A new evolutionary genetics of aging. I. What is aging?

Michael R. Rose[1*], Thomas Flatt[2], Joseph L. Graves[3], Lee F. Greer[4], Daniel E. Martinez[5], Margarida M. Matos[6], Laurence D. Mueller[1], Robert J. Shmookler Reis[7], and Parvin Shahrestani[1].

[1]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA*

[2]*Institute of Population Genetics, Department of Biomedical Sciences, Vetmeduni Vienna, Vienna, Austria*

[3]*Joint School of Nanoscience & Nanoengineering, North Carolina A&T State University, UNC Greensboro, NC, USA*

[4]*Department of Biology, La Sierra University, Riverside, CA, USA*

[5]*Department of Biology, Pomona College, Claremont, CA, USA*

[6]*Universidade de Lisboa, Faculdade de Ciencias da Universidade de Lisboa, Centro de Biologia Ambiental, Departamento de Biologia Animal, Campo Grande, Lisboa, Portugal*

[7]*Central Arkansas Veterans Healthcare System; and Department of Geriatrics and Biochemistry/Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Correspondence:
Dr. Michael R. Rose
Department of Ecology and Evolutionary Biology
University of California, Irvine
321 Steinhaus Hall,
Irvine, CA, 92697-2525, USA
mrrose@uci.edu
949-824-8121, FAX (Dept): 949-824-2181

Recent research on the evolutionary genetics of aging has led to significant changes in the views that were held *circa* 1990. As part of the development of this journal, we will be examining central questions in the evolution and genetics of aging, with a view to developing new lines of thought for research in this area, broadly conceived. We begin with a seemingly innocuous question, what is aging? Aging can be operationally defined in terms of declining adult life-history characters, such as the probability of survival from one age to another. But such definitions do not identify what *kind* of phenomenon it is. Recent demographic and evolutionary research suggests that aging is not due to unrelenting, cumulative, physiological processes. In particular, the discovery that aging stops has led to a substantive reformulation of the evolutionary biology of aging. Closer theoretical examination of the evolutionary genetic consequences of Hamilton's declining forces of natural selection suggests that aging can come to an end under sufficiently benign conditions, among some species. Experiments with *Drosophila* have shown that changing the ages at which survival and reproduction are truncated in the evolutionary history of a population leads to corresponding evolutionary changes in the age at which aging stops, as predicted by such Hamiltonian theory. Instead of a cumulative physiological process, aging is better conceived of in terms of declining age-specific adaptation. In this context, late-life plateaus in mortality and fecundity reflect the stabilization of adaptation after Hamilton's forces of natural selection have leveled off. In sum, we propose that aging is a multifaceted phenomenon that is a derivative feature of the evolutionary biology of adaptation, *not a single* physiological process.

Keywords: definition of aging; [forces of] natural selection; *Drosophila*; evolution of aging; late life; senescence; fitness; adaptation

Main Thesis: Aging is De-tuned Adaptation

In 1991, the book *Evolutionary Biology of Aging* offered the following definition of aging: *a persistent decline in the age-specific fitness components of an organism due to internal physiological deterioration* (Rose, 1991). This definition has since been used by others a number of times. However, it was only a modest generalization of a definition proffered by Alex Comfort over three editions (1956-1979) of his key book *The Biology of Senescence* (Comfort, 1979): "a progressive increase throughout life, or after a given stadium, in the likelihood that a given individual will die, during the next succeeding unit of time, from randomly distributed causes." The 1991 definition chiefly added reproductive fitness components to Comfort's definition, while adding the qualifiers that the fitness-component decline should be persistent and should be "due to internal physiological deterioration," where the latter phrase was meant fairly broadly. Thus increases in mortality with age due to chronic infections such as HIV/AIDS were excluded by the 1991 definition.

Yet a mere definition doesn't necessarily tell a scientist what causally underlies the phenomenon that is so defined. The latter issue is much broader, implicitly raising fundamental scientific questions regarding mechanisms. As a simple but related example, the term *adaptation* can be defined as an attribute that enhances the net reproduction of members of a particular population or species, and yet many deeper issues are invoked by a question like, "*What is adaptation*?" A creationist, for example, could view adaptation as an attribute that is so defined, yet further assume that all such adaptations are specifically bequeathed to organisms by an omnipotent creator. By contrast, an evolutionary biologist would instead assume that such adaptations are necessarily products of natural selection, acting directly or indirectly.

