A mini-review of the evolutionary theories of aging.
Is it the time to accept them?

Éric Le Bourg

Table of Contents

A mini-review of the evolutionary theories of aging. Is it the time to accept them?

Éric Le Bourg [1]

Abstract

This article reviews some studies testing evolutionary theories of aging and shows that they are not always confirmed. Nevertheless, many gerontologists consider now that these theories provide a general explanation of the aging process. In such conditions, we may wonder whether time has come to provisionally accept these theories in order to redirect the research efforts of gerontologists towards other directions, such as the search for new means to modulate the aging process.

[1] Laboratoire d'Éthologie et de Psychologie Animale, E.R.S. C.N.R.S. n° 2041, Université Paul-Sabatier, 118 route de Narbonne, F-31062 Toulouse cedex 4, France.
(fax: 33 5 61 55 61 54, e-mail: lebourg@cict.fr)

1. Introduction

After twenty years or so of experiments testing the evolutionary theories of aging, they are now considered by many gerontologists as the basis of the explanation of the aging process. These theories explain the ultimate causes of aging (why aging occurs?), while other theories explain the proximate causes of aging (what mechanisms do explain aging?). These two kinds of theories operate at different levels of explanation and thus theories describing proximate causes of aging do not contradict evolutionary theories. For some scientists, proximate causes of aging deal with the deleterious actions of free radicals (see for instance all the articles of the Sohal's team). Others favor the imbalance of long-term low intensity stressors and of protective and repair processes (Masoro 1996), or other mechanisms as those described by Medvedev (1990) in his famous article compiling more than 300 theories of aging.

The matter of the present article is to pay some attention to the evolutionary theories of aging. This article describes in a few words the theory of the accumulation of mutations at old age (Medawar 1952), the theory of antagonistic pleiotropy (Williams 1957), the disposable soma theory (Kirkwood 1999), and reviews some results purporting to test them.

Even if these theories are very appealing, we may think that the final word about their validation process has not still be said. Yet, in a recent past, it has become clear that there is a risk for some gerontologists to consider these theories as definitively validated, which seems premature. For instance, Keller and Genoud (1997) have explained the long life span of queens of ants by relying on evolutionary theories of aging. Le Bourg (1998) considered that Keller and Genoud (1997) showed "that evolutionary theories of aging are consistent with the high longevities of queens, but …only apply a theoretical explanation to what is observed in the wild". Following this article, a debate occurred between these authors (Keller and Genoud 1999, Le Bourg and Beugnon 1999).

The present article proposes to provisionally accept evolutionary theories of aging - even if the review will show that one may have some doubts about their full validity - to redirect a part of the research efforts towards other questions. In other words, we could accept the evolutionary theories of aging, because they currently offer the most plausible explanation of aging and, at the same time, not accept all claims that experiments support them. This behavior allows to consider that testing these theories is no longer a top priority.

2. Evolutionary theories of aging: a brief overview

According to these theories, aging is a by-product of natural selection. Any individual has a probability to reproduce. It is zero at birth and reaches a peak in young adults. Then, it decreases due to the increased probability of death linked to various external (predators, illnesses, accidents) and internal causes (aging). In such conditions, deleterious mutations expressed at young age are severely selected against, due to their high negative impact on fitness. Conversely, the same mutations, if they are expressed at old age only, are rather neutral to selection, because their bearers have already transmitted their genes to the next generation. Note that these mutations can affect fitness directly or not. For instance, a mutation increasing the risk for leg fracture, due to a low fixation of calcium, may be as deleterious to fitness as one impairing the nesting of the egg in the uterus. In both cases, the animal is at risk not to reproduce, either because many precocious abortions occur or because it becomes an easy prey for a predator.

This theory of the accumulation at old age of mutations (Medawar 1952) seems to be in accordance with common sense. It may be easily understood that persons loaded with a deleterious mutation at young age have less or no chance to reproduce; for instance, progeria patients live for about 12 years (Turker 1996). By contrast, people expressing a mutation only at older ages can reproduce before the illness occurs, as it is the case with the Huntington's disease. In such conditions, the autosomal dominant genetic disease progeria stems from de novo mutations and not from the genes of parents. As an outcome, progeria is less frequent than late diseases such as the Huntington's disease, because the deleterious alleles are not removed from the gene pool and can accumulate in successive generations. Obviously, the most important conceptual problems with the theory of the accumulation of mutations at old age are that it predicts that diseases are more common at old age than at young age, which is a mere tautology, and that the risk of dying increases with age. However, the fact that mortality rates can decelerate at old ages is at variance with the theory (Pletcher, Houle and Curtsinger 1998).

In 1957, Williams added that pleiotropic genes with favorable effects on fitness at young age and deleterious ones at old age could exist and explain the aging process. Such genes could be selected due to their positive effect on fitness at young age, despite their negative effects at old age: these negative effects are the aging process. For instance, let us suppose that a gene favoring the fixation of calcium in bones does exist. This gene could have positive effects at young age, because the risk of fracture and thus of death is decreased, and negative effects at old age, because the risk of osteoarthritis is increased. In the wild, such a gene has no actual negative effect because most of animals die before its negative effects can be observed. There is then a trade-off

between an *actual* positive effect at young age and a *potential* negative one at old age: this negative effect may become effective if animals live in a zoo, which is free of predators and supplied with a veterinarian and enough food.

The main difference between these two theories is that, for the former, genes with negative effects at old age *passively* accumulate from one generation to the next, while for the latter these genes are *actively* kept in the gene pool by selection. More experiments have tried to experimentally test the antagonistic pleiotropy theory than the theory of accumulation of mutations at old age, because the former theory allows more easily such tests.

More recently, Kirkwood (1993, 1999) accepted these two theories and considered that it is useless to invest too much energy in the soma maintenance if the chances to live long are low. In such conditions, it is more appropriate to favor fast reproduction. Thus, there is a balance between maintenance and reproduction for all species. When living conditions improve, and thus the chance for a longer life, it is useful to switch the balance more towards maintenance, because reproductive life increases, and the aging rate will decrease. When living conditions worsen, it is time to invest more in fast reproduction to increase fitness, which increases the aging rate since maintenance is deserted. This theory thus implies that it is possible to allocate energy either to maintenance or reproduction. Furthermore, this allocation does not only occur between successive generations, i.e., it is not only an evolutionary process. Rather, this process is also considered to explain individual variability of life histories, as it will be shown in the following.

These three theories, which are more complementary than antagonist (see e.g. Kirkwood and Rose 1991), shape modern thinking in gerontology. It is correct to stress that they offer a convincing explanation of the aging process. The hot question is to know whether they may be considered as validated or not. Indeed, in the past, other theories were considered to readily explain the aging process, before to be eventually given up, as for instance the Pearl's rate of living theory (1928). As emphasized by Kirkwood (1999, p. 58), "the theory of natural selection is one of the best tools we have to understand the living world". We may share that view (I share) and consider that it is yet needed to test the predictions of evolutionary theories of aging. Such tests are of help to refine these theories and it is well known that a deeper knowledge can be gained from studies trying to falsify or to confirm theories than from impassioned speeches claiming that the right theory has been discovered. To speak clearly, the review of existing data will show that evolutionary theories have not been fully validated, which does not mean that these theories have to be put to the trash. Indeed, for the time being, these theories are probably the best ones we have.

