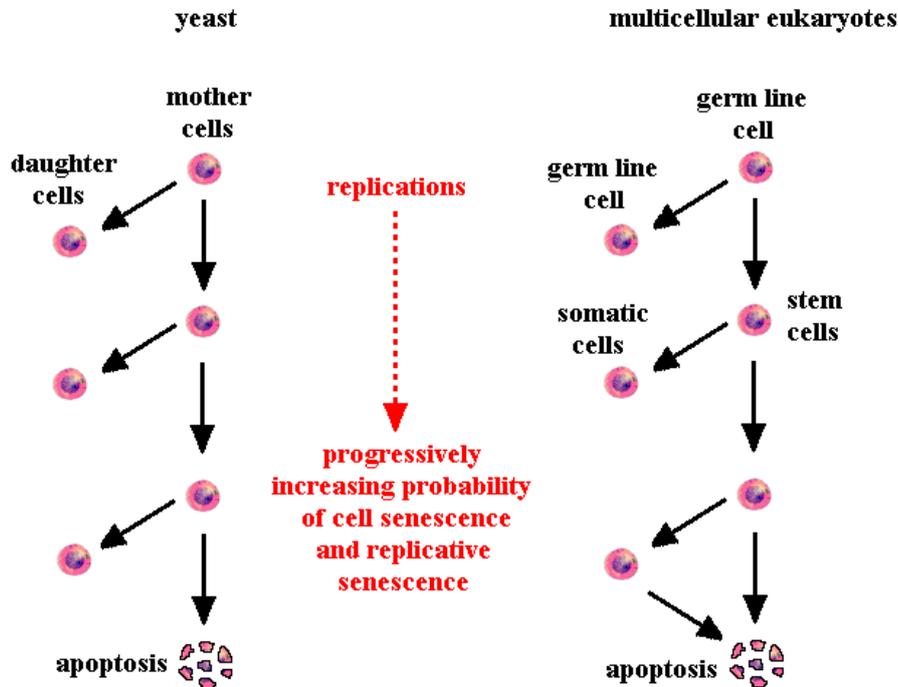


Figure 6 - A schematic comparison for apoptosis between yeast and multicellular eukaryotes.

EVOLUTIONARY INTERPRETATIONS

C) IN MONOCELLULAR EUKARYOTES

C-1) Apoptosis

Apoptotic patterns in yeast have been interpreted as adaptive because they are useful to the survival of the clone, which is likely made up of kin individuals [Fabrizio et al., 2004; Herker et al., 2004; Longo et al., 2005; Mitteldorf, 2006; Skulachev, 1999, 2002, 2003; Skulachev and Longo, 2005]. An exception is apoptosis triggered by toxin secreted by competing yeast tribes, where apoptotic mechanisms are exploited by competitors for increasing their fitness [Büttner et al., 2006].

The adaptive hypothesis appears plausible when a species is divided in many small demes, each of which is made up of one or a few clones, previously derived from as many individuals, and in conditions of K-selection, that is with population size “at or near [or over] carrying capacity of the environment” [Pianka, 1970]. In fact, in such conditions, the sacrifice of part of the population increases the survival probabilities of the remaining individuals, which are kin individuals (coefficient of relationship, r , equal to 1 in the case of a deme made up of a single clone, and greater than zero in the case of a deme made up of few clones). In terms of inclusive fitness [Hamilton, 1964, 1970; Trivers, 1971; Trivers and Hare, 1976], suicide individuals - by action of a hypothetical gene C - reduce their individual fitness but increases it for surviving kin individuals, in which there is a probability r of the existence of a copy of C . The inclusive fitness of C (F_C) is given by the sum of individual fitness reduction of suicide individuals plus the

sum of individual fitness increase of survivors each multiplied by the probability that C is present in them:

$$FC = \sum_{x=1}^{n_1} (r_x S_x) + \sum_{x=1}^{n_2} (-S'_x) \quad (1)$$

where n_1 = number of surviving individuals; S_x = advantage for a surviving individual; r_x = coefficient of relationship between a surviving individual and suicide individuals; n_2 = number of suicide individuals; $-S'_x$ = disadvantage for each suicide individual.

If F_C is positive, C is favoured by selection.

The possibility that the suicide of an individual, called “phenoptosis” by analogy to the term apoptosis [Skulachev, 1999], is also favoured by natural selection in prokaryote organisms, is necessary to explain the existence of “programmed death in bacteria” [Lewis, 2000; Skulachev, 2003]: e.g., bacterial phytoplankton mass suicide as defence against viruses [Lane, 2008], bacterial suicide triggered by phage infection “thereby curtailing viral multiplication and protecting nearby *E. coli* from infection” [Raff, 1998] and the “built-in suicide module” activated by antibiotics in *E. coli* [Engelberg-Kulka et al., 2004]. Interestingly, these mechanisms have been defined as “proapoptosis” and hypothesised as phylogenetic precursors of eukaryotic apoptosis [Hochman, 1997], as they share with it various features: “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].

C-2) Cell senescence and replicative senescence

In yeast, increasing vulnerability to apoptosis in relation to the number of duplications, a feature of cell senescence [Fabrizio and Longo, 2008; Herker et al., 2004], determines, or contributes to determining, which cells will die in conditions in which the sacrifice of part of the population may allow the survival of the others.

Cell senescence and replicative senescence may be explained by a mechanism similar to that justifying apoptosis but with a different evolutionary advantage. In fact, Büttner et al. 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 a new argument. Yeast ecological life conditions, if they are of the K-selection type, allow one to hypothesise that cell senescence and replicative senescence are adaptive and explainable with the same evolutionary mechanism proposed for age-related fitness decline in multicellular species subject to K-selection [Libertini, 1988, 2006]. This is the same above-mentioned suggestion of Büttner et al., but formulated in terms of individual selection.

In short, the diffusion of a gene G is dependent both on its advantage S over a neutral allele and on the inverse of the mean duration of life (ML), or generation time (fig. 7).

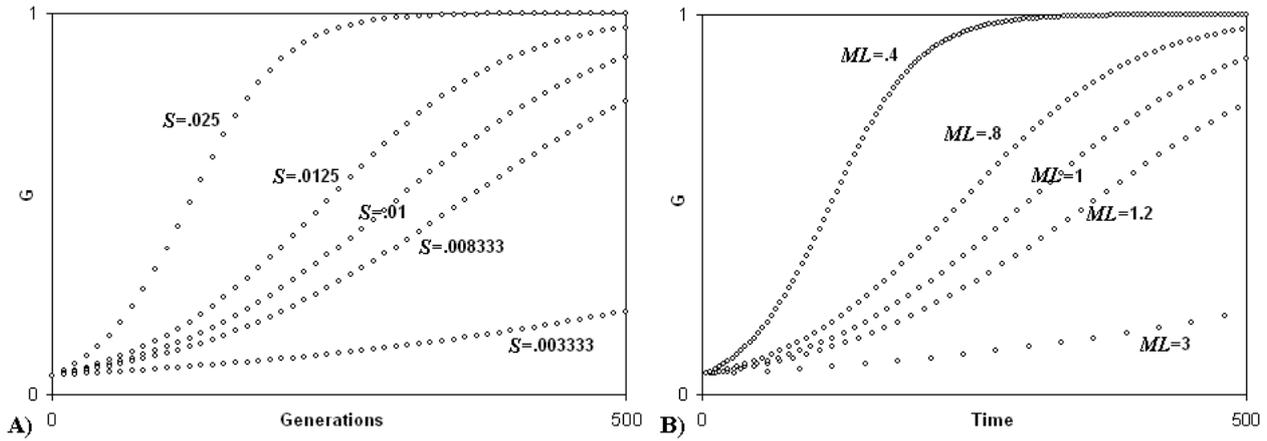


Figure 7 - A) Spreading of a gene (G) according to the variation of S ; B) Spreading of a gene according to the variation of ML . An increase / decrease of S or of $1/ML$ have the same effects on the spreading of a gene G within a species [Libertini, 2006].

A gene C that causes the premature death of an individual I , where C is present, and so reduces its ML and causes a disadvantage S' , accelerates the spreading of any favourable gene in the individual (I') that takes the place of I . If I' is kin to I , the inclusive fitness (F_C) will be positive and C will be favoured by selection, if:

$$F_C = r \cdot \sum_{x=1}^n (S_x) \cdot (1/ML_C - 1/ML_{C'}) - S' > 0 \quad (2)$$

where: ML_C and $ML_{C'}$ are the ML of individuals with the gene C and the neutral allele C' , respectively; $\sum(S_x)$ is the summation notation of the advantages of the n favourable genes spreading within the species; $-S'$ is the disadvantage of a smaller ML ; r is the mean coefficient of relationship between I and I' . (The use of kin selection to explain the age-related fitness decline should not be confused with the use of the same type of selection to explain the survival in the post-reproductive period, as suggested in other papers [Lee, 2008].)

This hypothesis was formulated for multicellular organisms, but there is no theoretical argument against its application to monocellular eukaryotes. Büttner et al. do not express alternative evolutionary explanations for cell senescence and replicative senescence besides the above-mentioned suggestion [Büttner et al., 2006], which is a short reformulation of the theory described.

In contrast with this hypothesis, Lewis argues against the “suggestion that yeast cells provide a precedent for programmed death” [Lewis, 2000], proposed by others AA. [Sinclair et al., 1998], with the following observation: if a yeast cell of the mother lineage dies after n duplication ($n = 25-35$ in laboratory conditions [Jazwinski, 1993]), the death of a single individual among 2^n descendants ($= 10^7-10^{10}$ individuals) appears insignificant for any theory of programmed death that is somehow favoured by natural selection. In fact, in natural conditions the probability that an individual of the mother lineage dies by apoptosis after n duplications is practically zero and the phenomenon, being observable in laboratory conditions only, cannot have selective value. However, this argument misses a pivotal point: it is important not the death after n duplications of a single individual among innumerable descendants, but the exponentially progressive - in relation to the number of duplications - increasing probability of apoptosis, coupled with a difference in mortality rates and capability of having offspring between “younger” and “older” individuals (“in a population of [yeast] cells the lifespan distribution follows the Gompertz law” [Laun et al., 2007], that is an age-related

exponential 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]) and, therefore, a faster generation turnover caused by the preferential death of “older” individuals. If cell senescence and replicative senescence manifest themselves in natural conditions and reduce significantly wild yeast *ML*, Lewis’ objection does not invalidate the hypothesis that yeast fitness decline related to duplication number may have a selective value and may be favoured by natural selection. However, Lewis’ objection is very interesting because it echoes a similar argument against programmed aging theories for multicellular organisms that will be discussed in the next section.

D) IN MULTICELLULAR EUKARYOTES

D-1) Apoptosis

In multicellular organisms, apoptosis is essential for many physiological functions as outlined above. The evolutionary justification for these phenomena is evident and will not be discussed.

D-2) Cell senescence and replicative senescence

As underlined in the preliminary remark, an age-related increasing mortality, or fitness decline, is documented for many species in wild conditions (fig. 8).

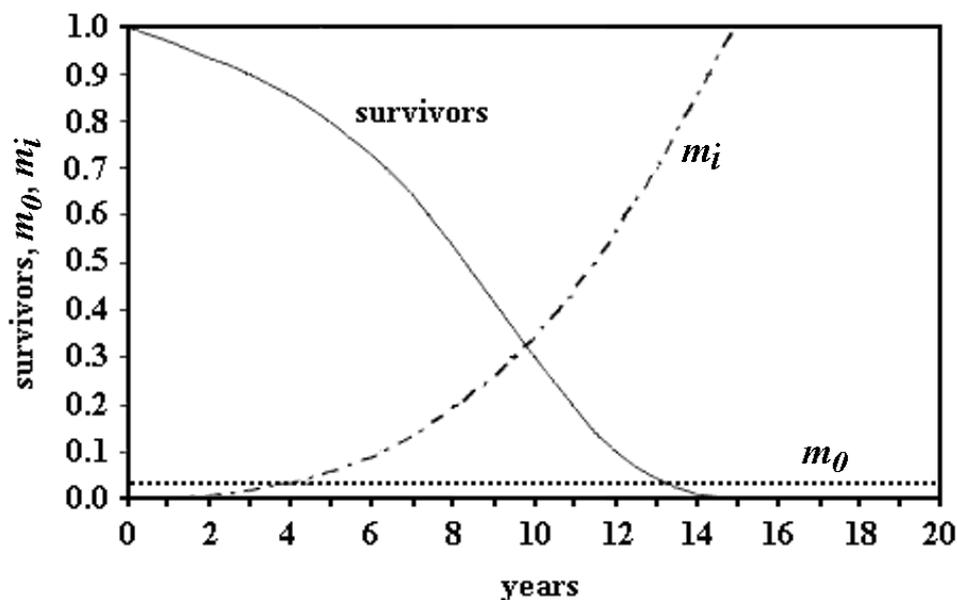


Figure 8 - An example of species with age-related fitness decline in the wild. Life table of *Panthera leo* after the early stages of life: survivors, basal mortality (m_0) and age-related increasing mortality (m_i). Weibull’s equation ($m_t = m_0 + \alpha \cdot t^\beta$) and data ($m_0 = 0.032$, $\alpha = 2.52E-4$, $\beta = 3$), utilised to define the curves, are from Ricklefs [Ricklefs, 1998].

A plausible mechanism for this fitness decline is the progressive slowdown of cell turnover, that is a progressive prevalence of programmed cell death (PCD), by apoptosis or other forms of PCD, on cell substitution by duplication of stem cells (Fossel’s “cell senescence general model of aging” [Fossel, 2004; Libertini, 2006]). A hypothesis of this type was suggested for the first time by Weissmann [Kirkwood and Cremer, 1982] while the concept of senescence as a result of decrease in cellularity of organs was discussed by Szilard [Szilard, 1959], although in the context of a theory that attributed

the cell loss to the accumulation of somatic mutations. In support of this thesis, for some species, as Rockfish and lobsters, both telomere length and mortality rate are unvaried with the age [Klapper, Heidorn et al., 1998; Klapper, Kühne et al., 1998].

There is empirical evidence for an adaptive meaning of the age-related fitness decline phenomenon [Libertini, 2008], which in its more advanced expression, common in protected conditions, is usually called ‘aging’, an imprecise term [Libertini, 2006]. A theory, the same as the above-mentioned to elucidate cell senescence and replicative senescence in yeast, explains this fitness decline as evolutionarily advantageous by a mechanism of kin selection that, in consequence of a quicker generation turnover, allows a faster spreading of any advantageous mutations. According to this theory, the advantage exists in conditions of K-selection (species divided in demes, populated by kin individuals, and with saturated habitats in which only the death of an individual gives space to a new individual) [Libertini, 1988, 2006].

The main objection against this theory, analogous to Lewis’ argument above-mentioned, 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.” [Kirkwood and Austad, 2000]. This objection, analogous to Lewis’ argument, misses a pivotal point: the existence or absence in the wild of “old” individuals (e.g., individuals of *P. leo* older than 15 years) is not important. Individuals of *P. leo* younger than 15 years are “not old” individuals, yet they show an increasing fitness reduction at ages present in the wild: this significantly reduces *ML* with a consequent faster generation turnover and a possible selective advantage.

“Senescence reduces average life span ... by almost 80% when $m_0 = 0.01 \text{ yr}^{-1}$ ” [Ricklefs, 1998]. For the fraction of a population that survived the high mortality risk of the early stages of life, the ratio between the residual *MLs* without and with age-related increasing mortality has been estimated to be in the range 2.5-5 for eight mammal species in wild conditions. Without the subtraction of the early stages of life, the ratio has been estimated in the range 1.55-3.21 [Libertini, 1988].

In short, in wild conditions, *ML* reduction caused by age-related increasing mortality is not irrelevant, although the equivalents of septuagenarian or older men for animal species are likely inexistent in the wild.

PHYLOGENETIC CORRELATIONS

The empirical evidence and the above-mentioned arguments suggest a phylogenetic correlation between phenomena observed in colonies of kin yeast cells and analogous phenomena in multicellular organisms. These phenomena require the formulation of a general phylogenetic hypothesis of apoptosis, telomere-telomerase system, cell senescence, replicative senescence and the age-related fitness decline, which is commonly but imprecisely called “aging”. Correlated phenomena in bacteria must be also considered in the phylogenetic model.

In particular (see Table 1 and fig. 9):

a) Phenomena described in eubacteria as “proapoptosis”, activated in particular conditions and probably favoured by the mechanism of kin selection (e.g., for bacterial phytoplankton: “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]), have been interpreted as plausible phylogenetic precursors of eukaryotic apoptosis [Hochman, 1997]. Proapoptosis, a form of “suicide useful in critical conditions”, is necessarily derived from a previous condition in which this pattern was inexistent.

b) Eubacteria evolved in monocellular eukaryotes. Apoptosis of monocellular eukaryotes, likely derived from a form of eubacterial proapoptosis, is in yeast triggered

by starvation, damaged cell conditions, unsuccessful mating, etc. In these cases, it is favoured by kin selection because cell suicide increases survival probability of kin cells [Herker et al., 2004] (“suicide useful in critical conditions”).

c) Both for proapoptosis and for apoptosis, a mechanism that triggers the suicide pattern, but kills only a part of the population - proportional to the severity of stress condition - is indispensable. Yeast evolved an efficient mechanism based on the number of previous duplications and a telomere-telomerase-ERCs clock [Büttner et al., 2006; Fabrizio and Longo, 2008; Herker et al., 2004; Laun et al., 2007].

d) Apoptosis of multicellular eukaryotic species has a clear phylogenetic relationship with monocellular eukaryotic apoptosis [Longo and Finch, 2003]. In most species, the evolved clock does not use ERCs [Fossil, 2004]. Considering each multicellular individual as a clone having all cells with the same genes (coefficient of relationship, r , equal to 1) but with differentiated functions, apoptosis of less fit cells may be considered as favoured by analogous mechanisms of kin selection.

e) In multicellular organisms, apoptosis as part of morphogenetic mechanisms (e.g., embryogenesis, tissue development or reshaping, tissue turnover) and of lymphocyte selection is clearly a derived function, being impossible in monocellular organisms;

f) In yeast, apoptosis, cell senescence and replicative senescence, genetically determined by mechanisms based on telomere-telomerase system, appear to contrast “genetic conservatism” [Büttner et al., 2006]. Furthermore, these phenomena might be explained as favoured by kin selection, as for multicellular organisms [Libertini, 1988, 2006]. Suicide-predisposition passes from a pattern useful only in emergency conditions to a pattern useful in non-stress conditions too (“suicide useful in non-critical conditions”).

g) In multicellular organisms, apoptosis, cell senescence and replicative senescence, cause age-related limits in cell turnover with consequent age-related fitness decline [Fossil, 2004; Libertini, 2006] (“senile state” in its more advanced expressions [Libertini, 2006]), and this has been explained by kin selection in conditions of K-selection [Libertini, 1988, 2006].

In short, “aging” mechanisms in yeast, a monocellular eukaryote, and in multicellular eukaryotic species, separated by about 600 millions of distinct evolution, are incredibly similar in their basic physiological components and selective explanations. Moreover, apoptosis, the core of these mechanisms, has its phylogenetic roots in eubacterial proapoptotic phenomena.

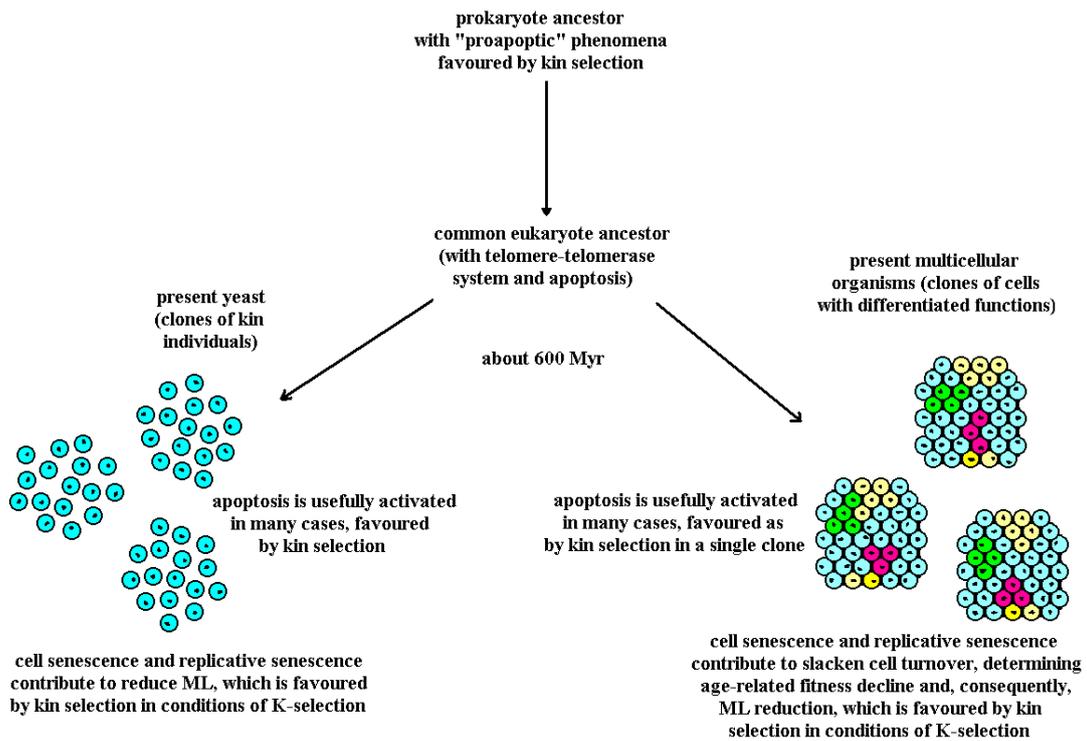


Figure 9 – Proapoptosis, apoptosis, cell senescence and replicative senescence in a phylogenetic scheme.

Table 1 - Comparison of three functions in bacteria, yeast (monocellular eukaryote) and multicellular eukaryotes

Phenomenon	Description	Function in bacteria	Function in yeast (and other monocellular eukaryotes)	Function in multicellular eukaryotes
Proapoptosis	Various type of bacterial self-destruction mechanisms	Activated by various conditions (Note 1)	-	-
Apoptosis	Ordinate process of self-destruction with modalities allowing the use of cell components by other cells	-	Activated when nutrients are scarce, mating is not successful and in old individuals (Note 1)	Eliminates damaged cells (Note 2) Essential for morphogenesis and similar phenomena (Note 2) Essential to determine cell turnover whose progressive impairment contributes to age-related fitness decline (Note 1)
Cell senescence and replicative senescence	In relation to the number of replications, in a cell culture, progressive impairment of cell functions, increasing probability of apoptosis and of losing duplication capacity, determined by the repression of subtelomeric DNA	-	Reduce <i>ML</i> , causing a faster generation turnover (Note 1)	Contribute to progressively slacken cell turnover, determining age-related fitness decline (defined "senile state" in its more advanced expressions) and, therefore, <i>ML</i> reduction and faster generation turnover (Note 1)
<p>Note 1 = altruistic behaviour(s) favoured by kin selection in conditions of K-selection Note 2 = altruistic behaviour considering the multicellular individual as a clone</p>				

CONCLUSION

Aging in yeast is considered adaptive while, for multicellular eukaryotes, this idea is excluded by the current gerontological paradigm [Kirkwood and Austad, 2000], which is contrasted both by theoretical arguments and empirical evidence [Goldsmith, 2003; Libertini, 1988, 2006, 2008; Longo et al., 2005; Mitteldorf, 2006; Skulachev, 1997]. Figure 10 shows that even authoritative Authors, not restrained by current paradigm, do not state openly that apoptosis is part of aging mechanisms in our species, while for other species this is maintained [Longo and Finch, 2003; Longo et al., 2005].

Apoptosis and the telomere-telomerase system are sophisticated mechanisms, necessarily determined and highly regulated by genes forged by natural selection. They are ubiquitous in the eukaryotic world and the many variations among the different phyla do not obscure their single origin [Longo et al., 2005].

This strongly suggests that they have significant evolutionary meanings that are related to cell senescence (Fossel's "cell senescence limited model" [Fossel, 2004]) and, likely, to the age-related fitness decline of the whole organism (Fossel's "cell senescence general model of aging" [Fossel, 2004; Libertini, 2006]). In contrast with this evidence, current gerontological theories state that age-related fitness decline, a phenomenon certainly observable at ages existent in the wild [Libertini, 2008], is determined by random factors (harmful mutations, unpredictable effects of pleiotropic genes or of conflicting evolutionary exigencies [Edney and Gill, 1968; Hamilton, 1966; Kirkwood, 1977; Kirkwood and Holliday, 1979; Medawar, 1952; Mueller, 1987; Partridge and Barton, 1993; Rose, 1991; Williams, 1957]). This excludes the aforementioned mechanisms, which are sophisticated and highly regulated, as causes of the phenomenon.

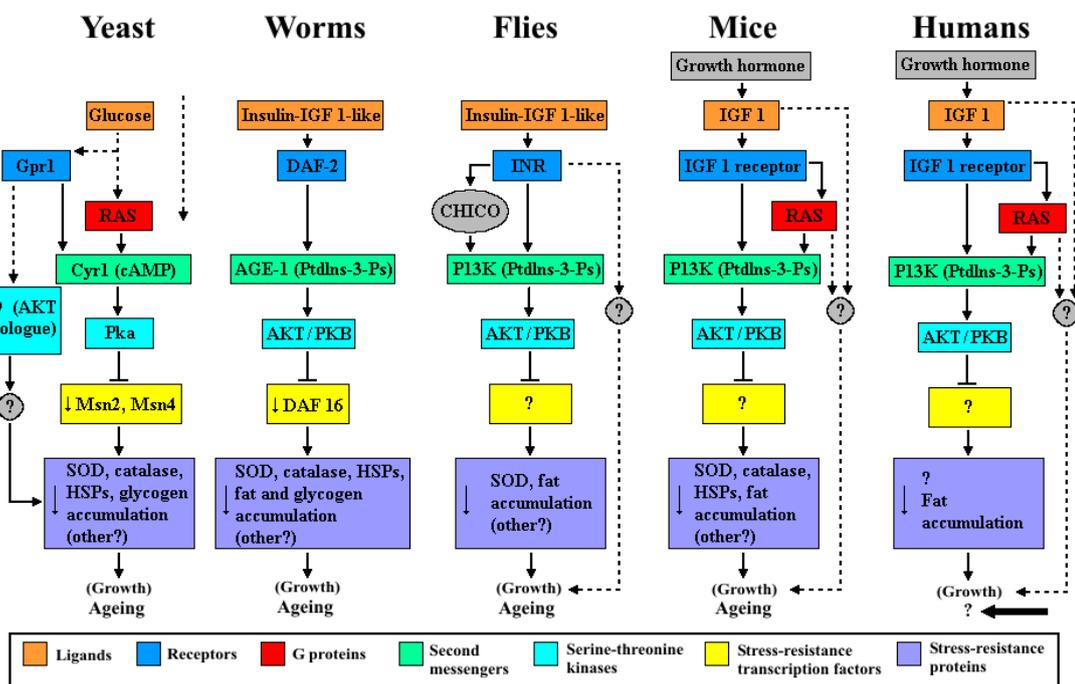


Figure 10 – Scheme of trigger mechanisms for apoptosis in various eukaryotic phyla. Part of a figure (redrawn) from Longo et al. [Longo et al., 2005], obtained with modifications from Longo and Finch [Longo and Finch, 2003]. Apoptosis is considered part of the aging mechanism, but only for our species this is considered doubtful (see the horizontal arrow, added to the original scheme) without a rational explanation.

It is important to underline that the life-limiting effects of telomere-telomerase system are currently explained as a general defence against cancer [Campisi, 1997, 2003; Troen, 2003; Wright and Shay, 2005] but there are strong arguments and evidence against this hypothesis [Fossel, 2004; Libertini, 2008; Milewski, 2010] (e.g., senescent cells secrete substances that increase mutation rates and the risk of oncogenesis [Parrinello et al., 2005; Coppé et al., 2008]). The steady affection to defence-against-cancer hypothesis by the supporters of non-adaptive aging theories may be explained by the fact that there is no other proposed explanation compatible with non-adaptive hypotheses and using philosophical and historical knowledge [Milewski 2010].

Current gerontological theories contrast strongly with the functions of apoptosis, the telomere-telomerase system, cell senescence and replicative senescence, in their phylogenetic schematisation outlined in this paper, which is based on the concept that all these phenomena are certainly adaptive. This contrast should be solved by current gerontological theories or, on the other hand, these theories should be dropped and

substituted by the alternative paradigm that the age-related fitness decline is a function with an evolutionary advantage and its physiological mechanisms. Moreover, the thesis maintained in this paper, namely that genetically regulated active mechanisms, based on telomere-telomerase system and determining the death of an organism, have a very ancient phylogenetic history should not be considered a surprise if we consider the numberless well-known cases of phenoptosis through rapid senescence and sudden death widely described elsewhere [Finch, 1990].

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A page (http://www.programmed-aging.org/theory-2/aging_theory_chronology.html) from the site www.programmed-aging.org.

The site is organized by Theodore Goldsmith, Giacinto Libertini & others and is sponsored by Azinet LLC © 2010.



Aging Theories – Historical Chronology of Theories and Discoveries

Traditional evolutionary mechanics theories and dependent non-adaptive aging theories	Alternative evolutionary mechanics theories and dependent adaptive aging theories	Experimental evidence applicable to aging theory
Before Darwin - Some believe organisms gradually wear out and deteriorate in the same manner as non-living things	Before Darwin - No evidence that origin of life span different from any other organism characteristic that differs between species, i.e. organisms appear to be designed to have a species-specific life span	
1859 Darwin's evolutionary mechanics theory proposes that the force of evolutionary selection is toward the longest possible life span and maximal reproductive capacity, i.e. immortality		
1859+ - Darwin's critics note the contradiction between the Darwinian concept of natural selection favoring individuals with maximal survival and reproductive capacity and the observed progressive decline of vital and reproductive capacities, i.e. a limited life span that differed greatly between similar species (see Goldsmith 2004)	1859+ - Darwin suggests that a limited life span must convey some unknown benefit that offset its otherwise adverse effect. He did not specify the nature of the benefit. (see Goldsmith 2004)	
	1889 - Weismann hints that the death of old individuals was beneficial because this gave more space to new generations and that this was useful for the evolution of species (Weismann 1889 ; see Kirkwood & Cremer 1982)	1889 - Weismann observes that the cells of the various organs and tissues are renewed continuously and that when this turnover slackens or stops, the organs or the tissues reduce or lose their functionality with negative effects on fitness (see Kirkwood & Cremer 1982)
	1892 - Weismann disavows his 1889 idea (Weismann 1892 ; see Kirkwood & Cremer 1982)	

- Wear and tear hypotheses and stochastic hypothesis about aging (reviews of these hypotheses in: Kowald & Kirkwood 1996 ; Kirkwood & Kowald 1997 ; Kirkwood 1998, 2002 ; Fosel 2004)		1913 - Carrel "demonstrates" that cells explanted and cultivated <i>in vitro</i> multiplied an unlimited number of times and so Weissmann's hypothesis is unacceptable (Carrel 1913)
1945+ - Modern synthesis and neo-Darwinism codify traditional mechanics theory		
1952+ - Mutation accumulation theory (Medawar 1952 ; Hamilton 1966 ; Edney & Gill 1968 ; Mueller 1987 ; Partridge & Barton 1993)		
1957+ - Antagonistic pleiotropy theory (Williams 1957 ; Rose 1991)		
		1961+ - Discovery of cell limits in duplication ("Hayflick limit") (Hayflick & Moorhead 1961 ; Hayflick 1965 ; Schneider & Mitsui 1976)
	1962+ - Group selection theory (Wynne-Edwards 1962, 1986)	
	1964+ - Kin selection theory (Hamilton 1964 ; Hamilton 1970 ; Trivers 1971 ; Trivers & Hare 1976 ; Wilson 1975)	
		1972 - "Apoptosis" phenomenon is described (Kerr et al. 1972)
		1972 - DNA polymerase cannot replicate a whole molecule of DNA and a little part of an end of the molecule is unreplicated at each duplication (Watson 1972)
		1973 - Olovnikov hypothesizes that the shortening of DNA molecule at each duplication after a certain number of times blocks cell replication capacity, i.e. determines Hayflick limit (Olovnikov 1973)
1977+ - Disposable soma theory (Kirkwood 1977 ; Kirkwood & Holliday 1979)		1977 - Discovery of complex octopus suicide mechanism (Wodinsky 1977)
	1979+ - Many examples of animals that do not show age-related increasing mortality are documented (Comfort 1979 ; Finch 1990)	

		1980+ - Programmed cell death by apoptosis, selectively triggered for some cells in specific times, causes cell turnover in healthy adult organs (Wyllie et al. 1980 ; Lynch et al. 1986 ; Israels & Israels 1999 ; Medh & Thompson 2000) (as documented for many tissues and organs: see Libertini 2006)
	1983+ - Kin selection based aging theory (Libertini 1983, 1988, 2006, 2009a)	1983+ - The final incapability of a cell to duplicate (replicative senescence) is shown to be not an abrupt event but a progressive reduction of cell duplication potential which depends on the reduction of telomere length (Pontèn et al. 1983 ; Jones et al. 1985)
		1985 - Discovery of telomerase, an enzyme capable of adding new segments of telomeric repetitive sequence (Greider & Blackburn 1985)
		1990 - Telomere shortening at each replication is demonstrated (Harley et al. 1990)
		1990 - Various alternatives to aging in its common definition (i.e., age-related increasing mortality) are documented (Finch 1990)
		1991 - Telomere is shown to be a highly conserved repetitive sequence (Blackburn 1991)
		1991 - A review of studies on decreasing survival in laboratory conditions at ages not existing in the wild and for species that in the wild do not show age-related fitness decline (Rose 1991)
		- 1993+ - Various studies prove that "aging" in <i>C. elegans</i> is influenced strongly by many genes (Kenyon et al. 1993 ; Dorman et al. 1995 ; see other studies at: http://www.programmed-aging.org/theory-3/kenyon.html)
	1996+ - Evolvability theories (Wagner & Altenberg 1996)	
1997+ - To justify evolutionarily their existence, replicative senescence, cell senescence and,	1997+ - Evolvability-based aging theories (Skulachev 1997 ; Goldsmith 2003, 2004, 2006 ,	1997+ - "Proapoptosis" (Hochman 1997), a form of eubacterial cell suicide, is shown to be clearly

<p>in general, the limits imposed by telomere-telomerase system are hypothesized as a general defense against cancer (Campisi 1997, 2000; Wright & Shay 2005).</p>	<p>2008)</p>	<p>related to eukaryotic apoptosis (Koonin & Aravind 2002) and this indicates a very old evolutionary persistence and similarity</p>
		<p>1997+ - Description of an apoptosis-like phenomenon in yeast (Madeo et al. 1997), elicited by factors present in multicellular organisms (Longo et al. 1997; Ligr et al. 1998)</p>
<p>1998 - A well-publicized study claims to discern a cost of reproduction in a historic database of British nobility, thus supporting Disposable Soma theory (Westendorp & Kirkwood 1998)</p>		<p>1998 - Old Lobsters and rainbow trouts, “animals with negligible senescence”, are shown to have, in the wild, the same levels of telomerase activity as young individuals (Klapper, Heidorn et al. 1998; Klapper, Kuhne et al. 1998)</p>
		<p>1998 - Telomerase introduction in somatic cells makes them able to innumerable duplications and recerts manifestations of cell senescence (Bodnar et al. 1998, Counter et al. 1998, Vaziri & Benchimol 1998, de Lange & Jacks 1999)</p>
	<p>1998+ - Ricklefs documents an inverse relation between extrinsic and intrinsic mortality in natural conditions (Ricklefs 1998, 2008)</p>	<p>1998+ - Inactivated telomerase and short telomeres increase the probability of apoptosis (Ozen et al. 1998; Seimiya et al. 1999; Holt et al. 1999; Ren et al. 2001; Fossel 2004)</p>
	<p>1999 - The death of an individual, when not determined by accidents or diseases and as event provoked by particular mechanisms genetically regulated, namely programmed, and therefore somehow favoured by natural selection, is defined for the first time by a specific neologism, "phenoptosis" (Skulachev 1999)</p>	<p>1999+ - For yeast apoptosis-like phenomenon a common phylogenetic origin with apoptosis in multicellular eukaryotes is indicated (Madeo et al. 1999; Longo et al. 2005; Kaeberlein et al. 2007)</p>
		<p>1999+ - Apoptotic patterns in yeast are interpreted as adaptive because they are useful to the survival of the clone, which is likely made up of kin individuals (Skulachev 1999, 2002, 2003; Fabrizio et al. 2004; Longo et al. 2005; Skulachev & Longo 2005; Mitteldorf 2006b)</p>
<p>2000 - Kirkwood & Austad quote Ricklefs' 1998 data as supporting</p>		<p>2000 - A too short telomere length does not allow further replications</p>

non-adaptive aging theories without realizing that the contrary is true, as stated by the same Ricklefs (Kirkwood & Austad 2000)		(Shay & Wright 2000)
2000 - Shanley and Kirkwood formulate a Disposable Soma model in mice that try to explain the effects of calorie restriction on aging (Shanley & Kirkwood 2000)		2000- Blackburn's model explains the graduality of cell senescence in a culture (Blackburn 2000)
		2000+ - When telomeres are shortened, there is a great vulnerability to cancer as a consequence of dysfunctional telomere-induced instability (DePinho 2000 ; Artandi 2002)
	2001 -Mitteldorf shows that the Shanley & Kirkwood 2000 model is hardly tenable (Mitteldorf 2001)	
2002 - Historical hypothesis (De Magalhães & Toussaint 2002)		
2004 - Vaupel et al. try to explain negative senescence with non-adaptive theories (Vaupel et al. 2004)		2004 - Telomere shortening is related to the progressive decay of cell functions (cell senescence) and to the progressive reduction of cell duplication capacities (replicative senescence) (Fossel's "cell senescence limited model") (Fossel 2004)
	2004+ - The hypothesis that replicative senescence, cell senescence and telomere-telomerase system are a general defence against cancer is challenged (Fossel 2004 ; Libertini 2006 , 2009a , 2009b)	2004+ - Cell senescence, replicative senescence and aging are related (Fossel's "cell senescence general model" of aging) (Fossel 2004 ; Libertini 2006 , 2009a , 2009b)
		2005 - Cell senescence is defined as a "fundamental cellular program" (Ben-Porath & Weinberg 2005) as the changes that define it are stereotyped and predictable. Replicative senescence is part of cell senescence phenomenon. The degree of senescence of a culture is determined by the fraction of cells in cell senescence state.
	2006 - Libertini underlines the contradiction between Ricklefs' data and traditional aging hypotheses (Libertini 2006)	
	2006 - Group selection based aging	

	theory (Mitteldorf 2006a)	
	2007 - In a meta-analysis of 115 studies, the antioxidant supplements were significantly associated with increased mortality, in strong contradiction with ROS theories of aging (Bjelakovic et al. 2007)	
	2008 - The empirical evidence in support of adaptive theories of aging and against non-adaptive hypotheses is highlighted (Libertini 2008)	
	2009 - Westendorp & Kirkwood 1998 study is shown to have used an inappropriate statistical test while standard linear correlation on the same database reveals a positive correlation, thus falsifying Disposable Soma theory (Mitteldorf 2009)	
	2009 - Evolutionary classification of diseases and of disease-like phenomena (as aging). Definition of a research program to master aging with the modification of its physiological mechanisms (Libertini 2009b)	
	2010 - Strong empirical and theoretical arguments in support of ageing adaptive theories: "Several lines of evidence suggest that [telomere-cell senescence system] was selected first and foremost to cause ageing" (Milewski 2010)	
	2010 - Age-related fitness decline is defined "slow phenoptosis" (Skulachev 2010)	
2010 - Most gerontologists continue to believe non-adaptive aging theories	2010 - Extensive and increasing interest in alternative mechanics but no consensus. Steadily increasing observational evidence for adaptive aging theories	2010 - Telomerase reactivation in aged mice with artificially blocked telomerase shows a marked reversal of degenerative manifestations, even for nervous system (Jaskelioff et al. 2010)
		2011 - The first (prudent) attempt of a mild stimulation of telomerase activity to preserve health conditions (Harley et al. 2011)

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SECTION II

EVOLUTION AND DISEASE PHENOMENON

Chapter V (Evolutionism and Pathology) and Interlude (between chapters II and III) in: G. Libertini, **Evolutionary Arguments on Aging, Disease and Other Topics**, 2011, Azinet LLC (ISBN-13 978-0-9788709-2-8), English reprint of G. Libertini, **Ragionamenti Evoluzionistici**, 1983, Società Editrice Napoletana, Napoli

[For the full text both of the Italian and in the English version, and for the simulation programs, see my personal site www.r-site.org/ageing]

Chapter V - Evolutionism and Pathology

1) Topic

On the basis of the empirical data, as a first attempt at description, it is possible to define the concept of disease as a state of deviation - in a pejorative sense - from the norm of one or more functions of a living organism. Clearly, the norm will be established in reference to the totality of the individuals of the population. With the opportune modifications and specifications - and discussions as regards the borderline with the "state of health", - this definition could be pacifically accepted by the clinician, the physiologist, the pathologist, etc.. In this chapter, I investigate a definition of the concept of disease that is not uncritically descriptive but that is, likewise, set within the phenomenon of evolution, which is also, it should be noted, firmly based on empirical data. I will, therefore, compare the definition expressed here, which I would say is peculiar to the non-evolutionary empiricist, with that which might be expressed by an empiricist who takes evolution into due account.