Before 1992, almost every scientist who thought about aging assumed that it progressed without remit to the point of death. Evolutionary biologists further thought that this inexorable deterioration was brought about by the progressive decline in Hamilton's forces of natural selection (Hamilton, 1966; Rose, 2007). With respect to the underlying physiological machinery of aging, the only difference between most evolutionists and most gerontologists at that time was that evolutionists overwhelmingly expected that there were likely to be many physiological mechanisms of deterioration, rather than a few (Williams, 1957; Rose, 1991). Thus the aforementioned 1991 book accommodated commonly inferred physiological mechanisms of aging within an overarching evolutionary framework, thus delineating an "evolutionary biology of aging" that subsumed conventional gerontological thinking, rejecting only those parts that were inconsistent with evolutionary theory.

This synthesis of evolutionary biology and gerontology survived for only one year before being undermined by two 1992 papers, Curtsinger et al. (1992) and Carey et al. (1992), in which demographic aging was shown to subside in late-life among cohorts of *Drosophila* and the medfly. Some initial attempts to accommodate their results focused on possible density artifacts (Nusbaum et al., 1993), but a substantive series of experiments from the Curtsinger lab (Khazaeli et al., 1995, 1998) demolished such quibbling. By 1995, it was clear that the cessation of aging was a genuine phenomenon rather than an experimental artifact.

Yet another possibility remained, one that had been discussed in 1939 by Greenwood and Irwin (1939) in their article showing that human aging stopped demographically: lifelong heterogeneity. This is a concept that has been mathematically developed, particularly by Vaupel

(Vaupel et al., 1979; Vaupel, 1988), but it is fairly easy to convey as a verbal argument. If a cohort consists of sub-cohorts that differ radically in their lifelong robustness, then the less robust will be eliminated early, leaving only the much more robust individuals. If these surviving sub-cohorts are robust enough, demographic aging should greatly slow at very late ages.

Many have looked for evidence of such an association between lifelong robustness and the cessation of aging (Khazaeli et al., 1998; Drapeau et al., 2000; Mueller et al., 2003; Rauser et al., 2005). It *is* possible to produce such a mortality-rate flattening by artificially constructing cohorts out of very different sub-cohorts (Brooks et al., 1994), but no one has yet found enough naturally-occurring lifelong heterogeneity to generate demographic plateaus in age-specific mortality or fecundity (reviewed in Mueller et al., 2001). Indeed, there are good evolutionary genetic reasons to expect that such lifelong heterogeneity will rarely arise: natural selection will oppose the maintenance of such heterogeneity, whether it is due to genetic polymorphism or extreme non-genetic plasticity (Mueller et al., 2011, Chapter 7). Natural selection instead favors the maintenance of genetic variation affecting fitness-components when that genetic variation has opposed effects at different ages, in other words antagonistic pleiotropy, not lifelong effects that are consistent in direction (Rose, 1985; Mueller et al., 2011).

Thus it appears that the cessation of aging occurs at the individual level, and is not just an artifact of population structure. Yet this is clearly paradoxical, if we think of the machinery of aging in terms of such physiological processes as steadily cumulative damage. If it is supposed that some process of cumulative damage or disharmony is supposed to underlie aging, why should that process abruptly stop at the very point, late in adult life, when it has greatly reduced the ability of the surviving individuals to sustain life and reproduction?

Mueller, Rauser, and Rose instead developed very different models for the evolution of late-life plateaus in mortality and fecundity (Mueller and Rose, 1996; Mueller et al., 2011), using the eventual plateaus in Hamilton's forces of natural selection as their core explanatory principle for mortality and fecundity plateaus late in adult life. These formal mathematical models, founded in evolutionary genetics, show that it is perfectly reasonable for natural selection to produce late-life plateaus in life-history characters, especially with finite population sizes, once the forces of natural selection have fallen to very low values. Of greater significance for strong-inference science, they further demonstrated that experimental evolution can tune the timing of the cessation of *Drosophila* aging in conformity with these theoretical results (Rose et al., 2002; Rauser et al., 2006). Indeed, the discovery that aging stops turns out to be a powerful corroboration of Hamilton's original results for the forces of natural selection (Hamilton, 1966; Mueller et al., 2011), all the more dramatic because it was counterintuitive and hence unexpected.