In the following, the article focuses on the tests of evolutionary explanations of aging. Firstly, it reviews the results on trade-offs between longevity and fecundity in

humans and other primates, secondly, studies selecting directly or indirectly for an increased longevity in flies and, thirdly, the very few experiments modulating extrinsic mortality rates.

3. Studies of trade-offs in human beings and primates

Evolutionary theories of aging are genetic theories: they try to explain aging by relying on the selection of genes with positive or negative effects on aging (Williams 1957) or on the accumulation of mutations at old age (Medawar 1952). At a first sight, it could be considered that it is nearly impossible to test these theories in human beings and other primates, since they are not easily amenable to genetic studies. This point is correct.

However, since both Williams' and Kirkwood's theories predict that trade-offs between longevity and fitness, particularly early reproduction, exist, it is valuable to look for their existence. The existence of trade-offs in humans or other primates would not prove that the theories are valid, since it would remain impossible to know whether trade-offs are due to genetic or environmental causes (or both). Nevertheless, discovering such trade-offs would obviously stimulate the search of their causes.

3.1 Human beings

A difficulty with the study of life histories in human beings is that modern people strongly limit their progeny number, which is not the best condition to study trade-offs between reproduction and longevity. Obviously, not limiting the progeny number in conditions where infant mortality is very low would give very large families, which is not the wish of most people. Consequently, studying ancient populations is of interest. It seems that only four studies have been done: Canadian women living in Quebec during the XVII-XVIIIth centuries (Le Bourg et al. 1993), British aristocrats living in the 740-1875 period (Westendorp and Kirkwood 1998), Germans living in the 1720-1870 period (Lycett, Dunbar and Voland 1999), European aristocratic and Finnish rural families living in the XVIII-XIXth centuries (Korpelainen 2000). Table 1 summarizes the results.

Le Bourg et al. (1993), using the parish registers of old Quebec have correlated various life history parameters in two populations: the French immigrant women arriving in Quebec before 1680 (range of birth dates: 1603-1666) and the first French-Canadian women born before 1700 in Canada (range: 1620-1699). At that time, contraception was unheard of and healthy living conditions spared people from many

Table 1: *Summary of the studies of trade-offs between fecundity and longevity in human beings.*

Authors	Time	Place	Trade-off?	Comments
Le Bourg et al. 1993	17-18th centuries	Quebec	No in immigrants No in first native Canadians	Natural fertility conditions
Westendorp and Kirkwood 1998	740-1875	United Kingdom	Yes in women and men (British aristocrats)	Database strongly male-biased, number of legitimate children used in computations, rather than the actual number (case of fathers), low progeny number
Lycett, Dunbar and Voland 1999	1720-1870	Germany	Yes, but very weak: r = – 0.07	
Korpelainen 2000	1700-1899	Europe and Finland	No (European aristocrats and rural Finns)	Trade-off with total number of children in Finns, but not with number of children reaching adulthood

diseases. Most women were married at least once and around 8% of immigrants remained sterile, this proportion being lower in the first Canadians (Charbonneau et al. 1987). Only women having at least one child during their life were considered in the analysis. The main difference between the two populations was that immigrants lived for 7 years longer than Canadians did, probably because they were a highly selected population. However, the two groups had the same mean number of children.

No clear relationship between early fecundity and longevity was observed in immigrants, while the most longevous Canadians had a higher early fecundity. However, this relationship was due to death at young age of some women, which obviously ends reproduction. Considering only women reaching the age of menopause made the relationship to disappear.

To sum up, it could be concluded that in the two groups of women, those who have their first child at a young age have also a high fecundity peak and a high number of children during a long reproductive life. There is thus no trade-off between fecundity, early or late, and longevity.

Westendorp and Kirkwood (1998) have also tried to discover trade-offs using the database of British aristocracy. They reported that, among women living at least to 60 years, the age at first child was positively correlated with longevity and the number of progeny was negatively correlated with longevity. That relationship held either for women living before 1700 and for those living in the 1700-1875 period, when the

progeny number was lower than before 1700. Furthermore, the same negative correlation was observed between progeny number and longevity of fathers.

This study seems thus to show that there is a trade-off between fecundity, early or not, and longevity, both in men and women. However, the problems of that study are huge.

Firstly, it is clear enough that British aristocrats strongly limited their progeny number since more than one third of women remained childless and, if we take into account only mothers, the progeny number was around 3.5. This is in sharp contrast with the study of Canadians for whom that number was 8. Therefore, British aristocrats are probably not the best sample to study the relationships between fecundity and longevity, since their fecundity is low. It is of interest that Ligtenberg and Brand (1999) showed that, when only mothers are considered in the analysis, there is no negative correlation between fecundity and longevity.

Secondly, Westendorp and Kirkwood (1998) when correlating progeny number with father's longevity used the legitimate number of children, and not the actual one. Unless to hypothesize that British aristocrats did not use their dominant social position to obtain intercourses with maidservants, a very strong hypothesis, their legitimate number of children is probably poorly connected to their actual number. Thus, the negative correlation between progeny number and father's longevity is surely spurious. It probably reflects an environmental component, as emphasized by Promislow (1998).

Thirdly, contrarily to Rose (1989), Le Bourg et al. (1993) and Promislow (1998), Westendorp and Kirkwood (1998) considered that the existence of trade-offs, as deduced from phenotypic correlations, would support "the interpretation that the decrease in progeny number in long-lived women has its basis in evolutionary genetics". This is maybe a too liberal attitude, since phenotypic correlations mix genetic and environmental influences, as already noticed. It cannot be argued that Westendorp and Kirkwood (1998) made a clumsy turn of phrase. Westendorp and Kirkwood (1999) answered to a criticism by Ligtenberg and Brand (1999) that the weakness of a correlation between spouse's life span "strongly argues against environmental factors playing a major role in the trade-off (between longevity and reproductive success), and supports the hypothesis that genetic factors are important". Finally, a correspondence between the author and Dr Westendorp confirms he thinks that "the data support the idea that there is a genetic variation within the human population for genes that affect life span, and genes for fertility and that there is a trade-off between the two".

Fourthly, the quality of the database of British aristocrats has been severely criticized (Gavrilova and Gavrilov 1999), particularly because the base is strongly male-biased (19,380 men and 13,667 women) and women's birth dates are unknown in much cases. Gavrilova and Gavrilov (1999) concluded, "this British database unfortunately can not be used in the scientific analysis in its present form".

Therefore, the problems with that study are so numerous that it cannot provide firm conclusions.

Lycett, Dunbar and Voland (1999) studied the relationships between fecundity and longevity in the Krummhörn population (northwest Germany) during the 1720-1870 period. Contrarily to British aristocrats, only 10% of women remained childless, and the mean number of children in the whole population was around 5. There was no negative correlation between the number of children and mother's longevity (only women reaching 50 years of age were used in analysis).

The authors then controlled for the duration of marriage, which was positively correlated with women's longevity, and claimed that, for the poorest group of the population only (the "landless"), there was a negative correlation between the number of children and women's longevity (r = – 0.139, p = 0.040, n = 223). However, no correlations were observed in two richer groups ("farmers" and "smallholders"). Considering the whole population resulted in a significant negative correlation (r = – 0.072, p = 0.041, n = 820). The negative correlation in the poorest group was not connected to a higher number of children in this group, since that number was similar in the three groups.