* * *

This work is based, among other things, on the concept - already stressed by others - that evolution is the most general theory of all biology: each biological phenomenon, which is not strictly contingent, is ultimately an aspect, an expression of the evolutionary process. With such an approach, the question arises of whether the pathology can be formulated in its outline in evolutionary terms. In other words, if the disease is an anomaly, an exception or rather a phenomenon that is an integral part of the evolutionary process. In this chapter, I look for possible answers to this question, maintaining among other things that:

a) From an evolutionary point of view, diseases are not something that breaks out of the mould but are, rather, a whole series of categories of phenomena which are evolutionarily "predictable" in their general essence. "To predict", I stress once again, means to make certain deductions starting from the theory of evolution, with the suggestion, confirmation and confirmation in natural and experimental data: it is common practice, in scientific methodology, to obtain accepted interpretations and classifications of the actual phenomena deductively from a theory, looking then at empirical data for possible confirmation of the validity of the interpretations and classifications.

b) The evolutionary approach to the concept of disease is the most rational and general one possible. Any other more limited approach, even one which is more useful as regards a single pathological problem, simply because it is more limited and selectively oriented, should not be conceived in terms that run contrary to the theory of evolution. There must be no substantial contradiction, either basic or practical, between an evolutionary and non-evolutionary approach to the concept of disease because both points of view are correct and the object of study is one. Contradictions may originate where one wishes to make illicit generalizations and interpretations from contingent empirical data or from reasoning in evolutionary terms.

- c) To reason about pathological topics in evolutionary terms does not necessarily mean expressing things never spoken of before in the non-evolutionary approach, but largely to repeat things that have already been said, which are known and are empirically and inductively proven and accepted, with a view to unifying them.
- d) The evolutionary approach to the concept of disease spontaneously raises suggestions and basic questions about the prevention and cure of the various categories of diseases.

2) The starting point

To formulate the immense complexity of the real world in terms which are necessarily simplified, inevitably entails a certain amount of loss and a flattening of the information that one wishes to get across, if we fail to consider that the simplicity and shortness of a word does not imply the simplicity of the concept expressed.

The term 'species' is a very good example of how an incredibly complex reality may be summarized in such a brutal manner that it may deceptively seem to be something much simpler than it really is. The subject requires us to consider the concept of species to an extent that is more detailed and complex, but certainly closer to reality.

I now wish to define better the concept of a hypothetical species A.

Let us imagine a whole series, unlimited numerically and temporally, of generations of mutually fertile individuals (read: I am not suggesting that this happens to an unlimited extent), which live in a whole series of ecological niches, varying (read: may not vary) from generation to generation, from individual to individual, from time to time; these ecological niches, of which the constitutive elements are both the *modus vivendi* of the individual and the physical environment in absolute in which they live, and the whole series of individuals of the species B, C, ..., Z, with which the species A is in relation (read: no limitation of relation types has been assumed). Let us consider each individual in terms of genome (read: it is not necessary for the definition to specify the physical substrate of genetic information) received by other individuals of the preceding generation and that can be handed down to other individuals of the next generation to an entirely or *nearly* accurate extent, a genome that, in the interaction with the *n* ecological niches that follow, one upon the other, for the individual *x*, expresses a certain phenotype (read: which therefore changes gradually over time) which is more or less suitable for survival in the various subsequent ecological niches.

This, or any similar formulation is certainly not practical to be repeated whenever the concept of species is used. However, this definition is indispensable for the following argument and is by no means negligible in its details and implications. From this formulation indeed, as spontaneous, natural and empirically confirmable facts, certain categories of events will arise, each with its own distinct definition, but which can be covered by a single, overall definition under the term "disease".

Each of the paragraphs following immediately hereafter will discuss one of these categories of events.

3) Diseases deriving from alterations of the genotype

The transmission of the genetic information from the individuals, or from the individual, of the parental generation to the offspring is not accurate to a total extent. This has been proven experimentally and, moreover, the transmission inaccuracy - read: mutations, chromosome alterations, etc. - is the precondition for the evolutionary mechanism by natural selection, because on the basis of individual variety, by definition the fitter "mutants" prevail. On the other hand, as the genome of any living being is a highly ordered structure and as - see Appendix 5 - in an ordered system the entropy always tends to increase by the action of random forces, it follows from this that the greater

part of the “transmission inaccuracies”, that are not mute, will not improve but will alter in various ways the equilibrium of the system - read: living being – that depends on the genome.

A threshold having been arbitrarily set, I will define as sick, with the origin of the sickness in alterations of the genotype, any mutant of a species that is less fit than the norm - statistically and arbitrarily defined - to the persistence in the ecological niche to which a species is adapted.

It is important to emphasize that if many mutations are presumably harmful in any ecological niche, a part of the mutations are likewise disadvantageous only in connection with some, and not all, ecological niches and that the reference to an ecological niche, i.e. the ecological niche to which a species is adapted, is, therefore, indispensable, (or, as a questionable alternative, an arbitrary ecological niche, making the concept of disease deriving from alterations in the genotype even more arbitrary). Some considerations:

a) The factors - read: mutagenic agents in a broad sense - that increase the degree of inaccuracy in the transmission of the genetic information, likewise increase the incidence of diseases deriving from genotype alterations, provided that the arbitrary parameters of reference assumed are not changed.

b) If we take the expression “complexity of a function” to mean the extent of the genetic information that is necessary to define the function, and assume that, as proposed on an experimental basis, the mutation frequency of the various genes is roughly equal, it follows from this that, if we consider a particular function, the existence of diseases deriving from alterations of the genotype concerning the function is predictable, and the more complex the function, the greater the number of mutations altering it.

c) The frequency of an x alteration of the genotype is limited by the selective pressure deriving, to a proportional extent, from the degree of reduced fitness that the alteration entails. On the one hand, there will be deadly alterations or those that cause sterility, the frequency of which will only be that of the specific mutations that arise at each generation. On the other hand, it is necessary to consider those alterations that cause minimal damage and whose frequencies will, therefore, be greater than those of the frequencies of the specific mutations, as the subjects with such alterations are eliminated by the selective process over several generations (see Fig. V 3-1). It should now be noted that the more advanced the age - relative to the longevity of the species – in which an alteration causes damage, the lesser the selective pressure expressed against it. Thus, the frequency expected for this type of disease is not low (see Fig. II 6-1). Such diseases are defined as “senile diseases”, with the specification that this category of events must be kept well distinct from senescence. As examples of human senile diseases, I might mention: senile cataract and glaucoma, Parkinson's disease, atherosclerosis. An essential characteristic of senile diseases is that they may affect a large percentage, but never the totality of the ageing individuals of a species. (It should be noted that this it is not true for hypersenescent individuals because, as these are not found, or almost not found, in natural conditions, it follows by definition that there is no selection). Finally, note that, and this will be emphasized later, the concept of senile disease is not limited only to the category of diseases deriving from alterations of the genotype. See paragraph 7 for a further discussion of the concept.

d) The weakening of the selective mechanisms towards an x alteration of the genotype, for example, in man, as a consequence of the increasing effectiveness of medical therapy, causes an increase over generations in the frequency of alteration x . The topic will be discussed again in paragraph 10.

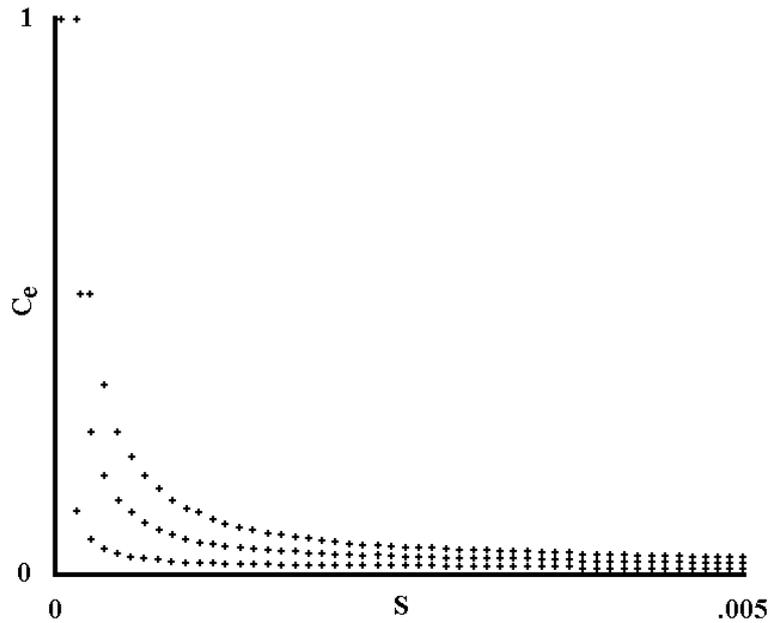


Fig. V 3-1 - Equilibrium frequencies of a gene with damage S, arising with frequency V (Theoretical model).

C and C' are alleles. C causes the damage S, while C' is inactive. C' changes into C with frequency V at each generation and the contrary happens with negligible frequency.

With the assumptions formulated, we have:

$$\begin{aligned}
 C_{n+1} &= \frac{C_n(1 - S) + C'_n V}{C_n(1 - S) + C'_n V + C'_n - C'_n V} , \\
 &= \frac{C_n(1 - S - V) + V}{1 - C_n S} \qquad \qquad \qquad (V-1)
 \end{aligned}$$

At equilibrium, as $C_{n+1} = C_n = C_e$, dividing by C_e and with the mathematical transformations already expressed for Fig. II 6-1, we obtain the two solutions:

$$C_e = 1 ; \quad C_e = \frac{V}{S} \qquad \qquad \qquad (V-2)$$

The first solution is applied if $V > S$, because $C_e \leq 1$.

The figure shows three curves with values of V, going from top to bottom respectively:

.0001 ; .00005 ; .00001.

The value of C_e is on the ordinates.

The damage S is expressed on the abscissas (0 on the abscissa 0; .005 on the extreme right of the abscissas; the difference for each interval is equal to $.005/50 = .0001$).

4) Diseases deriving from alterations of the ecological niche

As a consequence of the selective pressures, the totality of the individuals of a species is adapted, in so far as this has been possible, to the ecological niche - read: totality of

ecological niches - according to which the species lives. It is clear that a further adaptation, when there is a change in the ecological niche, cannot happen immediately, as the effects of the selection manifest themselves over several generations. It is likewise obvious that a new ecological niche may be better, neutral or worse in reference to the aptitude of a species and towards the preceding ecological niche. However, considering that the totality of the individual-ecological niche relations is a highly ordered structure, reminding again of that which was expounded in Appendix 5, an easy prediction is that a random modification of the ecological niche, given that it reduces the order of the system, alters, for the most part, the equilibrium between species and ecological niche - read: adaptation -, i.e. it entails a lesser aptitude for persistence in the individuals of the species. Lesser fitness means, by definition, damage or the possibility of damage for the individuals of the species.

A threshold having been arbitrarily set, I will define as sick, with the origin of the sickness in alterations of the ecological niche, any individual with one or more functions altered as a consequence of a modification in the ecological niche.

It must be stressed that the individuals of a species are not identical to each other - for various reasons: a) due to the existence of mutants; b) because the selective pressures in the ecological niches according to which the species lives, are numerous and various; etc. - and that a modification in the ecological niche is, therefore, not necessarily an alteration for all individuals of the species. Note also that:

a) For the aforementioned definition, reference to the whole individual-ecological niche was necessary and the origin of the pathological event was attributed to the ecological niche.

b) An ideal ecological niche does not exist: I have spoken about modifications in the ecological niche towards a preceding ecological niche, for which a species is, in so far as this has been possible, adapted.

c) A minor modification in the ecological niche entails minor aptitude variations and not, therefore, the disease, because it falls below the threshold value arbitrarily chosen. On the other hand, the disease arises when the change is significant and occurs over one or few generations (see Fig. V 4-1).

* * *

The human species - see, for documentation, the large number of publications - provides significant examples of great modifications in the ecological niche that have led to, either by themselves or in concomitance with other factors, the outbreak of real epidemics. I could mention:

a) Smoking and lung cancer and chronic bronchitis;

b) The high calorie diet and atherosclerotic disease and diabetes mellitus type II;

c) Diets low in vegetable waste and constipation, haemorrhoids, rhagades, anal fistulas, diverticulosis of the colon and, possibly, cancer of the rectum;

d) The gathering of the population in large urban areas and the tremendous infectious epidemics of the pre-industrial era (and the less dramatic ones of modern times);

e) The stress of urban and "civilized" life and mental and psychosomatic diseases;

f) The intake of and contact with drugs, industrial chemical substances, etc. and related pathologies.

An observation. To cure the individual of the aforementioned diseases directly, means to treat the consequences and not the cause of the problem. The decision of accepting the alterations of the ecological niche - instead of correcting them - by curing only the individual, is a choice that is political and/or personal and/or dictated by necessity. The evolutionary point of view coincides with that which has become ever more predominant over the last few decades - on the basis of experimental, ecological,

economic, etc. data - when stating that the core of the problem, on which it is necessary to focus our efforts, is the ecological niche (read: primary disease prevention).

* * *

There is an aspect that must be emphasized for which non-evolutionary and evolutionary points of view are substantially different.

For the non-evolutionary empiricist, a modification of the ecological niche must be subjected to judgment of observation in order to be considered harmful or insignificant. A prejudice concerning its harmfulness is dictated only by prudence or by preceding experiences. On the other hand, the evolutionary empiricist considers - on the basis of theoretical explanations - that a modification of the ecological niche is, more probably, an alteration, until evidence of the facts proves the contrary. This attitude is conservative, but I do not see any scientific reason why it should be rejected. Moreover, as regards certain types of modification of the ecological niche - see licence to use for new drugs -, the non-evolutionary empiricist is the first to maintain the correctness of this attitude.

I believe that, according to the theoretical reasons expressed, and with the support of painful past and present experiences, this attitude must rationally be extended to all types of ecological niche modification.

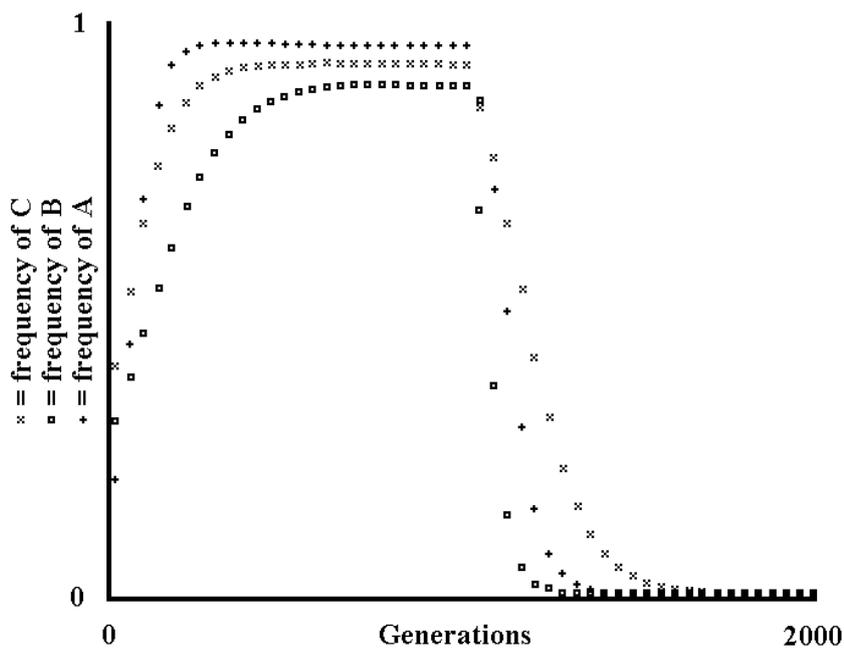


Fig. V 4-1 - Effects deriving from a sudden change in the ecological niche (Theoretical model).

Within a species, there are n genes - A, B, ..., Z – that manifest the advantages S_a, S_b, \dots, S_z towards their respective unique alleles - A', B', ..., Z'.

Assuming that each of these genes changes into its allele with frequency U_x at each generation and disregarding, for the sake of simplicity, the back-mutation, the iterative formula that allows us to calculate the spreading of any of these genes, is that expressed in the model of Fig. I 2-2:

$$X_{n+1} = \frac{X_n(1 + S_x - U_x)}{1 + X_n S_x} \quad (V-3)$$

Assuming that, at generation t , as a consequence of an abrupt change in the ecological niche, the values of the advantages change into S'_a, S'_b, \dots, S'_z , respectively, the formula to be applied remains the same, but S_x is substituted with S'_x . The figure was obtained using the same conditions as above, with $n = 3$, and with the frequencies of A, B and C indicated using crosses, squares and x, respectively.

As usual, the frequencies are on the ordinates and the generations on the abscissas (but with values from 0 to 2000 and with each interval representing 40 generations).

The assumed values are:

$$\begin{aligned} A_o &= .2 ; S_a = .03 ; S'_a = -.02 ; B_o = .3 ; S_b = .01 ; \\ S'_b &= -.03 ; C_o = .4 ; S_c = .015 ; S'_c = -.01 ; \\ U_a, U_b, U_c &= .001 ; t = 1000 . \end{aligned}$$

The figure shows that, with the selective pressures suddenly varying, the species is suddenly in a non-equilibrium condition - at least for some genes - and for a certain number of generations, the frequencies of the genes involved vary considerably until a new equilibrium is reached.

If the genes that are optimal for the preceding ecological niche likewise cause, in the new ecological niche, alterations in the individual to an extent greater than an arbitrarily established threshold, then we have a state of disease deriving from alterations in the ecological niche as defined in the text.

5) Diseases deriving from the relations with other living beings

A living being may get the energy it needs for its own persistence either from the inanimate world or from the energy resources of other living beings. In the second case, this relation between living beings can cause damage for the organism from which energy resources are removed. Disregarding the cases in which said damage is inevitably and univocally the death of the parasited organism (predator-prey, herbivore-grass cases, etc.) and, moreover, limiting the subject to the organisms from which the energies are removed:

A threshold having been arbitrarily set, I will define as sick, with the origin of the sickness in relations with other living beings, any organism damaged by the taking away of its own energy resources on the part of other living beings.

In Chapter III, this situation was addressed in general terms. I think that one point must be emphasized, because it is essential for the argument. A parasite that is well adapted – and not, therefore, in the eventuality of sudden modifications in the ecological niche - damages the host as little as possible. I have suggested that this really happens to a minimal extent - in evolutionary terms - despite the fact that an initial superficial examination of many empirical data suggests the contrary. See Chapter III, par. 5, for the arguments expressed in support of this statement. Moreover, I remind the reader that the concept of senile disease that I will discuss again in par. 7, also concerns the category of events defined in this paragraph.

6) Diseases deriving from 'excesses of the ecological niche'

The ecological niche of a species can also be constituted by events that are harmful for the individual, which:

a) are rare, that is to say, the selective pressure (which is proportional to the damage and to the frequency of the event) is not able to muster enough effective defences against such events, balancing the mutations that alter such defences. In other words, a defence

against these events proves to be relatively superfluous and does not exist or is lost (see second observation, Chapter I, par. 2).

and/or:

b) are such as to demand defences that cannot be developed in a species because it is impossible (see postulate of the potentiality, Chapter I, par. 3), or because it contrasts with other more pressing evolutionary needs.

In the occurrence of any of these events - which will be defined “excesses of the ecological niche” - the individual involved will be damaged in various ways.

A threshold having been arbitrarily set, I will define as sick with origin of the sickness in “excesses of the ecological niche” any individual damaged by an event such as that defined above.

* * *

Some examples of “excess of the ecological niche” are: being struck by a lightning or swept away by an avalanche, an exceptional drought or famine, a fall from a considerable height of a non-flying individual of significant weight, etc..

The origin of this category of events is in the ecological niche, albeit with different logic from that of the “diseases deriving from alterations of the ecological niche”. One observation needs to be made.

In an ecological niche that is notably different from that to which a species has adapted, and that is new, namely if the selective pressures have not had the possibility to have any effect, it is possible to classify an event as an “excess of the ecological niche” using criteria of analogy, given that it is impossible for the conditions described for defining the concept of excess to actually happen or be verified. For example, for man I will classify, as excesses of the ecological niche, a car accident, the amputation of a hand due to an accident at work, a sulphuric acid burn, a gunshot wound, etc.

* * *

The definition of this category of events, which is perhaps less interesting from an evolutionary point of view, is necessary to complete the picture of all those phenomena that I include under the single term "disease".

7) Disease and senescence

Senescence is not a disease.

The non-evolutionary empiricist may arrive at this conclusion after observing that it is a process which damages all individuals indiscriminately. The morphological and physiological analogies and identities between diseases and senile alterations become secondary compared to such a fundamental observation. (How could the non-evolutionary empiricist maintain that a process which damages all individuals is a disease if the disease is, likewise, defined as a deviation from the norm?).

On the contrary, the evolutionary empiricist may arrive at this conclusion after observing that senescence is a phenomenon that entails an evolutionary advantage - if what was said in Chapter II is true - and that therefore it is substantially different from the categories of events defined in the preceding paragraphs. The fact that senescence is a characteristic of all individuals of a species is, for the evolutionary empiricist, an outcome of the advantage entailed and not the fundamental criterion for judging it as a non-disease.

* * *

To maintain that senescence is not a disease does not automatically mean excluding or minimizing the importance that this phenomenon has in a discussion on diseases. First, there are the analogies of manifestation (and of the problems that derive from this from a medical point of view for the human species) between senescence and diseases. Furthermore, there is the phenomenon - already expounded in Chapter II, par. 6 (see in particular Fig. II 6-1) and stressed in par. 3 of this chapter - according to which the later in life a disease manifests itself in an individual, the less it causes selective pressure. These diseases, which have been defined as “senile diseases”, are an important subgroup in common between diseases with their origin in alterations of the genotype and diseases with their origin in relations with other living beings.

Now, I will give an explicit definition of senile disease:

It is a disease with its origin in alterations of the genotype or in relations with other living beings, which affects a part, even a great part, but never all of the senescent individuals of a species, and which is justified for its non-minimal frequency by the gradually decreasing selective pressure caused by an alteration, the later it manifests itself in the life of an individual.

Moreover, I should repeat that, as regards that subgroup of senescent individuals defined as “hypersenescent”, which show marked physiological and morphological alterations, it follows, given that such individuals are rarely or never observable in natural conditions (see Chapter II, par. 1), that:

a disease affecting only hypersenescent individuals does not exert, or hardly exerts, selective pressure and therefore may even concern the totality of hypersenescent individuals, and is sometimes indistinguishable from alterations of the senile process.

8) Diseases deriving from several causes. The epidemic

There is no theoretical explanation according to which we must exclude, in the genesis of the same pathological phenomenon, the coexistence of two or more of the events expressed in the preceding paragraphs. Likewise, the observation of human, and animal, pathology shows that the categories described must be considered as abstractions and idealizations of a reality where a combination of several factors is usual. I would mention, as an example of this statement, all the epidemics of plague or smallpox of past centuries. As an essential condition of any epidemic, there is a specific parasite involved. Among the factors that trigger the epidemic, there are the gathering of the population in urban centres, unresolved problems of removal and treatment of organic waste, etc., all of which are events that must be categorized as “great and fundamental modifications of the human ecological niche”. A complication of this is that there are individual differences, both genetic and those caused by senility and by senile diseases, in ability to endure infections. It must also be considered that the parasite, which is not a genetically static and unchanging entity, may tend to increase its aggressiveness in its serial passages from one host to another (see Gladstone, G. P., in Florey, 1970).

* * *

The concept of epidemic urges us to consider an aspect for which non-evolutionary and evolutionary points of view are different. If we define an epidemic as a pathological event, infective or otherwise, with a “high” incidence, for the non-evolutionary empiricist, the epidemic - without any other indications - differs from a disease with a “low” incidence only, and by definition, in its frequency. Observation must, then, define all other aspects.

Before observation, the evolutionary empiricist, can, perhaps, say only one thing in addition, but it is something with subtle implications:

If we define an epidemic as a pathological event with a high incidence and exclude senile diseases, epidemics are uncommon in an ecological niche to which the species has, as far as possible, adapted.

In fact, the harmful agents activate selective mechanisms that reduce the number of individuals damaged to a minimum. This minimum will be very small for the inanimate harmful agents, while for the parasites it must be less small, both in terms of number of individuals involved and in terms of threshold level, due to what was stated in Chapter III, par. 1 and 2. An epidemic could, likewise, happen only if the ecological niche changes suddenly (e.g., a new *modus vivendi*, a more virulent mutant parasite, etc.), or if the selection for a harmful gene is suppressed for many generations.

For the evolutionary empiricist, the phenomenon "epidemic" emerges as the marked breakdown of the equilibrium of the whole species-ecological niche: an event that is, therefore, distinguishable both qualitatively and quantitatively from the "non-epidemic". This stimulates us - and I think that this stimulus should not be undervalued - to a greater understanding and more active intervention towards the epidemic event that is, for the human species, no longer a consequence only of random factors, but the result of well recognizable "behaviours" - in a broad sense - that can be modified, if there is a will.

For example, the current high incidence of many diseases in the human species (atherosclerosis, diabetes mellitus, constipation, mental diseases, sight defects, etc.) are, from an evolutionary point of view, an anomaly of colossal dimensions to be imputed, even before we know the particular causes of each disease, to humans and not to nature. I think that a great deal of empirical data confirms this statement.

In short, I think that evolutionism implies a more critical and dynamic view of epidemiology, with a focus on prevention.

9) Evolutionary definition of 'disease'

For the non-evolutionary pathologist, disease is a deviation from the norm, where the norm is an entity statistically and arbitrarily defined on the basis of contingent experience. The non-evolutionary pathologist studies the causes of such deviations from the norm but - I would say - tends to conceive of such a norm as something static and unchanging and the causes of disease as something external that alters a "model". The reality of evolution teaches that the "model" is not at all unchanging and that, furthermore, the term "normality" in any way it is - arbitrarily - defined, is meaningless except in reference to an ecological niche. From the evolutionary point of view, a model attacked and damaged by causes of disease does not exist: "causes" and "model" are integral parts of the evolutionary process. I think that it is not possible to conceive of an evolutionary process disregarding the facts of the:

- a) transmission inaccuracy of the genetic information;
- b) adaptation of the species to the ecological niche;
- c) relations between living beings;
- d) impossibility of adaptation to any event.

I suggest the following evolutionary definition of the concept of disease:

Using, as the norm, the ecological niche to which a species X is adapted, disease is a state of deviation from the norm, in a pejorative sense, of one or more functions of an individual belonging to the species X, which happens in conditions when the whole individual-ecological niche deviates from the ideal state of perfect adaptation.

Note that the substantial difference of such a definition against the non-evolutionary definition of disease is the reference to the ecological niche and, more precisely, to an ecological niche that may certainly be different from that contingent where the individual lives.

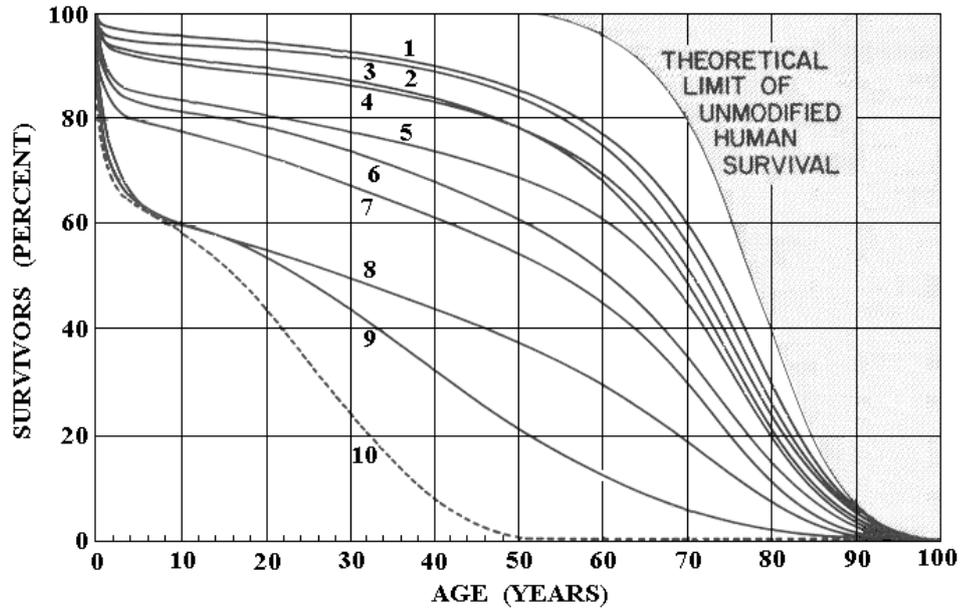
Moreover, even for the non-evolutionary empiricist, if a modification of the ecological niche causes alterations in a great part, or in all, individuals of a population, it is clear that we should more correctly refer to a different ecological niche in which the modification is not present, to define the “normality” and the deviation from the norm. The evolutionary definition gives a theoretical framework and a justification for this intuitive procedure.

10) Conclusions. Eugenics

The study of a species and of its pathology in a way that is detached from its ecological niche is wrong and misleading because the species depends on the characteristics of the ecological niche. This first point has already been stressed and here I only emphasize its importance. As a second point, I wish to stress the subject of “model” instability and the related subject of eugenics.

It is clear that the species is not something unchanging and that the events in which it is involved, given that they entail changes of the ecological niche, are, in themselves, sources of modifications of the selective pressures to which the species is subjected. On the other hand, the extent of these modifications, which might even be insignificant, should be weighed alongside the observation for each single event. Following this premise, we should ask to which degree, in which way and how fast the human species is modifying itself as an effect of the modifications of its own ecological niche. Without observation, is impossible to answer these questions. But there is an aspect which, on the basis of empirical data, I would like to stress.

It is common, among those who speak of eugenics, to refer to rare diseases and state that eugenic actions would have limited effectiveness and usefulness, maintaining, moreover, that, in any case, the eugenic problem will become significant only after several generations. This is, perhaps, a very wrong attitude. If it is true that the number of genes passed on is great, it must likewise be expected that the sum of all harmful mutations arising at each generation is relatively large - with no part of the genome spared - and that in, natural conditions, they are combatted by selection. The mortality curves for the man in the pre-medical era (see Fig. V 10-1), show a marked fall (of the order of 35-40%) in the first period of life, which leads us to suspect the removal of a large number of individuals with some genetic defect at each generation. The mortality curves in industrialized countries show, on the other hand, that this fall is currently very much reduced (and this is one of the biggest achievements of modern medicine). A simple examination of the curves does not tell us the extent to which genetically defective individuals are preserved, nor the type of defects. However, I suggest that: a) the portion affected is not minimal; b) the majority of genetic defects is not classified in the light of current knowledge; c) no function is spared (considering, of course, that many mutations are deadly). This would imply that eugenics is not a problem of generations in the distant future and for rare and exceptional diseases, but rather a problem of generations in the near future, concerning the totality of the human “model”.



- 1 NEW ZEALAND, 1934-1938
- 2 U.S. (WHITES), 1939-1941
- 3 U.S. (WHITES), 1929-1931
- 4 ENGLAND AND WALES, 1930-1932
- 5 ITALY, 1930-1932
- 6 U.S. (WHITES), 1900-1902
- 7 JAPAN, 1926-1930
- 8 MEXICO, 1930
- 9 BRITISH INDIA, 1921-1930
- 10 STONE AGE MAN

Fig. V 10-1 - Mortality of the first period of life in human species. Source: Comfort, A., 1979, p. 6.

The figure shows (curves 8 and 9) that about 37% of the population dies before age 6 in conditions that, perhaps, are closest to the original. The tenth curve is hypothetical.

Evolutionary definition of disease phenomenon

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Abstract

Disease is usually defined as the alteration of physiologic conditions. If it is true that evolutionary mechanisms are indispensable for the full understanding of any biologic phenomenon, it is necessary to investigate if and how disease phenomenon is explainable and classifiable in evolutionary terms and if from this approach useful indications can be deduced.

As the transfer of genetic information from a generation to the next is imperfect, a fact that is fundamental for the whole evolutionary theory, the modification of genetic information, if not neutral, is, as more probable event being a change in a very complex and ordinate system, a possible cause of physiologic dysfunctions (1 - diseases caused by alterations of the genotype).

Because a modification of the ecological conditions to which a species is adapted, if not neutral, is, as more probable event, being a change in a very complex and ordinate system, a possible cause of physiologic dysfunctions (2 - diseases caused by alterations of the ecological niche).

As there is continuous competition among the species with conflictual evolutionary exigencies and specifically between an organism and its parasites (bacteria, virus, fungi, worms, etc.), a third general cause of disease is predictable (3 - diseases caused by interactions with other species). In particular, being the relationship between an organism and its parasites analogous to that between a prey and its predators, it is predictable that, in a similar way to what happens in prey-predators case, to minimise disadvantages and maximise advantages both for host and parasite, parasites will damage more very young and old individuals and less intermediate ages. Conditions beyond the adaptation range of the species will cause physiologic dysfunctions (4 - diseases caused by conditions beyond adaptation range).

Age-related fitness decline, usually called "ageing" in its more advanced expression at ages not existing in the wild, is a peculiar function and not a disease in evolutionary terms. The simple fact that individuals in protected conditions (civilisation, captivity) survive at ages not existing in the wild is a predictable cause of greater expression of alterations underlying age-related fitness decline.

Evolutionary interactions between fitness decline and other category of diseases are important. In particular, diseases of categories 1 and 3 will increase their frequencies in relation with age and, if protected conditions increase life span, frequency and severity of these diseases will increase in proportion.

Interactions between diseases of categories 2 and 3 are very important too.

The evolutionary arrangement and classification of the diseases is not a useless theoretical exercise. On the contrary, it is essential for a better understanding of the epidemiology and to improve prevention and control of diseases. The knowledge of evolutionary mechanisms explaining disease origins should be an indispensable component of the medical education and of the definition of sanitary politics.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

Main concept:

Evolutionary Medicine is scarcely considered by Physicians and even by Evolutionary Biologists while should be a pivotal topic for both

“Disease” is commonly defined as the alteration of physiologic conditions. If we accept that evolutionary mechanisms are indispensable for the full understanding of any biological phenomenon [1], it is necessary to investigate if and how disease phenomenon is explainable and classifiable in evolutionary terms and if this approach could give us useful indications [2-4].

Four different evolutionary mechanisms can cause disease:

- 1) Alterations of the genotype: The transfer of genetic information from a generation to the next is imperfect, a fact that is fundamental for the whole evolutionary theory. As these modifications are a change in a very complex and ordinate system, they are, when not neutral, a possible cause of physiologic dysfunctions;
- 2) Alterations of the ecological niche: The modification of the ecological conditions to which a species is adapted is a change in a very complex and ordinate system and, when this change is not neutral, is a possible cause of disease (Fig. 1 and Table I);

Table I
Alterations of the ecological niche → Diseases ([2-9] + other 23 references)
Excessive ingestion of salt → Hypertension (→ heart hypertrophy, congestive heart failure, arrhythmia and sudden death)
Excessive time spent focusing close up or in improper conditions of vision → Myopia (up to 70-90% of a population affected) and other refractive defects (astigmatism, hyperopia)
Excessive ingestion of unsaturated fats, caloric foods, meat with high fat content → Obesity (→ renal cell carcinoma, heart hypertrophy, congestive heart failure, arrhythmia and sudden death), type 2-diabetes and increased vascular risk (→ myocardial infarct, cerebral ischemia, infarcts in all the vascular districts, heart hypertrophy and failure, etc.)
Excessive exposure to noise, smoking, high Body Mass Index → Hearing loss
Smoking and/or air pollution → Chronic bronchitis and respiratory diseases, emphysema, coronary heart and other cardiovascular disease, pregnancy complications, lung / larynx / bladder / kidney / pancreas carcinoma, peptic ulcer
Excessive ingestion of simple and refined carbohydrates (in particular sugar) and other dietary modifications → Dental caries, pyorrhoea, crowded teeth
Scarce ingestion of fibre → Constipation, colon diverticulosis, colon carcinoma, stomach carcinoma, type 2-diabetes, metabolic syndrome and cardiovascular disease, appendicitis
Scarce ingestion of calcium and reduced physical activity → Osteoporosis, back pain
Altered conditions of sociality, stress of civilised condition → Mental and psychiatric disorders, headache
Reduced exposure to natural allergens in the childhood → Allergies
Exposure to chemical substances artificially synthesised → Allergic diseases
Alcoholism → hepatic steatosis, steatohepatitis, cirrhosis, larynx carcinoma
Various factors → Increased incidence of many types of cancer



Fig. 1 – Upper side: ancestral dietary habits and “teeth ... excellent and free from dental caries”. Lower side: modern diets and multiple dental caries, “crowding of the teeth”, “changes in facial form”, pyorrhoea [5].

3) Interaction with other species: There is a continuous competition among the species with conflictual evolutionary exigencies and specifically between an organism

and its parasites (bacteria, virus, fungi, worms, etc.). In particular, as the relationship between an organism and its parasites is analogous to that between a prey and its predators, it is predictable that, such as it happens in prey-predators case, parasites will damage more very young and old individuals and less individuals of intermediate ages, to minimise disadvantages and maximise advantages both for host and parasite; 4) Conditions beyond adaptation range of the species.

Moreover, age-related fitness decline (Fig. 2), usually called “ageing” in its more advanced expression at ages not existing in the wild, is not a disease in evolutionary terms but “a specific biological function” [10], advantageous in certain conditions in terms of inclusive fitness [11-14] and not caused by the combined action of many harmful genes (Fig. 3). The simple fact that individuals in protected conditions (civilisation, captivity) survive at ages not existing in the wild is a predictable cause of greater expression of alterations underlying age-related fitness decline [13].

Evolutionary interactions between fitness decline and other category of diseases are important. In particular, diseases of categories 1 and 3 will increase their frequencies in relation with age and, if protected conditions increase life span, frequency and severity of these diseases will increase in proportion (Fig. 4). Interactions between diseases of categories 2 and 3 are very important too (Table II). Diseases classified in evolutionary terms are comparable to analogous categories of car breakdowns (Fig. 5). The evolutionary arrangement and classification of the diseases is not a useless theoretical exercise. On the contrary, it is essential for a better understanding of disease epidemiology and to improve their prevention and control. The knowledge of evolutionary mechanisms explaining disease origins should be an indispensable component of the medical education and of the definition of sanitary politics (Fig. 6).

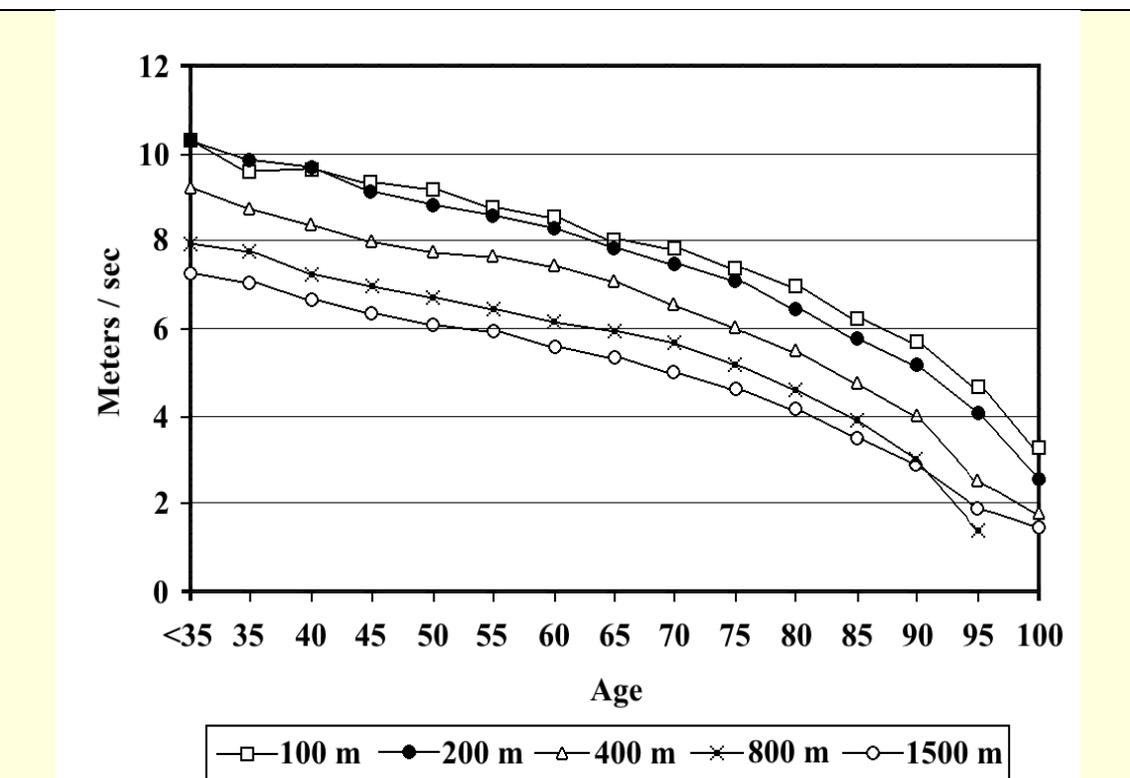


Fig. 2 – Age-related fitness decline. Source of data: for age group < 35 (world records) http://en.wikipedia.org/wiki/World_records_in_athletics; for other age groups http://www.world-masters-athletics.org/records_output/rec_list_outdoor_m.php.