These results call for some fundamental re-thinking of what aging is. Twenty years ago, evolutionary biologists imagined that once Hamilton's forces of natural selection reached zero, death should quickly follow due to the absence of natural selection opposing the effects of cumulative damage and/or regulatory disharmony. Now at least some have a very different vision (Mueller et al., 2011). As Hamilton's forces of natural selection decline during the first part of adulthood, we might say that age-specific elements of adaptation are de-tuned. This de-tuning in turn could be said to generate the demographic phenomena of aging, as well as the myriad physiological dysfunctions that we know as the seemingly, but actually secondary,

mechanistic foundations of aging. In species with sufficiently severe antagonistic pleiotropy between reproduction and adult survival, such as Pacific salmon, soybean, and mayflies, all members of a cohort may die without either a well-defined period of aging or late life, in the absence of human intervention. But under sufficiently benign environmental conditions, individuals from species as disparate as humans and fruit flies can survive a protracted aging period and reach a subsequent late-life respite in which fitness-component deterioration stops, a phase permitted by the complete attenuation of the forces of natural selection relative to the effects of genetic drift.

The above results suggest that aging is *not* inevitably a cumulative and unremitting process of deterioration. Instead, aging might be best conceived as a facet of adaptation, specifically its de-tuning during the first part of adulthood. This de-tuning is due to the steady declines in the forces of natural selection that occur after the start of adulthood in most populations. Once those declines stop, aging eventually ceases, and adaptation stabilizes albeit at a low level. There is little sign of a physiological "momentum" that necessarily advances aging until every member of a cohort has died; nor is there any *a priori* requirement for such constancy, despite the seductive analogy to Newtonian physics. An important corollary is that many of the standard biological intuitions about aging, particularly those that associate it with the Second Law of Thermodynamics, are not generally valid. Some functional declines of physiological characters continue into late-life, and some even accelerate, whereas other functional declines come to a halt (Shahrestani et al., 2012). There is thus no scientific justification for assuming that each and every type of physiological deterioration that has been associated with aging must continue without remit throughout late adult life.

This realization leads to another fundamental change in our thinking about "the process of aging": it is not actually a physiological *process,* in and of itself. Although it certainly involves physiological changes, the physiology of aging is molded and constrained according to the dictates of natural selection shaping adaptation. Some of the genetic foundations of adaptation serve to sustain survival and reproduction later in life, presumably because of age-independent benefits (Charlesworth, 2001). Other features of adaptation are apparently subject to age-specific and pleiotropic genetic effects which undermine age-specific mortality and fecundity, together with their underlying physiology, during middle adulthood (Rose, 1991; Rose et al., 2002; Mueller et al., 2011). In extreme cases of trade-offs between survival and reproduction, continued adult survival may be wholly sacrificed by natural selection, resulting in semelparous, univoltine, or annual life cycles (Rose, 1991). All these possibilities for patterns of aging are permitted by evolution.

The evolutionary biology of aging proposed in 1991 (Rose, 1991) provided some warrant for allowing gerontologists to conduct their research largely without evolutionary considerations. The falling forces of natural selection were supposed to ensure the cumulative and unremitting physiological deterioration commonly assumed by gerontologists. But now neither that evolutionary rationale nor that type of mechanistic thinking seem warranted, given what we know of the cessation of aging. At its very foundations, aging is a multifaceted phenomenon that is a derivative feature of the evolutionary biology of adaptation, *not a single* physiological process, even though adaptations generally involve physiology.