Furthermore, when the amount of time spent in a fecund marriage was controlled for, i.e. the time between marriage and menopause, the authors observed a similar negative correlation in the poorest group (r = – 0.074, p = 0.005, n = 276), while positive correlations were observed in the two richest groups. However, since these groups were less numerous, the significance levels of these correlations were lower (r = 0.099, p = 0.043, n = 73 and r = 0.068, p = 0.082, n = 119). Considering the whole population (n = 1073) resulted in a non-significant positive correlation.

Lycett, Dunbar and Voland (1999) concluded, "at least for the poorest social group, there is a trade-off between reproduction and longevity". It could be opposed to this rationale that the evidence for a trade-off between longevity and fecundity is not clear, since it is observed only in a given group, while positive or non-significant correlations are seen in other groups. More fundamentally, all correlations are weak and thus only explain a tiny part of the variance. It seems then that this study is not at variance with that of Le Bourg et al. (1993): in both studies no clear trade-off between reproduction and longevity does exist.

Finally, Korpelainen (2000) studied Finnish rural families and European aristocrats living in the XVIII and XIXth centuries. Women living longer than 80 years had a lower progeny number than those living for 50-79 years (respectively, 4.34 and 5.40 children). The number of offspring surviving to the age of 18 years was however not different (3.40 vs 3.88). No effect of longevity on progeny number was observed among fathers.

The author mixed in the analysis people experiencing very different living conditions, which can bias the results. As aristocrats had a lower number of offspring

and lived longer than rural families (table 1 in Korpelainen 2000), more Finns were in the short-lived group and more aristocrats in the long-lived one. A correspondence between the author and Dr Korpelainen indicated that the progeny number of aristocrat women (±SEM, n) was 4.35 (± 0.32, n = 100) for those dying in the 50-79 age range and 3.97 (±0.56, n = 29) for those living at least for 80 years, a non-significant difference.

Concerning Finn women, these numbers were respectively 5.92 (±0.21, n = 203) and 4.62 (±0.53, n = 39), a significant difference, but the number of offspring reaching adulthood did not depend on longevity (respectively for Finns living 50-79 years and more than 80 years: 3.99±0.17 and 3.51±0.38). Since mothers living more than 80 years had a higher proportion of surviving offspring (78.3%, table 2 in Korpelainen 2000) than those living for 50-79 years (71.9%), this could explain why there is a contrast between the effect of longevity on progeny number and on surviving progeny. Long-lived mothers have less children than shorter-lived ones, which is as trade-off, but they are more able to rear successfully their children, which is the contrary of a trade-off. In other words, there is no trade-off between fecundity and longevity because, with a lower total number of children, long-lived mothers had the same number of children reaching adulthood.

Considering the previous studies it seems that there is no clear trade-off between early and late fecundity in women, and no trade-off between fecundity, early or late, and longevity. These results do not refute the antagonistic pleiotropy theory, because the observed correlations are phenotypic, since they mix genetic and environmental influences (see, for a discussion of genetic and phenotypic correlations, Cheverud 1988, Stearns 1992, Roff 1995, 1996, Koots and Gibson 1996). However, they show that there is no ground to aver that trade-offs between fecundity and longevity do exist in human populations, as it could be expected from the antagonistic pleiotropy theory. It could be that antagonist pleiotropic genes dealing with fecundity and longevity do exist, but they do not seem to have a deep impact on life histories, since their influence does not outmatch the effect of other genes with no such pleiotropic effects or of environmental influences.

These four studies on human beings do not provide any clear evidence for trade-offs. It remains to know whether results gathered from other primates point in the same direction.

3.2 Primates

Studies focusing on reproduction and longevity in primates are scarce, to say the very least, and Table 2 summarizes them.

Table 2: *Summary of the studies of trade-offs between fecundity and longevity in primates.*

Authors	Species	Trade-off?	Comments
Bercovitch and Berard 1993	Rhesus macaques (*Macaca mulatta*)	No	Positive correlation between fecundity and longevity
Rhine, Norton and Wasser 2000	Baboons (*Papio cynocephalus*)	No	Positive correlation between fecundity and longevity, in optimal and bad living conditions (population decline)

In 1993, Bercovitch and Berard showed, in female rhesus macaques (*Macaca mulatta*) observed during 30 years, that macaques reproducing when 3 years-old ("rapid reproducers") did not live shorter than those reproducing when 5 years-old ("delayed reproducers", respectively: 9.0 ± 4.5 years, $n = 14$, vs 11 ± 4.5 years, $n = 11$). This result speaks against the existence of a trade-off between early fecundity and longevity. Considering only these two contrasted groups, longevity was independent of age at first parturition ($r = 0.077$, $n = 25$), but was strongly correlated with the number of offspring surviving to the age of sexual maturity ($r = 0.812$, $p < 0.001$, $n = 21$), or with the total number of offspring ($r = 0.891$, $p < 0.001$, $n = 25$). When age of death was kept constant, rapid reproducers gave birth to more offspring reaching age at maturity than delayed reproducers.

In summary, there is no trend for any trade-off between fecundity and longevity in female rhesus macaques, in accordance with results in humans. However, the sample size is rather low and considering the whole population, and not only rapid and delayed reproducers, would allow refining the picture. Furthermore, conclusions drawn from a single study need to be confirmed.

In 2000, Rhine, Norton and Wasser reported the results of a study of reproductive longevity and lifetime reproductive success in baboons (*Papio cynocephalus*). Lifetime reproductive success was defined as the number of offspring living at least to the age of sexual maturity. Reproductive longevity (highly correlated to longevity: $r > 0.98$) was the time interval between sexual maturity and death. The troop of baboons experienced

a severe population decline during the 24 years study, and it was possible to compare females born before the start of the study (n = 27) and those reaching adulthood in the years before the population decline (n = 45). The first sub-sample was less affected by the population decline and its reproductive longevity and lifetime reproductive success were higher than those of the second sub-sample (respectively, 13.58 vs 7.38 years and 3.13 vs 1.18 offspring). However, the correlation between lifetime reproductive success and reproductive longevity was positive in the two sub-samples (r = 0.70, n = 27, p < 0.0001; r = 0.73, n = 45, p < 0.0001). Even if this study did not differentiate between early and late fecundities, it clearly confirms a part of the conclusions of Bercovitch and Berard (1993) on macaques: there is no trade-off between fecundity and longevity.

3.3 Conclusions

This review of data correlating fecundity, particularly early fecundity, and longevity does not provide firm evidence in favor of the existence of trade-offs at the inter-individual level in human and non-human primates. However such trade-offs between life-history parameters do exist at the inter-specific (see, e.g., Stearns 1983) and, in some cases, at the intra-specific level (Stearns 1992).