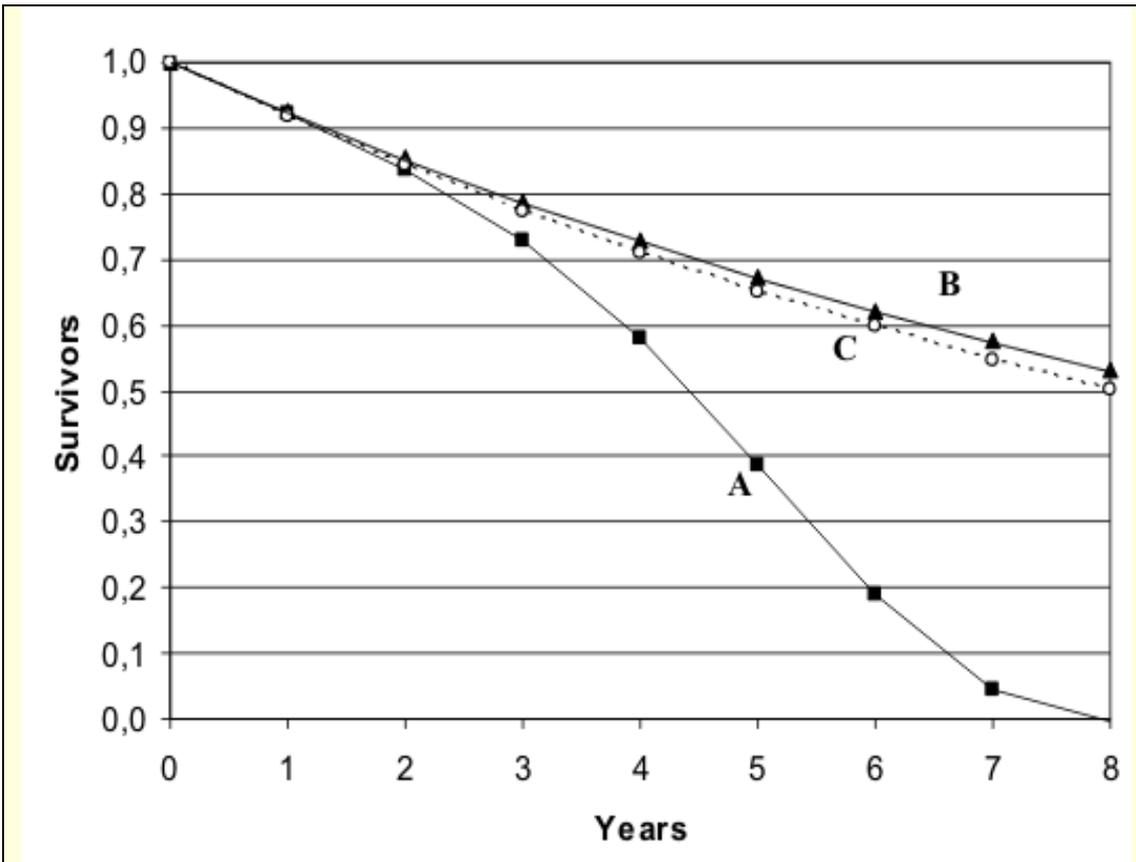


Fig. 3 – Curve A is the life table in the wild of a real species showing an age-related fitness decline. B is a hypothetical life table of the same species with only the extrinsic mortality at its lowest value and without the age-related fitness decline. C is a hypothetical life table with the same mortality of B plus the effects of a great number (500/year) of noxious genes acting at years t_1, t_2, \dots . Curve A is quite different from C and, so, it is unjustifiable as an effect of noxious genes insufficiently eliminated by natural selection [11-13].

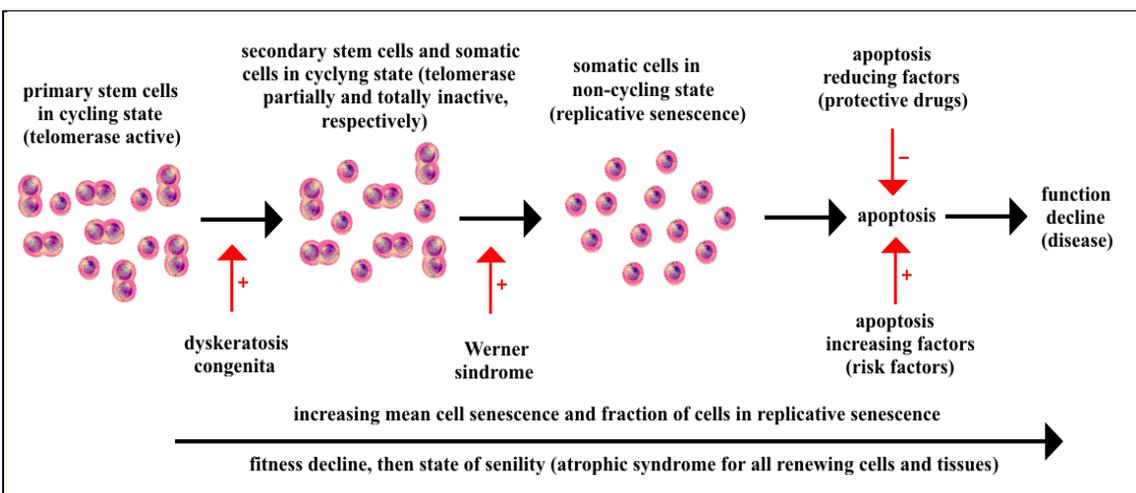


Fig. 4 – Risk factors [15,16] (and some genetic diseases [17]) increase apoptosis rate and cell turnover. Protective drugs contrast the effect of risk factors [15,18,19] but it is not documented the capacity of reducing physiological cell turnover rate.

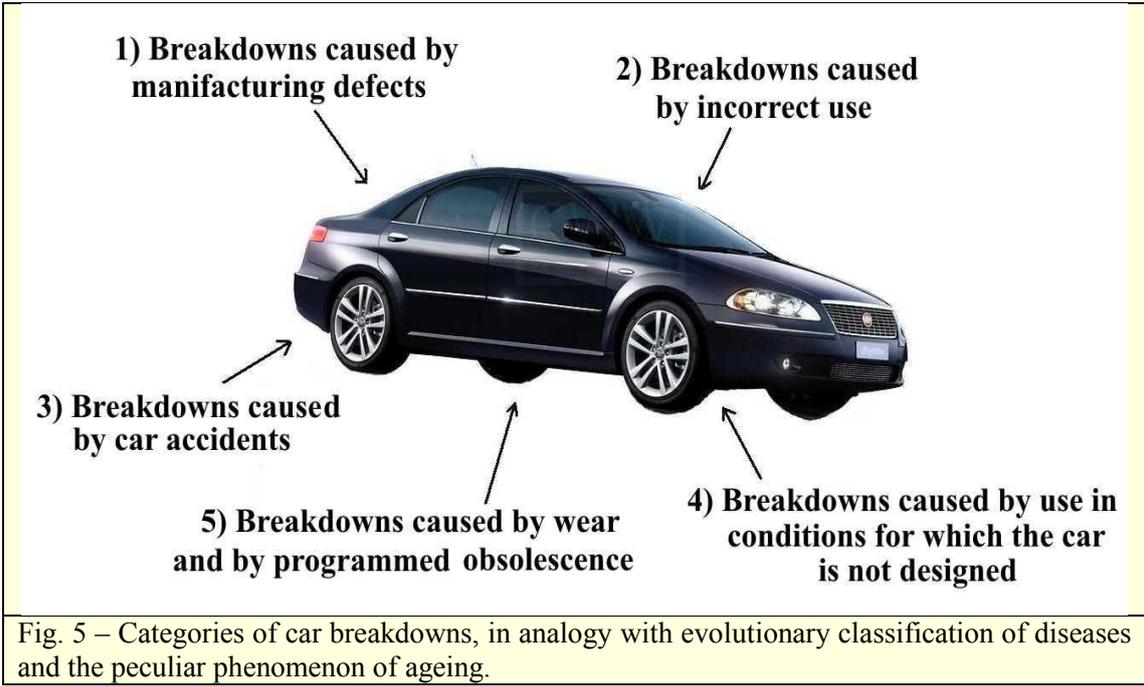


Table II
Alterations of the ecological niche → Diseases related to category 3 ([20-22] + other 24 refer.)
Extraordinary growth of human population and of its demographic density, cohabitation or proximity with bred animals, dangerous hygienic habits → dreadful and non-dreadful epidemics (black death, bubonic plague, smallpox, typhus, cholera, influenza, hepatitis A, tuberculosis, measles, parotitis, HIV, etc.)
Hygienic or iper-hygienic habits that restrict and delay first exposure to microbes and parasitic worms or make impossible infections or infestations → Gravity of first infection in adulthood (e.g., polyomyelitis), anomalous maturation of immunologic capacities (→ allergies and atopic diseases, autoimmune diseases as Crohn's disease, ulcerative colitis, multiple sclerosis, etc.)
Use and abuse of antibiotics → deadly infections by antibiotic resistant bacteria
Abuse of topic disinfectants → Alteration of normal microbial flora of epidermis and external mucosae (especially of armpits, genitals and hands) and consequent infections by pathogens, in particular fungi.

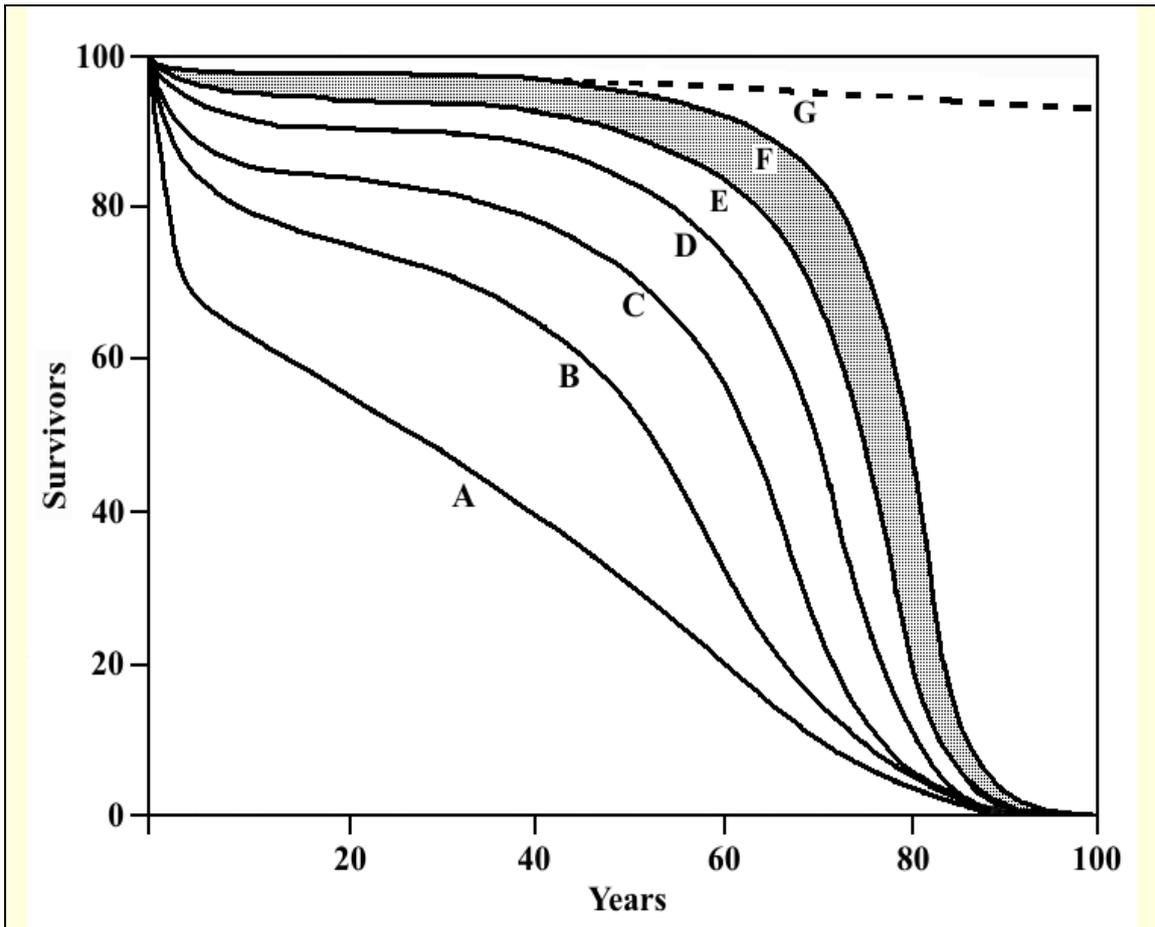


Fig. 6 – Life tables of human species illustrating a historical progressive increase of life span while longevity appears unchanged (curves A-E). Actual condition in developed countries is roughly indicated by curve E. With good preventive measures (due consideration of ancestral lifestyle to which our species is adapted!) and better health treatment, curve F is a likely outcome, with a little further increase of life span (dashed area) but not of longevity. Only with a modification of the progressive increase of mortality caused by intrinsic factors (ageing) a drastic increase of life span and longevity could be possible (curve G). Modified from [23] and redrawn.

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Prospects of a longer life span beyond the beneficial effects of a healthy lifestyle

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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.

Links:

Nova Science Publishers = https://www.novapublishers.com/catalog/advanced_search_result.php?keywords=libertini+longevity

Personal site = http://www.r-site.org/ageing/index_e.htm

Evolutionary definition of "normality"

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Abstract

The statistical concept of "normal range" (alias "reference values" or, shortly, "normality") is trivial and needs no particular explanation. But, applying this concept to a biological community that lives to a great extent in modified ecological conditions, the dysfunctions (diseases) deriving from conditions to which the species is not adapted become statistical normality.

It is therefore essential, in the study of biological phenomena, to use the concept of normality referred exclusively to the ecological niche (habitat, dietary habits, lifestyle, etc.) to which a species is adapted and to consider abnormal any different condition and the consequential diseases.

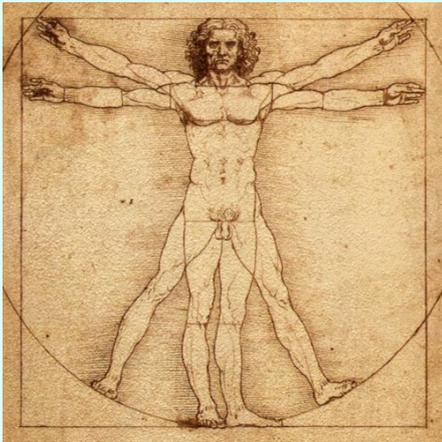
This evolutionary definition of normality is not at all a simple semantic curiosity, but a fundamental concept that must be the basis of a really scientific Medicine. For our species, many diseases that are regarded as consequence of the interaction between genes predisposing to a disease and environmental factors, if we consider their almost complete absence in populations living in primitive conditions, much closer to the ecological niche to which our species is adapted, turn out to be, in fact, the consequence of alterations of the normal (ancestral) ecological niche.

It follows that many common diseases (hypertension, cardiovascular diseases, obesity, type 1-diabetes, autoimmune diseases - type 2-diabetes included -, eye refractive defects, hearing loss, chronic respiratory diseases, dental caries, pyorrhoea, crowded teeth, constipation, haemorrhoids, anal rhagades, colon diverticulosis, appendicitis, nephrolithiasis, cholelithiasis, osteoporosis, back pain, allergies, mental and psychiatric disorders, practically all types of cancer, etc.) could be prevented almost completely by precautionary measures that correct as much as possible the alterations of our ecological niche, obviously in ways and manners compatible with the modern organization.

Actually, a rational health-care policy should be based first of all on the evolutionary concept of normality and only in the second place on the present medical practice that appears clearly more and more inadequate to contrast the exponential spreading of most of the diseases as direct consequence of the increasing alteration of our ecological niche. This implies a drastic redefinition both of sanitary and social framework: the main problem is that, for health operators, to cure is much more profitable - in terms of earnings, social advance and scientific success - than to prevent using evolutionary concepts. But, the future will compel to alternatives that are less self-interested and ineffective and more rational and fruitful.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm



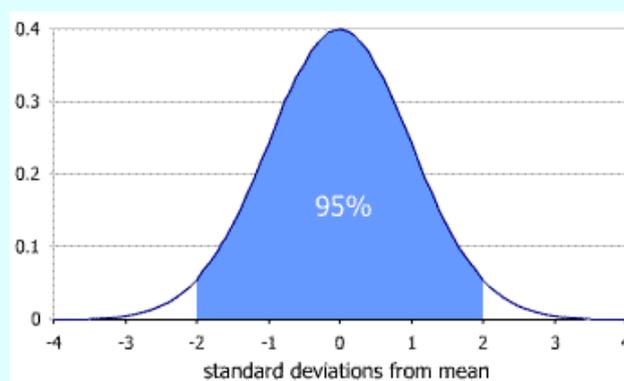
Evolutionary definition of “normality”

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A normal value, in its statistical meaning, is a value within the reference range (or normal range), which originates from an arbitrary mathematical definition.

For example, in the case of a "normal distribution", the normal range may be defined as all the values between 2 standard deviations added or subtracted to the mean.



But, it is essential to establish the population from which the mean is drawn!

It is in the “normal range”, in a mental hospital to be mad ...

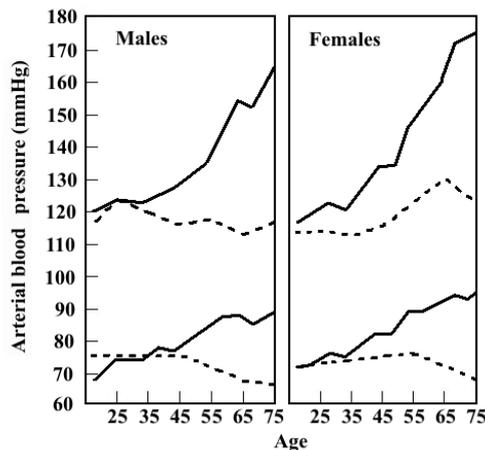


... and in a cemetery to be dead!

An intelligent listener could easily dispute these paradoxes with the simple observation that it is essential to consider a sufficiently large number of individuals and not a part of the whole population that is mad or dead.

But this solves only the surface of the problem ...

Thirty or forty years ago, it was usual, even for physicians, to say that the “normal” value of systolic blood pressure was roughly equal to: $100 + \text{age}$. Now, it is well-known that an age-related pressure increase is pathologic, but specific studies, based on statistical data, confirmed the trueness of this conviction, although **only in its statistical meaning and only for populations with a modern lifestyle.**



Arterial blood pressures in !Kung individuals (dashed lines) and in London citizens (continuous lines) [1].

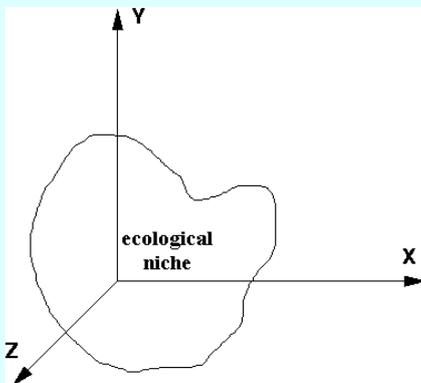
[1] Truswell AS et al. (1972) Blood pressure of !Kung bushmen in northern Botswana. *Am. Heart J.* 84, 5-12.

Therefore, even considering large parts of the human species (i.e., those with modern lifestyles), we obtain questionable results, as surely we cannot consider “normal” individual with pressure values that increase with age and so with increasing cardiovascular risks.

To escape this contradiction, it is generally used the concept of “optimal health range”, which is a reference range based on parameters that are associated with optimal health or minimal risk of related complications and diseases, rather than the standard range based on the values observed in a population.

But the concept of “optimal health range” is empirical and to obtain the “normal” value may require many years and generate many controversies (e.g.: see the long question about the definition of the normal values for arterial pressure and for cholesterolemia).

**It is indispensable a rational definition of “normality”
based on rational scientific bases**



The definition of “normality” may be immediately derived from a fundamental concept of evolutionary theory.

A species is adapted at best to its ecological niche (physical habitat, dietary habits, relations with other species, etc.), which we may represent as a part of a space with countless dimensions (in the picture, for obvious graphical limits, only three dimensions are depicted).

The statistical values related to the individuals living in the ecological niche to which the species is adapted are defined as “normal” conditions.

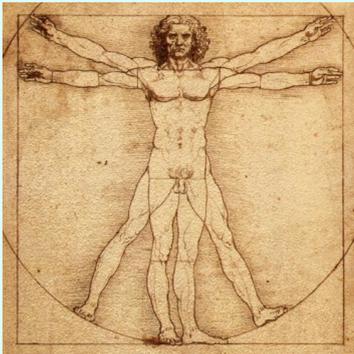
This definition has a very important implication:

As the adaptation a species to its ecological niche means a very delicate and sophisticated equilibrium, it is presumable that a random modification of this equilibrium causes – as more probable consequence – pathologic alterations, as well as a random modification of a symphony causes – as more probable consequence – an alteration of the melody.



These may seem a theoretical abstraction, not supported by experimental tests. But, it is current an immense unintentional experiment (NOT AUTHORIZED AND ETHICALLY QUITE UNACCEPTABLE!) in which the greater part of a species – our species! - lives in conditions that are very different from those of the ecological niche to which the species is adapted.

According to the theoretical predictions, the consequences are predicted to be terrible: from a state of optimal adaptation to its own ecological niche (“normality”, symbolized by the Leonardesque imagine of the vitruvian man [A]) the occurrence of a wide heap of diseases is expected [B].



A



B



This evolutionary definition of normality is not at all a simple semantic curiosity but a fundamental concept that must be the basis of a truly scientific Medicine.

For our species, many diseases are regarded as consequence of the interaction between genes predisposing to a disease and various environmental factors.

But, considering the almost complete absence of these diseases in populations living in primitive conditions, much closer to the ecological niche to which our species is adapted, they turn out to be, in fact, the consequence of alterations of the “normal” (ancestral) ecological niche.

Any change of the ecological niche to which a species is adapted must be considered potentially harmful until the contrary is proven.

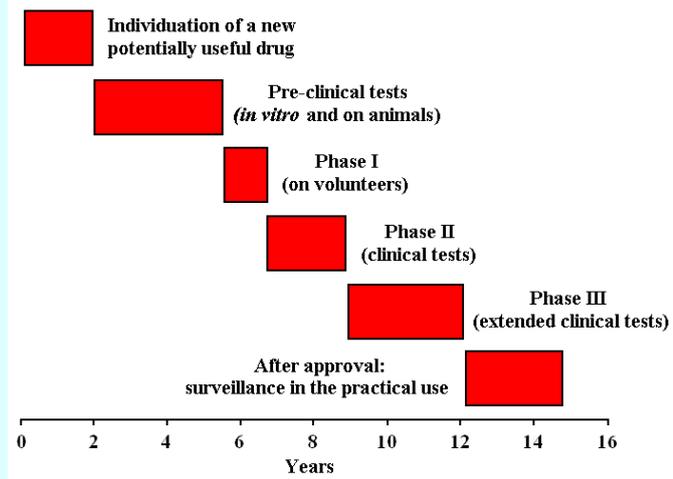
In the case of a new drug, this principle is observed!

[Precautionary principle]

But for other modifications of the ecological niche, no precaution is taken.

It is presumed – irrationally and stupidly, because of non-scientific evaluations – that a modification must not be considered harmful until the experience proves the contrary!

[Imprudence Principle]



A change in the ecological niche (e.g.: smoking, excessive salt intake and many other "new" dietary habits)

(After numberless cases of diseases and deaths)

The change is harmful!

Examples of: Alterations of the ecological niche -> Diseases

Excessive ingestion of salt -> hypertension [1,2,3] (-> heart hypertrophy, congestive heart failure, arrhythmia and sudden death [4])

Excessive ingestion of unsaturated fats, caloric foods, meat with high fat content -> obesity (-> renal cell carcinoma [5], heart hypertrophy, congestive heart failure, arrhythmia and sudden death [4]), type 2-diabetes and increased vascular risk (-> myocardial infarct, cerebral ischemia, infarcts in all the vascular districts, heart hypertrophy and failure, etc.) [1]

Occupational noise, smoking, high Body Mass Index -> hearing loss [6]

Excessive exposure to noise -> hearing loss [1,7]

[1] Eaton SB et al. (1988) *The paleolithic prescription: a program of diet & exercise and a design for living*. New York (USA), Harper & Row.

[2] Bragulat E & de la Sierra A (2002) Salt intake, endothelial dysfunction, and salt-sensitive hypertension. *J. Clin. Hypertens. (Greenwich)*. 4, 41-6.

[3] Rodriguez-Iturbe B et al. (2007) Pathophysiological mechanisms of salt-dependent hypertension. *Am. J. Kidney Dis.* 50, 655-72.

[4] Morse SA et al. (2005) The heart in obesity-hypertension. *Expert. Rev. Cardiovasc. Ther.* 3, 647-58.

[5] Lipworth L et al. (2006) The epidemiology of renal cell carcinoma. *J. Urol.* 176, 2353-8.

[6] Fransen E et al. (2008) Occupational noise, smoking, and a high Body Mass Index are risk factors for age-related hearing impairment and moderate alcohol consumption is protective: a European population-based multicenter study. *J. Assoc. Res. Otolaryngol.* 9, 264-76.

[7] Daniel E (2007) Noise and hearing loss: a review. *J. Sch. Health* 77, 225-31.

Continued ...

- Smoking -> coronary heart and other cardiovascular diseases, chronic respiratory diseases, pregnancy complications, and respiratory diseases in children [1], lung [1,2] / larynx [2,3] / bladder [2,4] / kidney [5] / pancreas [6] carcinoma, peptic ulcer [7,8]

- [1] Giovino GA (2007) The tobacco epidemic in the United States. *Am. J. Prev. Med.* 33, S318-26.
[2] Clavel J (2007) Progress in the epidemiological understanding of gene-environment interactions in major diseases: cancer. *C. R. Biol.* 330, 306-17.
[3] La Vecchia C et al. (2008) Alcohol and laryngeal cancer: an update. *Eur. J. Cancer Prev.* 17, 116-24.
[4] Janković S & Radosavljević V (2007) Risk factors for bladder cancer. *Tumori* 93, 4-12.
[5] Lipworth L et al. (2006) The epidemiology of renal cell carcinoma. *J. Urol.* 176, 2353-8.
[6] Hart AR et al. (2008) Pancreatic cancer: a review of the evidence on causation. *Clin. Gastroenterol. Hepatol.* 6, 275-82.
[7] Halter F & Brignoli R (1998) *Helicobacter pylori* and smoking: two additive risk factors for organic dyspepsia. *Yale J. Biol. Med.* 71, 91-9.
[8] Parasher G & Eastwood GL (2000) Smoking and peptic ulcer in the *Helicobacter pylori* era. *Eur. J. Gastroenterol. Hepatol.* 12, 843-53.

Continued ...

- Scarce ingestion of fibres -> constipation, colon diverticulosis, colon carcinoma, stomach carcinoma, type 2-diabetes, metabolic syndrome and cardiovascular diseases [1], appendicitis [2,3]
- Altered conditions of sociality, stress of civilized condition -> mental and psychiatric disorders [4,5]
- Scarce ingestion of calcium and reduced physical activity -> osteoporosis [4,5], back pain [4]

- [1] Trepel F (2004) [Dietary fibre: more than a matter of dietetics. II. Preventative and therapeutic uses][Article in German] *Wien. Klin. Wochenschr.* 116, 511-22.
[2] Arnbjörnsson E (1983) Acute appendicitis and dietary fiber. *Arch. Surg.* 118, 868-70.
[3] Adamidis D et al. (2000) Fiber intake and childhood appendicitis. *Int. J. Food. Sci. Nutr.* 51, 153-7.
[4] Nesse RM & Williams GC (1994) *Why we get sick.* New York (USA), Times Books.
[5] Eaton SB et al. (1988) *The paleolithic prescription: a program of diet & exercise and a design for living.* New York (USA), Harper & Row.

Continued ...

- Various factors -> increased incidence of many types of cancer [1,2]
- Alcoholism -> hepatic steatosis, steatohepatitis, cirrhosis [3], larynx carcinoma [4]
- Smoking and/or air pollution -> chronic bronchitis [5], emphysema [6]
- Exposure to chemical substances artificially synthesized -> allergic diseases [7]

[1] Eaton SB et al. (1988) *The paleolithic prescription: a program of diet & exercise and a design for living*. New York (USA), Harper & Row.

[2] Greaves MF (2000) *Cancer: The Evolutionary Legacy*. Oxford (UK), Oxford University Press.

[3] Adachi M & Brenner DA (2005) Clinical syndromes of alcoholic liver disease. *Dig. Dis.* 23, 255-63.

[4] La Vecchia C et al. (2008) Alcohol and laryngeal cancer: an update. *Eur. J. Cancer Prev.* 17, 116-24.

[5] Viegi G et al. (2006) Epidemiology of chronic obstructive pulmonary disease: health effects of air pollution. *Respirology* 11, 523-32.

[6] Taraseviciene-Stewart L & Voelkel NF (2008) Molecular pathogenesis of emphysema. *J. Clin. Invest.* 118, 394-402.

[7] Kirchner DB (2002) The spectrum of allergic disease in the chemical industry. *Int. Arch. Occup. Environ. Health.* 75, S107-12.

Continued ...

- Excessive time spent focusing close up or in improper conditions of vision -> myopia [1] (up to 70–90% of a population affected [2,3]), refractive defects (myopia, astigmatism, hyperopia) [4]

But, studying two groups of 6- and 7-year-old school children of Chinese ethnicity, the first living in Singapore and the other in Sydney, with only two significant differences (Sydney children made more near-work activity and spent more time in outdoor activities), it was observed that the prevalence of myopia was only 3.3% in Sydney children and 29.1% in Singapore children [5].

The idea that the direct exposition to natural light was the key factor has been confirmed by other studies [6,7].

[1] Fredrick DR (2002) Myopia. *BMJ.* 324, 1195-9.

[2] Chow YC et al. (1990) Refractive errors in Singapore medical students. *Singapore Med J.* 31, 472–3.

[3] Wong TY et al. (2000) Prevalence and risk factors for refractive errors in an adult Chinese population in Singapore. *Invest. Ophthalmol. Vis. Sci.* 41, 2486-94.

[4] Kee CS & Deng L (2008) Astigmatism associated with experimentally induced myopia or hyperopia in chickens. *Invest. Ophthalmol. Vis. Sci.* 49, 858-67.

[5] Rose KA et al. (2008) Myopia, lifestyle, and schooling in students of Chinese ethnicity in Singapore and Sydney. *Arch. Ophthalmol.* 126, 527-30.

[6] Rose KA et al. (2008) Outdoor activity reduces the prevalence of myopia in children. *Ophthalmol.* 115, 1279-85.

[7] Dirani M et al. (2009) Outdoor activity and myopia in Singapore teenage children. *Br. J. Ophthalmol.* 93, 997-1000.

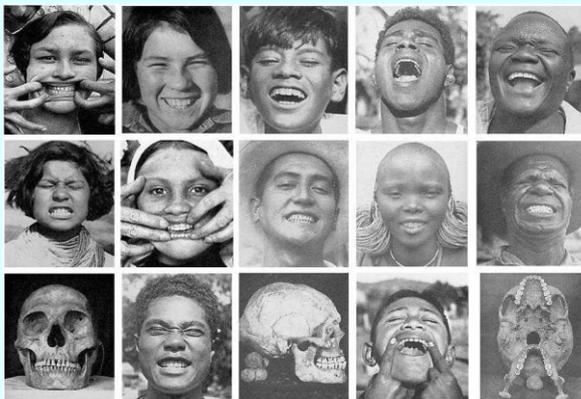
Continued ...

- Reduced exposure to natural allergens from bacteria, viruses, helminths ->
- a) Alterations of TH1-mediated immune response (autoimmune diseases as Crohn's disease, ulcerative colitis, diabetes type I, multiple sclerosis, Guillain-Barrè syndrome, Hashimoto's disease and other thyroiditis, psoriasis, rheumatoid arthritis, temporal arteritis, etc.)
- b) Alterations of TH2-mediated immune response (allergic diseases as hay fever, allergic asthma, eczema, etc.)

[Hygiene Hypothesis] [1-5]

Helminthic therapy (deliberate infestation with a helminth, or with its ova) is currently being studied as a promising treatment for several autoimmune diseases including Crohn's disease, multiple sclerosis, asthma, and ulcerative colitis.

[1] Strachan DP (2000) Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax* 55: S2-10.
[2] Grammatikos AP (2008) The genetic and environmental basis of atopic diseases. *Ann Med.* 40:482-95.
[3] Folkerts G et al. (2000) Do common childhood infections 'teach' the immune system not to be allergic? *Immunol Today* 21: 118-20.
[4] Bufford JD & Gern JE (2005) The hygiene hypothesis revisited. *Immunol Allergy Clin North Am* 25: 247-62.
[5] Janeway C et al. (2001). *Immunobiology* (5th Ed.). New York and London, Garland Science.



Continued ...

Excessive ingestion of simple and refined carbohydrates (in particular sugar), calcium deficiency and other dietary modifications -> dental caries, pyorrhoea, changes in facial form, crowded teeth [1,2]

Ancestral dietary habits and "teeth ... excellent and free from dental caries" [2].

Modern diets and multiple dental caries, "crowding of the teeth", "changes in facial form", pyorrhoea [2].

[1] Eaton SB et al. (1988) *The paleolithic prescription: a program of diet & exercise and a design for living.* New York (USA), Harper & Row.
[2] Price WA (1939) *Nutrition and Physical Degeneration.* New York - London, Paul B. Hoeber.



From dr. Price's book [1], published in 1939:

In the quite isolated Swiss Loetschental valley, with ancient dietary habits: only 2.3% teeth with caries.
Analogous results in other less isolated Swiss valleys.

On the contrary, in almost all not isolated parts of Switzerland 95 to 98 per cent of the people suffered for dental caries.
“In the modernized districts of Switzerland tooth decay is rampant.”
In St. Moritz valley, in a class of 16 children, there were 9.8 cavities per person.
In the same valley: “When parents were asked to permit their children to have one meal a day reinforced, according to a program that has proved adequate with my clinical groups in Cleveland, the objection was made that there was no use trying to save the teeth of the girls.
The girls should have all their teeth extracted and artificial teeth provided before they were married ...”



“Normal design of face and dental arches”



“very badly formed dental arches with crowding of the teeth. This deformity is not due to heredity”

[1] Price WA (1939) *Nutrition and Physical Degeneration*. New York – London, Paul B. Hoeber.

Problems with wisdom teeth:

A consequence of the slowness of evolution?

“Wisdom teeth are vestigial third molars that human ancestors used to help in grinding down plant tissue. The common postulation is that the skulls of human ancestors had larger jaws with more teeth, which were possibly used to help chew down foliage to compensate for a lack of ability to efficiently digest the cellulose that makes up a plant cell wall. As human diet changed, a smaller jaw was selected by evolution, yet the third molars, or ‘wisdom teeth’, still commonly develop in human mouths. (Dubrow TJ et al. (1988). “Detailing the human tail”. *Annals of plastic surgery* 20, 340–4. 1)” [1]

Or a consequence of dietary alterations of the ecological niche?

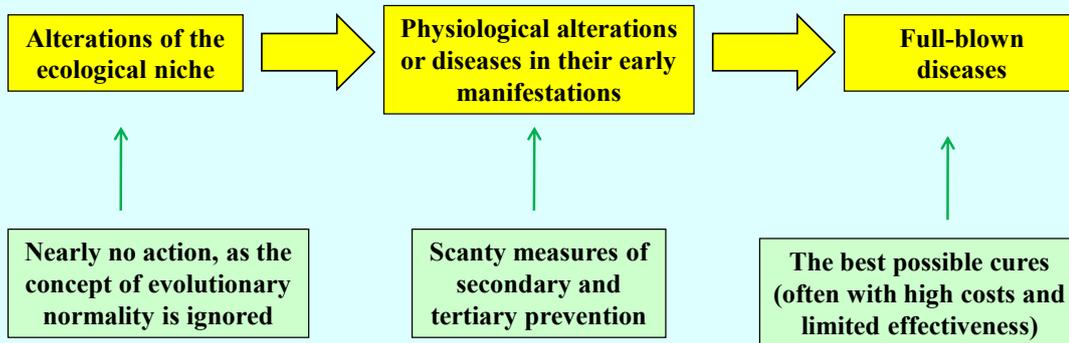
“... from 25 to 75 per cent of individuals in various communities in the United States have a distinct irregularity in the development of the dental arches and facial form ... In a study of 1,276 skulls of ... [pre-Colombian] Peruvians, I did not find a single skull with significant deformity of the dental arches.” [2]

“I was able to examine a number of skulls from this cave which apparently represented a pre-Spanish period. ... broad sweep of the dental arches and freedom from tooth decay. The third molars (wisdom teeth) are well developed and in normal position for mastication. ... It is very evident that these individuals were provided with an adequate nutrition throughout the formative and growth periods, as well as during their adult life.” [2]

[1] Wikipedia, *Human vestigiality*.

[2] Price WA (1939) *Nutrition and Physical Degeneration*. New York – London, Paul B. Hoeber.

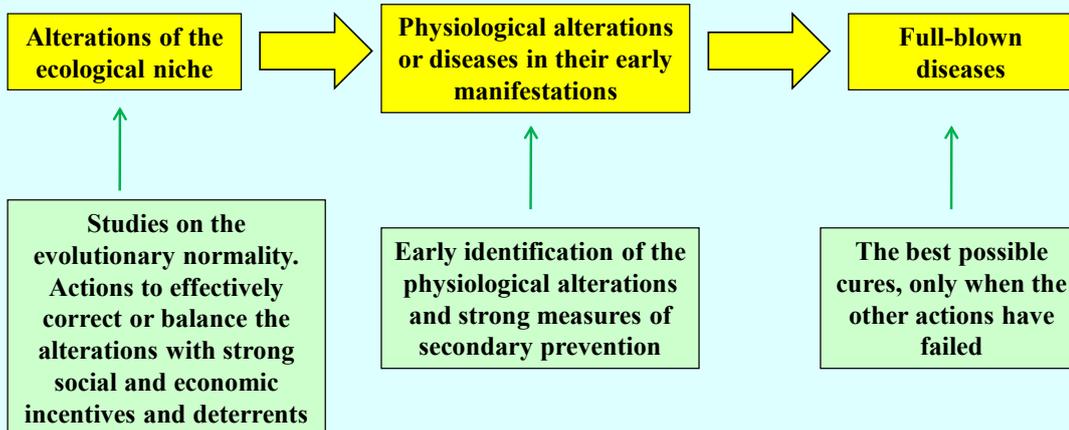
Modern (irresponsible) Medicine and Sanitary Policy



Effects of this sanitary policy:

- a) Increasing and unrestrained alterations of our ecological niche
- b) Exponential spreading of most of the diseases and of the related deaths
- c) Exponential increase of the related costs with a bad cost / efficacy ratio

Future (desirable) Medicine and Sanitary Policy



Effects of this sanitary policy:

- a) Increasing “normalization” of our ecological niche
- b) Reduction of most of the diseases and of the related deaths
- c) Reduction of the related costs with a better cost / efficacy ratio

Conclusion

Modern Medicine is only partially scientific since, to all intents and purposes, it ignores Evolutionary Medicine, and in particular the concept of “normality” rationally defined in evolutionary terms.

Evolutionary Medicine is not at all a form of alternative medicine but, on the contrary, the pivotal chapter of a medicine truly grounded on scientific bases.

Nowadays, the physician is entirely lacking in the knowledge of the most elementary concepts of Evolutionary Medicine.

In fact, the sanitary policy of all modern states is managed in the total ignorance of the most elementary concepts of evolutionary theory.

...

Conclusion - Continued

...

At the same time, evolutionary biologists are unaware of the extreme importance of these concepts for a rational organization of a system that should prevent, control and handle diseases.