As such, aging is best studied in light of the methodological strictures and theoretical scaffolding supplied by evolutionary biology. Some of those elements were sketched in 1991

(Rose, 1991), but the analysis offered then was far too simplistic. We now know that aging is much more complex than was understood then, both genomically (Rose and Burke, 2011) and demographically (Mueller et al., 2011), and it is inseparable from adaptation itself (Rose, 2009). This makes it a hazardous proposition to study aging without significant attention to evolutionary genetics. An evolutionary-genetic perspective on aging raises several points of concern, including the difficulty of studying aging under conditions in which adaptation has been undermined or distorted, such as breeding regimes that create inbreeding depression, highly artificial genotype-by-environment interactions, and obscure evolutionary history (Rose et al., 2011). As aging is neither more nor less than the deterioration of adaptation with adult age, obscuring the features of adaptation by performing experiments with laboratory cohorts of an abnormally inbred and/or mutated strain with a poorly-documented history of laboratory culture has created and will perpetuate significant difficulties of interpretation.

This vision of what underlies aging may be off-putting for some, given its theoretical complexities and difficulties for experimental design. No doubt many physicists felt the same way about the destruction of the elegant late 19th Century version of Newtonian mechanics by the advent of relativistic and quantum mechanics, in the period from 1905 to 1945. But paradigm transitions in science are generally like that, requiring that we abandon comfortable theories in favor of those that are significantly less wrong.

The genetics of aging cannot go on as it did before 1992. We need not jettison every lesson gleaned from past research, whether evolutionary or mechanistic, though conclusions reached under the quondam paradigm now require re-examination within our current, broader understanding. We will be able to salvage those parts that can be reintegrated within a scientific framework for the evolutionary genetics of aging, developed in light of its fundamental nature: de-tuned adaptation during the first part of adulthood. But a new evolutionary genetics of aging must now be built.

Initial Discussion

I do not fundamentally differ with Michael Rose's definition of aging and his main thesis. For him aging is defined as a decline or loss (a "detuning") of adaptation with increasing age, caused by a time-progressive decline of Hamilton's forces of natural selection. To my mind, this definition is consistent with most previous definitions of aging used by the majority of evolutionary biologists.

Most evolutionary biologists define aging as an age-dependent or age-progressive decline in intrinsic physiological function, leading to an increase in age-specific mortality rate (i.e., a decrease in survival rate) and a decrease in age-specific reproductive rate (e.g., Medawar, 1955; Williams, 1957; Rose, 1991; Partridge and Barton, 1996; Tatar, 2001; Promislow and Bronikowski, 2006; Flatt and Schmidt, 2009; Bronikowski and Flatt, 2010; Fabian and Flatt, 2011). Rose (1991), for example, in his seminal book on the evolution of aging defines aging as "*...a persistent decline in the age-specific fitness components of an organism due to internal physiological degeneration*". At level of the individual, the intrinsic physiological state at a specific age determines, among other things, whether an individual is dead or alive and how much it reproduces. At the level of the cohort, the underlying physiological states of the individuals translate into the age-specific rates of mortality and reproduction. We therefore

diagnose demographic aging to occur if we observe an increase in age-specific mortality and a decrease in age-specific reproductive rate in the cohort (e.g., Bronikowski and Flatt, 2010).

In particular, the notion that aging is a de-tuning of adaptation is completely consistent with the standard definition of aging. Adaptation by natural selection is based on heritable variation in fitness among individuals, and survival and reproduction are the most important determinants or components of fitness (e.g., Stearns, 1992). Thus, in agreement with Rose's definition, the age-dependent decline of age-specific survival and reproductive rates represents an age-progressive loss or de-tuning of fitness or the *state of adaptation.* Moreover, it is well-accepted among evolutionary biologists that this decline is due to the declining forces of natural selection with increasing age. So where exactly do Rose's definition and main thesis differ from the standard definition of aging?

The crux of Rose's thesis is not so much about the fact that adaptation and the forces of selection *decline* but about the fact that the forces *plateau* (Rose et al., 2007). Although this is well-known from both theory and data, the important point is that, under protected conditions (low extrinsic mortality), age-specific mortality and fecundity *also* exhibit plateaus late in life. This means that the age-specific rates of survival and reproduction do not necessarily decrease to zero toward the end of life, as had previously (mostly implicitly yet incorrectly) been assumed. In fact, the existence of such plateaus has often been missed because observing them requires very large cohort sizes. The work by Rose and others now posits that these late-life mortality and fecundity plateaus are a direct, causal consequence of the plateaus in the forces of selection.