On the one hand, this absence of trade-offs does not disprove the Williams' antagonistic pleiotropy theory of aging, but only shows that one of its predictions is not fulfilled. In such conditions, it is premature to wonder what part of the variation of longevity is due to pleiotropic genes. It has to be said that Williams (1957) stated that "most of the genes or gene combinations that favor vigor early in life probably also favor longevity" and that "only a small proportion of the genes need be of the sort that produce opposite effects on fitness at different ages". In such conditions, it could be argued that it would be always impossible to discover any trade-off between early fecundity and longevity at the inter-individual level. The only means to confirm the antagonistic pleiotropy theory of aging in humans could be to discover a human genetic disease due to an allele conferring the illness at old age and a selective advantage at young age (Albin 1993).

On the other hand, the absence of trade-offs disproves the Kirkwood's disposable soma theory of aging, because the theory does not only apply to the evolutionary level, but also to the inter-individual one.

In conclusion, the current evidence does not confirm evolutionary theories of aging in primates, as far as inter-individual trade-offs are concerned. Other studies of trade-offs between early fecundity and longevity in *Drosophila melanogaster* (e.g. Le Bourg et al., 1988) or in medflies (Carey et al. 1998) gave similar results.

However, selection for longevity in a species well amenable to genetic studies, such as *D. melanogaster*, could provide different conclusions about the validity of evolutionary theories of aging.

4. Direct and indirect selections for longevity in *Drosophila melanogaster*

Williams (1957) predicted that "successful selection for increased longevity should result in decreased vigor in youth", such as for instance a decreased early fecundity. This is a testable prediction, which explains that many gerontologists have tried to discover such a trade-off in *D. melanogaster*. Obviously, the goal of these studies was not only to study the possible genetic trade-off between longevity and early fecundity, but also, if not mainly, to create lines with contrasted longevities. Two selection procedures have been used: direct selection for life span and indirect selection via reproduction at old age. Studies using the first procedure are less numerous than those using the second one are. Tables 3 and 4, respectively, summarize the results.

4.1 Direct selection for longevity

Lints et al. (1979) tried to select directly for increased longevity. When 20% of the pairs of the line selected for increased longevity were dead, the authors kept the virgin progeny of surviving pairs. When only 25% of these pairs were still alive, their progeny was crossed to give the next generation and the progeny of the other pairs was discarded. Therefore, only the progeny of long-lived flies produced the next generation and this process was done for 8 generations.

There was no longevity increase in the selected line when compared to two control lines and the realized heritability was only 0.034 (Baret, Beckers and Lints 1995). However, longevity increased in the three lines during selection.

Zwaan, Bijlsma and Hoekstra (1995) also selected directly for longevity. They started selection for increased longevity in two replicate lines and for decreased longevity in two other lines, two control lines being used. In all lines, a part of the virgin progeny was kept at 29°C to measure longevity and the other part was stored at 15°C to produce the next generation. When all 29°C flies were dead, brothers and sisters of the most longevous ones were mated to produce the next generation of the long-lived lines. A similar procedure was used to give the next short-lived generation, and flies of the control lines were randomly paired. The whole process was done for 6 generations.

Table 3: *Summary of the studies selecting directly for longevity in Drosophila melanogaster.*

Authors	Longevity	Trade-off?	Comments
Lints et al. 1979	No increase (8 generations of selection for increased longevity)	Not studied	Longevity increased to the same extent in selected and control lines
Zwaan, Bijslma and Hoekstra 1995	Longevity increased, (6 generations of selection for increased or decreased longevity), when compared to control lines	Fecundity decreased at all ages in long-lived lines	The actual longevity did not really increase (0 day in females, 3 days in males)

Table 4: *Summary of the studies selecting indirectly for longevity in Drosophila melanogaster, by reproducing flies at old age.*

Authors	Longevity	Trade-off?	Comments
Lints and Hoste 1974, 1977	No increase (10 generations of reproduction at old or young age)	No	Erratic longevity variations between generations
Rose and Charlesworth 1981, Rose, 1984	Old lines live longer	Early fecundity lower and late fecundity higher in old lines	Longevity measured only in one generation
Luckinbill and Clare 1985, Clare and Luckinbill 1985	Longevity increases more in old lines than in young lines	Early fecundity lower in old lines	Longevity measured regularly during the 21 generations
Partridge and Fowler 1992	Old lines live longer	Early fecundity similar in young and old lines	Longevity measured only in one generation
Engström, Liljedahl and Björklund 1992	Old lines live longer	Early fecundity similar in young and old lines	Longevity measured only in one generation
Partridge, Prowse and Pignatelli 1999	Old lines live longer, but longevity only measured in mated females	Early fecundity lower in old lines	Longevity measured only in one generation
Buck et al. 2000	Old lines live longer, but sex and mating status are unknown	Developmental viability is negatively correlated with longevity in all lines	Longevity measured regularly during 50 months

Zwaan, Bijlsma and Hoekstra (1995) concluded that longevity increased in the long-lived lines, and that the selection was less successful in the short-lived ones. Consequently, they reported high realized heritabilities (up to 0.517).

These conclusions were based on the comparison of selected and control lines. However, if the actual mean longevity values are considered, longevity decreased during selection in both short-lived and control lines (by 40% in one of the control lines), while a slight longevity increase was observed in long-lived males (ca 3 days when compared to the parental generation) and no increase at all in females. The authors also measured longevity at 25°C in virgin flies. Long-lived lines derived from the fourth generation of selection lived longer than control ones, but the pattern was less consistent in flies coming from crosses between the two replicate lines. However, the authors did not indicate whether the longevity has decreased or not when compared to the parental generation. Finally, the progeny was lower in long-lived lines at all ages, and not only at young age, while viability of eggs, development time, body weight and starvation resistance were similar in control and long-lived lines.

On the one hand, Lints et al. (1979) failed to increase longevity in their selected line, but longevity increased in both selected and control lines during selection. Zwaan, Bijlsma and Hoekstra (1995) increased longevity in the long-lived lines, when compared to the control lines, but this pattern was only due to the decreased longevity of these latter lines during selection. It is difficult to accept that selection was successful when mean longevity does not increase.

The whole evidence of the two experiments selecting directly for longevity is inconclusive and we may hope that studies using indirect selection for longevity will clarify the issue.

4.2 Indirect selection for longevity

The rationale of the indirect selection for longevity procedure is that reproduction at old age in successive generations could increase longevity, provided this trait is partly heritable, since only long-lived flies may reproduce.

If the antagonistic pleiotropy theory is correct, we may expect to observe a decreased early fecundity in lines reproduced at old age and, maybe, an increased late fecundity. Conversely, if flies are repeatedly reproduced at young age, their longevity is expected to decrease, as well as their late fecundity, while their early fecundity increases.

If the theory of the accumulation of mutations at old age is correct, a trade-off between early and either late fecundity or longevity is not mandatory.

Numerous studies have tried to confirm these expectations. The most famous experiment is that of Luckinbill and Clare (1985) after the early work of Luckinbill et al. (1984). They measured longevity in successive generations and, after 21 generations of reproduction, longevity had regularly increased in lines reproduced at old age (hereafter called the OLD lines) and, to a lesser extent, in lines reproduced at young age (YOUNG lines). Furthermore, early fecundity decreased in OLD lines (Clare and Luckinbill 1985).