No evolutionary biologist is included in the rolls of any national health system in any operating level.

The unwitting ignorance is deplorable but without direct blame.

On the contrary, it is culpable neglect to be inert, whereas one has the awareness that his own contribution is essential to prevent and contrast effectively diseases, sufferings and deaths.

**Thanks
for your attention**

This presentation is on my personal pages too:

www.r-site.org/ageing

(e-mail: giacinto.libertini@tin.it)

A test about the knowledge of some concepts of Evolutionary Medicine in the medical field

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Abstract

The correct knowledge in the medical field of some basic concepts of Evolutionary Medicine is tested with the answers of physicians of various professional qualifications to a group of 14 questions. The answers obtained show not only that there is an almost complete ignorance of some basic concepts of Evolutionary Medicine, but that current opposite ideas are prevalent. The consequence is that diseases are prevented and cured assuming an erroneous framework in a health maintenance system organized on unscientific assumptions with disastrous consequences for the rates of morbidity and mortality.

Keywords: evolutionary medicine, hygiene hypothesis, antibiotics, vaccines.

Introduction

Evolutionary Medicine is not an alternative medicine, but a Medicine that wants to be thoroughly and consistently based on sound scientific ideas and therefore duly integrated with concepts derived by Evolutionism. In fact, as Medicine is an applicative branch of Biology and Evolutionism is the backbone of Biology, it is not conceivable that Medicine may be organized without taking into due account the results and the logic of Evolutionism.

According to the current view and teaching of Medicine, Evolutionism is of little or no practical importance to this discipline: it is useful to understand the anatomy of certain organs, to explain better the physiology of some functions. Yet, considering the nature of the disease, which is something that does not evolve and hits as an anomaly the living being, Evolutionism is irrelevant for the understanding and the treatment of diseases.

On the contrary, the thesis of Evolutionary Medicine is that only taking into account that the living being is the result of evolution it is possible to understand the primary causes of the diseases and their real essence and from this to derive the most effective preventive and curative measures.

Those unfamiliar with the Evolutionary Medicine could argue that diseases are currently understood in their primary causes, preventive measures are mostly well-known, and treatments are certainly improvable, but that a good knowledge of Evolutionism in the medical field could be of little use for this purpose. Yet, this opinion is heavily wrong, as the careful Reader will observe in the next pages.

This paper wants to pinpoint on some biological-medical problems for which the discrepancy between the traditional medical view and that of Evolutionary Medicine is strong, with serious consequences for the prevention and the treatment of many diseases.

Method

A series of 14 questions with multiple answers (all in Italian language) was submitted by e-mail to over 200 Italian physicians of various professional qualifications (general practitioners, specialists of various type, hospital workers, researchers and scholars,

etc.). The choice of the person addressed was random and no information was given about the aims of the test before the answers. Only 48 doctors replied to the questions, expressing as requested one answer for each question. The goal was to detect if there was convergence or divergence between the traditional medical view and that of Evolutionary Medicine. Each question had four possible answers of which one was correct and the others wrong. In one case (question 11) two answers were correct, and in another case (question 3) three answers were correct. In these last cases, the fact that two or more answers were correct was not communicated.

Results for each question

For each question, it is now presented the formulation of the question and of the possible answers (all in the English translation) for which it was asked to make a single choice for the one deemed most appropriate. Immediately after, I report the responses obtained and then synthetic considerations that expose why the proposed answers are correct or not.

In each of the tables of the answers, it is also shown the expected correctness of random answers (“ECRA”), given by the number of correct answers divided by four.

The overall results are shown in the next section.

Question 1) Vaccines stimulate organism defences against bacteria, viruses and other pathogens and constitute the more effective medical means of defence against the diseases caused by them. Disregarding the cases when vaccines are ineffective (e.g., when they do not activate defences against appropriate antigens) or harmful (e.g., when they provoke allergic reactions or because sometimes the pathogen survives and rouses a noxious infection), in the other cases vaccines ...:

Answers:

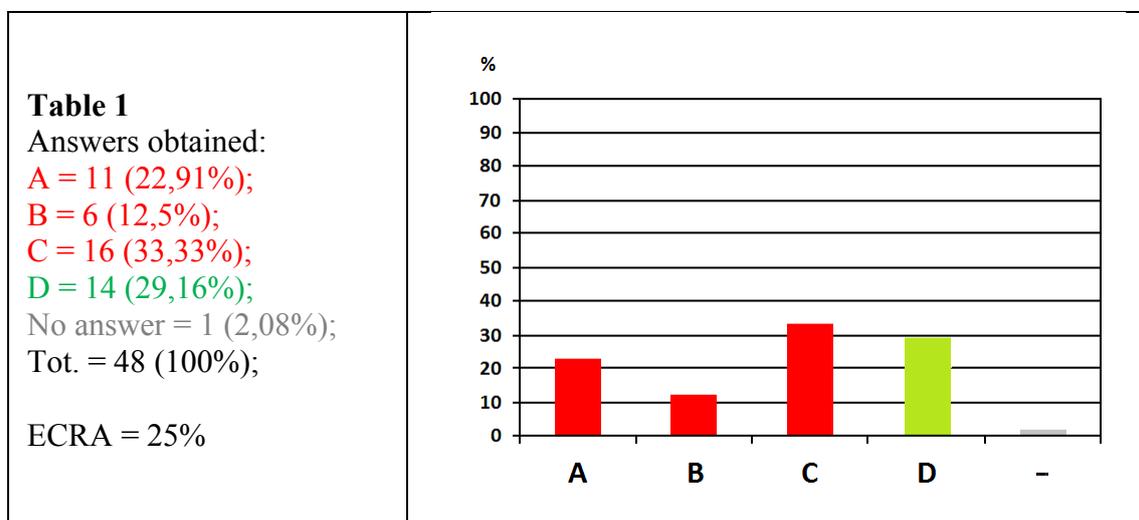
A) as they cause undue risks for vaccinated subjects, should be used in restricted ways, should never be compulsory and their use should be subordinated to the consent of the subjects or of their tutors;

B) are always advantageous, and pathogen germs cannot develop more or less virulent mutants because totally blocked in their reproduction;

C) in addition to an immediate advantage, cause the selection of less virulent mutants and, therefore, in prospect may eliminate even the future danger of severe infections;

D) are advantageous against infections, but are potentially harmful if not well designed as they may provoke the selection of more virulent pathogens;

[For all the questions only one answer was required: A / B / C / D]



Correct answer: D

Vaccines are generally advantageous against infections, but it is a serious error to overlook that they act on entities that evolve and not on inanimate objects [Read and Mackinnon, 2008]. It is not true that pathogens against which vaccines are aimed are always totally blocked in their reproduction and, therefore, cannot develop mutations making them more or less virulent: e.g., in the case of hepatitis B virus, mutants not blocked by vaccine-induced immunologic defences are spreading [Francois et al., 2001; Hsu et al., 2004; FitzSimons et al., 2005] (Answer B is false). Moreover, it is not true that vaccines always favour the prevalence of less virulent mutants and so make less and less dangerous the pathogen opposed: e.g., in a well-documented case, Marek's disease virus, a disease caused by a herpes virus that provokes a form of poultry cancer, two generations of vaccines, after an initial phase of seeming success, have provoked the onset of more virulent and harmful stocks [Witter, 2001; Davison and Nair, 2004, 2005] (Answer C is false).

In shorts: "Rightly, vaccination is viewed as a medical triumph. Yet it is argued that the long-term control of acute childhood diseases like smallpox, polio, and measles does not mean vaccines are evolution-proof. The pathogens now being targeted are quite different from the organisms responsible for those diseases, and some of the vast evolutionary experiments currently being conducted with vaccines are generating pathogen evolution. As shall be seen, a variety of evolutionary responses to vaccination are possible, including the evolution of more virulent pathogens." [Read and Mackinnon, 2008]

In spite of these limits and reservations, which impose a careful evaluation of the consequences on the evolution of pathogens opposed by vaccines, advantages deriving from their use are generally much greater than risks caused by them. For certain types of diseases, in particular, we should not make exceptions to vaccine compulsoriness or trust in the free will of people that have no qualification to evaluate correctly about the best decision and that could decide from a personal point of view even in contrast with the public good (Answer A is false).

This question requires a critical evaluation of vaccine action in an evolutionary scenery, avoiding the serious error to consider pathogen germs as simple inanimate targets that cannot evolve. Carelessness of this concept can induce to serious errors of evaluation, which can jeopardise vaccine effectiveness or even cause harsh consequences (Answer D is correct).

Question 2) In subjects that are not elderly or suffering for a disease, refraction defects that reduce the eyesight (myopia, astigmatism, hypermetropia) are very frequent. Such defects ...:

Answers:

A) are caused by the interaction of pathological genes predisposing to myopia / astigmatism / hypermetropia and environmental factors (weariness of the eyesight, unfit lighting, etc.);

B) are caused by pathological genes while environmental and nutritional factors are rarely the cause;

C) are caused by environmental factors while pathological genes and nutritional factors are rarely the cause;

D) are caused by the interaction of pathological genes predisposing to myopia / astigmatism / hypermetropia, environmental (weariness of the eyesight, unfit lighting, etc.) and nutritional (scarcity of particular vitamins and micronutrient) factors;

Table 2

Answers obtained:

A = 10 (20,83%);

B = 7 (14,58%);

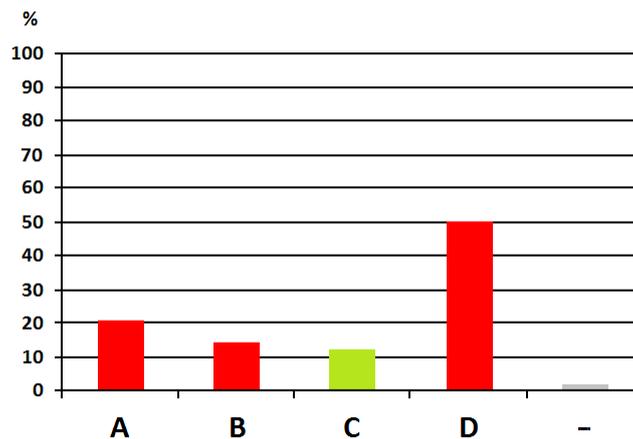
C = 6 (12,5%);

D = 24 (50%);

No answer = 1 (2,08%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: C

“Animal models in multiple species show that early visual experience affects growth of the eye and eventual refraction. ... It has been hypothesised that prolonged reading or the retinal blur of prolonged near work leads to the development of myopia. This is supported by evidence showing an increase in the prevalence of myopia from near 0% to rates found in the Western population in aboriginal peoples exposed to a Western curriculum of education. ... Whether using primates (monkeys, marmosets, or tree shrews) or chickens, investigators have shown that when a clear, formed image is not allowed to be focused on the retina (by suturing up eyelids or placement of translucent goggles) high myopia will develop in the eyes of young animals.” [Fredrick, 2002]

In chickens forced from 5 to 12 days of age to use spherical lenses with -10 to $+10$ of dioptric value, this visual manipulation caused astigmatism, myopia or hyperopia in 66-100% of the animals [Kee and Deng, 2008].

Myopia frequency varies by country and by ethnic group, from near 0% in Eskimo populations living in ancestral conditions [Young et al., 1969] and 0.8% in some Solomon Islands in 1966 [Verlee, 1968] up to 70-90% in some Asian populations [Chow et al., 1990; Wong et al., 2000].

“The marvellous vision of these primitive people [Australian Aborigines] is illustrated by the fact that they can see many stars that our race cannot see. In this connection it is authoritatively recorded regarding the Maori of New Zealand that they can see the satellites of Jupiter which are only visible to the white man's eye with the aid of telescopes. These people prove that they can see the satellites by telling the man at the telescope when the eclipse of one of the stars occurs. It is said of these primitive Aborigines of Australia that they can see animals moving at a distance of a mile which ordinary white people can not see at all.” [Price, 1939]

“Epidemiological surveys have shown that myopia is more prevalent in individuals who spend more time reading or performing close work than those who spend more time not using their eyes at near. Myopia has been correlated with the amount of school work and level of educational attainment. The process continues into the third decade of life with graduate students, microscopists, and military conscripts becoming more myopic with more near work. Studies of Aboriginal peoples and Inuits have shown increasing incidence of myopia correlating to the increased near work demands.” [Fredrick, 2001]

In shorts, evidence indicates that with the prolonged and frequent use of the eyes to see too much near objects in the childhood and in the juvenile age (reading of books, use of monitors, etc.), physiologic genes that have the beneficial function of regulating precisely eyeball growth to optimise the eyesight, cause an abnormal eyeball growth

determining refraction defects. Such refraction defects increase their frequencies insofar as the eye is used for too much near objects or, however, forced in ways for which the eye is not adapted.

But, studying two groups of 6- and 7-year-old school children of Chinese ethnicity, the first living in Singapore and the other in Sydney, with only two significant differences (Sydney children made more near-work activity and spent more time in outdoor activities), it was observed that the prevalence of myopia was only 3.3% in Sydney children and 29.1% in Singapore children [Rose et al., 2008b].

The idea that the direct exposition to natural light was the key factor has been confirmed by other studies [Rose et al., 2008a; Dirani et al. 2009].

In the absence of conditions of vision to which our species is not adapted, refraction defects are not developed, as shown by the case of Inuit populations living in ancestral conditions with near 0% myopia frequency [Young et al., 1969] and its increased frequency in correlation with near work demands [Fredrick, 2001]. “The increase in myopia prevalence observed in Hong Kong, Taiwan, Japan, and Singapore over the past few decades suggests an environmental risk factor, since the gene pool has not changed.” [Saw et al., 1996]

Consequently, except rare particular cases, the so-called “pathological” genes predisposing to myopia or to other refraction defects are indeed physiologic beneficial genes, favoured by natural selection, which on the contrary determine refraction defects in non-physiologic conditions to which the organism is not adapted.

Nutritional lacks (e.g., vitamin A deficiency) are important only in rare and particular cases.

The above-said considerations indicate that answers A-B-D are wrong and mirror well-established opinions according to which a great part of the human population would be unlikely afflicted by “pathological” genes causing refraction defects, whereas human populations living in non-modern conditions would be oddly exempt from such “pathological” genes. Moreover, these well-established opinions do not explain the extraordinary increase of refraction defects frequency observed in many populations in few decades and therefore without an impossible genetic general degeneration.

Negative definitions as (pathological) gene “predisposing to the defect of refraction X” should be substituted by positive definitions as (physiologic) gene “regulating ocular development”.

Evolutionary Medicine discriminates between “evolutionary” or “primary” causes (e.g., in the case of refraction defects: prolonged and frequent use of the eyes to see too much near objects in childhood and in juvenile age, and other conditions to which our eyes are not adapted) and “proximate” or “near” causes (in our case, physiologic genes that regulate precisely eyeball growth to optimise the eyesight and that in altered conditions determine refraction defects).

The meaning of genes as causes of refractive defects should be clearly specified. Except particular cases, physiologic and not pathological genes are essential in the pathogenesis, in combination with altered conditions that are the “primary” causes. Therefore, to document and maintain that those genes (without other specifications) are essential in refraction defects pathogenesis is misleading: this creates the wrong idea that there are largely diffuse pathological genes needing medical treatment while, on the contrary, pathological alterations of our ecological niche are the “primary” cause of refraction defects and preventive measures should be studied and applied. For evolutionary medicine: 1) refraction defects are the consequence of the inexistence or of the bad application of preventive measures; 2) medical treatment of refraction defects is the second-rate treatment of a medical failure.

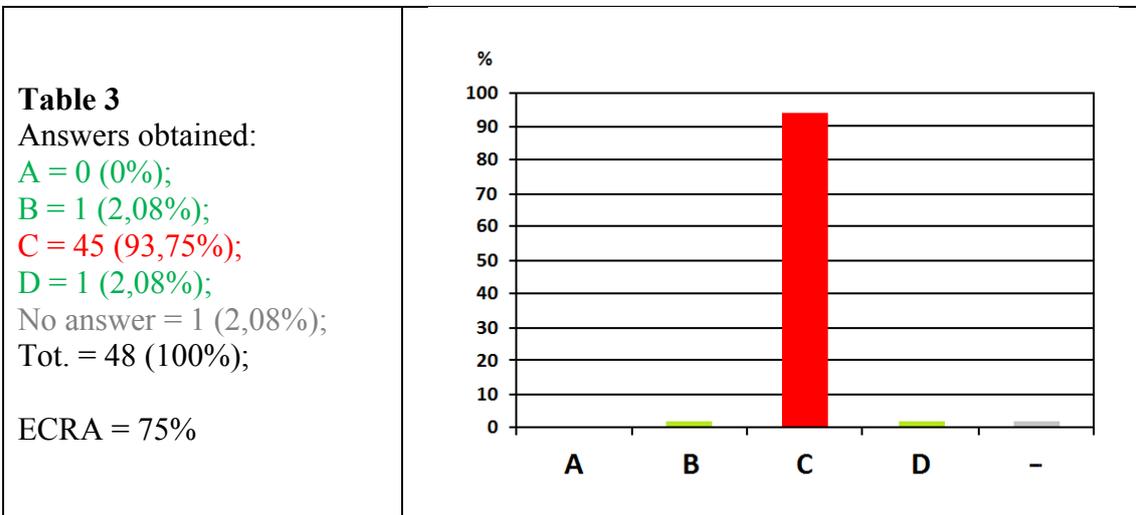
In shorts, the present extraordinary epidemic of refraction defects is not a consequence of insufficient medical treatment, but an outcome of the absence of preventive

measures, which would be an application of an essential statement of evolutionary medicine: it is absolutely necessary to identify and to modify alterations of ecological niche for which our genes are mismatched and that therefore cause diseases!

Question 3) Decays, crowded teeth and related complications are common diseases. Which of the following statements is true?

Answers:

- A) In 1932, in St. Moritz valley, girls were advised to extract all the teeth and to use a beautiful denture because with the marriage they would have lost all their teeth;
- B) In 1935, in Fiji islands, because the lack of dentists, the only cause of suicide was toothache;
- C) Modern dentistry and common rules of oral hygiene have greatly improved teeth conditions in comparison with preceding centuries and prehistoric age;
- D) It is possible to have healthy teeth practically for a whole population without dental treatments and without the use of toothpaste, toothbrush and any form of oral hygiene, in particular, not removing the tartar and the remainders of food between the teeth;



Correct answers: A, B and D

The correct answers are given with exceptional evidence by the extraordinary book of Dr. Price [Price, 1939] that, although published in 1939, is of real weight and relevance to the present and should be read with great care by all. It is documented that in prehistoric populations the teeth were practically exempt from caries, while in Neolithic populations, with the introduction of agriculture and the consequent modifications of diet, the teeth conditions worsened [Richards, 2002]. In modern populations, with the wide use of sugary drinks, foods with high sugar contents and poor in fibres, etc., the mean condition of teeth has further worsened in a catastrophic way. In the last decades, with the diffusion of modern dental treatment, the mean state of the teeth is in part improved without reaching the optimal condition of the Paleolith neither that partially deteriorated of the Neolithic.

Some quotations from Price's book (see also fig. 1):

(Chapter 2 – The progressive decline of modern civilization):

<In South Africa> “In not one of a very large collection of teeth from skulls obtained in the Matjes River Shelter (Holocene) was there the slightest sign of dental caries. The indication from this area, therefore, bears out the experience of European anthropologists that caries is a comparatively modern disease and that no skull showing

this condition can be regarded as ancient. [DRYER, T. F. Dental caries in prehistoric South Africans. *Nature*, 136:302, 1935.]”

(Chapter 5 – Isolated and modernized Eskimos):

“The excellence of dentitions among the Eskimos has been a characteristic also of the skulls that have been excavated in various parts of Alaska.

It might be expected that such wonderfully formed teeth would maintain so high an immunity to dental caries that their proud possessors would never be troubled with tooth decay. This, unfortunately, is not the case, a fact of great significance in evaluating our modern theories of the causes of dental caries. When these adult Eskimos exchange their foods for our modern foods, which we will discuss in Chapter 15, they often have very extensive tooth decay and suffer severely. This is clearly illustrated in Fig. 11, for these Eskimos' teeth had been seriously wrecked by tooth decay. They had been living on modern foods and were typical of a large number who are in contact with the Bering Sea ports. Their plight often becomes tragic since there are no dentists in these districts.

...

It is a matter of great significance that the Eskimos who are living in isolated districts and on native foods have produced uniformly broad dental arches and typical Eskimo facial patterns. Even the first generation forsaking that diet and using the modern diet, presents large numbers of individuals with marked changes in facial and dental arch form. In Fig. 12 will be seen four Eskimo girls who are of the first generation following the adoption of modernized foods by their parents. All have deformed dental arches. It is important to note the pattern of the settling inward of the lateral incisors and the crowding outward of the cuspids. This facial design is currently assigned to a mixing of racial bloods. These girls are pure-blooded Eskimos whose parents have normally formed dental arches.”

(Chapter 6 – Primitive and modernized North American Indians):

“This collection contains also skulls from several places and from prehistoric periods. The teeth are all splendidly formed and free from dental caries. The arches are very symmetrical and the teeth in normal and regular position.

It was important to study the conditions of their successors living in the same general community. Accordingly, we examined the teeth and general physical condition of the Indians in a reservation in North Vancouver, so situated that they have the modern conveniences and modern foods. In this group of children between eight and fifteen years of age, 36.9 per cent of all the teeth examined had already been attacked by dental caries. No people were found in this group who were living largely on native foods.”

(Chapter 10 – Isolated and modernized Australian aborigines):

“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.”

For modern Australian aborigines: “Those individuals, however, who had adopted the foods of the white man suffered extremely from tooth decay as did the whites.”

(Chapter 12 – Isolated and modernized New Zealand Maori):

“Since over 95 per cent of the New Zealanders are to be found in the North Island, our investigations were limited to this island. Our itinerary started at Wellington at the south end of the North Island and progressed northward in such a way as to reach both the principal centers of native population who were modernized and those who were more isolated. This latter group, however, was a small part of the total native population. Detailed examinations including measurements and photographic records were made in twenty-two groups consisting chiefly of the older children in public schools. In the

examination of 535 individuals in these twenty-two school districts their 15,332 teeth revealed that 3,420 had been attacked by dental caries or 22.3 per cent. In the most modernized groups 31 to 50 per cent had dental caries. In the most isolated group only 2 per cent of the teeth had been attacked by dental caries. The incidence of deformity of dental arches in the modernized groups ranged from 40 to 100 per cent. In many districts members of the older generations revealed 100 per cent normally formed dental arches. The children of these individuals, however, showed a much higher percentage of deformed dental arches.

These data are in striking contrast with the condition of the teeth and dental arches of the skulls of the Maori before contact with the white man and the reports of examinations by early scientists who made contact with the primitive Maori before he was modernized. These reports revealed only one tooth in 2000 teeth attacked by dental caries with practically 100 per cent normally formed dental arches.”

(Chapter 13 – Ancient civilizations of Peru):

“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.”

(Chapter 3 – Isolated and modernized Swiss):

“When parents were asked to permit their children to have one meal a day reinforced, according to a program that has proved adequate with my clinical groups in Cleveland, the objection was made that there was no use trying to save the teeth of the girls. The girls should have all their teeth extracted and artificial teeth provided before they were married, because if they did not they would lose them then.” (Answer A is correct!)

(Chapter 7 – Isolated and modernized Melanesians):

<in the years 1934-1936, in Fiji islands> “Abscessed teeth often cause suicide.” “No dentists or physicians are available on most of these islands. Toothache is the only cause of suicide.” (legend of fig. 32). (Answer B is correct!).

Modern dentistry has improved the teeth conditions in comparison with the dreadful state of the previous centuries, but certainly has not led back to prehistoric condition (Answer C is false). Lastly, it is true that Paleolithic populations, and those modern populations that live in conditions Paleolithic-like, have had, and have, healthy teeth without using any specific oral hygiene or dental treatment (Answer D is correct).

It is well documented that dental caries is caused by acid-producing bacteria (e.g., *Lactobacillus species*, *Streptococcus mutans*, etc.) acting in the presence of fermentable carbohydrates such as glucose, sucrose, and fructose [Rogers, 2008]. Evolutionary Medicine discriminates between “primary” causes (diet alterations) and “proximate” causes (acid-producing bacteria, which are physiologic commensal microbes and which without diet alterations do not cause dental caries): oral hygiene contrasts partially “proximate” causes and not “primary” causes and therefore its effectiveness is limited.

This question is peculiar because only one of the possible answers is wrong while the other three are correct. Therefore, a random reply had a 75% probability to be correct. In spite of this, the rate of wrong answers was 93,75%!

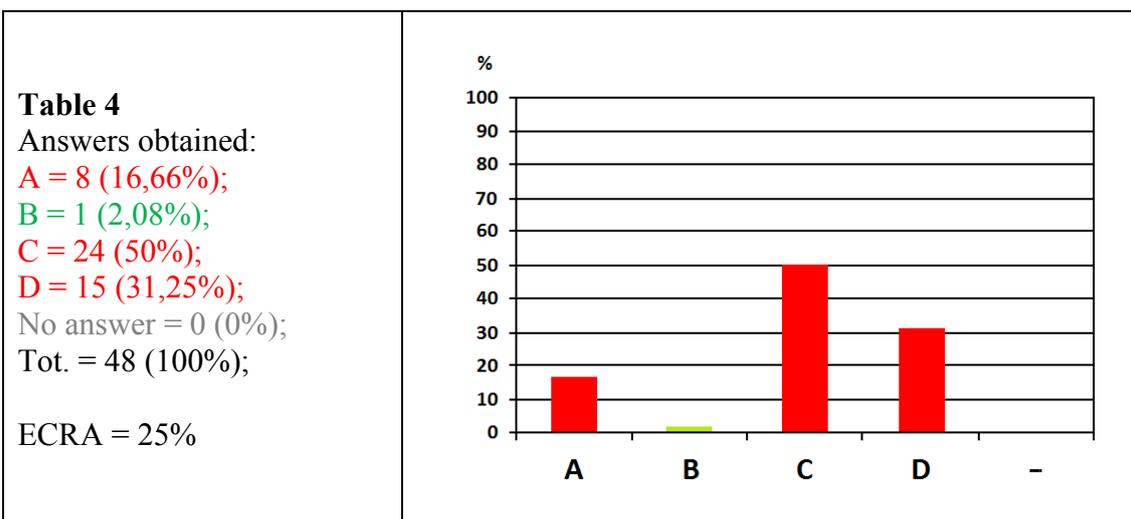


Figure 1 – A collage of photos from Price’s book. In the upper side, living natives and skulls of individuals from people observing ancestral dietary habits (without any form of oral hygiene), while in the lower side natives following modern diets.

Question 4) There are various pathological genes (and some malformations determined by genes) predisposing to atherosclerotic diseases in non-old subjects and to arterial hypertension.

Answers:

- A) This is true and the known number of such genes increases from year to year;
- B) Except for sporadic cases, the above-said diseases are not caused by pathological genes;
- C) The above-said diseases are caused by the interactions between pathological predisposing genes and excesses in alimentary habits;
- D) A mild hypertension and small atherosclerotic lesions are common and practically normal even in young subjects. Unfit drinks and foods in subjects with pathological genes predisposing to the above-said diseases increase this basic vulnerability up to pathological conditions;



Correct answer: B

In modern populations living in Paleolithic-like conditions, that is hunting and gathering peoples (e.g., some Australian aborigine populations, Hadza in Tanzania, !Kung of Kalahari desert in Botswana, Ache of Paraguay, Efé of the Democratic Republic of Congo, Agta of the Philippines), atherosclerotic diseases in non-old subjects and arterial hypertension are practically unknown [Trevathan et al., 2008]. This is not a consequence of short life spans as in these peoples more than 8% of the population exceeds 60 years of age [Blurton Jones et al., 2002]. Systolic arterial blood pressure found in !Kung populations is practically always about 120 mmHg at all ages [Truswell et al., 1972] [see fig. 2].

Excessive salt intake, hypercaloric and hyperlipidic diet, stress (in shorts, the so-called risk factors for hypertension and atherosclerosis) are all conditions to which our species is not adapted. Genes that are physiologic and favoured by selection in natural condition (otherwise their existence would not be justifiable) in such anomalous conditions provoke the above-said diseases [Eaton, Konner et al., 1988; William and Nesse, 1991; Nesse and Williams, 1994; Trevathan et al., 1999, 2008]. The use of negative terms as (pathological) genes “predisposing to atherosclerotic diseases / hypertension” is misleading and should be substituted with terms that describe positively their functions in the ecological conditions to which our species is adapted. Disease origin is in the alterations of the ecological niche and not in genes that are not pathological but physiologic and advantageous in natural conditions.

For these considerations, with certain exceptions, the attribution of the above-said diseases to the action of “pathological” genes or to the interaction between

“pathological” genes and risk factors is misleading. In the pathogenetic mechanisms underlying atherosclerotic diseases and hypertension, physiologic genes are certainly involved, but they are “proximate” and not “primary” causes and, however, they are physiologic and not “pathological” in the natural conditions to which our species is adapted (Answers A, C and D are wrong). Except for sporadic cases, the above-said diseases are always caused by alterations of the ecological niche to which our species is adapted (Answer B is correct).

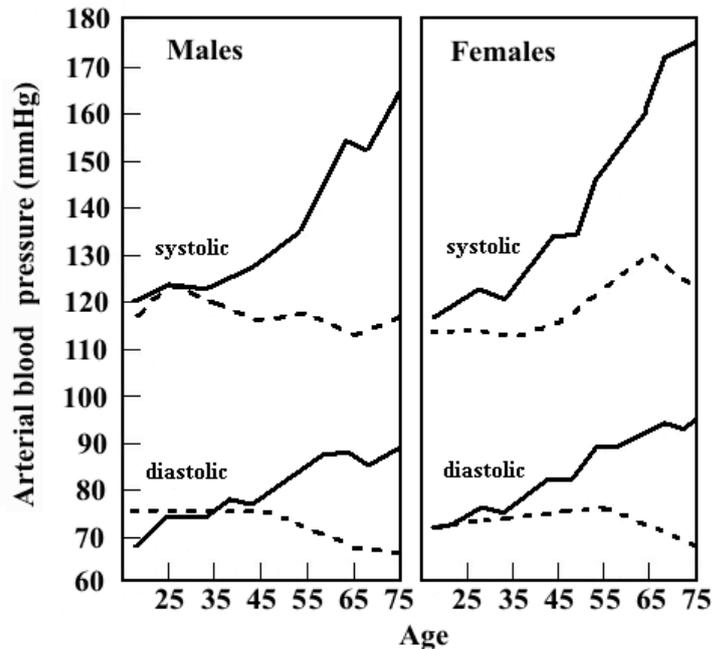


Figure 2 - Arterial blood pressures in !Kung individuals (dashed lines) and in London citizens (continuous lines) [Truswell et al., 1972] (partially redrawn).

Question 5) Type 2 diabetes or mature diabetes is determined by the interaction of pathological genes predisposing to diabetes and bad alimentary and lifestyle habits (excess of calories and fats in the food, sedentary lifestyle, etc.). Type 1A diabetes or immune mediated juvenile diabetes (the most common form of type 1 diabetes) is exclusively caused by predisposing pathological genetic factors. This is ...:

Answers:

- A) completely true;
- B) completely false. Except for sporadic cases due to genetic defects, both types of diabetes are caused by changes of life conditions to which man is adapted;
- C) true only for statements about type 1A diabetes. For type 2 diabetes, except for sporadic cases due to genetic defects, it is caused by changes of life conditions to which man is adapted;
- D) true only for statements about type 2 diabetes. For type 1A diabetes, except for sporadic cases due to genetic defects, it is caused by changes of life conditions to which man is adapted;

Table 5

Answers obtained:

A = 25 (52,08%);

B = 1 (2,08%);

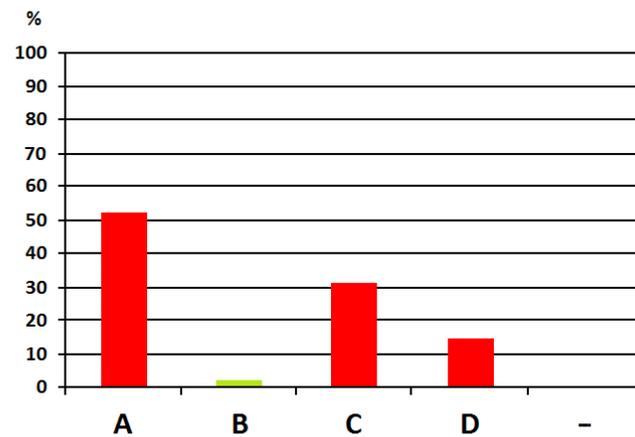
C = 15 (31,25%);

D = 7 (14,58%);

No answer = 0 (0%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: B

Worldwide diabetes frequency is high and rapidly increasing. It has been estimated to be 2,8% in 2000 (171 million cases) and 4,4% in 2030 (366 million cases) [Wild et al., 2004]. On the contrary, in populations living in Paleolithic-like conditions (e.g., “Broaya pastoralists who maintain a traditional nomadic life in the Sahara”) diabetes is practically unknown [Eaton, Shostak and Konner, 1988].

The rapid increase in diabetes frequency cannot be justified by a rapid increase in a single generation of the frequency of pathological genes. Likewise, the extreme difference in diabetes frequencies between populations living in ancestral-like conditions and those living in modern conditions cannot be caused by hypothetical pathological genes absent in some populations and largely present in others.

Hypercaloric and hyperlipidic diets, the use of food with high glycemic index (namely nourishment that provokes a rapid absorption of sugar), the scarcity of physical activity are all modern conditions to which our species is not adapted. Genes that are physiologic, advantageous and favored by selection in natural conditions (otherwise their existence would not be justifiable) cause in such anomalous conditions type 2 or mature diabetes [Eaton, Shostak and Konner, 1988].

The assertion that some genes (not defined as pathological) are involved in type 2 diabetes pathogenesis is correct but misleading as these physiologic genes are “proximate” and not “primary” cause of the disease.

Less known alterations of the ecological niche cause autoimmune diseases that provoke the destruction of beta cells of Langerhans and so type 1A or immune mediate juvenile diabetes.

“A wide (over 400-fold) variation exists in worldwide incidence rates of type 1 diabetes, with the highest occurring in Finland (over 45 per 100,000 under the age of 15 years) and the lowest in parts of China. In many countries (e.g. in Europe, the Middle East, Australia) the incidence of autoimmune-mediated type 1 diabetes in children <15 years of age has risen by 2-5% per annum.” [Silink, 2002]

“Type 1 diabetes is associated with other autoimmune conditions; the most common association is with thyroid disease. The Belgian Diabetes Registry indicated that the prevalence of thyroid peroxidase autoantibodies is 22% in patients with type 1 diabetes. Approximately 1 in 10 patients with type 1 diabetes express transglutaminase IgA autoantibodies, and more than half of these patients have coeliac disease on intestinal biopsy. Approximately 1 in 50 people with type 1 diabetes have 21-hydroxylase autoantibodies, and approximately 25% of these patients progress to Addison's disease.” [Devendra et al., 2004]

“The steady rise in the incidence of T1D [Type 1 Diabetes] in developed countries over the last three decades is a matter of major public health concern (Gale, 2002). Taken together with a very uneven distribution of the disease worldwide, with a North–South gradient, this increase in incidence prompted epidemiologists to determine the factors that could possibly explain this unfortunate trend. The fact that similar epidemiological features have been observed for other autoimmune diseases, notably multiple sclerosis, and for allergic diseases (Bach, 2002), suggests that the explanation is probably not essentially specific to T1D. There is epidemiological evidence for the causal role of the decrease of infections in the increase of incidence of T1D.” [Feillet and Bach, 2004]

According to “hygiene hypothesis”, helminth eradication and a too clean environment for young infants lead to an abnormal immunoregulation that causes autoimmune diseases as type 1A diabetes and allergy [Gale, 2002; Bach, 2002].

The weight of certain genes in type 1A diabetes pathogenesis is well known [Dejckhamron et al., 2007] but these genes are “proximate” and not “primary” causes of the disease. They cannot be defined “pathological” genes, as in the ancestral conditions to which our species is adapted both type 1A and type 2 diabetes are practically unknown [Eaton, Shostak and Konner, 1988].

The use of negative concepts as “genetic predisposition” to type 1A diabetes [Dejckhamron et al., 2007] and “genes responsible for type 2 diabetes” [Virally et al., 2007] is misleading and they should be substituted with terms that describe in a neutral or positive way their function in the ecological conditions to which our species is adapted. Type 1A diabetes origin, or “primary” cause, is in alterations of the ecological niche and not in genes that are not pathological in natural conditions.

For these considerations, to attribute, with certain exceptions, the two types of diabetes to the action of “pathological” genes or to the interactions between “pathological” genes and risk factors is misleading (Answers A, C and D are wrong). Except for sporadic cases, the two types of diabetes are always caused by severe alterations of the ecological niche to which our species is adapted (Answer B is correct).

Question 6) The theory of evolution, or evolutionism, has a huge weight to understand biological phenomena. Moreover, it has real importance for the comprehension of the origin of various genetic diseases (e.g., thalassemia). However, beyond its great weight for the scientific knowledge in general, and excluding some particular case as the explanation of the origin of some genetic diseases, which is the effective importance for everyday life and for ordinary medical practice?

Answers:

A) It is indispensable for medical daily practice and for any branch of medicine. A profound knowledge of evolutionary mechanisms is essential for health safeguard;

B) It has no direct importance for the ordinary medical practice and for health safeguard. However, the knowledge of evolutionary mechanisms is fundamental to the biological essential bases of medical knowledge.

C) It is important only in the case of antibiotic therapy strategies and in the treatment of infections and of worm parasitosis;

D) It is important only in the treatment of allergies and of autoimmune diseases;

Table 6

Answers obtained:

A = 22 (45,83%);

B = 24 (50%);

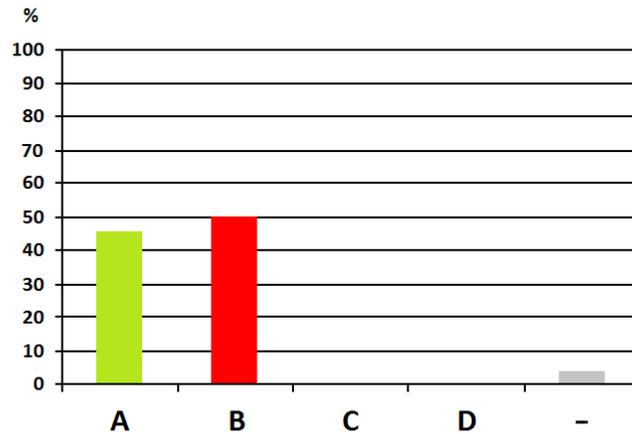
C = 0 (0%);

D = 0 (0%);

No answer = 2 (4,16%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: A

“*Evolution in Health and Disease* describes how evolutionary thinking gives valuable insights and fresh perspectives into human health and disease, establishing evolutionary biology as an essential complementary science for medicine. Integrating evolutionary thought into medical research and practice helps to explain the origin of many medical conditions, including diabetes, obesity, cardiovascular disease, asthma, allergies, other autoimmune diseases, and aging. It also provides life-saving insights into the evolutionary responses of pathogens to antibiotics, vaccinations, and other human interventions.” (from the back cover of the book of Stearns and Koella [Stearns and Koella, 2008]).

Evolutionary or Darwinian Medicine [Williams and Nesse, 1991; Nesse and Williams, 1994], a modern development of the work of Charles Darwin (1809-1882), is the practical application of evolutionary concepts to medicine.

With a shrewd reference to a famous statement of Dobzhansky [Dobzhansky, 1973], Nesse and Williams concluded their book saying “... nothing in medicine makes sense except in the light of evolution.” [Nesse and Williams, 1994]

Other clever statements from the same source are:

(Medical Education) “When evolution is included, it will give students not only a new perspective on disease but also an integrating framework on which to hang a million otherwise arbitrary facts. Darwinian medicine could bring intellectual coherence to the chaotic enterprise of medical education.”

(Clinical Implications) “An evolutionary approach does, however, suggest that many such treatments are unnecessary and that we should do the research to see if the benefits are worth the costs.”

(Public Policy Implications) “New ways of organizing medical care may finally provide incentives for dedicating substantial clinical resources to preserving health based on principles of Darwinian medicine.”

In 1983, it was written [Libertini, 1983]:

“... any biological phenomenon that is not strictly contingent, is after all an aspect, a side of the evolutionary process. With such assumption, the question about the possible definition of pathology in evolutionary terms comes out spontaneously. In other terms, the question is if disease phenomenon is an anomaly, an exception or, on the contrary, a phenomenon that is integral part of the evolutionary process. ...

From an evolutionary point of view, diseases are not something outside the norms, but, on the contrary, a set of various categories of phenomena, each evolutionarily “predictable” in its general essence. ...

Evolutionary approach to the concept of disease is the most rational and general possible. Any other more limited approach, even if more useful as regards a specific pathological problem, just because more limited and specifically oriented, must not be conceived in terms in contrast with evolution theory. ...

Evolutionary approach to the concept of disease originates spontaneously suggestions and basic interrogatives about prevention and treatment of the various categories of diseases.” (Ch. V — Evolution and pathology; translated from Italian; original text available at Library of Congress; digital copy at: http://www.r-site.org/ageing/index_e.htm.)

Many arguments about the practical utility of Evolutionary Medicine are expounded in the books of: a) Eaton et al. [Eaton, Shostak and Konner, 1988]; b) Nesse and Williams [Nesse and Williams, 1994]; c) Trevathan et al. [Trevathan et al., 2008]; d) Stearns and Koella [Stearns and Koella, 2008], in particular in the introductory chapters of c) and d). Evolutionary Medicine is essential for a correct understanding of “primary” causes of diseases, which are well distinct from “proximate” causes, and therefore for an effective primary prevention. For example, in the case of type 1A immune-mediated diabetes, in a review of the disease [Dejckhamron et al., 2007], the weight of genetic factors in pathogenesis is well documented, but the Authors do not state that these factors are only “proximate” causes and ecological alterations are the “primary” causes of the disease (and of other autoimmune diseases), a concept that explains the extreme variation of disease frequency (from practically zero in populations living in ancestral conditions [Eaton, Shostak and Konner, 1988] up to 45 per 100,000 under the age of 15 years in Finland [Silink, 2002]) and that could indicate efficacious preventive measures.

The correct answer is clearly A that is nearly the contrary of B. Answer C and D are true without the wrong limitation to particular arguments.

Question 7) In pregnant women, especially in the first months of gestation, nausea and refusal of particular foods as vegetables and meat are frequent. This phenomenon (morning sickness) is ...:

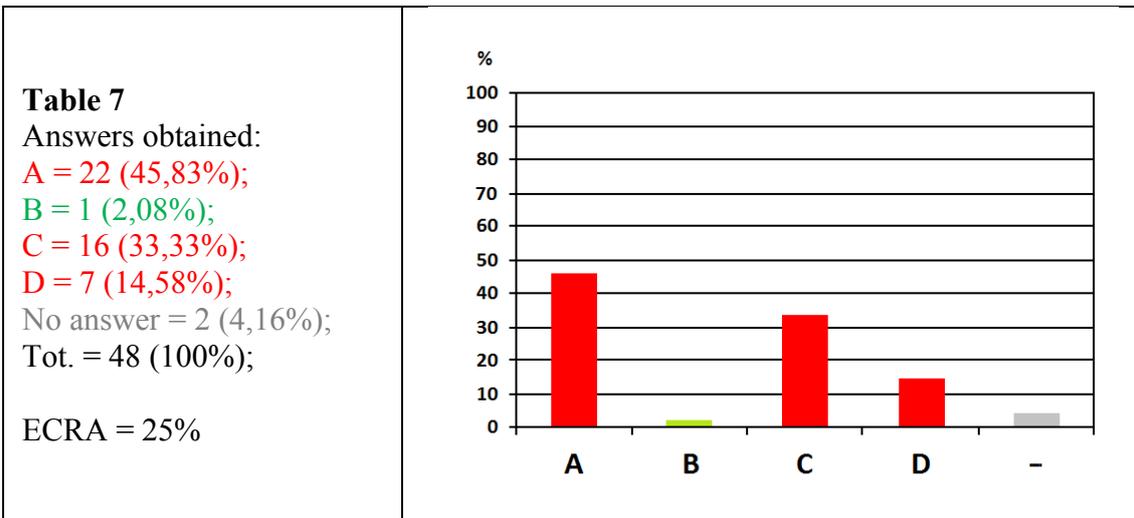
Answers:

A) a consequence of bradykinins increment caused by raised progesterone rates produced by placenta during foetal growth;

B) a phenomenon that is advantageous and therefore favoured by natural selection;

C) a phenomenon with many supposable causes, but always without any particular advantage for mother or foetus. Analogous phenomenon is the menstrual syndrome;

D) the consequence of contrasting evolutionary exigencies between foetus and mother in the first phases of gestation. Only for the following phases evolution has been able to develop an adequate equilibrium with no trouble and fitness alteration for the mother;



Correct answer: B

Nausea in pregnancy (morning sickness), provoked by particular foods, as strong-tasting vegetables, caffeine, alcohol, fish, meats, poultry, eggs, and in general foods with unusual smell or taste, is a defence of the mother against possible damages to the foetus deriving from potentially teratogen substances or infectious agents that could be present in particular foods [Flaxman and Sherman, 2000]. Therefore, the phenomenon is favoured by natural selection, being important for progeny safeguard (Answer B is correct). In support of this thesis, proposed by Margie Profet (1992) [Profet, 1992]: “(i) symptoms peak when embryonic organogenesis is most susceptible to chemical disruption (weeks 6-18), (ii) women who experience morning sickness are significantly less likely to miscarry than women who do not (9 of 9 studies), (iii) women who vomit suffer fewer miscarriages than those who experience nausea alone ... Animal products may be dangerous to pregnant women and their embryos because they often contain parasites and pathogens, especially when stored at room temperatures in warm climates. Avoiding foodborne microorganisms is particularly important to pregnant women because they are immunosuppressed, presumably to reduce the chances of rejecting tissues of their own offspring (Haig 1993). As a result, pregnant women are more vulnerable to serious, often deadly infections.” [Flaxman and Sherman, 2000] Answers A and D are false statements that try to seem likely. Answer C could be accepted by those not accepting the strong evidence in support of B.

Question 8) In many infectious diseases, there is a condition of iron deficiency causing anemia. Such condition ...:

Answers:

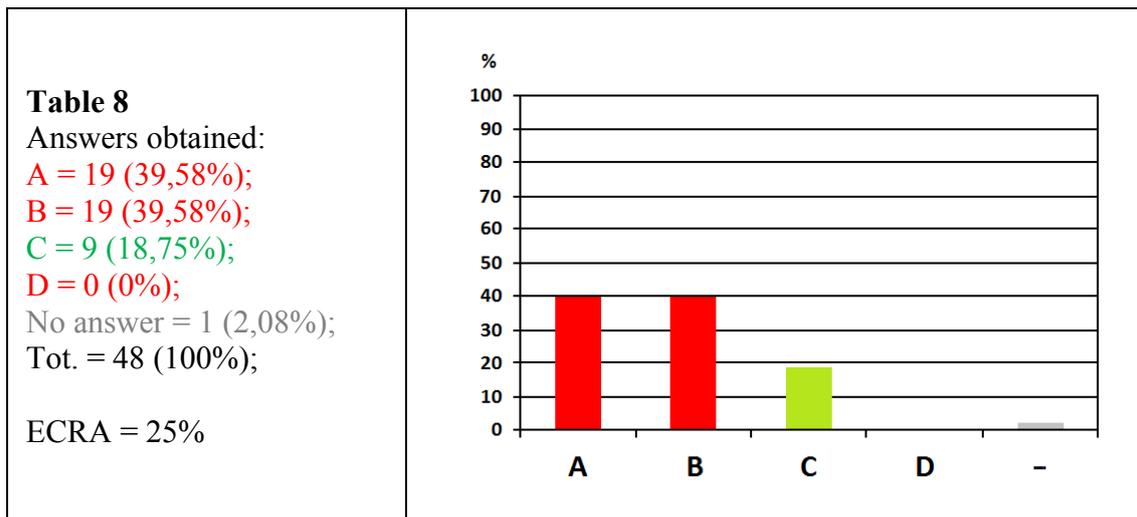
A) is benign and a moderate iron supply has no consequence about possible complications and mortality rate;

B) must be cured with a suitable iron supply as to remedy the observed lack because this reduces the probability of possible complications and, consequently, disease severity and mortality;

C) must not be counterbalanced with an iron supply because this increases the probability of possible complications and, consequently, disease severity and mortality;

D) must be treated with strong doses of iron so that to pass from a state of want that weakens the organism to a state of saturation that optimises organism defensive capacities. In wild conditions, iron scarceness has been evolutionarily one of the greater tie for organism efficiency and such a scarceness manifests itself crucially in critical

circumstances when an external help is useful and sometimes indispensable for the survival;



Correct answer: C

“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]

“There is convincing evidence that iron deficiency protects against many infectious diseases such as malaria, plague, and tuberculosis as shown by diverse medical, historical, and anthropologic studies.” [Denic and Agarwal, 2007]

Iron administration in Polynesian infants increased dramatically gram-negative neonatal sepsis cases and when iron administration was stopped sepsis decreased [Barry and Reeve, 1977].

“In a malaria-endemic population of Zanzibar, significant increases in serious adverse events were associated with iron supplementation” [Iannotti et al., 2006]

“Recent evidence from a large, randomized, controlled trial has suggested that the universal administration of iron to children in malaria-endemic areas is associated with an increase in adverse health outcomes.” [Prentice et al., 2007]

“In northeastern Tanzania, where malaria and iron deficiency are common, we found that placental malaria was less prevalent (8.5% vs. 47.3% of women; $P < .0001$) and less severe (median parasite density, 4.2% vs. 6.3% of placental red blood cells; $P < .04$) among women with iron deficiency than among women with sufficient iron stores, especially during the first pregnancy. Multivariate analysis revealed that iron deficiency ($P < .0001$) and multigravidity ($P < .002$) significantly decreased the risk of placental malaria.” [Kabyemela et al., 2008]

“Oral iron has been associated with increased rates of clinical malaria (5 of 9 studies) and increased morbidity from other infectious disease (4 of 8 studies). In most instances, therapeutic doses of oral iron were used. No studies in malarial regions showed benefits ... Experimental studies in laboratory animals uniformly show reversible deleterious effects of iron administration on tests of functional immunity. These may occur even in mild deficiency. ... Experimental and in vitro animal studies suggest that organisms that spend part of their life cycle intracellularly, such as plasmodia, mycobacteria and invasive salmonellae, may be enhanced by iron therapy.” [Oppenheimer, 2001]

In a large prospective, randomized, double-blind, placebo-controlled trial in the early 1980s, iron supplementation in Papua New Guinea infants was correlated with more

frequent clinical malaria, severe lower respiratory infections, acute otitis media, measles [Oppenheimer, 2001]

“Unless the host immune response is impaired by severe iron deficiency, there is rarely an urgency to supplement iron and it is likely to contribute little to host iron status due to the block on absorption associated with inflammation. In the presence of intracellular infections such as tuberculosis or chronic inflammatory or immunosuppressive diseases (e.g. HIV), the decision to supplement iron must be considered on an individual basis, because the potential exists to benefit a pathogen rather than the host.” [Doherty, 2007]

“Our bodies have a related defense mechanism, of which most people are unaware and which physicians sometimes unwittingly attempt to frustrate. Here are some clues about how it works. A patient with chronic tuberculosis is found to have a low level of iron in his blood. A physician concludes that correcting the anemia may increase the patient’s resistance, so she gives him an iron supplement. The patient’s infection gets worse.” [Nesse and Williams, 1994]

In shorts, iron availability is a limiting factor for the growth of pathogens. Host organism tries to protect itself from the infections reducing iron quantity that is available by pathogens and, consequently, blood iron levels are actively reduced in the case of infections. It follows that the answer C is correct and that the actions described in the other answers, which are unfortunately habitual for medical practice, increase the risks for treated subjects.

Question 9) The synthesis of the sulphamides, the discovery and/or the synthesis of penicillins, cephalosporins and of many other powerful antibiotics are a series of fundamental events in the history of medicine. The USA statistics show that ...:

Answers:

A) sulphamides and antibiotics have barely influenced mortality rates for infective diseases. Moreover, bacterial antibiotic-resistance phenomena are reducing their effectiveness and even causing an increasing number of undue deaths, especially in hospital;

B) only penicillins and cephalosporins have influenced in a drastic way the mortality reduction for infective diseases. On the contrary, for sulphamides no important effect is documented for their limited effectiveness. For other categories of antibiotics (macrolides, streptomycin, rifampicin, quinolones, etc.), the beneficial effect on mortality reduction results important but inferior to that observed for penicillins and cephalosporins;

C) sulphamides and antibiotics have enormously reduced the mortality for infective diseases. Their use is by far the most important progress in the history of medicine and without them we would still be victims of the dreadful infective epidemics of the past centuries. Unfortunately, bacterial antibiotic-resistance phenomena are reducing their effectiveness and even causing an increasing number of undue deaths, especially in hospital;

D) only antibiotics, and not sulphamides, have enormously reduced the mortality for infective diseases. Their use is by far the most important progress in the history of medicine and without them we would still be victims of the dreadful infective epidemics of the past centuries. Unfortunately, bacterial antibiotic-resistance phenomena are reducing their effectiveness and even causing an increasing number of undue deaths, especially in hospital;

Table 9

Answers obtained:

A = 0 (0%);

B = 4 (8,33%);

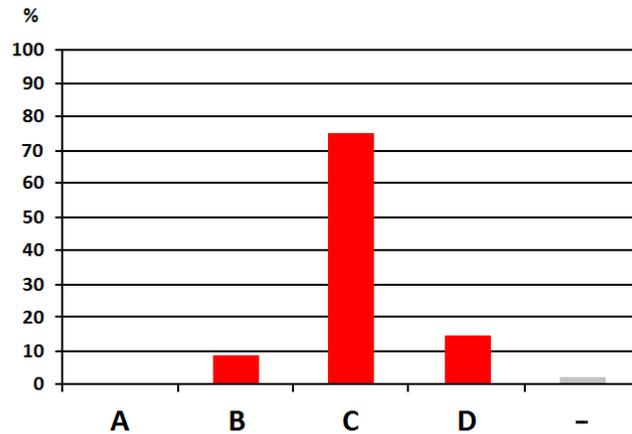
C = 36 (75%);

D = 7 (14,58%);

No answer = 1 (2,08%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: A

Everyone would be ready to maintain that the use of penicillins, and of other antibiotics afterwards developed, has saved the life to an enormous number of people. The USA statistics from the beginnings of '900 up to our days show a very different picture (fig. 3 and fig. 4) [Armstrong et al., 1999]. Mortality for infectious diseases was very high at the beginning of the past century because of very poor social, economic, house, alimentary, etc. conditions. In the next years, mortality has fallen with a strong annual rate that is not at all changed with the use neither of sulphamides nor of penicillins nor of more modern antibiotics. In spite of the availability of the best modern antibiotics, "In the United States, mortality due to infectious diseases increased 58% from 1980 to 1992, a trend that was unforeseen." a phenomenon "mainly due to the emergence of the acquired immunodeficiency syndrome (AIDS)" [Armstrong et al., 1999], favoured by particular harmful habits (AIDS is partially opposed by antivirals and better checked by preventive measures).

Statistics indicate that factors of social, economic, alimentary, etc. advance and lifestyle have a very strong and decisive action, whereas the effects of antibiotics are not evident and, no matter how, they are much small in comparison with these factors. Likely, most of the decline in mortality in the nineteenth century was caused by improved condition of life and not by direct medical actions [Generaux and Bergstrom, 2005].

Moreover, it is true too that the improper use of antibiotics, especially in hospital, has caused and causes the selection of mutant stocks of pathogens with greater virulence and often practically not treatable with antibiotics. As an example of how substances developed to fight the diseases caused by pathogens can provoke considerable damages: "In the United States alone, at least 200,000 people and probably far more suffer from a hospital-acquired infection every year. The associated mortality is considerable; the Center for Disease Control has estimated that 90,000 U.S. residents die each year from nosocomial infections. To place this number in context, AIDS/HIV kills approximately 17,000 per year in the United States, influenza 37,000 per year, and breast cancer roughly 40,000 per year. As large as these numbers are, some estimates suggest that the actual magnitude of the problem could be up to tenfold higher." [Bergstrom and Feldgarden, 2008].

These data prove that the correct answer is A. The mechanisms that are behind this seemingly paradoxical answer will not be comprehensible if a correct use of evolution knowledge is not applied.

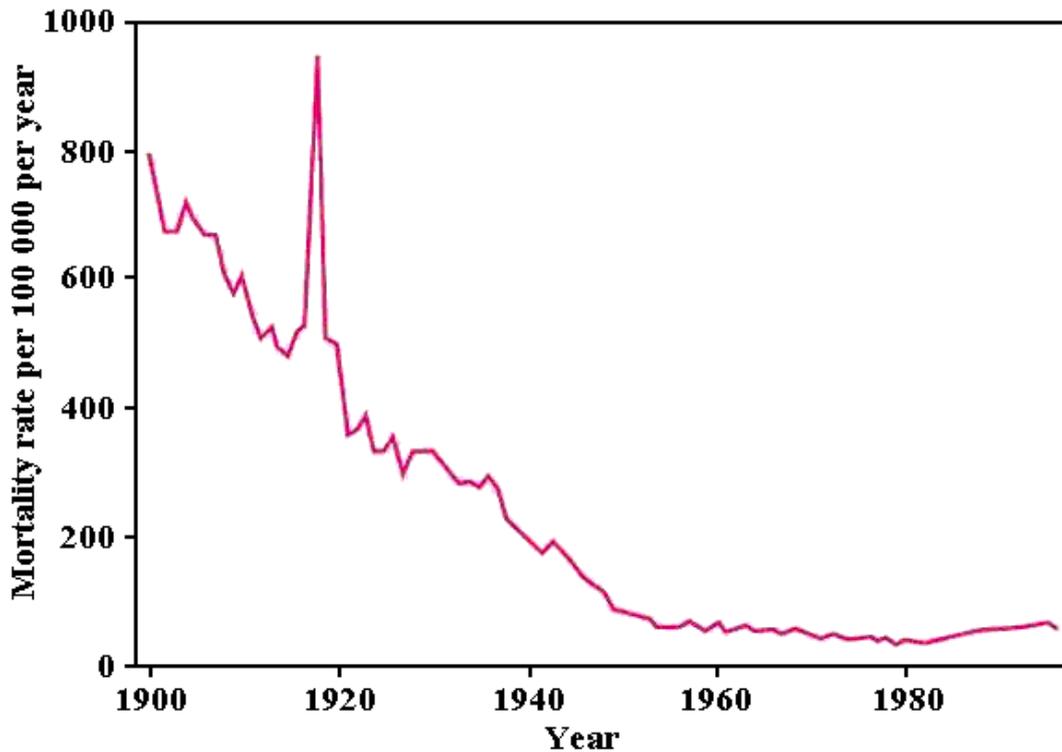


Figure 3 - Crude infectious disease mortality rate in the USA from 1900 through 1996 [Armstrong et al., 1999].

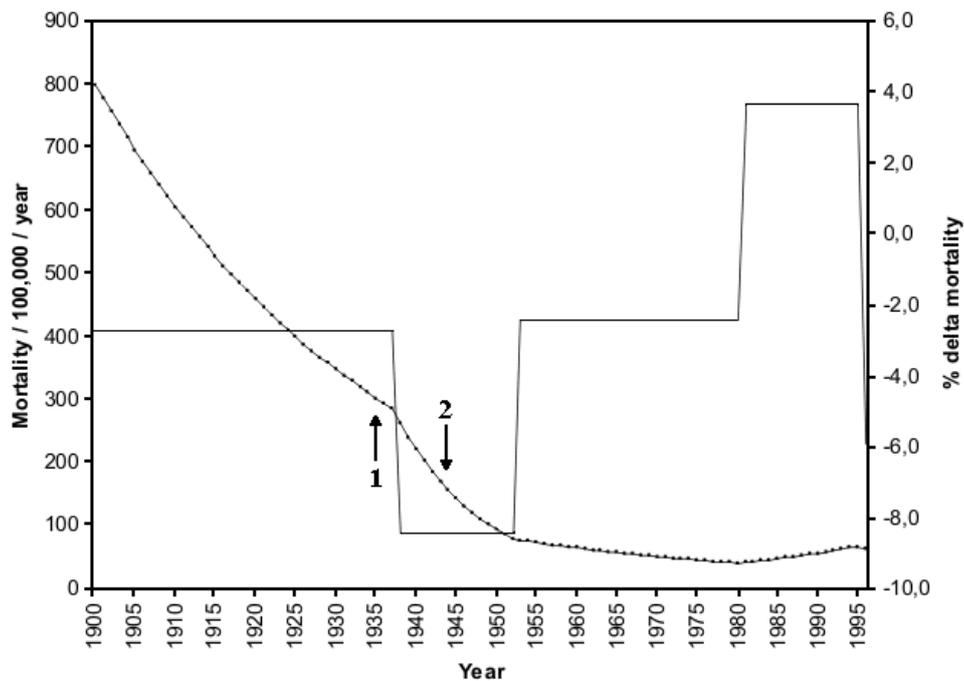


Figure 4 - Overall trends in infectious disease mortality rate and per cent variation of mortality rate in the USA from 1900 to 1996 [Armstrong et al., 1999]. The episodic strong increase of mortality due to 1918 influenza pandemic has been disregarded. Sulphonamides were released in 1935 (arrow 1) and the beginning of clinical use of penicillin was in 1943 (arrow 2) but there is no clear effect of their use on mortality rates.

Question 10) About 300 species of worms parasite for the man are known and there are many diseases caused by them. Worm infestations ...:

Answers:

A) are opposed effectively by appropriate antibiotics (vancomycin, macrolides and spiramycin, in particular) together with appropriate dietetic measures and, in some cases, laxatives and surgical remedies (e.g., in the case of hydatid cysts);

B) must be always opposed as causes of dangerous diseases and because compromise normal somatic and psychic development;

C) only in some cases must be opposed as they are useful for normal somatic development and for health;

D) are effectively opposed with drastic laxatives together with strong doses of orally administered sulphamides;

Table 10

Answers obtained:

A = 20 (41,66%);

B = 25 (52,08%);

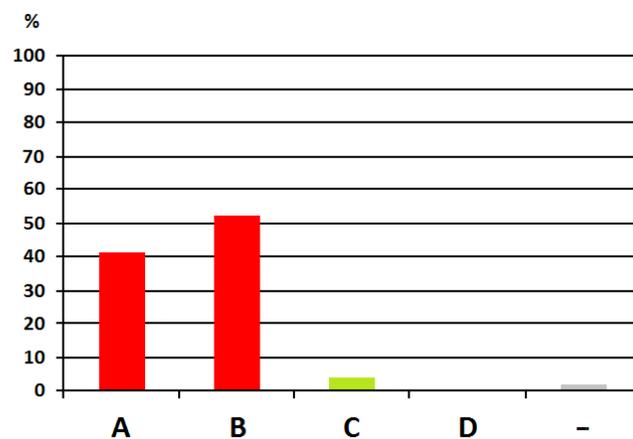
C = 2 (4,16%);

D = 0 (0%);

No answer = 1 (2,08%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: C

According to “hygiene hypothesis”, children exposed to viruses, bacteria and parasitic worms are protected against the onset of atopic diseases [von Mutius, 2002] and allergies [Cooper, 2004].

In general, in the western world, worm infestations do not cause severe troubles and rather result indispensable for a correct development of the immunologic system because our species has coevolved with the practically constant presence of worm infestations.

An example: “The most successful human helminth of the western world is the pinworm *Enterobius vermicularis*, and some 50% of young children in Europe and North America may have been infested around the middle of the twentieth century. Pinworms are benign, usually asymptomatic, and may have immunomodulatory properties that protect against the development of immune-mediated disorders including diabetes and asthma. Their decline in response to improved living conditions might explain a number of features of the epidemiology of childhood atopy and diabetes.” [Gale, 2002]

In particular, some intestinal worms secrete chemicals that suppress the immune system to prevent the host from attacking the parasite [Carvalho et al., 2006]. Without these substances, the immune system becomes oversensitive and unbalanced [Yazdanbakhsh et al., 2002].

Modern mass worm disinfestation has altered the delicate balance between our species and worms, causing serious autoimmune diseases. At present, some therapeutic techniques in experimentation try to treat autoimmune diseases modulating the

immunologic system with the deliberate infestation of sick subjects with a parasitic worm. “Helminthic therapy” seems a promising treatment for allergies [Falcone and Pritchard, 2005] and for several autoimmune diseases, as Crohn's disease [Hunter et al., 2004; Summers et al., 2005; Croese et al., 2006], allergic asthma [Falcone and Pritchard, 2005; Leonardi-Bee et al., 2006], multiple sclerosis [Correale and Farez, 2006], rheumatoid arthritis [Osada et al., 2008], etc., whose increasing incidence is greater in industrialised countries in comparison with developing countries with less strict hygienic habits [Leonardi-Bee et al., 2006; Zaccone et al., 2006; Rosati, 2001; Silman and Pearson, 2002; Weinstock et al., 2004].

However, it is true that many types of worm infestations cause severe and deadly diseases, but, as possible evolutionary explanation, this is caused by great alterations of our ancestral ecological niche provoked by human actions, in particular in the case of high demographic densities with polluted rivers used for drinking. This means that the primary causes of worm infestations epidemics are serious alterations of the habitats or of lifestyles and not a mere consequence of the existence of parasitic worms.

Consequently, only answer C is acceptable, but it should be supplemented with the necessity of the correction of altered habitats or lifestyles, where this is opportune.

Question 11) For the best definition of the normal parameters of the health conditions for man, which should be measured in the conditions when a population is on average in an optimal condition of health, it is necessary to consider (excluding subjects that are clinically sick or suffering from violent causes) a significant sample of the population ...:

Answers:

A) of the USA;

B) of the !Kung (Botswana);

C) of the Efé (Democratic Republic of the Congo);

D) of Todi, model town for the quality of life, and of towns similar in excellent quality of environment, food and lowest stress;

Table 11

Answers obtained:

A = 12 (25%);

B = 1 (2,08%);

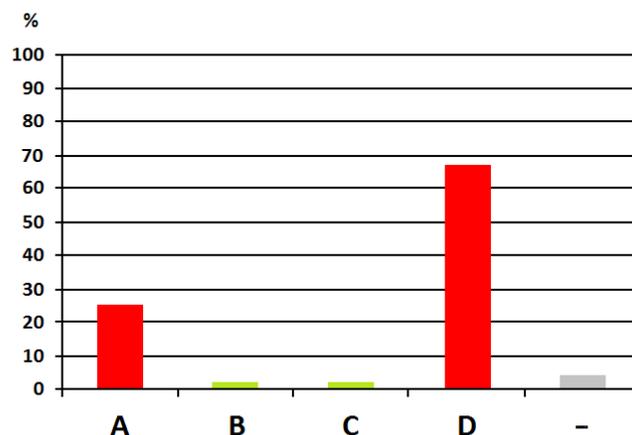
C = 1 (2,08%);

D = 32 (66,66%);

No answer = 2 (4,16%);

Tot. = 48 (100%);

ECRA = 50%



Correct answers: B and C

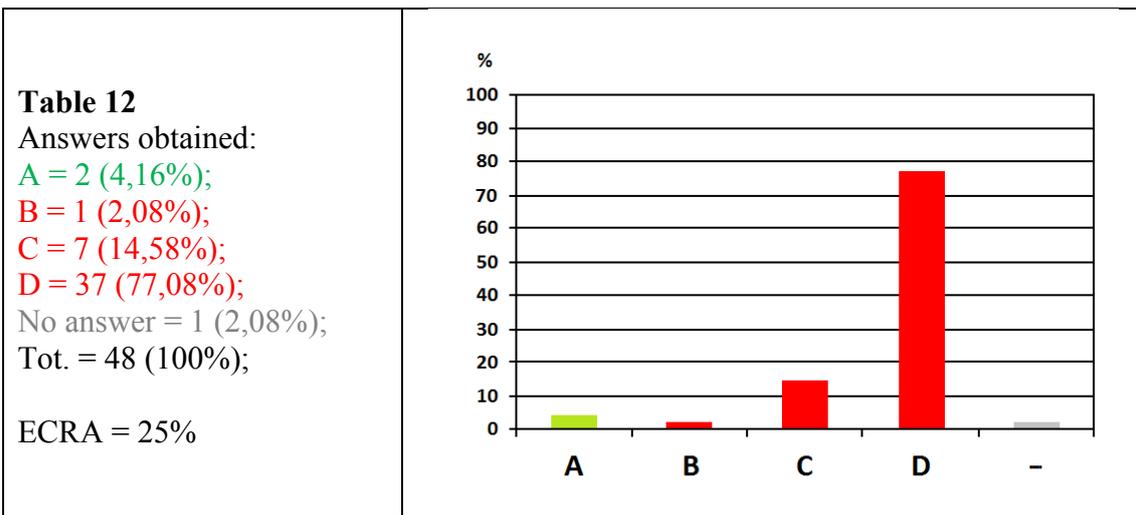
The genes of our species are adapted to Paleolithic-like conditions of life, that is of hunter-gatherer populations, and only partially to conditions of more recent ages (e.g., use of dairy products for some populations as a consequence of cattle-breeding and use of milk; use of starchy food as a consequence of agriculture developed by Neolithic populations; etc.) [Eaton, Shostak and Konner, 1988]. Only few populations of the

modern age live according to life conditions analogous to those of the Paleolith (e.g., the !Kung of Botswana, the Efé of the Democratic Republic of Congo, and few other populations [Eaton, Konner and Shostak, 1988]). Because of evolutionary mechanisms, the best health state should be found in life conditions analogous to those for which our genes are adapted and, in fact, among the !Kung and the Efé diseases as diabetes, hypertension, atherosclerotic diseases, caries, constipation and its complications, appendicitis, myopia, astigmatism, most types of cancer and of mental diseases, etc. are exceptional or practically inexistent [Eaton, Konner and Shostak, 1988]. Consequently, in such populations the normal or optimal values of all vital parameters must be measured (Answer B and C are correct) and not in populations as those of the USA living in conditions that are largely different from those that are the best for our genes. Therefore, answer A is wrong as (although less) answer D.

Question 12) Our species is afflicted by a great number of cancer types, with an increasing morbidity in the last decades because of the greater life span, and has been defined as “especially vulnerable” to cancer (Mel Greaves, 2008) in comparison with other animal species.

Answers:

- A) It is not true that our species is particularly vulnerable to cancer;
- B) It is true that our species is particularly vulnerable to cancer, as widely proved by statistics beyond any reasonable doubt;
- C) The vulnerability of our species to cancer is a probable consequence of our considerable longevity. Other species with considerable longevity (e.g., certain species of Rockfish that reach 100 years of age) show similar cancer frequencies in older individuals;
- D) It is true that our species is particularly vulnerable to cancer, but minimising with appropriate preventive measures the cases of cancer caused by polluting substances, environmental factors, bad life habits (e.g., smoke) cancer incidence could be reduced by about 70%;



Correct answer: A

There are species with considerable longevity in natural conditions that show mortality rates not increasing with age (“animals with negligible senescence”, e.g., some species of Rockfish with a longevity in wild of 100 years [Finch, 1990; Finch and Austad, 2001]) and therefore cannot have an age-related increment of mortality rate for cancer

[Libertini, 2008]. This demonstrates that cancer mortality is not necessarily in function of age (Answer C is wrong).

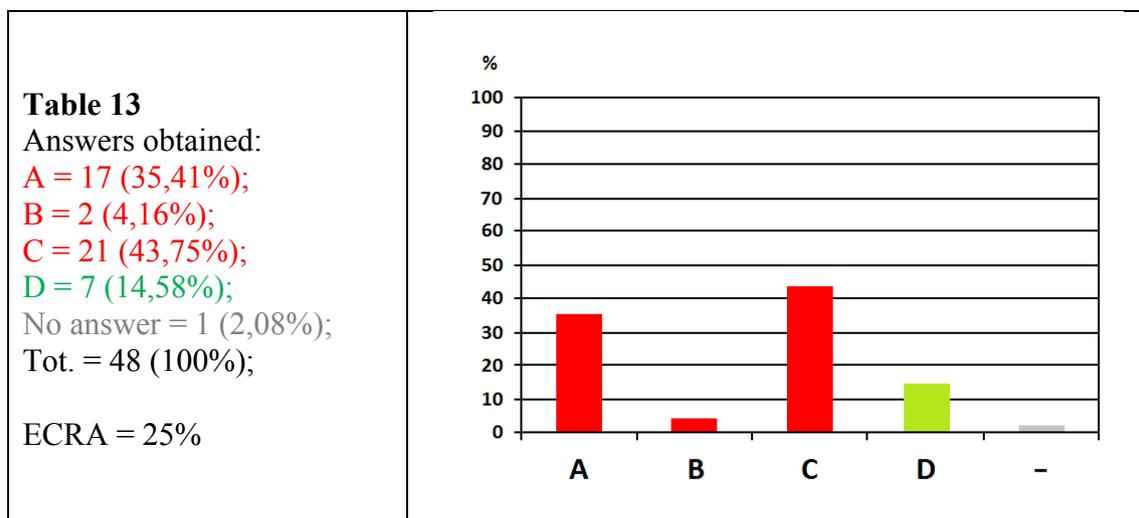
In the comparison of human populations living in modern conditions with populations of animal species that live in natural (or natural-like) conditions, the documented result is that our species is afflicted by many types of cancer while wild animal species seem to be almost exempt [Greaves, 2008]. This can create the impression that our species is particularly vulnerable to cancer [Greaves, 2008]. But, in the comparison of human populations living in Paleolithic-like conditions (e.g., !Kung of Botswana, Hadza in Tanzania, etc. [Trevathan et al., 2008, introduction]) with animal species reared in particular conditions, analogous to those of our species in civilised conditions, the opposite is documented: the above-said human populations are nearly exempt from cancer [Eaton, Konner and Shostak, 1988], a phenomenon not due to short life expectancy as more than 8% of individuals exceed 60 years of age [Blurton Jones et al., 2002], whereas reared animals show many cancer types [Greaves, 2008]. Greaves observes that for our species at least 90% of the cancers are caused not by genetic inheritance but by deliberate choices about diet and lifestyle [Greaves, 2000]. Populations living in Paleolithic-like conditions for which our genes are adapted and showing “near-absence of cancer” [Trevathan et al., 2008, introduction] demonstrate that cancers are caused almost exclusively by diets and lifestyles modified in comparison with those to which our genes are adapted.

All this proves that alterations of the ecological niche to which the species is not adapted is the primary condition causing cancer and not a specific genetic vulnerability to cancer of our (or of other) species. Therefore, the answer B is false and the answer D underestimates the importance of the alterations of our ecological niche, while the right answer is A.

Question 13) An accurate hygiene and a careful and continuous cleaning of all the rooms of daily life is ...:

Answers:

- A) one of the few achievements of the civilisation that has determined only advantages for the health;
- B) a sure way to cause many diseases, without any advantage;
- C) a sure way to prevent many diseases, without any disadvantage;
- D) a sure way to cause many diseases and to prevent many other diseases;



Correct answer: D

Comparing modern hygienic conditions with those of the previous last centuries, a huge improvement with consequent reduction of mortality due to infectious diseases is unquestionable. However, the correct term of comparison is not the dreadful urban conditions of the seventeenth or eighteenth centuries but the Paleolithic conditions to which our genes are adapted [Eaton, Shostak and Konner, 1988; Williams and Nesse, 1991; Nesse and Williams, 1994].

According to “hygiene hypothesis”, alterations of exposure modalities, especially in childhood, to infectious agents, symbiotic microorganisms, as gut and skin flora, and parasites, jeopardize the correct development and modulation of the immune system [Strachan, 2000; Gale, 2002; Bach, 2002; Bufford and Gern, 2005].

“Epidemiologic data provide strong evidence of a steady rise in the incidence of allergic and autoimmune diseases in developed countries over the past three decades. The incidence of many diseases of these two general types has increased: asthma, rhinitis, and atopic dermatitis, representing allergic diseases, and multiple sclerosis, insulin-dependent diabetes mellitus (type 1 diabetes) - particularly in young children - and Crohn’s disease, representing autoimmune diseases.” [Bach, 2002]

“Western countries are being confronted with a disturbing increase in the incidence of most immune disorders, including autoimmune and allergic diseases, inflammatory bowel diseases, and some lymphocyte malignancies. Converging epidemiological evidence indicates that this increase is linked to improvement of the socio-economic level of these countries, posing the question of the causal relationship and more precisely the nature of the link. Epidemiological and clinical data support the hygiene hypothesis according to which the decrease of infections observed over the last three decades is the main cause of the incessant increase in immune disorders.” [Bach, 2005]

The modern extreme hygiene does not allow a correct development of the immunologic system and provokes an increasing incidence of allergic [Leonardi-Bee et al., 2006] and autoimmune diseases, sometimes severe and deadly as type 1 diabetes or juvenile diabetes [Silink, 2002], rheumatoid arthritis [Silman and Pearson, 2002], ulcerative colitis and Crohn's disease [Weinstock et al., 2004], multiple sclerosis [Rosati, 2001], etc., which are practically nonexistent in ancestral conditions [Eaton, Shostak and Konner, 1988] while in modern populations are often combined (e.g., thyroiditis and type 1 diabetes [Huber et al., 2008]; celiac disease and autoimmune thyroiditis, type 1 diabetes, autoimmune liver diseases, inflammatory bowel disease [Ch’ng et al., 2007]; type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and Crohn’s disease [Zaccone et al., 2006]).

Consequently, answer D is correct, while B and C are incomplete and A is surely false and misleading.

Question 14) The great infective epidemics of the previous centuries were ...:

Answers:

- A) a consequence of the almost total absence of medical cures and by the inexistence of antibiotics, antiviral drugs and vaccines;
- B) an indirect consequence of the introduction of agriculture and of craftsmanlike and industrial technologies;
- C) caused mainly by the arrival of deadly infections from America;
- D) caused by the increasing pollution determined by craftsmanlike and industrial activities;

Table 14

Answers obtained:

A = 37 (77,08%);

B = 6 (12,5%);

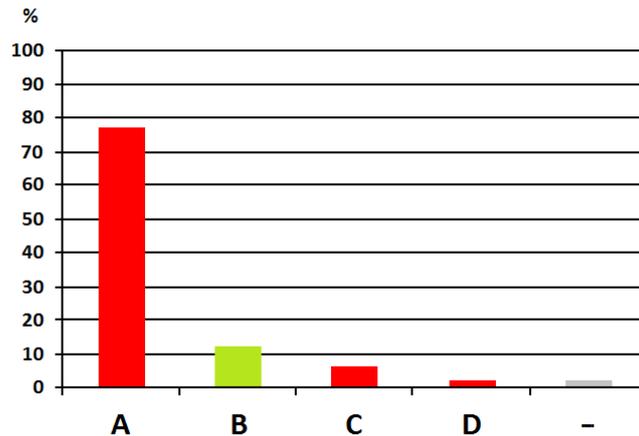
C = 3 (6,25%);

D = 1 (2,08%);

No answer = 1 (2,08%);

Tot. = 48 (100%);

ECRA = 25%



Correct answer: B

Changes in human demographics and society, contamination of food sources or water supplies, changes in land use or agricultural practices are indicated among the main causes of infections and epidemics [Woolhouse and Gowtage-Sequeria, 2005].

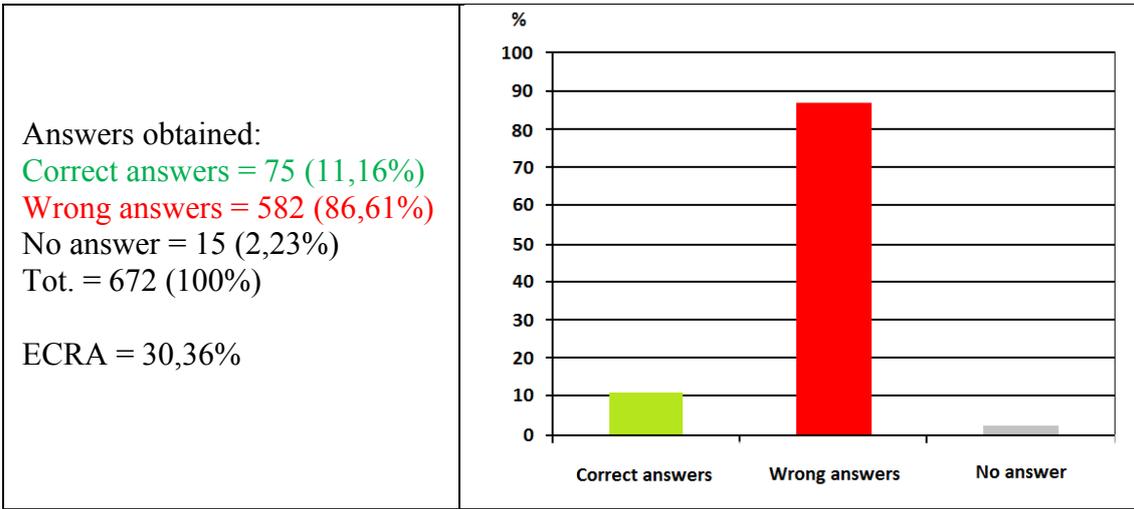
The great epidemics of the previous centuries were caused by a huge increase of demographic density and by population gathering in urban centres without sewer systems and with water frequently infected. Frequency and harshness of the epidemics of the previous centuries fell drastically with the construction of sewer and water supply systems and with the improvement of economic and alimentary conditions before the discovery and the use of sulphamides and antibiotics. For example, in the USA, the mortality deriving from infectious diseases has fallen before the introduction of sulphamides and antibiotics [Armstrong et al., 1999]. Smallpox vaccine has been one of the few great exceptions of a medical treatment that has had effectiveness and importance before the origin of modern medicine [Hopkins, 2002]. Consequently, answer A is false. With the discovery of America many dangerous infections (e.g., smallpox, measles, influenza, pertussis, parotitis, diphtheria, etc.) were transferred from the populations of the Old World (which were partially adapted to them for the dreadful epidemics endured in the previous centuries) to the populations of the New World that had no evolutionary experience about them and so sustained devastating and mortal epidemics [McNeill, 1976; Meltzer, 1992]. The opposite happened only for syphilis that became after Colombo a heavy scourge for European populations [McNeill, 1976] (Answer C is false).