This work confirmed the results of Rose and Charlesworth (1981) and Rose (1984). However, these last authors measured longevity and fecundity only once, in the last generation of selection, and erratic differences between OLD and YOUNG lines could explain their differences. As a matter of fact, Lints and Hoste (1974, 1977), who have reproduced YOUNG and OLD lines for ten generations, observed large longevity and fecundity variations between generations. However, there was no increased longevity in OLD lines when compared to YOUNG ones.

After Luckinbill and Clare (1985), Partridge and Fowler (1992) and Engström, Liljedahl and Björklund (1992) also reported that OLD lines live longer than YOUNG or control ones, but longevity was measured only once during the selection process. However, they failed to show a decreased early fecundity in OLD lines.

By contrast, a decreased early fecundity was observed in the OLD lines of Partridge, Prowse and Pignatelli (1999), that lived longer than YOUNG lines (longevity measured only once during the selection process). However, since these authors measured longevity in mated females only, a cost of reproduction could explain the low longevity of YOUNG lines.

When all these results are considered, it seems clear that reproduction at old age does increase longevity. However, Baret and Lints (1993) reanalysed the Clare and Luckinbill's results (1985). They noticed that, since YOUNG and OLD lines are reproduced at different ages, the same generation number occurs at a different calendar time in the two lines. If longevity differs along months, as in Lints et al. (1989), it could bias the results. As a matter of fact, Baret and Lints (1993) showed that, when the Clare and Luckinbill's results (1985) are expressed as a function of calendar time, and not of generation number, the difference between YOUNG and OLD lines is erased. In other words, when YOUNG and OLD flies living at the same moment are compared, there is no longevity difference between them. This article provoked considerable attention (see the debate in Gerontology: Fukui, Pletcher and Curtsinger 1995; Arking and Buck 1995; Baret, Le Bourg and Lints 1996). Arking and Buck (1995) published new results using the lines of Luckinbill et al. (1984) showing clear differences between OLD and control lines reproduced at random age. However, these new results also showed that these differences appeared very late, not before the 30th month of selection, which confirms that no increased longevity in OLD lines was observed in the article of

Luckinbill and Clare (1985). Reproduction of OLD lines has finally increased their longevity, but it is hard to reconcile the late and sudden longevity increase with the effects due to a selection of quantitative genes. However, Buck et al. (2000) reported a new selection experiment with a different issue. They selected for increased longevity in lines also selected for the speed of development (flies emerged during days 1-4 of eclosion or during days 6-10 of eclosion) and measured longevity regularly during selection. The OLD lines showed a regularly increasing longevity in the two cases, with a clear difference from control lines 20 months after the start of selection in the fast-developing line and after 12 months in the slow-developing line. The problem with that study is that the authors did not specify the sex and mating status of flies used in longevity measurements.

While it can be now safely accepted (but see below) that reproduction at old age increases longevity, it remains that a decrease in early fecundity was not always observed, which casts some doubt on the antagonistic pleiotropy theory. Furthermore, it has been shown that the trade-off between early fecundity and longevity could be lost (Leroi, Chippindale and Rose 1994). An absence of trade-off between longevity and other traits indirectly connected to fitness has also been reported. For instance, Service et al. (1985) reported that OLD lines have throughout life a higher resistance to starvation and desiccation than YOUNG lines. Arking et al. (2000) reported that OLD lines have at young and old ages a higher superoxide dismutase activity level than control lines, no difference being observed at intermediate ages. Indeed, the results seem more compatible with the accumulation of mutations at old age, since it could be argued that reproduction at old age has purged the gene pool from deleterious alleles, which could explain the increased longevity as well as the increased resistance to some stresses.

However, the debate about the increased longevity of OLD lines could be soon revitalized. Buck et al. (2000) have produced new OLD lines and showed that they have up to twice the developmental lethality as have control lines. Using 8 OLD and control lines, they reported that longevity increased when developmental viability decreased (r = – 0.79). This could thus explain a part of the increased longevity of OLD lines since only the fittest, and possibly the most longevous, flies reach adulthood in OLD lines, while most of flies do so in the control lines. In such conditions, a part of the increased longevity could be a mere statistical artifact, due to a demographic selection process, since the longevity of (short-lived?) flies dying during development cannot be observed. It is of interest to note that Partridge and Fowler (1992) showed that larvae of OLD lines were less able to compete with larvae of a mutant than were larvae of YOUNG lines. For instance, if one third of larvae in a vial were YOUNG and two thirds were mutant, one third of emerging imagoes were YOUNG. This proportion was only 20% in OLD lines. When larval crowding increased, the differences were more important. For

the same proportion of YOUNG larvae, the proportion of YOUNG imagoes was 45%, while it was only 15% in OLD lines. Roper, Pignatelli and Partridge (1993) confirmed these results, but Partridge, Prowse and Pignatelli (1999) did not. Curtsinger (pers. comm.) has indicated that the viability is currently 85% in the OLD Luckinbill's lines and 88% in the YOUNG ones, with respective longevities of 70-80 and 35-40 days. The small viability difference does not appear to explain the huge longevity difference.

4.3 Conclusions

This review of studies using reproduction at old age to increase longevity in *D. melanogaster* and of those attempting to directly increase longevity was not intended to cover all results. Particularly, other studies using different species have been done (e.g. Tucic et al. 1996 in the bean weevil *Acanthoscelides obtectus*). The goal of this review was simply to show whether longevity has been increased, and how this result is in accordance with evolutionary theories of aging.

Direct selection for longevity has failed to increase longevity. Zwaan, Bijlsma and Hoekstra (1995) increased longevity in their long-lived lines, when compared to the control ones, but this pattern was only due to the decreased longevity of these latter lines. Indeed, the mean longevity of long-lived lines did not increase.

Reproduction at old age was more successful and it can be accepted that it increased longevity. However, at least in what concerns the Luckinbill's lines for which we have regular longevity measurements during the experiment, it is difficult to explain why no increase occurred before 30 months of selection. The Buck's et al. (2000) lines suffer less from this problem, but a high developmental lethality could explain a part of the longevity increase in OLD lines.

The study of early fecundity has shown that trade-offs with longevity have been observed only in some cases. Furthermore, other traits, such as stress resistances, were not involved in trade-offs. The whole evidence does not totally confirm the antagonistic pleiotropy theory but shows that, at least in some cases, pleiotropic mechanisms could be at play.

To sum up, even if one could consider that these experiments have failed to unequivocally confirm the Williams' theory (1957), it remains that creating long-lived lines of flies has provided the community of gerontologists with a useful research tool. However, as emphasized by Harshman and Hoffmann (2000), inconsistent correlated responses to selection can be observed.

Another way of testing evolutionary theories of aging is to experimentally impose low or high extrinsic mortality rates to modify longevity.

5. Extrinsic mortality rates and longevity

When extrinsic mortality rates increase, there is less time to reproduce and an evolutionary response is expected to preserve the species from eventual extinction. As a matter of fact, a higher extrinsic mortality rate means a higher selection for a fast reproduction and, if evolutionary theories of aging are correct, we expect an early fecundity increase at the expense of late fecundity and lifespan. This is the rationale of the very few studies imposing higher extrinsic mortality rates as a means to test evolutionary theories of aging (see for a discussion Reznick 1997). These studies do not select directly for longevity, nor they impose an age at reproduction. Simply, they use contrasted extrinsic mortality rates.