The increasing pollution caused by craftsmanlike and industrial activities may have contributed somehow to historically more recent epidemics, but cannot have caused preceding epidemics (Answer D is false).

The introduction of agriculture and of craftsmanlike and industrial technologies, from Neolithic age on, has allowed an increasing demographic growth and, in parallel, the origin of greater and greater urban centres. These conditions are the requirement and the real or primary cause of infective epidemics of the past and present time. Therefore, the correct answer is B.

Overall results

The correct answers were only 11,16% and this low result should be assessed by considering that the value of the expected correct random answers (30,36%) was almost triple!



Only for two questions (1 and 6) the percentage of correct answers was greater than ECRA (29,16% and 45,83%, respectively). But, the relatively high number of correct answers to question 6 (Is evolutionism useful to practical medicine?) was in contradiction with the other answers expressed where possible indications of Evolutionary Medicine were disregarded or contradicted. The small number of correct answers to most question (3, 4, 5, 7, 9, 10, 11, 12) was outstanding, in particular for the two questions (3 and 11) were two or more answers were correct.

Conclusion

The percentage of correct answers, which was greatly lower than ECRA, indicates not only that in the medical field there is crass ignorance of Evolutionary Medicine, but also that there are deep-rooted prejudices against its basic concepts. The confusion between primary and secondary or proximate causes of diseases, the ignorance of the concept of mismatch between our adaptation and current lifestyles and ecological conditions, the thaumaturgic qualities attributed to antibiotics and similar substances are some examples of this ignorance and of the related prejudices. Unfortunately, the ignorance of the principles of Evolutionary Medicine is not just a problem of insufficient scientific knowledge, but something that has very serious consequences for the organization of health systems and for the prevention and treatment of diseases. The difference between knowing and not knowing Evolutionary Medicine is not a problem of higher or lower academic scores for health professionals, but a difference that is measured in a countless number of preventable diseases and deaths. It is absurd that malpractice cases, in which single individuals die or get worse, arouse a great deal of interest while there is a news blackout about the ignorance of the medical world of the principles of Evolutionary Medicine, a giant malpractice case with serious consequences for the health of billion people.

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SECTION III

EVOLUTION AND SEX

A simulation model for the evolutionary advantage of sex

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(SIBE, Società Italiana di Biologia Evoluzionistica)

Abstract

Evolutionary advantage of sex is a widely discussed topic with multiple and clashing conclusions. With the aim to show in which conditions sex is advantageous, it is formulated a simulation model based on the classic hypothesis that sex is advantageous because it allows a quicker attainment of favourable genetic combinations.

The model shows the substitution of two (or three) genes with two (or three) advantageous alleles and calculates in which conditions a further gene allowing recombination (R+) is advantaged or disadvantaged in comparison with an allele not allowing recombination (R-).

The simulation is based on the fact that real population are composed by a finite number of individuals and, therefore, any frequency variation – due to mutations, advantage, genetic drift, recombination, etc. – means a discrete variation in the numbers of involved individuals.

Mutation onset and genetic drift are simulated with the help of random numbers. Randomization is time-dependent so that a repetition of random sequence is practically impossible. Discreteness in the number of individuals is simulated with an algorithm that, in comparison with a binomial distribution, gives the same mean value and a variance almost identical except for values of the mean near 1.

In a very large population, where this discreteness has negligible importance, sex results neutral with any value of the advantage (S) of substituting alleles or of the mutation rate (U) with which they appear. In the case of an interaction (epistasis) between the advantages of the two substituting alleles, with no epistasis ($K=1$) sex is neutral, with positive epistasis ($K>1$) is disadvantaged, and with negative epistasis ($K<1$) is advantaged.

With two genes, the model shows that in small populations ($N = \log_{10}[\text{no. indiv.}] < 3.5$) and in large populations ($N \geq 10$) sex is not favoured, while in populations of intermediate size ($N > 3$ and < 10) sex is favoured. With three genes, the upper limit is shifted to >12 . Variations of U and S values modify the range in which sex is advantageous: a reduction / increase of U reduces / increases both lower and upper limits of the range; a reduction / increases of S reduces / increases only the upper limit.

Moreover, considering a population divided in z demes, each with N individuals, with a limited interdemetic gene flow, in the model the population is practically equivalent to a single population with $N \cdot z$ individuals.

In the simulations, if to a fraction p of R- individuals it is allowed to recombine, with small values of p, the advantage of sex rapidly fades.

Because time and energy is necessary for mating, namely that sex involves a disadvantage and that this advantage is greater in certain ecological conditions and lower in others, it is possible to predict in which ecological conditions sexual / asexual species of the same phylum / taxonomic group (or sexual / asexual stages of the same species) will prevail.

Predictions of the classic theory with the above said specifications are compared with predictions of other hypothesis (best-man, tangled bank, red queen, Muller's ratchet) and with the data from natural observation.

The classic theory results confirmed by empirical evidence while the other theories are contradicted.

Links:

Personal site = http://www.r-site.org/ageing/index_e.htm

A simulation model for the evolutionary advantage of sex

Giacinto Libertini

www.r-site.org/ageing

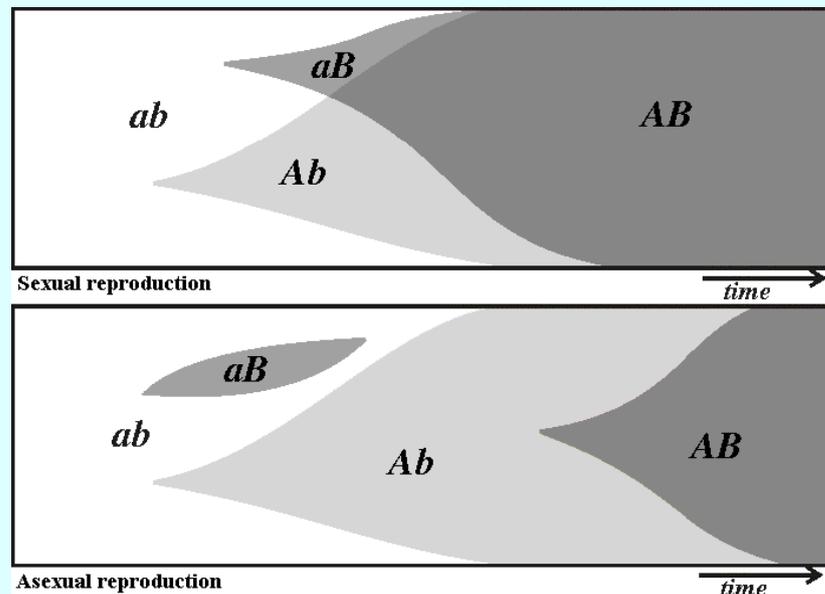
www.programmed-aging.org

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The term “sex”, synonymous of “mixis”, means the recombination of genes between two individuals.

The term “recombination” is not a synonymous of “sex”, because there are species that recombine genes within a single individual and without mating (e.g., autogamy, Bell 1982)

The “classic” theory (Weismann 1889; Guenther 1906; Fisher 1930; Muller 1932, 1958 and 1964; Crow & Kimura 1965) hypothesizes that sexual reproduction is advantageous because it allows the combination of new advantageous alleles while for asexual reproduction this is not directly possible.



Maynard Smith (1968) criticised the “classic” hypothesis with the following argument:

In a haploid species, we have two genes (a, b), with alleles (A, B, respectively) having an advantage (s_A, s_B) on a and b, respectively. The frequency of a combination xy at the generation n+1 (P'_{xy}) is in function of combination frequency at generation n (P_{xy}):

$$P'_{ab} = P_{ab} / T; \quad P'_{Ab} = P_{Ab} \cdot (1 + s_A) / T$$

$$P'_{aB} = P_{aB} \cdot (1 + s_B) / T; \quad P'_{AB} = P_{AB} \cdot (1 + s') / T$$

where:

$$s' = [(1 + s_A)(1 + s_B) - 1] \cdot k;$$

k = interaction (epistasis) between the fitnesses;

T = the sum of numerators.

If, at generation zero, there is no linkage disequilibrium, namely: $P_{ab} \cdot P_{AB} = P_{Ab} \cdot P_{aB}$

and no epistasis ($k = 1$), in the next generation it will be always: $P'_{ab} \cdot P'_{AB} = P'_{Ab} \cdot P'_{aB}$

with or without recombination, which can only halve linkage disequilibrium at each generation. Therefore, with these conditions sex is not advantageous.

With negative linkage disequilibrium ($D = P_{ab} \cdot P_{AB} - P_{Ab} \cdot P_{aB} < 0$) sex would be advantageous, while with positive linkage disequilibrium sex would be disadvantageous.

With positive epistasis ($k > 1$) between s_A and s_B , sex results disadvantageous because it breaks the more advantageous combination AB. The opposite happens if there is negative epistasis ($k < 1$).

Maynard Smith tried to overcome his argument observing that it was valid for infinite populations, but that “linkage disequilibrium is bound to arise by chance in a finite population” and in conditions of negative linkage disequilibrium sex would be advantageous (Maynard Smith 1976), as previously observed by Felsenstein (1974).

Many scholars did not accept the arguments of Maynard Smith (e.g., Crow & Kimura 1969, Williams 1975) and, however, why conditions of negative linkage disequilibrium, favorable for sex, should prevail over positive occurrences?

The doubts about the validity of the “classic” explanation of sex caused the flourishing of alternative hypotheses:

- Muller’s Ratchet (Muller 1964; Felsenstein 1974)
- Best-Man hypothesis (Williams 1966; Emlen 1973; Treisman 1976)
- Hitch-hiker hypothesis (Hill & Robertson 1966; Felsenstein 1974)
- Tangled Bank (Ghiselin 1974; etc.)
- Red Queen (Van Valen 1973; Glesener & Tilman 1978; Bell 1982; Ridley 1993, etc.)
- Sex is advantageous because it slows down evolution and excessive specialization (Williams 1975; Stanley 1976)
- Historical hypothesis (Williams 1975)

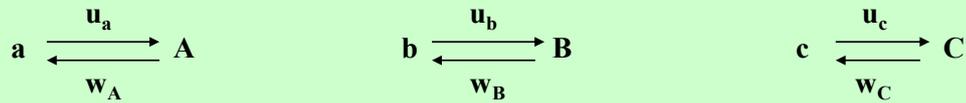
The crisis of the “classic” theory and the absence of a valid and undisputed alternative require the development of a reliable simulation model capable to confirm or falsify the classic theory:

- confirming the valid theoretical argument of Maynard Smith about the absence of advantage of sex in populations in linkage equilibrium;
- showing that sex is advantageous in finite populations;
- avoiding any bias on the prevalence of sex favoring conditions as negative epistasis ($k < 1$) or negative linkage disequilibrium
- avoiding any hypothesis about advantages of sex for the species or for successive generations.

The simulation model

We hypothesize two (a, b) or three (a, b, c) genes present in all the haploid individuals of a population composed by N individuals.

From these genes, more advantageous alleles (A, B, C, respectively) originate with frequency u_x . The frequency of inverse mutation is w_x :



The advantages of A, B, C on a, b, c are s_A, s_B, s_C , respectively.

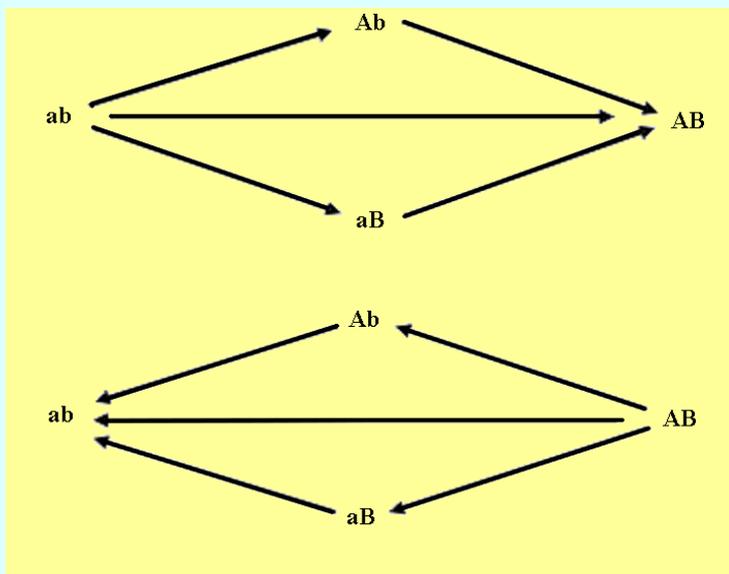
For simplicity, we suppose:

$$u = u_a = u_b = u_c$$

$$w = w_A = w_B = w_C$$

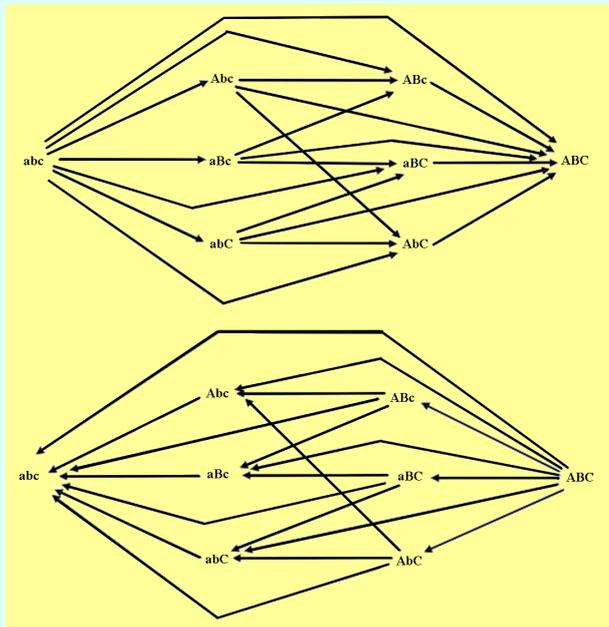
$$s = s_A = s_B = s_C$$

In the case of only two genes (a, b), the transformation of a combination to another is illustrated by the following schemes:



From	To	Prob.
aB	AB	u
Ab	AB	u
ab	aB	$u-u^2$
ab	Ab	$u-u^2$
ab	AB	u^2
aB	ab	w
Ab	ab	w
AB	aB	$w-w^2$
AB	Ab	$w-w^2$
AB	ab	w^2

In the case of three genes (a, b, c), the transformation of a combination to another is illustrated by the following schemes:



From	To	Prob.
ABc	ABC	u
aBC	ABC	u
AbC	ABC	u
Abc	ABc	u-u ²
Abc	ABC	u ²
aBc	ABC	u ²
abC	ABC	u ²
abc	Abc	u-2u ²
abc	aBc	u-2u ²
abc	abC	u-2u ²
abc	ABc	u ² -u ³
abc	aBC	u ² -u ³
abc	AbC	u ² -u ³
abc	ABC	u ³

From	To	Prob.
Abc	abc	w
aBc	abc	w
abC	abc	w
ABc	Abc	w-w ²
ABc	abc	w ²
aBc	abc	w ²
abC	abc	w ²
ABC	ABc	w-2w ²
ABC	aBc	w-2w ²
ABC	AbC	w ² -w ³
ABC	aBc	w ² -w ³
ABC	abC	w ² -w ³
ABC	abc	w ³

The advantage (s') for a combination with 2 more advantageous alleles is given by the formula:

$$s' = [(1 + s)^2 - 1] \cdot k$$

where k is the interaction (epistasis) between the two advantages.

By Maynard Smith, sex is predicted to be advantaged with positive epistasis ($k > 1$) and with positive linkage disequilibrium:

$$D = P_{ab} \cdot P_{AB} - P_{Ab} \cdot P_{aB} > 0$$

and disadvantaged with opposite conditions.

The advantage (s'') for the combination with all the three more advantageous alleles (ABC) is given by the formula:

$$s'' = [(1 + s)^3 - 1] \cdot k^2$$

In the population there are two alleles:
 R- -> not allowing recombination
 R+ -> allowing recombination with other R+ individuals.

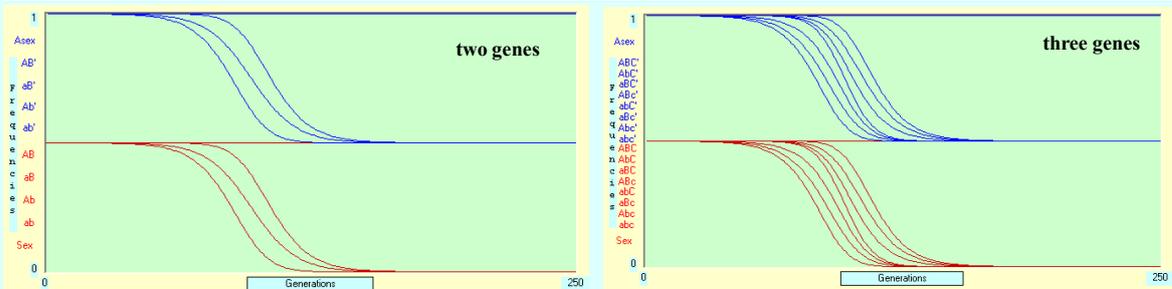
In the model, any disadvantage of sex is disregarded.

At zero generation, the frequencies of R+ and R- (R_{+0} , R_{-0}) are always 0.5

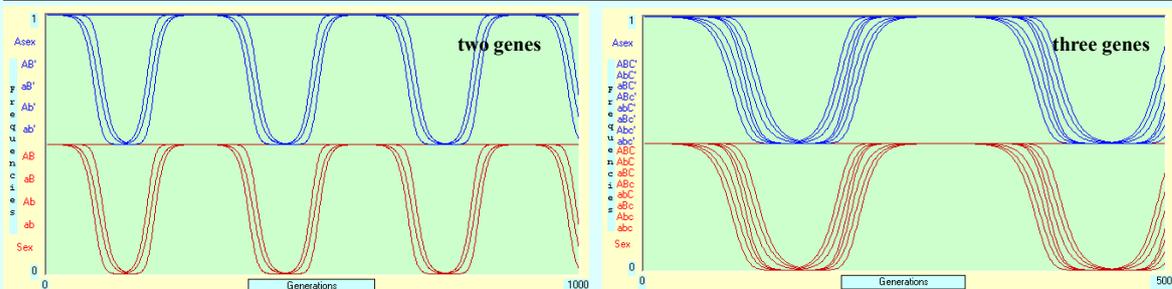
The simulation model should show whether after a certain number of generations (e.g., 250) the frequency of R+ (R_{+250}) is greater than R- frequency (= sex advantageous) or less (= sex disadvantageous).

With these conditions and using the above said formulas and others that simulate recombination for R+ individuals, a first simulation model has been created. No expedient has been used to simulate finiteness of population. The results are very simple and confirm Maynard Smith's observations.

With $k=1$ (no epistasis), sex is neutral with any value of u , w or s

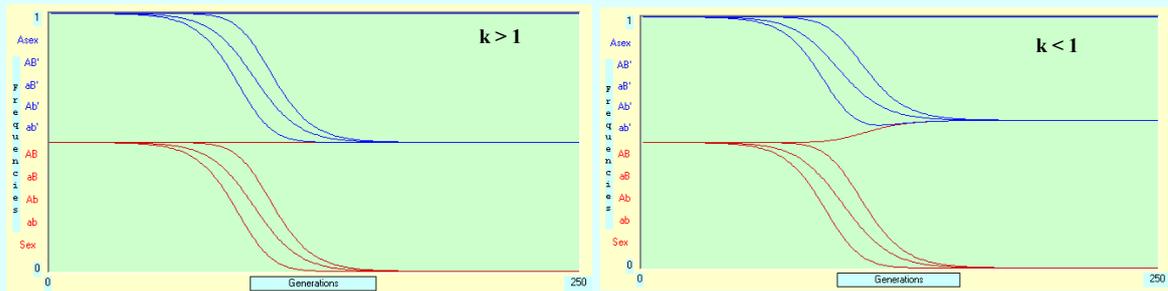


With varying values of s (in the simulations, s oscillates from -0.1 to $+0.1$ each 150 generations), sex is neutral too

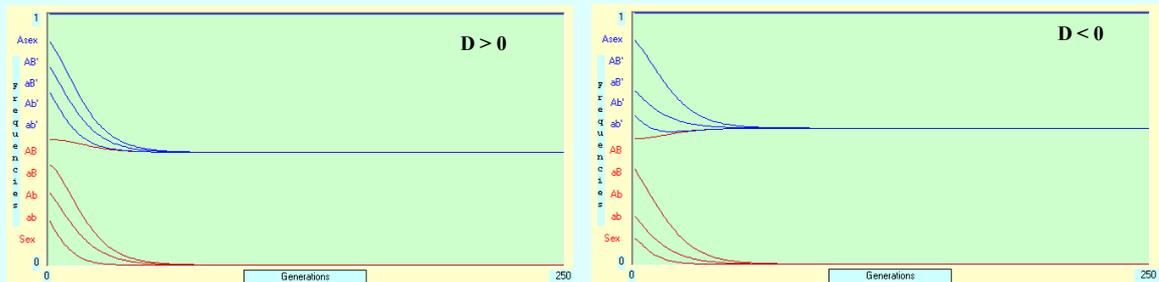


This means that Red Queen hypothesis, which explains sex as due to oscillating values of advantages caused by continuous interactions with other species, is insufficient to justify sex.

If $k > 1$ (positive epistasis), sex is disadvantageous, and, on the contrary, if $k < 1$ (negative epistasis), sex is advantageous, as predicted by Maynard Smith ($k = 1.03$ in the left figure; $k = 0.97$ in the right figure):



With positive linkage disequilibrium sex is disadvantaged, while with negative linkage disequilibrium sex is advantaged, as predicted by Maynard Smith ($D = +0.04$ in the left figure; $D = -0.04$ in the right figure):



However, it is unlikely and undocumented to justify sex as caused by prevailing conditions of negative epistasis or of negative linkage disequilibrium

As a matter of fact, it is indispensable a modification of the model, because real population are finite and discrete, namely not composed by fractions of individuals but by integer and finite numbers of individuals:

if for mutation, advantage, recombination, genetic drift or other, the frequency of a combination passes from a frequency XY_n at generation n to a frequency XY_{n+1} in the next generation with an increment Δ_{XY} , this increment must be always an integer number.

In the 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 method of “rbinom emulation” is used in the program to simulate the variations of frequencies due to:

- Mutations (e.g., a -> A)
- Inverse mutations (e.g., A-> a)
- Advantage
- Recombination
- Genetic drift
- Diffusion of combinations among demes (when the population is composed by more than one deme)

At each generation, rbinom emulation is used many times as described in the following table, which illustrates simulation times too.

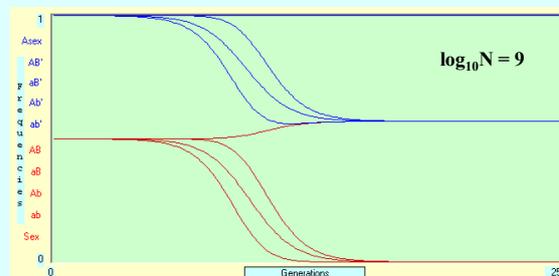
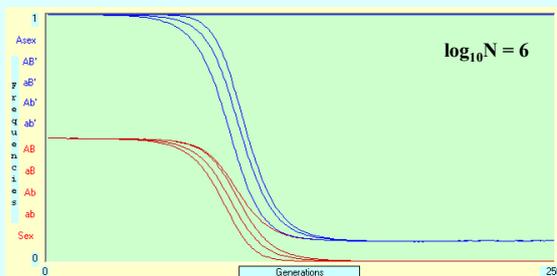
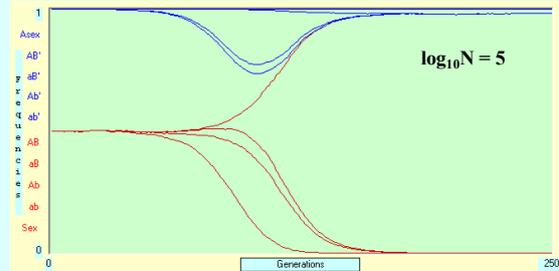
	Calls of rbinom emulation program routine		Simulation times* for 23 groups (with log ₁₀ N = 1 to 12, step 0.5) of 1,000 simulations for each group	
	2 genes case	3 genes case	2 genes case	3 genes case
1 deme	38 calls	114 calls	7 minutes	20 minutes
10 demes	460 “	1,300 “	52 “	166 “
100 demes	4,600 “	13,000 “	491 “	1,618 “

* on a 2.13 GHz PC

(RESULTS)

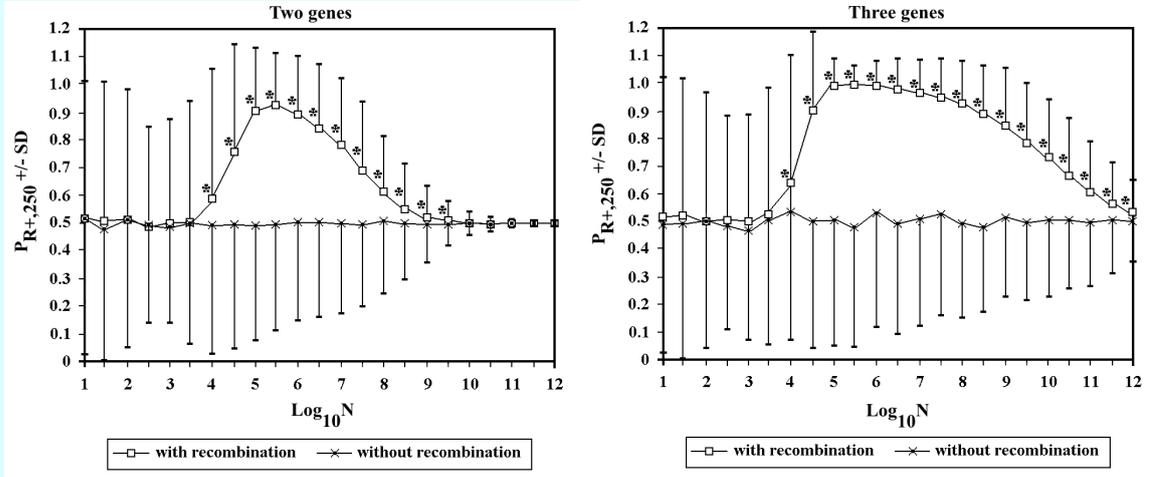
Examples of single simulations

(with $u = w = 0.00001$; $s = 0.1$; $k = 1$; and $\log_{10}N = 3, 5, 6, 9$ respectively)



(RESULTS)

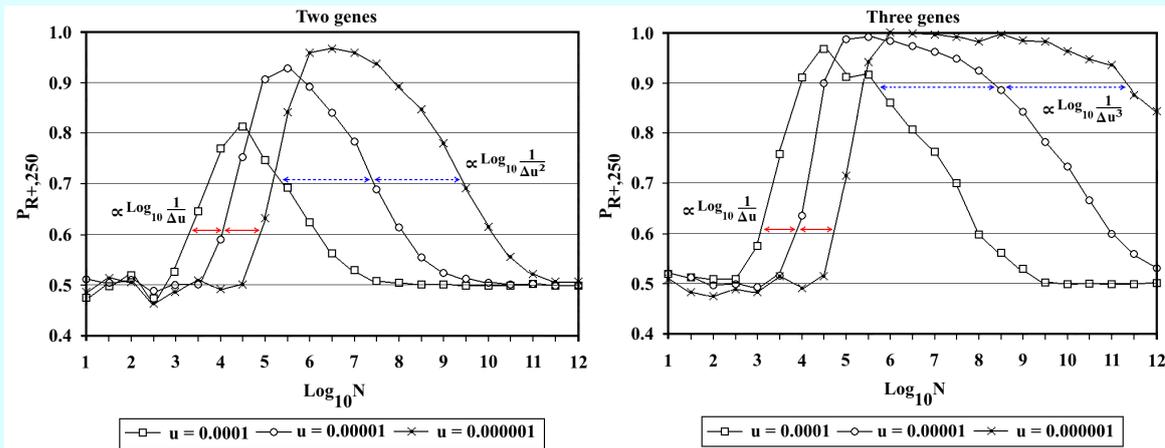
Series of simulations with $\log_{10}N$ varying from 1 to 12 step 0.5 (means and S.D. of 1,000 simulations for each point) and with $u = w = 0.00001$; $s = 0.1$; $k = 1$



*** = $p < 0.001$**

(RESULTS)

Series of simulations
Same conditions of the first series, but varying the value of u

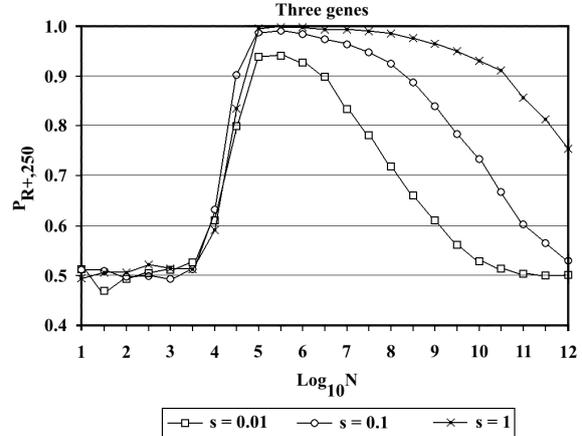
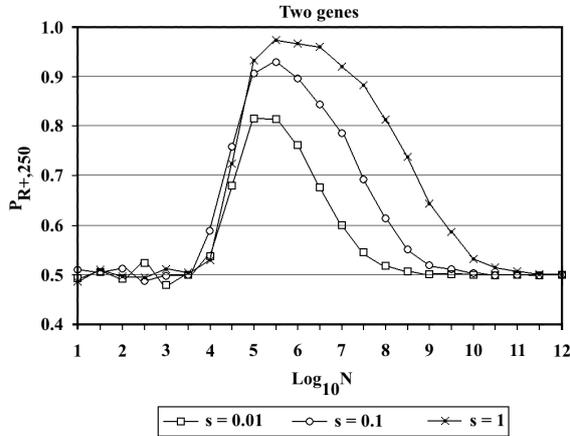


In logarithmic unities, an $[u]$ variation of 1 shifts the left side of the curve of 1 and the right side of 2 (two genes) or 3 (three genes)

(RESULTS)

Series of simulations

Same conditions of the first series, but varying the value of s

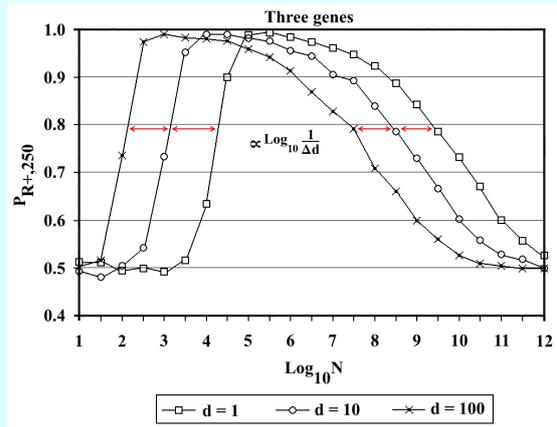
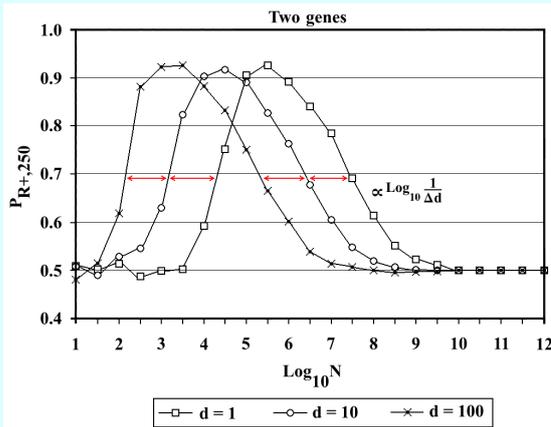


Note: with $s = 0.01$, simulations have been extended to 2,000 generations.

(RESULTS)

Series of simulations

Same conditions of the first series, but with the population (now metapopulation) divided in d demes each with N individual and with an interdemec interchange of individuals equal to 0.1 for generation



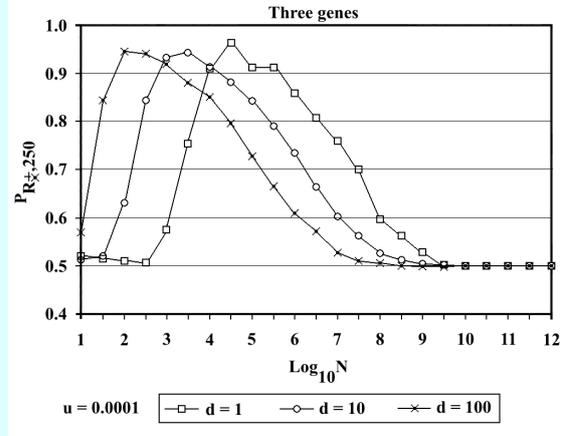
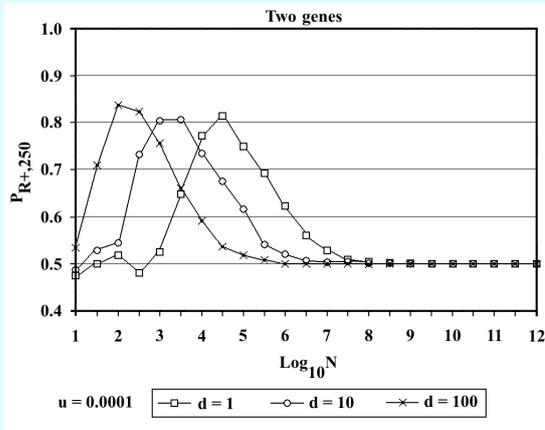
In logarithmic unities, a $[d]$ variation of 1 shifts the curve of 1.

Note: d demes, each with N individuals, for the advantage of sex are practically equivalent to a single deme with $N d$ individuals.

(RESULTS)

Series of simulations

Same conditions of the previous simulations, but the value of u is 0.0001 instead of 0.00001



Note: if $d = 100$, even with $\log_{10} N = 1$ sex is advantaged.

So, in the simulations sex is advantageous practically for any size of the metapopulation. However, to predict the diffusion of sex among the various species, in alternative to asexual reproduction, or to predict the occurrence of sexual phases for species alternating sexual and asexual phases, it is necessary to consider the disadvantage deriving from sexual reproduction:

Advantage of sex
(recombination of genes)



Disadvantage of sex
(time and energy to find a partner and to mate)

The prediction of “classic” theory is very simple and immediate:
when disadvantage of sex is greater, asexual reproduction is favoured by natural selection, and vice versa.

A theory is valid or not valid whether its predictions are confirmed or falsified by empirical data.

Therefore, it is necessary to verify if predictions of the “classic” hypothesis of sex evolutionary advantage are confirmed or falsified by data from natural observation.

It is useful to do the same for two other theories (Best Man hypothesis, Tangled Bank) that make precise predictions about the diffusion of sex (Bell 1982).

Legenda: red = wrong prediction; green = right prediction

	PREDICTIONS OF				
	Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	Classic hypothesis	Data from natural observation
PART 1: INTERSPECIFIC COMPARISON					
Correlation with different habitats (for Best-Man and Tangled-Bank hypothesis' predictions and for Data from Natural Observation, see Bell, pp. 359-65)					
Freshwater, Higher latitudes, Severely disturbed environments, r-selection, Ecological periphery of a species range, Novel habitats, Recently glaciated areas, Xeric environments	Sexual	Asexual	Asexual	Asexual	Asexual
Ocean, Lower latitudes, Constant environment, K-selection, Ecological center of a species range, Ancient habitats, Unglaciated areas, Non-xeric environments	Asexual	Sexual	Sexual	Sexual	Sexual



	Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	Classic hypothesis	Data from natural observation
Other conditions (see Bell, pp. 378-83 and 364)					
Parasitism	The same as observed in nature	The same as observed in nature but thelitoky is expected not rare	The same as observed in nature	The same as observed in nature	Sexual whenever possible. Thelitoky extremely rare, more common in free-living form
Very small size of soma	Sexual	-	Asexual	Asexual	Asexual
Large size of soma	Asexual	-	Sexual	Sexual	Sexual
Recombination (see Bell, pp. 411-35)					
Correlation between achiasmy and Ocean, Lower latitudes, constant environment, K-selection, etc.	Expected negative	Expected positive	Expected positive	Not expected	Not found
Correlation between chromosome number and proclivity for vegetative reproduction	Expected negative	Expected positive	Expected positive	Not expected	Not found
Correlation between crossing over freq. and proclivity for vegetative reproduction	Expected negative	Expected positive	Expected positive	Not expected	Not found

	Best-Man hypothesis	Tangled-Bank hyp.	Red Queen hypothesis	Classic hypothesis	Data from nat. observ.
PART 2: INTRASPECIFIC COMPARISON					
Intermittent sexuality (see Bell, pp. 365-70)					
During growing season (exponential growth of population)	Asexual	-	-	Asexual	Asexual
Before climatic changes	Sexual	-	-	-	-
At times of high population density	Asexual	Sexual	Sexual	Sexual	Sexual
At times of minimal population density	Sexual	Asexual	Asexual	Asexual	Asexual
Elicitation of sex in laboratory (see Bell, pp. 370-71)					
Signals of a change in environment	Sex elicited	-	-	-	-
Crowding and starvation	-	Sex elicited	Sex elicited	Sex elicited	Sex elicited
Dispersal and dormancy (see Bell, pp. 371-77)					
Actively dispersing stage	Sexual	Sexual (with some reservation)	-	Sexual	Sexual
Dormant stage	Sexual (for most Best-Man models)	Sexual / Asexual	-	Sexual (Asexual if the change of environment conditions is abrupt)	Sexual / Asexual

	SUMMARY			
	Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	Classic hypothesis
Differences	12	4	3	0
Concordances	3	7	8	14
No prediction	1	5	5	2
	 DISPROVED	 DISPROVED	 DISPROVED	 CONFIRMED

CONCLUSION

Matt Ridley wrote (The Red Queen, 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.'

Now, to this statement we can reply with:

The advantage of sex is rationally explained by "classic" theory and, considering the disadvantage of sex too, it is possible to formulate predictions about its diffusion in nature that are confirmed by data from natural observation.

No other theory is justified by sound theoretical arguments and/or confirmed by empirical data.

**Thanks
for your attention**

Concordance of the predictions of a simulation model for the evolutionary advantage of sex with observational evidence

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Abstract

Evolutionary advantage of sex is a widely discussed topic with multiple and clashing theories. A simulation model is proposed, based on the “classic” hypothesis that sex is advantageous because it allows faster attainment of favourable genetic combinations. The model shows the substitution of 2 (or 3) genes with advantageous alleles and calculates in which conditions a further gene allowing recombination is advantaged or disadvantaged in comparison with an allele not allowing recombination. With no epistasis, in infinite population sex results neutral, while in finite populations, in particular if the population is divided in demes, sex results advantageous. Considering the disadvantages caused by mating necessities, “classic” theory predicts the trends of ecological conditions in which sexual/asexual species of the same taxonomic group (or sexual/asexual stages of the same species) will prevail. Predictions of the “classic” theory with the above-mentioned specifications are compared with predictions of other hypotheses and data from natural observation: only the “classic” theory is confirmed by empirical evidence.

Keywords: sex; evolution; epistasis; red queen; finite populations.

Links:

WebmedCentral site: http://www.webmedcentral.com/article_view/1787

Personal site = http://www.r-site.org/ageing/index_e.htm

Introduction

The evolutionary justification of gene recombination between two individuals, defined with the technical term “mixis” but usually referred to using the popular word “sex”, is a widely discussed topic [Ghiselin, 1974; Williams, 1975; Maynard Smith, 1978; Bell, 1982; Ridley, 1993].

The “classic” hypothesis (alias Fisher-Muller hypothesis) that sexual reproduction is evolutionarily advantageous because it allows a continuous rearrangement of genes (Fig. 1), which Bell called “The Vicar of Bray” [Bell, 1982], was first expressed by Weismann [Weismann, 1889] and later by Guenther [Guenther, 1906]. Afterwards, it has been formulated in terms of population genetics by Fisher [Fisher, 1930] and Muller [Muller, 1932] and later, with greater mathematical formalism, by Muller [Muller, 1958, 1964] and Crow and Kimura [Crow and Kimura 1965].