Stearns, Ackermann and Doebeli (1998) defined two extrinsic mortality rates in *D. melanogaster*. The authors rear flies in population cages since 1993 and kill 90% of them twice a week (probability to survive a week: 0.01, HAM condition), or 20% (probability to survive a week: 0.64, LAM condition). In 1996, the probability to survive under the LAM condition was risen to 0.81 (10% of flies killed twice a week). In order not to mix the effects of mortality rates and those of density, killed flies are replaced by flies of the same condition (HAM or LAM) and age. Flies are thus kept under contrasted mortality rates and their life history parameters are observed regularly. After three years of experiment (75 generations in the high mortality conditions and 40 in the low ones) the mean longevity was about 30 days in HAM flies, and 34 days in the LAM condition (mean longevity computed from fig.4 in (Stearns, Ackermann and Doebeli 1998). Furthermore, HAM flies eclosed earlier and had a higher early fecundity than LAM ones. However, no difference was observed regarding late fecundity (Stearns et al. 2000, Gasser et al. 2000). After five years of experiment (Stearns et al. 2000), it was observed that the early fecundity differences had reached a plateau 30 months after the beginning of experiment, and the same was true for development time. Oddly enough, early fecundity decreased regularly under both HAM and LAM conditions (from 70 eggs at days 13-15 to 20), from the very beginning of experiment, while development time variations were more erratic. The longevity had also strongly increased under both conditions between 1996 and 1998, since the median longevity was above 50 days in 1998. However, the HAM flies lived shorter than LAM flies (mean longevities unknown, but median longevity estimated from fig.3 in Stearns et al. 2000), as it was observed earlier. Stearns et al. (2000) did not provide explanations for the increased longevity between 1996 and 1998, nor they explained why early fecundity decreased throughout experiment. In a correspondence with the author, Dr Stearns said that he prefers not to draw "any quantitative conclusions from the trends over time"

because he is "much more certain of the differences between the two treatments than… of the changes within a treatment from, say, 1995 to 1998".

Other experiments modifying extrinsic mortality rates have been done, for instance in guppies. Early fitness varies according to mortality rates, in accordance with evolutionary theories of aging, but experiments recording longevity are still in progress (Reznick 1997).

The study on flies seems to indicate that, even if some questions remain unanswered, contrasting extrinsic mortality rates makes longevity and early fecundity to differ, in accordance with evolutionary theories of aging. Obviously, more results are needed before to conclude unequivocally that these theories are confirmed. However, there is a good chance that they will be eventually confirmed, since their expectations concerning the effect of extrinsic mortality rates are very similar to those of life history studies (Stearns 1992).

6. Are the evolutionary theories of aging valid?

This review shows that, on the one hand, there is no clear trade-off at the individual level between fecundity and longevity in human beings, but it could be rightly argued that the absence of phenotypic correlations does not disprove the antagonistic pleiotropy theory (Williams 1957). This is correct, but it has been shown above that Westendorp and Kirkwood (1998) consider inter-individual trade-offs as arguments in favor of the Kirkwood's disposable soma theory.

On the other hand, indirect selection for longevity in *D. melanogaster* was eventually successful, but it remains difficult to explain the kinetics of the selection response. A progressive longevity increase was expected, but no increase was observed for a long time in the Luckinbill's lines. It remains that lines differing by their longevity and originating from the same genetic background are available. However, direct selection for longevity appears to fail.

Finally, experiments that modify extrinsic mortality rates seem a promising tool to test evolutionary theories of aging.

Some conclusions about the validity of the evolutionary theories of aging may now be drawn.

6.1 The theory of the accumulation of mutations at old age

The theory of the accumulation of mutations at old age is in accordance with common sense, since it states that mutations occurring at old age, i.e. after the reproductive age,

are likely to be kept in the gene pool. However, Pletcher and Curtsinger (1998) are right when stressing that "a theory involving deleterious mutation pressure alone as the cause of senescence is not consistent with mortality plateaus far below 100%".

If this theory is considered as the main or unique cause of aging, it may be ruled out. By contrast, if one considers that it describes one cause of aging among others, the theory may be valuable. Indeed, some features of aging could be due to such alleles with a late expression while other ones could be best explained by different mechanisms. If the mutations at old age explain only a part of the aging process, "plateaus far below 100%" could be explained.

6.2 The theory of the antagonistic pleiotropy

The theory of the antagonistic pleiotropy has been submitted to many tests. Maybe the most important result is that lines with contrasted longevities have been created when reproducing flies at old age.

However, the crucial idea of the theory is that "successful selection for increased longevity should result in decreased vigor in youth" (Williams 1957). This correlated response to selection has been observed in some cases, but not in each selection experiment. It is however not difficult to imagine that some alleles with antagonistic pleiotropic effects on early fitness and longevity could be at play in some strains, but not in others. If so, a decreased early fecundity, as a correlated response to selection on longevity, can be observed or not. In the same way, positive genetic correlations between early fecundity and longevity are not unexpected, too. For instance Clark and Guadalupe (1995) reported such a genetic positive correlation (correlations between line means: $r = 0.286$, $p = 0.0398$) in 52 lines of flies containing a P-element inserted at random into a common genetic background. Therefore, it may be that genetic variation for longevity is associated with a decreased early fecundity in some strains, an increased early fecundity in others, or not associated. It remains that observing a decreased early fecundity shows that the antagonistic pleiotropy mechanism is at play in some cases.

Thus, there is no debate about the validity of the antagonistic pleiotropy hypothesis: the hypothesis is valid. The debate concerns the importance of that mechanism for the aging process, because it can be shown only in some cases that a genetic trade-off exists between early fecundity and longevity (see Table 4). Thus, it may be concluded, as it was done for the theory of the accumulation of mutations at old age, that the antagonistic pleiotropy theory is correct but, probably, does not describe the main cause of aging.

6.3 The disposable soma theory

Concerning the disposable soma theory, we have to take into account the level of explanation of the theory, because it can be applied either at the evolutionary level or at the individual level.

When applied at the evolutionary level, the theory is not really different from the antagonistic pleiotropy theory. As emphasized by Kirkwood and Rose (1991), the disposable soma theory is a causal subset of the antagonistic pleiotropy theory, "if the genes responsible for somatic maintenance functions are regarded as … (to) prolong survival and … consume resources which might be used for reproduction". In fact, "the difference between the disposable soma theory and the antagonistic pleiotropy is partly a difference between an optimality approach… and a quantitative genetics approach", but the two theories do not differ in their predictions regarding trade-offs between reproduction and longevity.

The disposable soma theory is also explicitly applied at the individual level, because trade-offs can also operate at the individual level. For instance, Kirkwood and Rose (1991) consider the case when "reproductive activity reduces survival by increased risk exposure"; in that case, "rescheduling fecundity to later ages will increase survivorship". This kind of non-metabolic trade-off operates at the individual level. However, we may be skeptical about some "direct metabolic trade-offs … that can happen if reproduction and maintenance draw directly from the same supply of resources within the organisms". If such trade-offs were so important at the individual level, we would observe a clear trade-off between the number of children and longevity in women, particularly at periods when only breastfeeding, a costly metabolic activity, was possible. It has been shown (Table 1) that it is not the case.

Finally, it has to be recalled that it is not possible to use phenotypic correlations to directly conclude in favor of a genetic hypothesis, as Westendorp and Kirkwood (1998) did (see 3.1). Therefore, it may be concluded that the disposable soma theory is probably not correct when applied at the individual level.

7. Can we accept the evolutionary theories of aging?

In conclusion, the whole evidence shows that the evolutionary theories of aging explain a part of the results but not all of them. In such conditions, we may wonder whether time has come to accept them provisionally, even if one may have doubts about some of their expectations, because they explain a part of the aging process. In 1989, Rose and Graves wrote, "if other biologists could accept the sufficiency of the evolutionary

theory as the general theory of aging, then there might be a relaxation of efforts to find general physiological theories of aging". Nowadays, evolutionary theories have not any obvious challenger. In some cases, these theories well fit to the results and, in other cases, they fail; but many gerontologists consider that they offer, more or less, a plausible explanation of the aging process by integrating natural selection as a main cause of aging. In such conditions, accepting the general frame of these theories would relax efforts to test all their expectations.

Evolutionary theories of aging have faced the opposition of Sacher (1978). He considered that "the implication that… organisms are mortal only because of the accumulation of adventitious senescence genes, is more easily reconciled with a cosmology of special creation than with current scientific conceptions". Lints (1983, 1985) strongly criticized, not the evolutionary theories of aging, but rather the first experiments claiming to confirm them, and particularly that of Rose and Charlesworth (1980) expanded in Rose and Charlesworth (1981). The Le Bourg's et al. (1988) article provoked a hard debate with Rose (1989). Later on, Le Bourg et al. (1993) criticized Rose's (1991) logical flaws about theories testing. Since that time, new experiments have been done to test the theories and many gerontologists seem to consider that evolutionary theories are the current best candidates to explain the occurrence of the aging process. Particularly, connecting the evolutionary theories with the life history theory was probably a step toward this beginning of a consensus since "trade-offs have a central role in life history theory" (Stearns 1992). In fact, longevity and early fecundity are life history parameters among other ones, such as number of offspring, time to maturity, and so on. Thus, they have to be considered just like these other parameters. In this way, it could be said that evolutionary theories of aging are just a subset of the life history theory.

During the last twenty years, some gerontologists have tried to confirm or invalidate evolutionary theories of aging. We may wonder whether it is useful to spend the next twenty years to test them again, since it is clear that they explain, at least partly, the aging process. These theories probably still need to be refined. New tests of them are surely useful. However, testing them is probably no longer a top priority. This priority could be to find new means to modulate the aging process in animal models (worms, flies, rodents or primates), using genetic or environmental manipulations, to finally improve everyday life of elderly. This is of importance because the number of elderly is growing rapidly.

That does not mean that we must *definitively* accept the evolutionary theories of aging, while we may think they are not so good, but simply that testing them could be only a secondary goal or our research. Debates about the validity of evolutionary theories will continue for the best of gerontology, but should not constitute the main interest of the community. Current evolutionary theories have probably not told the

final word about the aging process and articles favoring them, even when alternative hypotheses are available, may be criticized (Le Bourg 1998). Therefore, it is possible to provisionally accept these theories while not being a dogmatic supporter of them.

It is indeed possible that new studies will challenge current evolutionary theories up to the point that they will be given up. It is also possible that, eventually, these new studies will confirm these theories. As scientists, we have to be ready to accept any outcome. However, even if testing evolutionary theories is no longer a top priority, previous attempts were not wasted time and effort, because gerontologists have reached the beginning of a consensus. To be perfectly clear, these theories give a plausible explanation of the aging process, but in many occasions they do not fit to the results: since better theories are not available, we could accept them provisionally and concentrate our efforts in other directions.

8. Acknowledgements

References

Albin TL (1993). Antagonistic pleiotropy, mutation accumulation, and human genetic disease. *Genetica* 91: 279-286.

Arking R, Buck S (1995). Selection for increased longevity in *Drosophila melanogaster*: a reply to Lints. *Gerontology* 41: 69-76.

Arking R, Burde V, Graves K, Hari R, Feldman E, Zeevi A, Soliman S, Saraiya A, Buck S, Vettraino J, Sathrasala K (2000). Identical longevity phenotypes are characterized by different patterns of gene expression and oxidative damage. *Exp. Geront.* 35: 353-373.

Baret PV, Beckers F, Lints FA (1995). Realized heritability of longevity in *Drosophila melanogaster*. *Gerontology* 41: 82-85.

Baret PV, Le Bourg E, Lints FA (1996). Selection for increased longevity in *Drosophila melanogaster*: reflections on new data. *Gerontology*, 42: 14-17.

Baret P, Lints FA (1993). Selection for increased longevity in *Drosophila melanogaster*: a new interpretation. *Gerontology* 39: 252-259.

Bercovitch FB, Berard J.D. (1993). Life history costs and consequences of rapid reproductive maturation in female rhesus macaques. *Behav. Evol. Sociobiol.* 32: 103-109.

Buck S, Vettraino J, Force AG, Arking R (2000). Extended longevity in *Drosophila* is consistently associated with a decrease in developmental viability. *J. Geront. Biol. Sci.* 55A: B292-B301.

Carey JR, Liedo P, Müller HG, Wang JL, Chiou JM (1998). Relationship of age patterns of fecundity to mortality, longevity, and lifetime reproduction in a large cohort of mediterranean fruit fly females. *J. Geront. Biol. Sci.* 53A: B245-B251.

Charbonneau H, Desjardins B, Guillemette A, Landry Y, Légaré J, Nault F (1987). Naissance d'une population. Les Français établis au Canada au XVIIe siècle. Paris: Institut National d'Études Démographiques, Presses Universitaires de France; Montréal: Presses de l'Université de Montréal.

Cheverud JM (1988). A comparison of genetic and phenotypic correlations. *Evolution* 42: 958-968.

Clare MJ, Luckinbill LS (1985). The effects of gene-environment interaction on the expression of longevity. *Heredity* 55: 19-29.

Clark AG, Guadalupe RN (1995). Probing the evolution of senescence in *Drosophila melanogaster* with P-element tagging. *Genetica* 96: 225-234.

Engström G, Liljedahl LE, Björklund T (1992). Expression of genetic and environmental variation during ageing. 2. Selection for increased lifespan in *Drosophila melanogaster. Theor. Appl. Genet.* 85: 26-32.

Fukui HH, Pletcher SD, Curtsinger JW (1995). Selection for increased longevity in *Drosophila melanogaster*: a response to Baret and Lints. *Gerontology* 41: 65-68.

Gasser M, Kaiser M, Berrigan D, Stearns SC (2000). Life-history correlates of evolution under high and low adult mortality. *Evolution* 54: 1260-1272.

Gavrilova NS, Gavrilov LA (1999). Data resources for biodemographic studies on familial clustering of human longevity. *Demogr. Res.* (Online) 1. Available: http://www.demographic-research.org/volumes/vol1/4.

Harshman LG, Hoffmann AA (2000). Laboratory selection experiments using *Drosophila*: what do they really tell us? *TREE* 15: 32-36.

Keller L, Genoud M (1997). Extraordinary lifespans in ants: A test of evolutionary theories of aging. *Nature* 389: 958-960.