Maynard Smith [Maynard Smith, 1968] 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) having an advantage (s_A, s_B) over a and b, respectively, combinations frequencies in the next generation will be:

$$\begin{aligned} P_{n+1,ab} &= P_{n,ab} / T \\ P_{n+1,Ab} &= P_{n,Ab} (1 + s_A) / T \\ P_{n+1,aB} &= P_{n,aB} (1 + s_B) / T \\ P_{n+1,AB} &= P_{n,AB} (1 + s_{AB}) / T \end{aligned} \quad (1)$$

where:

$$\begin{aligned} P_{n,xy} &= \text{frequency of combination } xy \text{ at generation } n; \\ P_{n+1,xy} &= \text{frequency of combination } xy \text{ at generation } n+1; \\ k &= \text{interaction (epistasis) between the fitnesses;} \\ T &= \text{the sum of numerators;} \\ s_{AB} &= [(1 + s_A)(1 + s_B) - 1] k \end{aligned} \quad (2)$$

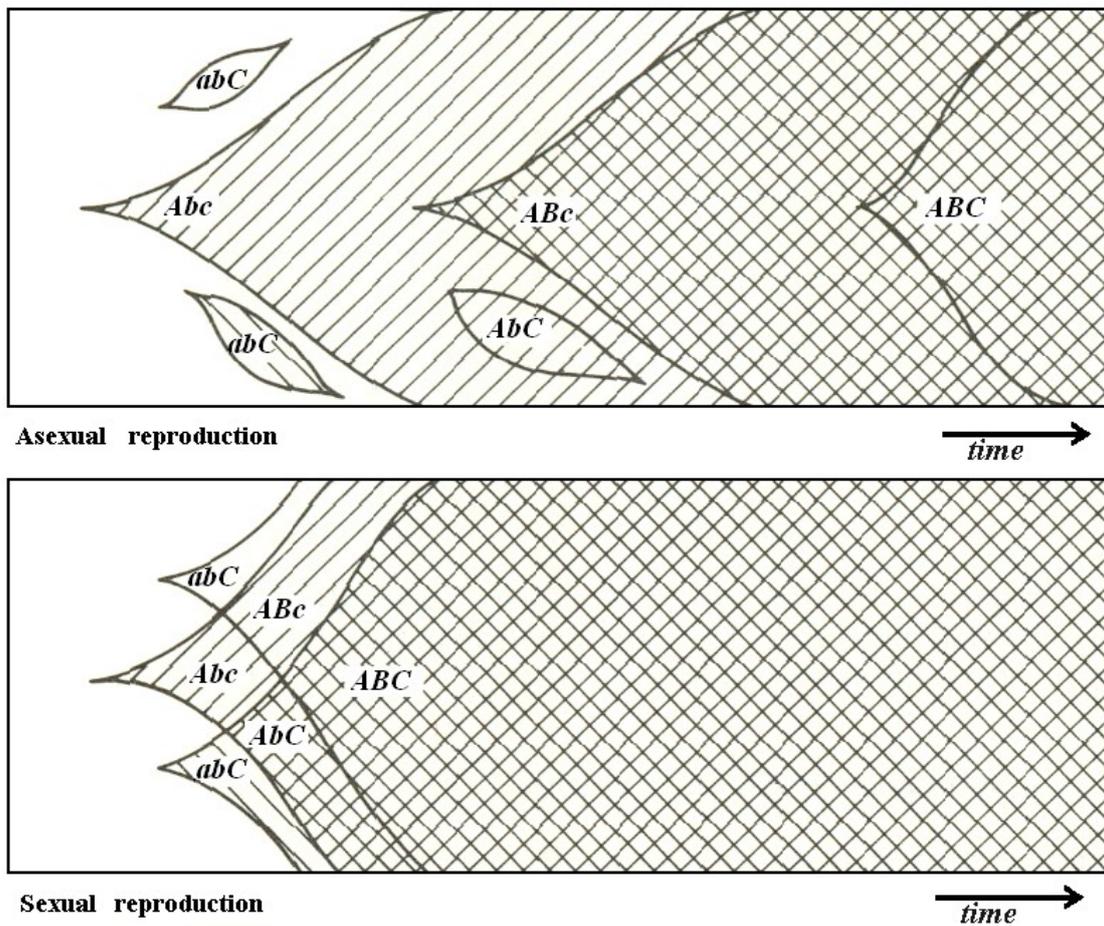


Fig. 1. For the “classic” hypothesis, sex is evolutionarily advantageous because it allows a continuous rearrangement of genes and therefore the attainment of the best combinations earlier than with asexual reproduction (from [Crow & Kimura, 1965]; partially redrawn).

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 ($k = 1$), Eq. (1) determine 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 is not 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 ($k > 1$) between the fitnesses, sex is disadvantageous because it breaks the more advantageous combination AB. The contrary happens if there is negative epistasis ($k < 1$).

Maynard Smith tried to overcome his argument [Maynard Smith, 1978], observing, in particular, that it was valid only for infinite populations, but that “linkage disequilibrium is bound to arise by chance in a finite population” (p. 15) and 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 it must be asked why conditions of negative linkage equilibrium, favourable for sex, should prevail over positive occurrences?

The doubts about the validity of Fisher-Muller “classic” explanation of sex caused the flourishing of alternative hypotheses such as, to use the eponyms of Bell [Bell, 1982]:

- Muller’s ratchet [Muller, 1964; Felsenstein, 1974; Butcher, 1995; Gordo and Charlesworth, 2000; Keightley and Otto, 2006; Gordo and Campos, 2008] (“sex ... facilitates the elimination of unfavourable mutations.” [Bell, 1982]; “In the absence of recombination, ... mutations will continually accumulate in the population, leading to the decline of its mean fitness.” [Gordo and Charlesworth, 2000]);

- Best-Man [Williams, 1966; Emlen, 1973; Treisman, 1976] (Recombination produces “a few individuals of extraordinarily high fitness. If only these individuals have any appreciable chance of surviving, then sexual parents will contribute a disproportionately large number of progeny to the next generation ...” [Bell, 1982]);

- Hitch-hiker [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 hitchhiked by these favourable alleles);

- Tangled Bank [Ghiselin, 1974; Burt and Bell, 1987; Ridley, 1993] (Sex diversifies progeny and its advantage is greater in conditions of environmental spatial heterogeneity, that is various “ecological niches in the same small geographical area – in an environment which does not change in time” [Bell, 1982]);

- Red Queen [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] (“The Red Queen hypothesis posits 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]);

- Historical hypothesis [Williams, 1975] (sex has no general evolutionary cause and sexual / asexual condition is mainly determined by ancestor sexuality / asexuality).

and, moreover, the hypotheses that:

- sex is advantageous because it slows down evolution and excessive specialization [William, 1975; Stanley, 1978];
- recombination eliminates the negative linkage disequilibrium generated by synergistic epistasis [Kondrashov, 1984; Charlesworth, 1990; Barton, 1995; Otto and Feldman, 1997];
- 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].

This paper originates both from the facts that many of these hypotheses are weakened by old serious criticisms [Bell, 1982] and that various subsequent 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], as well as from the conviction that sex evolutionary advantage must be investigated without hypothesizing artful and / or unduly limiting mechanisms.

I want to formulate a model that shows for sex - in terms of individual selection, as indicated by Felsenstein [Felsenstein, 1974] - both advantage in finite populations and no advantage in infinite populations. Moreover, the model must consider the important suggestion that natural populations are subject to genetic drift and are spatially structured [Otto and Lenormand, 2002].

In the first section, based on the classic Fisher-Muller hypothesis, stated in terms of individual selection and, for the sake of brevity, referred to as the “classic” hypothesis, I will illustrate a model for an infinite population that confirms Maynard Smith’s predictions [Maynard Smith, 1968, 1978].

In the subsequent section, I will insert in the model the condition of a finite population that demonstrates in this case an advantage for sexual reproduction, in accordance with a key observation on Fisher-Muller’s hypothesis expressed by Felsenstein [Felsenstein, 1974]: “ ... 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.” (p. 738)

The method utilized is the precise definition of a theoretical model and the following computer-aided verification, as discussed by Bell [Bell, 1982], pp. 79-84.

Finally, predictions of the “classic” hypothesis are compared with predictions of other theories and with data from natural observation.

The simulation model for infinite populations

Let us consider a species:

- a) that is 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 and a fraction z of R^- individuals. 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 mutation rates of R^+ in R^- , namely turning a sexual individual into an asexual individual, or vice versa, of zero frequency;

- e) with R+ and R- individuals having the same ecological niche and being by no means distinguishable except for the condition expressed in d;
- 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, considered negligible;
- g) with new alleles (A, B, C, ...) more advantageous than those prevailing in the species (a, b, c, ...), supposed at generation zero with frequency = 1;
- h) with independent gene transmission of any allele", i.e. the recombination fraction is assumed 0.5;
- i) with the mutation rate, at each generation, of an allele x in X equal to u_x and the back-mutation rate of X in x equal to w_x .

The question is, whether there is an advantage of sexual on asexual individuals or vice versa, that is 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) (“two genes case”), with four possible combinations (ab, Ab, aB, AB);

II) three genes (a, b, c) and the respective new alleles (A, B, C) (“three genes 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, the following is hypothesized:

$$u = u_a = u_b = u_c \tag{5}$$

$$w = w_a = w_b = w_c \tag{6}$$

$$s = s_A = s_B = s_C \tag{7}$$

With two and three genes, the possible cases of mutations from one combination into another are 10 (Fig. 2 and Table 1) and 38 (Fig. 3 and Table 2), respectively. The probabilities of transformations are indicated in the tables.

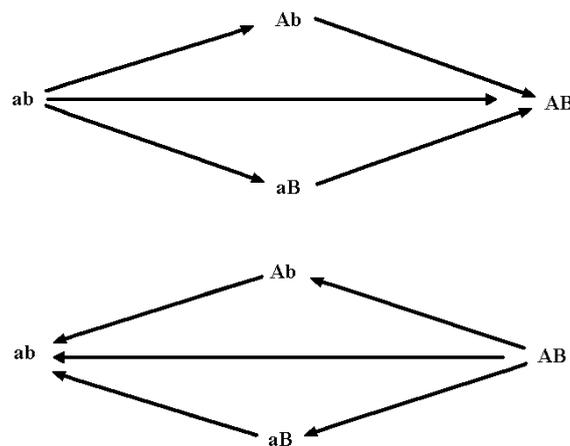


Fig. 2. Two genes case. Possible transformations of one combination into another.

Table 1. Two genes case. Possible transformations of one combination into another and their probabilities

	From	To	Probabilities
1	aB	AB	u
2	Ab	AB	u
3	ab	aB	$u - u^2$
4	ab	Ab	$u - u^2$
5	ab	AB	u^2
6	aB	ab	w
7	Ab	ab	w
8	AB	aB	$w - w^2$
9	AB	Ab	$w - w^2$
10	AB	ab	w^2

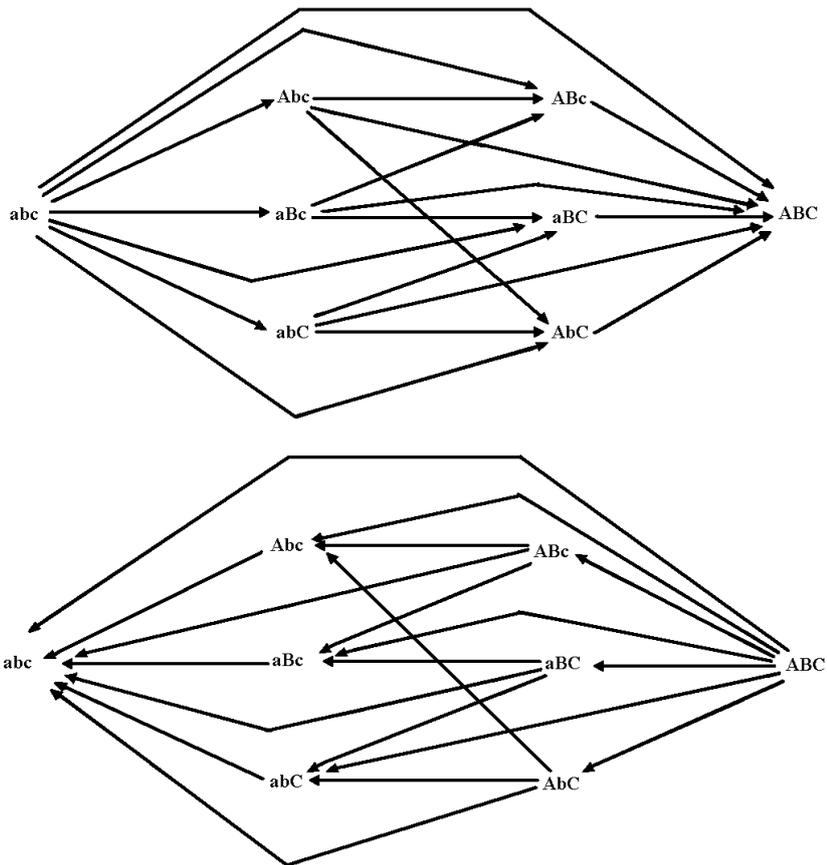


Fig 3. Three genes case. Possible transformations of one combination into another.

Table 2. Three genes case. Possible transformations of one combination into another and their probabilities

	From	To	Probabilities		From	To	Probabilities
1	ABc	ABC	u	20	Abc	abc	w
2	aBC	ABC	u	21	aBc	abc	w
3	AbC	ABC	u	22	abC	abc	w
4	Abc	ABc	$u - u^2$	23	ABc	Abc	$w - w^2$
5	Abc	AbC	$u - u^2$	24	ABc	aBc	$w - w^2$
6	aBc	ABc	$u - u^2$	25	aBC	aBc	$w - w^2$
7	aBc	aBC	$u - u^2$	26	aBC	abC	$w - w^2$
8	abC	aBC	$u - u^2$	27	AbC	Abc	$w - w^2$
9	abC	AbC	$u - u^2$	28	AbC	abC	$w - w^2$
10	Abc	ABC	u^2	29	ABc	abc	w^2
11	aBc	ABC	u^2	30	aBC	abc	w^2
12	abC	ABC	u^2	31	AbC	abc	w^2
13	abc	Abc	$u - 2u^2$	32	ABC	ABc	$w - 2w^2$
14	abc	aBc	$u - 2u^2$	33	ABC	aBC	$w - 2w^2$
15	abc	abC	$u - 2u^2$	34	ABC	AbC	$w - 2w^2$
16	abc	ABc	$u^2 - u^3$	35	ABC	Abc	$w^2 - w^3$
17	abc	aBC	$u^2 - u^3$	36	ABC	aBc	$w^2 - w^3$
18	abc	AbC	$u^2 - u^3$	37	ABC	abC	$w^2 - w^3$
19	abc	ABC	u^3	38	ABC	abc	w^3

The fitness for individuals with two advantageous alleles (F_{XY} ; XY means AB, for the two genes case; AB or BC or AC, for the three genes 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 $P_{xy',n}$, recombination for R+ individuals is simulated, in the two genes case, by calculating the frequencies of a, A, b, B, over the total of individuals with R+ (P_{R+}):

$$P_{a,n} = P_{ab,n} + P_{aB,n}; \dots \quad (10)$$

and, afterwards, by using the equations:

$$P_{ab,n+1} = \frac{1}{2} P_{ab,n} + \frac{1}{2} \frac{P_{a,n}}{P_{R+,n}} \frac{P_{b,n}}{P_{R+,n}} P_{R+,n}; \dots \quad (11)$$

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. The second part means that, in the remaining 50%, the allele present in I is substituted by other alleles from other individuals: the frequencies of the substituting alleles are given by the multiplication of the relative frequencies of each allele ($\frac{P_{x,n}}{P_{R+,n}}$), 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:

$$P_{ab',n+1} = P_{ab',n}; \dots \quad (12)$$

In the three genes 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+:

$$P_{a,n} = P_{abc,n} + P_{aBc,n} + P_{abC,n} + P_{aBC,n}; \dots \quad (13)$$

and, afterwards, by using the equations:

$$P_{abc,n+1} = \frac{1}{2} P_{abc,n} + \frac{1}{2} \frac{P_{a,n}}{P_{R+,n}} \frac{P_{b,n}}{P_{R+,n}} \frac{P_{c,n}}{P_{R+,n}} P_{R+,n}; \dots \quad (14)$$

while for R- individuals:

$$P_{abc',n+1} = P_{abc',n}; \dots \quad (15)$$

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 and not fractional 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 program, 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., $a \rightarrow A$);
- back-mutations (e.g., $A \rightarrow a$);
- 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 interdemetic diffusion of genes at each generation equal to f .

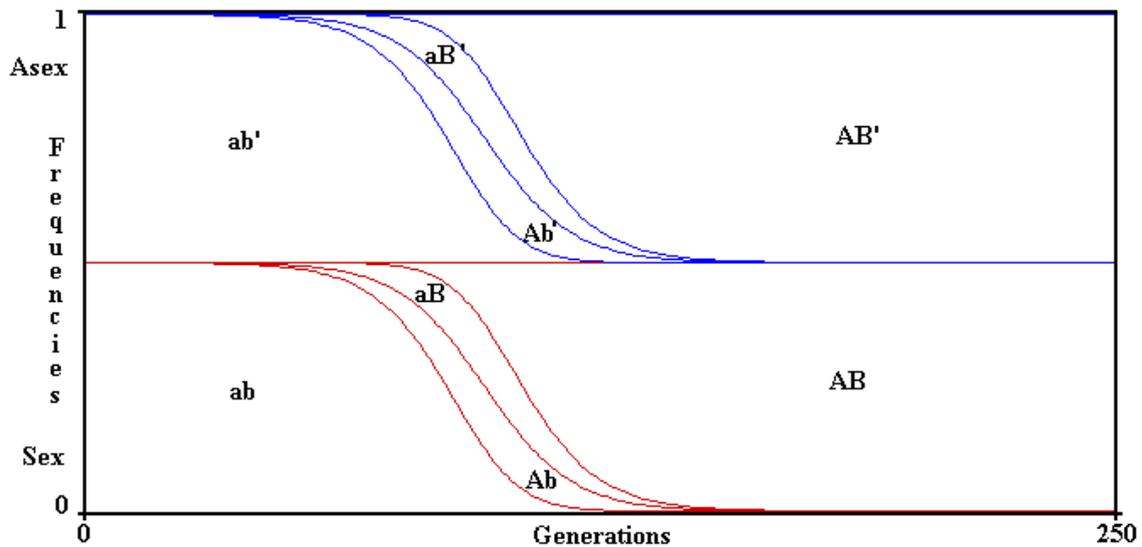
At each generation, the function is used several times (up to 13,000 times in 3 genes case and 100 demes).

Results for an infinite population

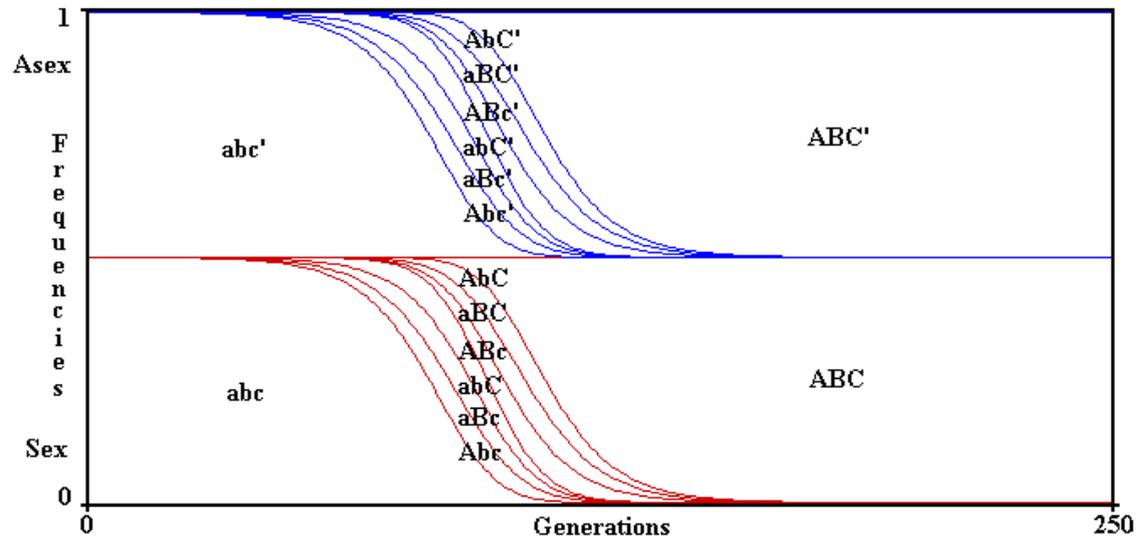
With no epistasis ($k = 1$) and no linkage disequilibrium ($D = 0$), sex is neutral with any value of u , w or s (Fig. 4).

With any time-dependent variation of the values of s , sex is neutral too (Fig. 5). This result needs to be remarked upon.

Red Queen theory rightly underlines that biotic are quantitatively more important than physical factors as selective forces. From this splendid idea ("Red Queen concept"), which is undoubtedly true considering the infinite interactions between predator-prey, parasite-host, herbivore-grass, competitors for the same resource of different species, intraspecific competitors, etc., and considering the fact that in many cases these interactions cause oscillating values of selective pressures, the theory deduces the evolutionary justification of sex [Bell, 1982; Otto and Nuismer, 2004; Kouyos et al., 2007].



A)



B)

Fig. 4. A) Two genes case; B) Three genes case. In both examples and in the following figures, if not specified otherwise: $u = w = 0.00001$; $s = 0.1$; $k = 1$; $D = 0$. The value of R^+ after 250 generations ($P_{R^+,250}$) is 0.499997620408316 in the first case and 0.499998194700698 in the second 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 asex 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 combination and R^+ values (except for fig. 14, simulation series with $s = 0.01$).

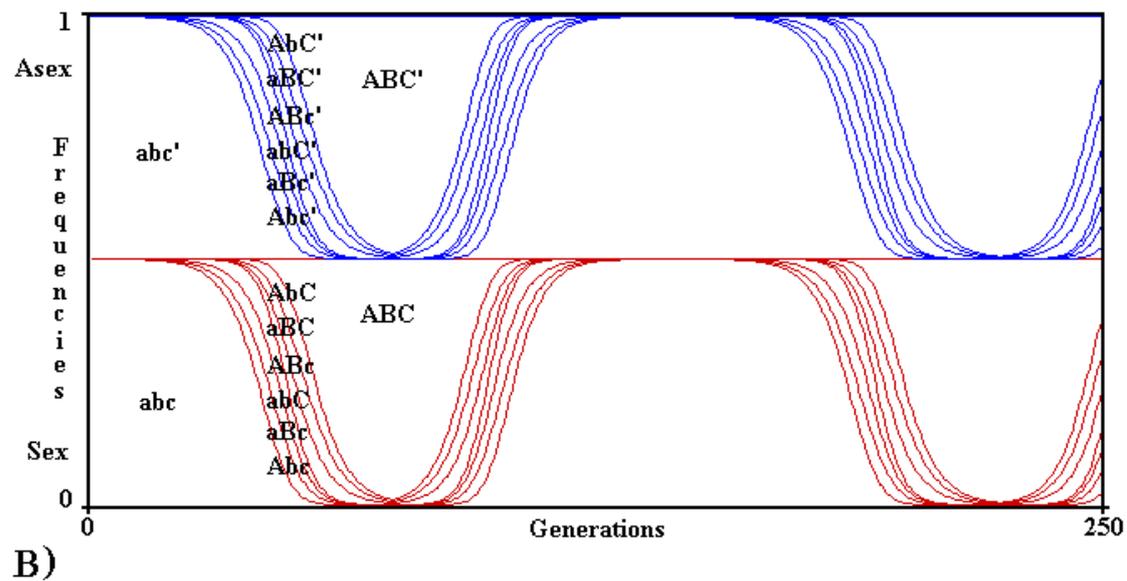
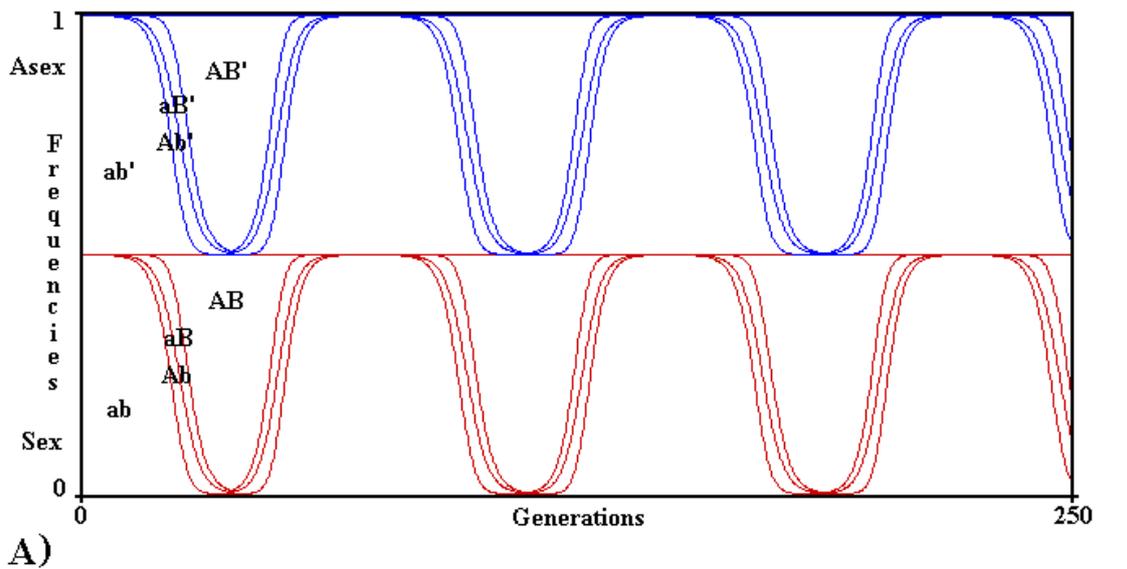
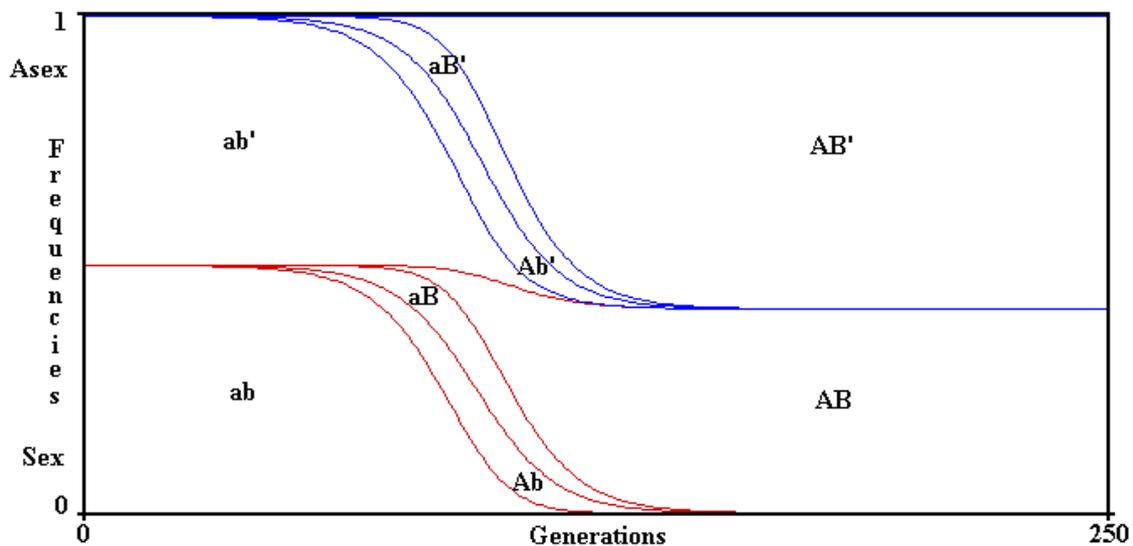


Fig. 5. Effects of the oscillations of s . A) Two genes case; B) Three genes case. In the simulations, s value oscillates from -0.1 to $+0.1$ every 150 generations.

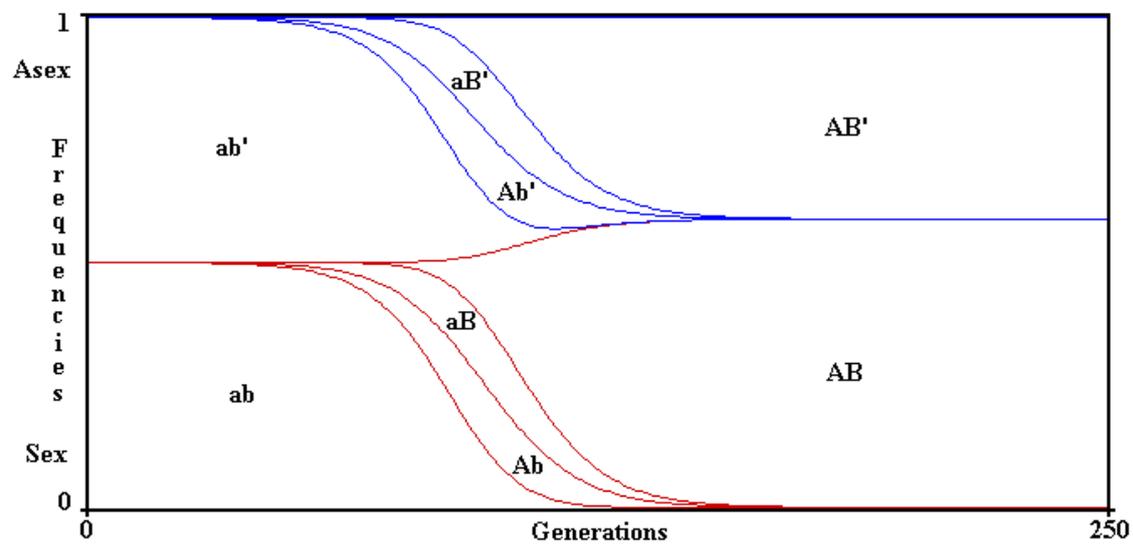
The model shows that in infinite populations any oscillating value of advantages cannot be sufficient to justify sex.

The results for finite populations (see subsequent section) show that sex is advantageous, but this 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 rejection or diminution of the Red Queen concept but as a theoretical 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 (Fig. 6).



A)



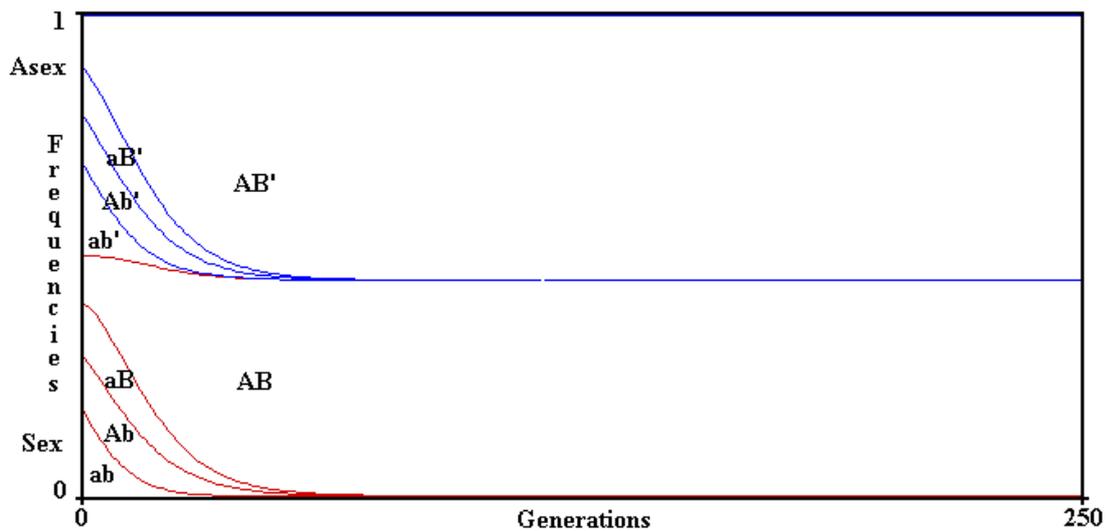
B)

Fig. 6. Effects of the variations of k . Two genes case. A) $k = 1.03$ (positive epistasis): sex is disadvantageous; B) $k = 0.97$ (negative epistasis): sex is advantageous. If the absolute value of $1-k$ is greater, the disadvantage / advantage of sex increases proportionally.

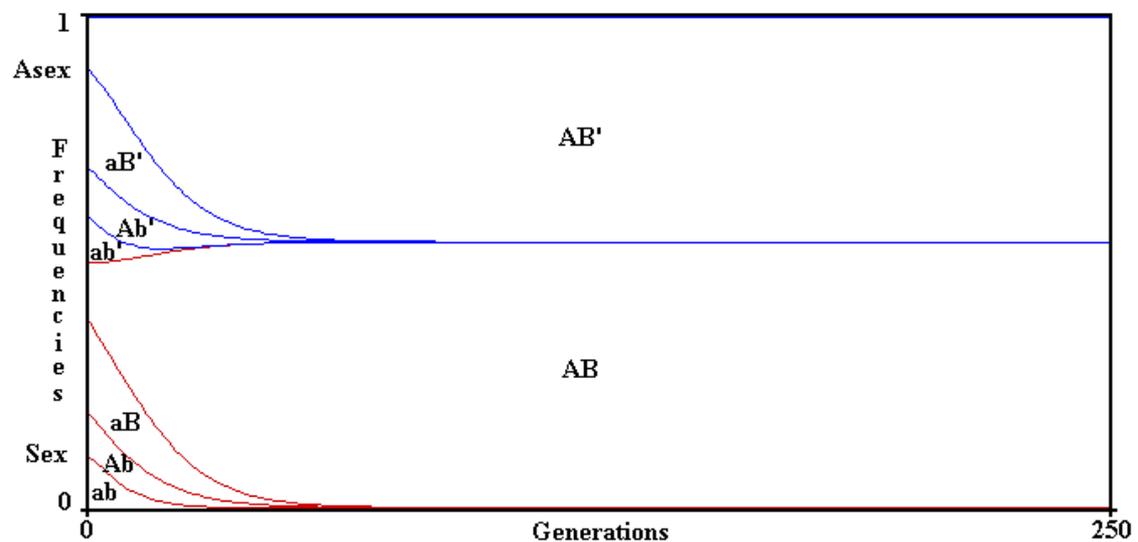
With positive linkage disequilibrium sex is disadvantageous, while with negative linkage disequilibrium sex is advantaged (Fig. 7).

In an infinite population, the results of simulations confirm considerations obtained through analytical arguments by other AA. [Felsenstein, 1965; Maynard Smith, 1968; Eshel and Feldman, 1970; Karlin, 1973].

However, a justification of sex as caused by prevailing conditions of negative epistasis or of negative linkage disequilibrium is unlikely and undocumented.



A)

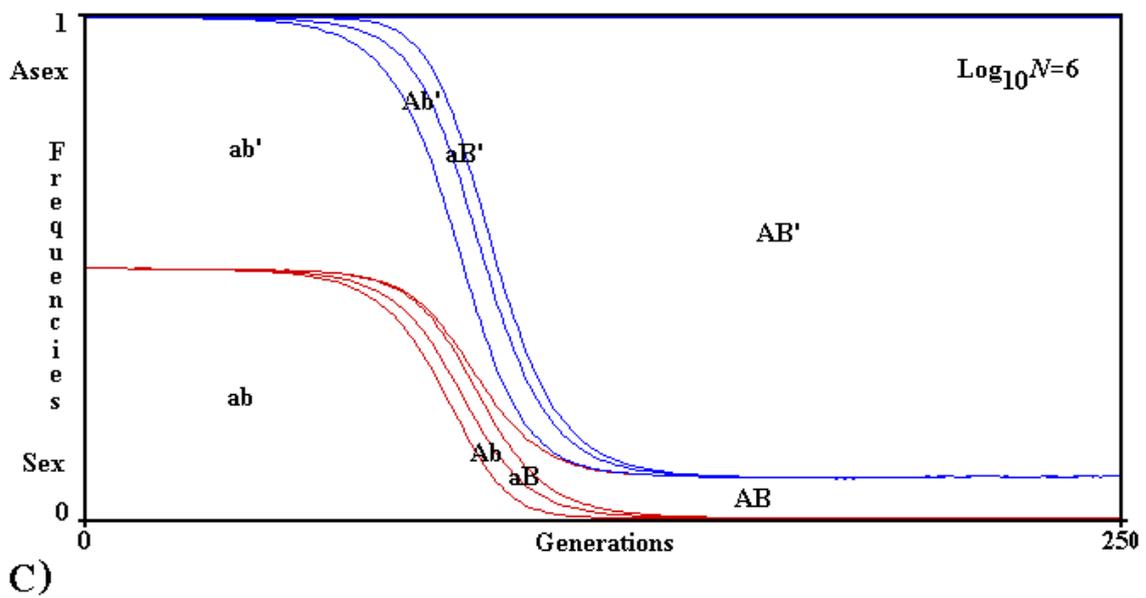
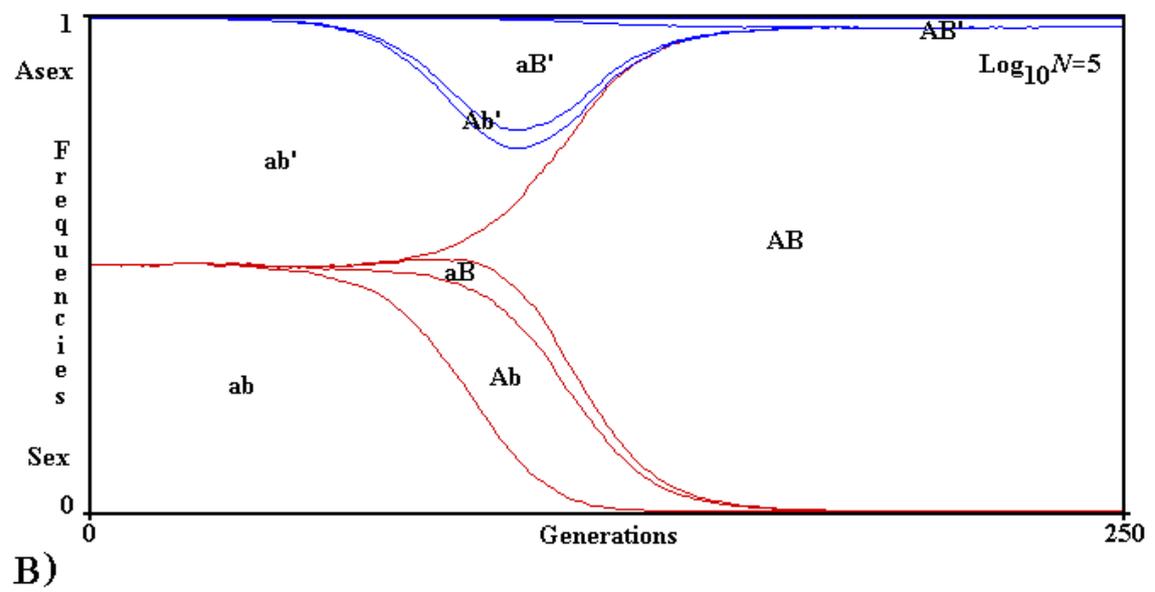
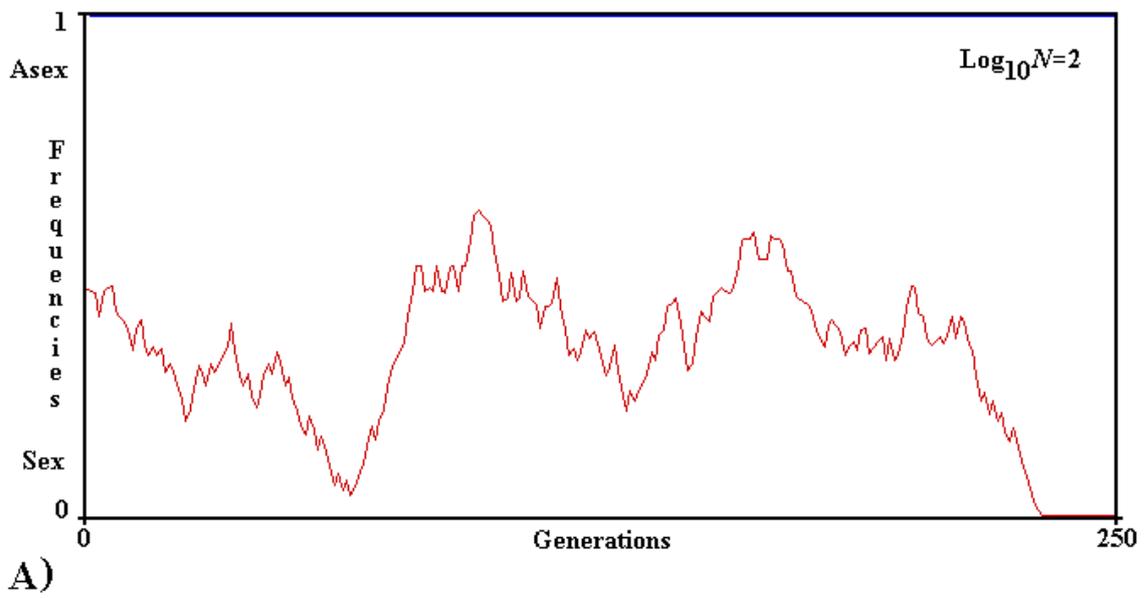


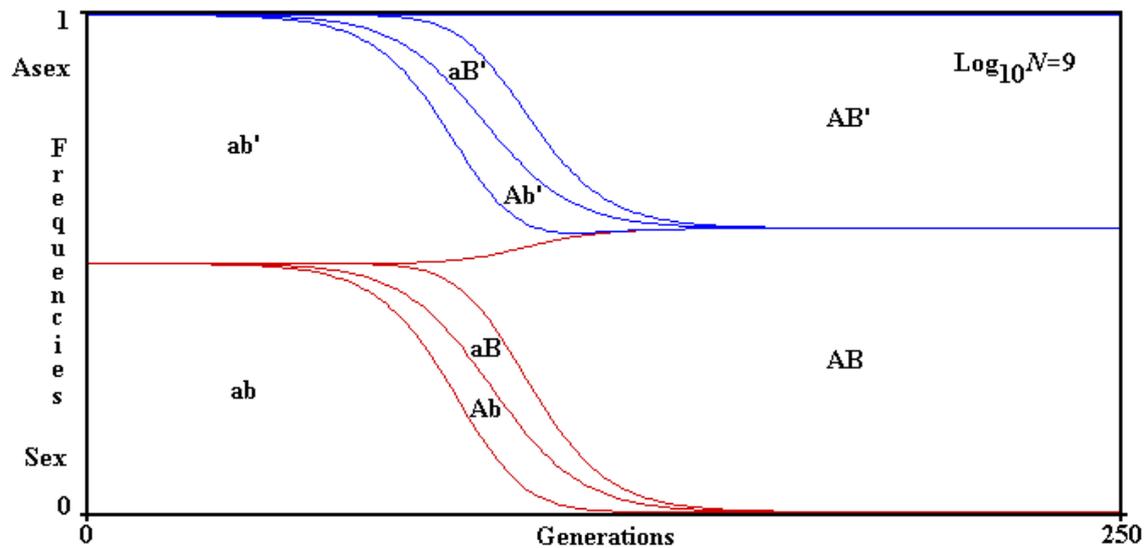
B)

Fig. 7. Effects of the variations of D . Two genes case. A) $D = +0.04$ (positive linkage disequilibrium): sex is disadvantageous; B) $D = -0.04$ (negative linkage disequilibrium): sex is advantageous. If the absolute value of D is greater, the disadvantage / advantage of sex increases proportionally.