Keller L, Genoud M (1999). Evolutionary theories of aging. 1. The need to understand the process of natural selection. *Gerontology* 45: 336-338.

Kirkwood TBL (1993). The disposable soma theory of aging: evidence and implications. *Neth. J. Zool.* 43: 359-363.

Kirkwood TBL (1999). Time of our lives. London: Weidenfeld and Nicolson.

Kirkwood TBL, Rose MR (1991). Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. Lond.* B 332: 15-24.

Koots KR, Gibson JP (1996). Realized sampling variances of estimates of genetic parameters and the difference between genetic and phenotypic correlations. *Genetics* 143: 1409-1416.

Korpelainen H (2000). Fitness, reproduction and longevity among European aristocratic and rural Finnish families in the 1700s and 1800s. *Proc. R. Soc. Lond.* B 267: 1765-1770.

Le Bourg E (1998). Evolutionary theories of aging: Handle with care. *Gerontology* 44: 345-348.

Le Bourg E, Beugnon G (1999). Evolutionary theories of aging : 2. The need not to close the debate. *Gerontology* 45: 339-342.

Le Bourg E, Lints FA, Delincé J, Lints CV (1988). Reproductive fitness and longevity in *Drosophila melanogaster. Exp. Geront.* 23: 491-500.

Le Bourg E, Thon B, Légaré J, Desjardins B, Charbonneau H (1993). Reproductive life of French-Canadians in the 17-18th centuries: a search for a trade-off between early fecundity and longevity. *Exp. Geront.* 28: 217-232.

Leroi AM, Chippindale AK, Rose MR (1994). Long-term laboratory evolution of a genetic life-history trade-off in *Drosophila melanogaster*. 1. The role of genotype-by-environment interaction. *Evolution* 48: 1244-1257.

Ligtenberg T, Brand H (1999). Longevity – Does family size matter? *Nature* 399: 522.

Lints FA (1983). Genetic influences on lifespan in *Drosophila* and related species. *Rev. Biol. Res. Aging* 1: 51-72.

Lints FA (1985). *Drosophila. Interdiscipl. Topics Geront.* 21: 91-119.

Lints FA, Hoste C (1974). The Lansing effect revisited – I. Life-span. *Exp. Geront.* 9: 51-69.

Lints FA, Hoste C (1977). The Lansing effect revisited – II. Cumulative and spontaneously reversible parental age effects on fecundity in *Drosophila melanogaster*. *Evolution* 31: 387-404.

Lints FA, Lints CV, Bullens P, Bourgois M, Delincé (1989). Unexplained variations in life span of the Oregon-R strain of *Drosophila melanogaster* over a four-year period. *Exp. Geront.* 24: 265-271.

Lints FA, Stoll J, Gruwez G, Lints CV (1979). An attempt to select for increased longevity in *Drosophila melanogaster*. *Gerontology* 25: 192-204.

Luckinbill LS, Arking R., Clare MJ, Cirocco WC, Buck SA (1984). Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* 38: 996-1003.

Luckinbill LS, Clare MJ (1985). Selection for life span in *Drosophila melanogaster*. *Heredity* 55: 9-18.

Lycett JE, Dunbar RIM, Voland E (1999). Longevity and the costs of reproduction in a historical human population. *Proc. R. Soc. Lond.* 267: 31-35.

Masoro EJ (1996). The biological mechanism of aging: Is it still an enigma? *Age* 19: 141-145.

Medawar PB (1952). An unsolved problem in biology. London: HK Lewis.

Medvedev ZA (1990). An attempt at a rationale classification of theories of aging. *Biol.Rev.* 65: 375-398.

Partridge L, Fowler K (1992) Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* 46: 76-91.

Partridge L, Prowse N, Pignatelli P (1999). Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Proc. R. Soc. Lond.* B 266: 255-261.

Pearl R (1928). The rate of living. London: Knopf.

Pletcher SD, Curtsinger JW (1998). Mortality plateaus and the evolution of senescence: why are old-age mortality rates so low? *Evolution* 52: 454-464.

Pletcher SD, Houle D, Curtsinger JW (1998). Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. *Genetics* 148: 287-303.

Promislow DEL (1998). Longevity and the barren aristocrat. *Nature* 396: 719-720.

Reznick DN (1997). Life history evolution in guppies (*Poecilia reticulata*): guppies as a model for studying the evolutionary biology of aging. *Exp. Geront.* 32: 245-258.

Rhine RJ, Norton GW, Wasser SK (2000). Lifetime reproductive success, longevity, and reproductive life history of female yellow baboons (*Papio cynocephalus*) of Mikumi National Park, Tanzania. *Amer. J. Primatol.* 51: 229-241.

Roff DA (1995). The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. *Heredity* 74: 481-490.

Roff DA (1996). The evolution of genetic correlations: an analysis of patterns. *Evolution* 50: 1392-1403.

Roper C, Pignatelli P, Partridge L (1993). Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster*. *Evolution* 47: 445-455.

Rose MR (1984). Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* 38: 1004-1010.

Rose MR (1989). To the editor. *Exp. Geront.* 24: I.

Rose MR (1991). Evolutionary biology of aging. Oxford, New-York, Tokyo: Oxford University Press.

Rose MR, Charlesworth B (1980). A test of evolutionary theories of senescence. *Nature* 287: 141-142.

Rose MR, Charlesworth B (1981). Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics* 97: 187-196.

Rose MR, Graves JL (1989). What evolutionary biology can do for gerontology. *J. Geront. Biol. Sci.* 44: B27-B29.

Sacher G (1978). Evolution of longevity. In: Schneider EL, editor. The genetics of aging. Plenum publ. Corp. pp.151-168.

Service PM, Hutchinson EW, Mackinley MD, Rose MR (1985). Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* 58: 380-389.

Stearns SC (1983). The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *OIKOS* 41: 173-187.

Stearns SC (1992). The evolution of life histories. Oxford, New-York, Tokyo: Oxford University Press.

Stearns SC, Ackermann M, Doebeli M (1998). The experimental evolution of aging in fruitflies. *Exp. Geront.* 33: 785-792.

Stearns SC, Ackermann M, Doebeli M, Kaiser M (2000). Experimental evolution of aging, growth, and reproduction in fruitflies. *Proc. Natl. Acad. Sci. USA* 97: 3309-3311.

Tucic N, Gliksman I, Seslija D, Milanovic D, Mikuljanac S, Stojkovic O (1996). Laboratory evolution of longevity in the bean weevil (*Acanthoscelides obtectus*). *J. Evol. Biol.* 9: 485-503.

Turker M. (1996). Premature aging. In: Birren JE, editor. Encyclopedia of gerontology. Age, Ageing, and the aged. Vol. 2: 341-354. Academic Press.

Westendorp RGJ, Kirkwood TBL (1998). Human longevity at the cost of reproductive success. *Nature* 396: 743-746.

Westendorp RGJ, Kirkwood TBL (1999). Longevity – Does family size matter? *Nature* 399: 522.

Williams GC (1957). Pleiotropy, natural selection and the evolution of senescence. *Evolution* 11: 398-411.

Zwaan B, Bijlsma R, Hoekstra RF (1995). Direct selection for life span in *Drosophila melanogaster*. *Evolution* 49: 649-659.