Results for a finite population

Examples of single simulations are illustrated in Fig. 8. With small values of N , 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. With intermediate values of N , sex is generally favoured, though sometimes it loses. With greater values of N , sex is almost always favoured, but the advantage (difference between $P_{R^+,250}$ and 0.5) becomes progressively smaller.





D)

Fig. 8. Two genes case, single simulations in finite populations. A) $\log_{10}N = 2$; B) $\log_{10}N = 5$; C) $\log_{10}N = 6$; D) $\log_{10}N = 9$. In case A, only genetic drift determines the fluctuation of R+ and R- values. In cases B, C, D, the prevalence of R+ or R- is determined by the antecedence of mutation onset in R+ or R-.

Fig. 9 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 S.D. are reported for each point and compared with another series of simulations (indicated by symbol x) where R+ individuals are not allowed to recombine, each point marked with an asterisk if the results are significantly different ($p < 0.001$, with t-test for two unpaired groups of data [Armitage et al., 2001]).

In Fig. 10, 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 (in Appendix, the modifications of equations (10-15), necessary when $z > 0$, are illustrated). Even with small values of z the advantage of R+ over R- individuals fades.

In Fig. 11, a variation of u modifies the curve of sex advantage. In particular, the left side is shifted to the left by an increase of u , and vice versa, in proportion to u (sex advantage is conditioned by mutation onset, which is proportional to u). The right side is shifted to the right / left in proportion to u^2 in the two genes case and to u^3 in the three genes case (sex advantage fades when two – in the two genes case – or three – in the three genes case – mutations arise at the same time and these events are proportional to u^2 and u^3 , respectively).

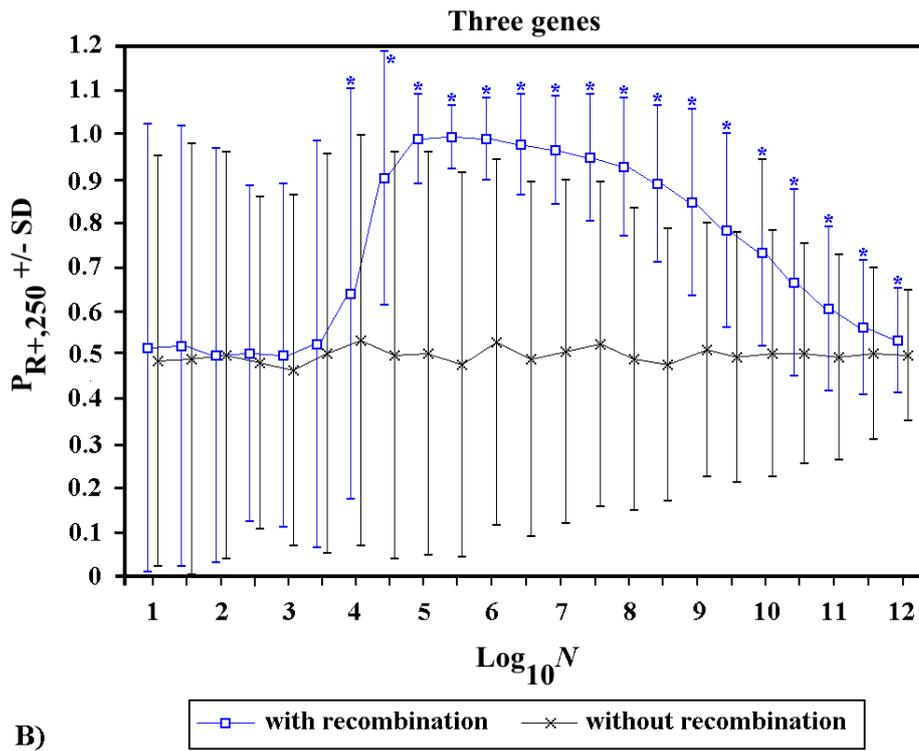
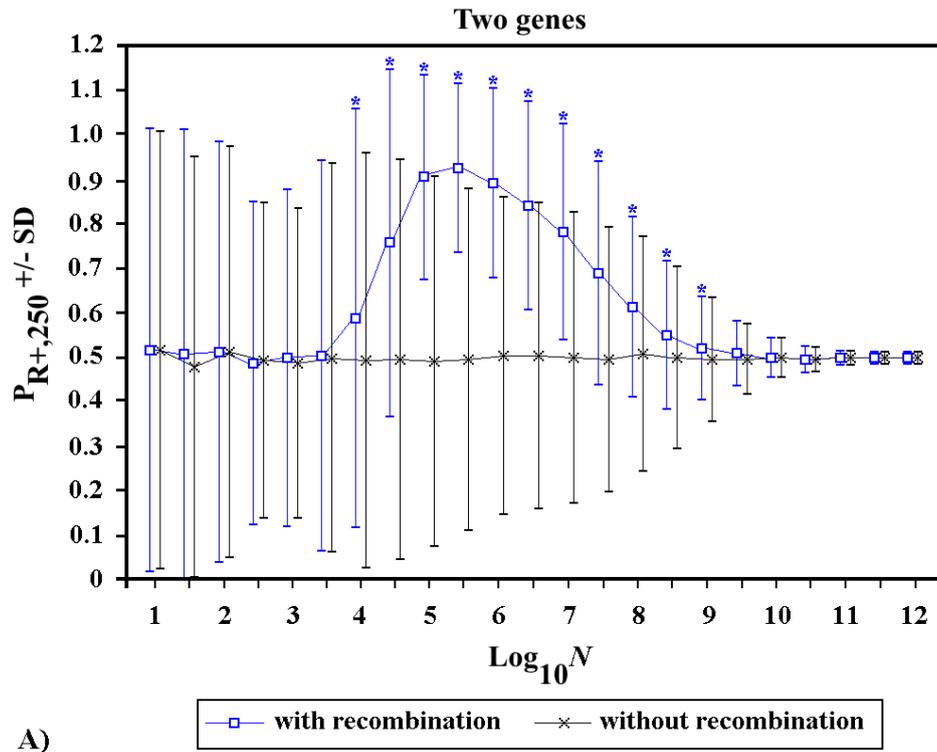


Fig. 9. Effects of recombination in finite populations. A) Two genes case; B) Three genes case. In these and in the following figures, if not specified otherwise: $u = w = 0.00001$; $s = 0.1$; $k = 1$; $D = 0$. Mean and standard deviation (SD) are reported for each point. For the series of simulations 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 last series have been shifted a little to the left and to the right, respectively; b) the results are always those obtained with the first run of simulations. Repetitions of the simulation runs for each series have given results equivalent to those of the first runs and these have not been used to substitute them.

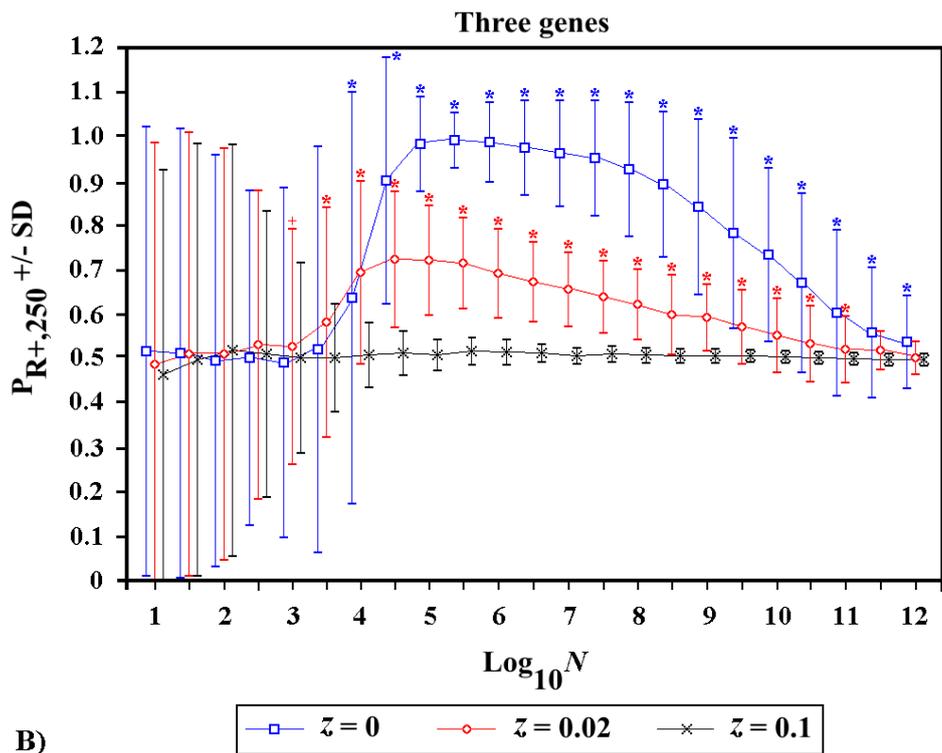
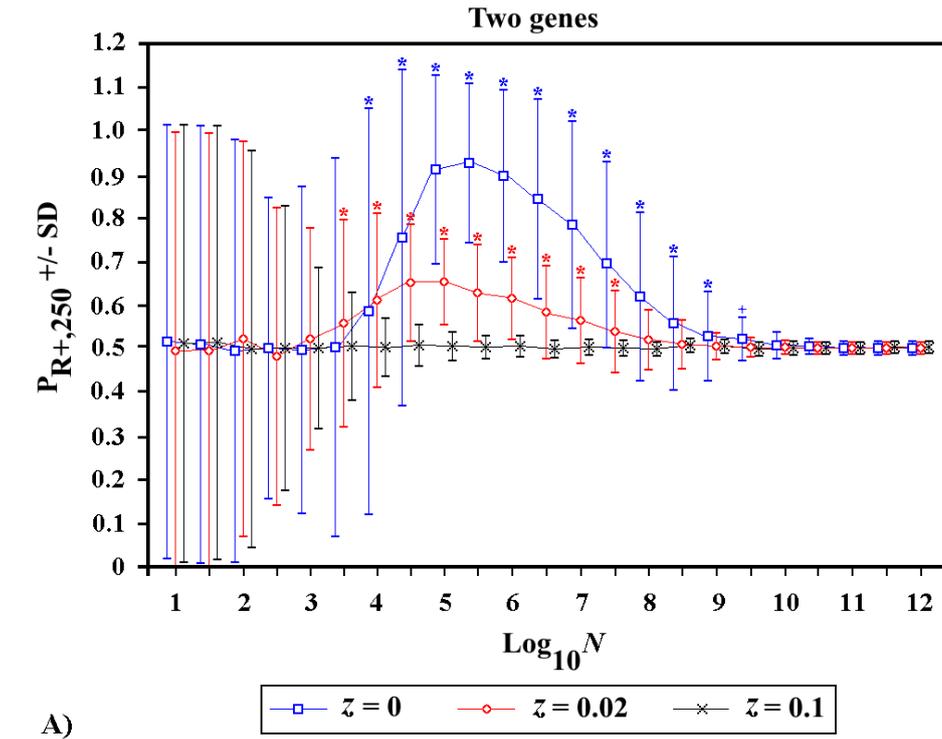


Fig. 10. Effects of the variations of z . A) Two genes case; B) Three genes case. The two series with $z = 0$ are the same as in Fig. 11 with recombination. With $z = 0.02$ the advantage for R+ individuals is greatly reduced and with $z = 0.1$ is practically cancelled. In these and in the following figures, 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 11-A and 11-B.

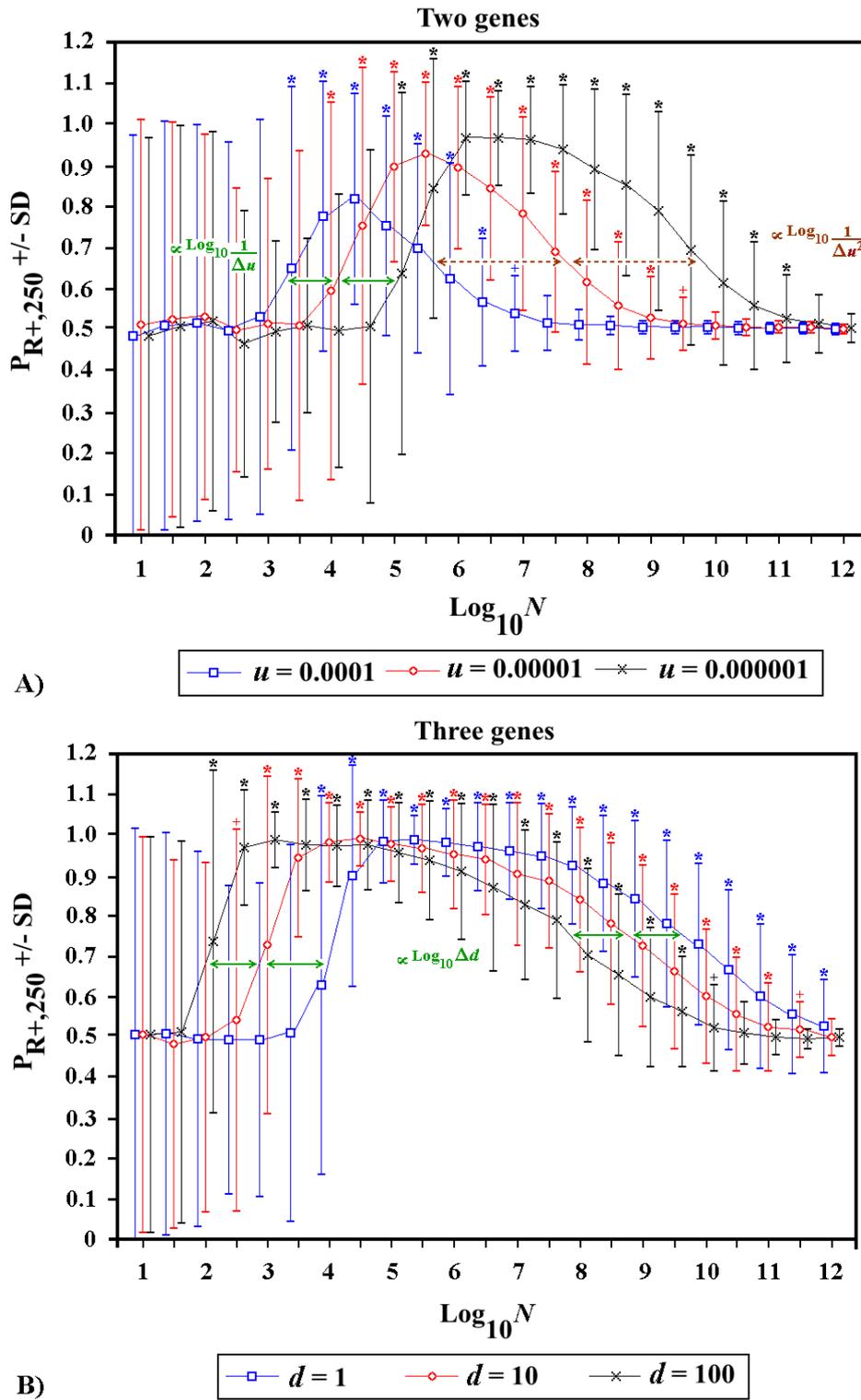
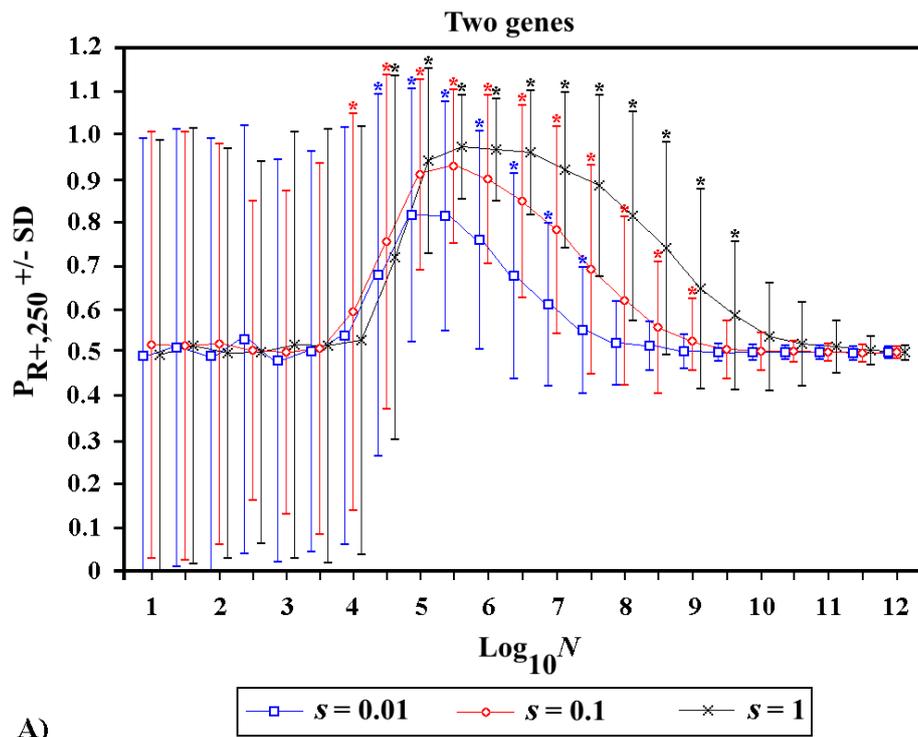
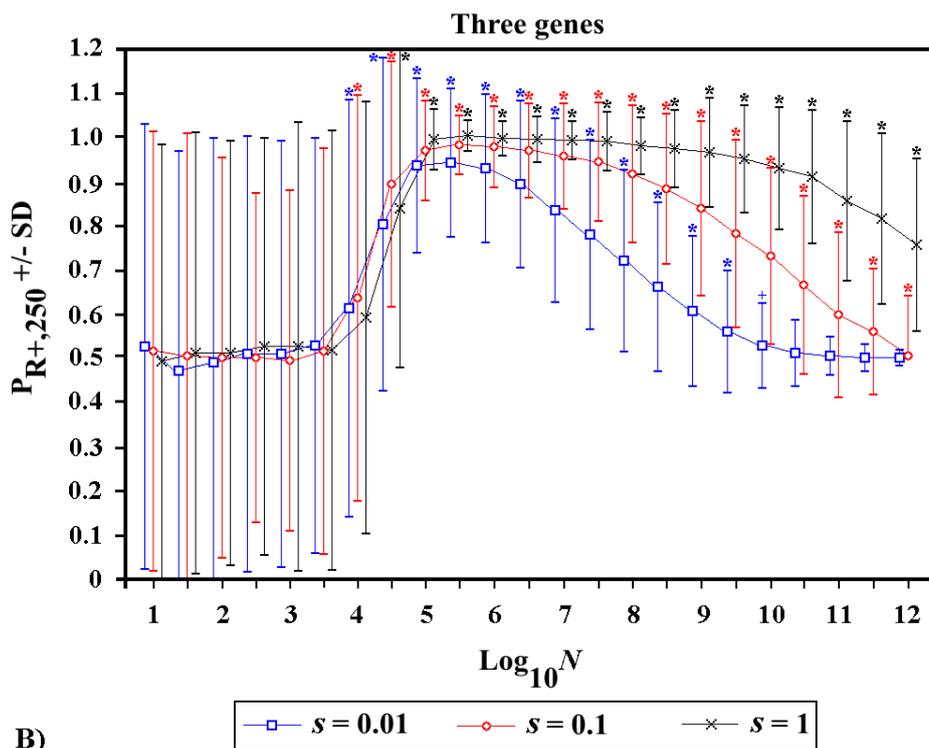


Fig. 11. Effects of the variations of u . A) Two genes case; B) Three genes case. The distance between the left sides of the curves is proportional to $\text{Log}_{10}(1/\Delta u)$, while for the right sides the distance is proportional to $\text{Log}_{10}(1/\Delta u^2)$ in A and to $\text{Log}_{10}(1/\Delta u^3)$ in B.



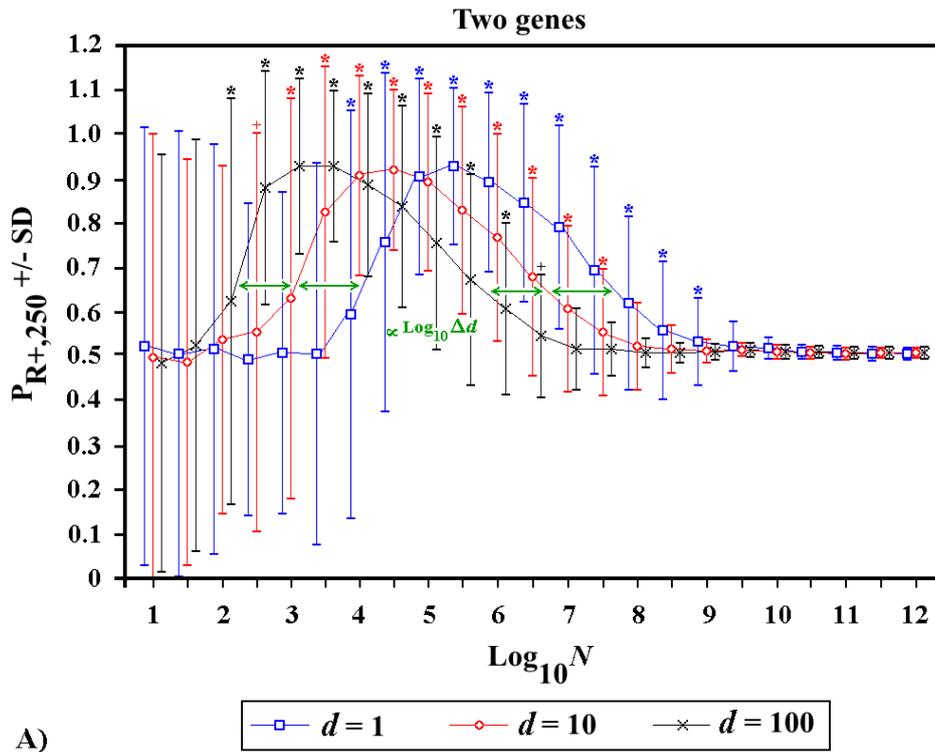
A)



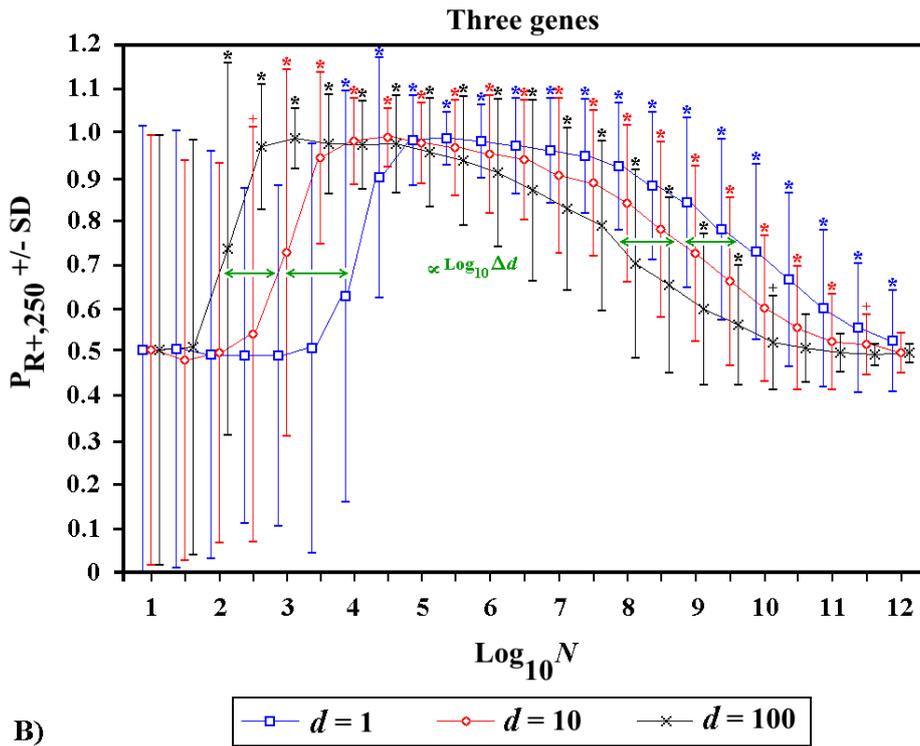
B)

Fig. 12. Effects of the variations of s . A) Two genes case; B) Three genes case. With $s = 0.01$, simulations have been extended to 2,000 generations.

In Fig. 12, a variation of s modifies, in proportion, only the right side of the curve of sex advantage.



A)



B)

Fig. 13. Effects of the variations of d . A) Two genes case; B) Three genes case. An increase of d shifts both sides of the advantage curves of sex to the left in proportion to $\text{Log}_{10} \Delta d$.

In Fig. 13, 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.

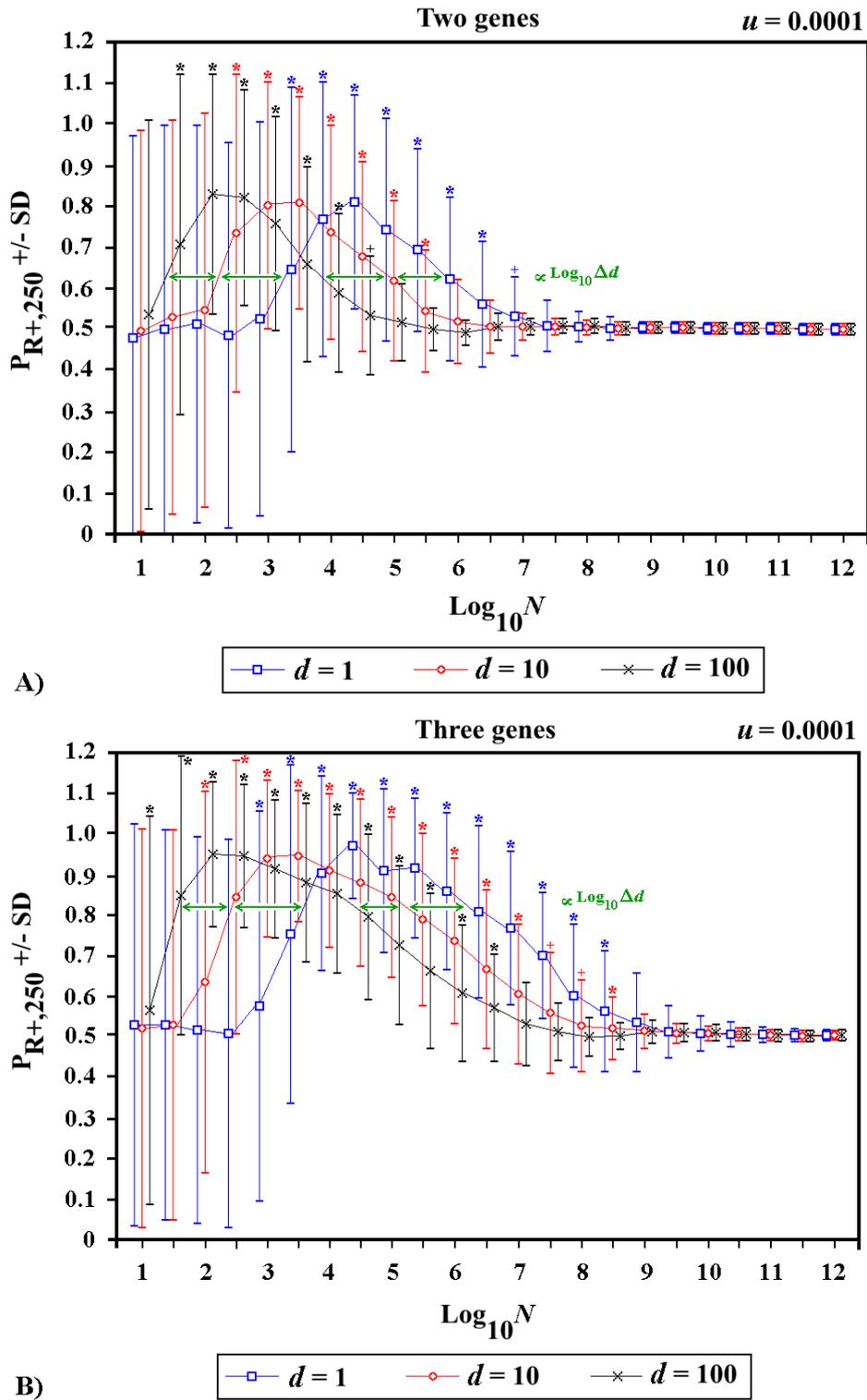


Fig. 14. Effects of the combined variations of u and d . A) Two genes case; B) Three genes case. The same conditions as in the previous figure, but the value of u is 0.0001 instead of 0.00001 and the curves are shifted to the left by a logarithmic unity. In these figures too, an increase of d shifts both sides of the advantage curves of sex to the left in proportion to $\log_{10} \Delta d$. For the three genes case, sex results advantageous even for values of $\log_{10} N = 1$.

In Fig. 14, it is shown that a contemporary variation of d and u have multiplicative effects and, so, with many demes and high values of u sex is advantageous even with small values of N .

Disadvantage of sex

The simple 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]. But, as an equally simple counter-argument, 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 that in other conditions ($B \geq 1$) the cost of sex is an advantage or is inexistent.

Moreover, if in the evolution from isogamy to anisogamy there are advantages / disadvantages caused by the second condition, selection should favour / contrast anisogamy and not sex in itself.

For these considerations, in an attempt to predict the trends of sex diffusion among the various species, as an alternative to asexual reproduction, or to predict the occurrence of sexual phases for species alternating sexual and asexual phases, I have disregarded the so-called “cost of sex”.

On the contrary, I deem it absolutely necessary to consider for sexual individuals the disadvantages deriving from the search of a mate and connected to the coupling. In this paper, I maintain that the counterbalance to the advantage of sex is intrinsic to the patterns of sex expression, as expressed by Bell [Bell, 1982]: “Amphimicts have one ... handicap: they must be able to find a mate, and this may be an expensive, risky and time consuming process.” (p. 357). Moreover, courtship and copulation take up more precious time. For the sake of brevity, I will call this set of handicaps the “disadvantage of sex” (DS).

Gerritson [Gerritson, 1980] maintains that DS is greater in conditions of low population density. On the contrary, I think that DS will be critically greater in severely disturbed habitats and in conditions of r-selection. Indeed, 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, I do not share the consequence of Gerritson’s opinion that “reproduction following long-distance dispersal should be parthenogenetic” [Bell, 1982], p. 357, because there is no severely disturbed habitat. Also in contrast with Gerritson’s opinion: “parthenogenetics insects very often live in small patches of high local population density” (*ibidem*), which is a condition of r-selection.

A comparative and experimental critique of the theories

(Empirical evidence for 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 about sex evolutionary advantage are confirmed or refuted by data from

natural observation. Moreover, according to the scientific method, a theory refuted by empirical data must be considered untenable and not presented as a valid hypothesis until its contradictions with empirical data have been explained or somehow resolved.

I have drawn a table (Table 3) in which predictions of the “classic” hypothesis on the evolutionary meaning of sex, along with those of three other theories (Best-Man, Tangled Bank and Red Queen) concerning the expected trends of the distribution in the nature of sex and related phenomena are compared with empirical evidence from natural observation.

This paragraph has the same name as chapter 4 of Bell’s book [Bell, 1982] and has its aims, methods and predictions for the Best-Man, Tangled-Bank and Red Queen hypotheses and references to data from natural observation, in common with it. Predictions for the aforesaid hypotheses are identical to those expounded by Bell, but in some cases, in the absence of Bell’s predictions, I have attempted a prediction explained in an appropriate note.

I have also formulated predictions of the “classic” hypothesis with one simple criterion: as the theory and the simulation model of this paper 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, crossing over frequency) and amphimixis or parthenogenesis is expected.

In various cases, predictions of the “classic” hypothesis and those of other hypotheses coincide, but 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, in my judgement, is an almost total correspondence between predictions of the “classic” hypothesis and data from natural observation.

The utter failure of the Best-Man hypothesis is remarkable and I share Bell’s negative opinion on this theory which, in Table 3, has the only function of showing 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, as 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 the Bell, himself, underlines [Bell, 1982].

As regards other theories not considered in the table:

- Muller’s Ratchet hypothesis. This 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 of this paper, could integrate the “classic” theory.

- 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 as a theory explaining sex, but as an inertial factor restraining a free passage from sexual to asexual reproduction, or vice versa, it should deserve a certain amount of attention.

- Hitch-hiker hypothesis. A R+ gene could be described as a gene that is advantaged because it hitchhikes favourable genes that are better spread because of by its action. The hitch-hiker hypothesis could, therefore, be defined as a different and indirect way of expounding the “classic” theory.

- Hypothesis that sex is advantageous because it slows down evolution and excessive specialisation. This theory makes no prediction.

Table 3. Comparison between predictions of four hypotheses on the evolutionary meaning of sex and data from natural observation. (Expected trends of prevalence of sex / asexual forms). Page numbers refer to Bell’s book [Bell, 1982].

	PREDICTIONS OF				
	Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	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	Sexual (p. 359, 364)	Asexual (p. 359, 364)	Asexual ¹	Asexual ²	Asexual (p. 359)
Ocean, Lower latitudes, Constant environments, K-selection, Ecological center of a species range, Ancient habitats, Unglaciated areas, Non xeric environments	Asexual (p. 359, 364)	Sexual (p. 359, 364)	Sexual ³	Sexual ⁴	Sexual (p. 359)
Other conditions (pp. 378-383 and 364)					
Parasitism	The same as observed in nature (p. 378-383)	The same as observed in nature but thelitoky is expected not rare (p. 378-383)	The same as observed in nature (p. 378-383)	The same as observed in nature ⁵	Sexual whenever possible. Thelitoky extremely rare, more common in free-living form (p. 378-383)
Very small size of soma	Sexual (p. 364)	-	Asexual ¹	Asexual ²	Asexual (p. 364)
Large size of soma	Asexual (p. 364)	-	Sexual ³	Sexual ⁴	Sexual (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)	Asexual (p. 365)	- (p. 367)	-	Asexual ²	Asexual (p. 368)
Before climatic changes	Sexual (p. 365-366)	- (p. 367)	-	-	Not related (p. 367)
At times of minimal population density	Sexual (p. 368)	Asexual (p. 367)	Asexual ¹	Asexual ²	Asexual (p. 367)
At times of high population density	Asexual (p. 368)	Sexual (p. 367)	Sexual ³	Sexual ⁴	Sexual (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	Sexual (p. 371)	Sexual (with some reservation) (p. 371)	-	Sexual ⁷	Sexual (p. 373)
Dormant stage	Sexual (for most Best-Man models) (p. 377)	Sexual / Asexual (p. 377)	-	Sexual ⁷ (Asexual if the change of environment conditions is abrupt ⁸)	Sexual / Asexual (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;					
Summary					
	Best-Man hypothesis	Tangled-Bank hypothesis	Red Queen hypothesis	Classic hypothesis	
Differences	12	4	3	0	
Concordances	3	7	8	14	
No prediction	1	5	5	2	

Conclusion

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).

I think that Williams' unlikelihood is now a likelihood and that the uncertainty of Maynard Smith has been solved: with theoretical arguments, the advantage of sex has been rationally explained by the "classic" theory in terms of individual selection and using only the "general properties ..." that Williams insisted on [Williams, 1975]. Moreover, if we consider the disadvantage of sex, it is possible to formulate predictions

about the trends of its diffusion in nature that are confirmed by data 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 – often with oscillating s values - are quantitatively more important than physical factors as selective forces in determining evolution (Red Queen concept), which is 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 this hypothesis.

Somehow, the "pluralist approach to sex and recombination" [West et al., 1999] seems to be the correct solution, but with this specification: "classic" theory is the trunk with the main branches and other theories complete the tree.

Supplementary documents

From the Internet address "http://www.r-site.org/ageing/sex_model.zip", it is possible to obtain an Excel© file with the raw data of figures 9-14 and the source and executable files of the simulation program.

A technical note for the program is necessary. With the option:

Loop with Log₁₀N = [...] to [...] step .5 *No. iterations (from 1 to 10000) [...]*

if the number of iterations is not small, the graphic display of simulations may disappear after some simulations (as a consequence of PC power limits) and program commands freeze. This does not mean that the program is blocked: it continues to run up to the end. Please, await the end and then see the results in ReportFile2.txt (result of each simulation) and ReportFile3.txt (mean and SD for each group of simulation).

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APPENDIX

If the condition [c] is attenuated, namely if $z > 0$, (10), (11), (12) become:

$$P_{a,n} = P_{ab,n} + P_{aB,n} + z (P_{ab',n} + P_{aB',n}) ; \dots \quad (10')$$

$$P_{ab,n+1} = \frac{1}{2} P_{ab,n} + \frac{1}{2} \frac{P_{a,n}}{T} \frac{P_{b,n}}{T} P_{R+,n} ; \dots \quad (11')$$

$$P_{ab',n+1} = P_{ab',n} - \frac{1}{2} z P_{ab,n} + \frac{1}{2} \frac{P_{a,n}}{T} \frac{P_{b,n}}{T} z P_{R-,n} ; \dots \quad (12')$$

and (13), (14), (15):

$$P_a = P_{abc,n} + P_{aBc,n} + P_{abC,n} + P_{aBC,n} + z (P_{abc',n} + P_{aBc',n} + P_{abC',n} + P_{aBC',n}) ; \dots \quad (13')$$

$$P_{abc,n+1} = \frac{1}{2} P_{abc,n} + \frac{1}{2} \frac{P_{a,n}}{T} \frac{P_{b,n}}{T} \frac{P_{c,n}}{T} P_{R+,n} ; \dots \quad (14')$$

$$P_{abc',n+1} = P_{abc',n} - \frac{1}{2} z P_{abc,n} + \frac{1}{2} \frac{P_{a,n}}{T} \frac{P_{b,n}}{T} \frac{P_{c,n}}{T} z P_{R-,n} ; \dots \quad (15')$$

with $T = P_{R+,n} + z P_{R-,n}$ in both cases.