The real issue at stake is therefore that the aging process is not sufficiently well defined if we describe it as a *persistent* age-progressive decline of physiological function leading to a *steady* age-progressive decline in survival and reproduction. Instead, demographic aging involves an age-progressive decline in survival and reproduction which is often (perhaps always) being followed by plateaus in the rates of mortality and reproduction. In other words, the age-dependent decline that is characteristic of the aging process is therefore not (at least not necessarily) persistent and steady but levels off. Note Rose's (1991) use of the word "*persistent*" in his classical definition of aging quoted above; clearly, in view of the by now well-documented demographic cessation of aging at advanced ages and under protected conditions, the usage of the term "persistent" is problematic. However, this being said, it should be pointed out that most evolutionary biologists do not include terms such as "persistent" or "steady" in their definitions of aging: most of them simply characterize demographic aging as an age-progressive increase in mortality rate and a decrease in reproductive rate. From my point of view, such a definition of aging is certainly not wrong, but one might say that it is not sufficiently precise. Obviously, in any finite cohort of individuals, the age-specific rates of survival and reproduction will eventually reach zero at some point of time: everyone has died and stopped reproducing. Thus, survival and reproduction *do decline* to zero levels *in a time-progressive manner*. However, as the existence of plateaus demonstrates, it would be wrong to assume that these rates decline *steadily* towards zero. If it turns out that such plateaus are general under benign conditions (the evidence so far suggests that they might be), then their existence must be integrated into the standard definition of the aging process.

Similar considerations apply when we talk about the age-progressive decline of physiological function. We might still say that demographic aging at the cohort level reflects the age-dependent decline or deterioration of physiological function of the individuals in the cohort;

however, we should not take this to mean that physiological function deteriorates *steadily* towards zero functionality. In a finite population with individuals of finite lifespan, physiological functionality will be zero at some point; however, before this point is reached, it might plateau. Although we still know next to nothing about the mechanistic details of physiology during late life, the plateauing of physiological decline might be a direct consequence of the plateaus of Hamilton's forces of natural selection. Thus, if Rose's model is correct, plateaus in the forces of selection cause plateaus in individual physiological state which in turn cause plateaus in mortality and reproduction.

The notion that Hamiltons' forces of natural selection plateau thus has major implications for our understanding of the aging process and, in particular, of late life: at advanced ages natural selection is unable to distinguish among individuals, be it at the level of physiological state or at the level of fitness components, so that demographic aging, under benign conditions, can come to a halt. While it remains to be seen how general the existence of such plateaus is across species, and although more theoretical and empirical efforts are required to fully understand the nature of the demographic cessation of aging, the phenomenon itself is now empirically well established.

T.F.

Science proceeds from its mistakes, just as well as its successes. We have argued above that it is "perfectly reasonable for natural selection to produce late-life plateaus in life-history characters." This is premised on the forces of natural selection having fallen to very low values. The reality of late-life mortality plateaus was a revelation for me, especially having been one of the earliest critics of their existence (see Nusbaum et al., 1993.) At that time I argued that aging consisted of an ever growing variety of physiological dysfunctions, which were ever increasing in their severity, leading to the eventual death of all individuals in a population.

Yet we now have both the well-corroborated observation of life mortality plateaus, as well as a series of theoretical developments and experiments demonstrating that antagonistic pleiotopy and mutation accumulation can account for these plateaus (Mueller and Rose, 1996; Reynolds et al., 2007; Mueller et al., 2011). This has led to a revolutionary recognition that aging is better described as the "detuning" of adaptation. Thus while a variety of physiological systems may detune during aging, there may be enough age-independent adaptations which allow some individuals to survive this life phase. For those that do, late life is now characterized as the phase in which adaptation re-stabilizes (as explained in Mueller et al., 2011) and thus their physiological performance is capable of allowing an undetermined length of additional life.

However this recognition has led us to entirely new and undiscovered country, specifically how does adaptation re-stabilize during late-life? Our previous work has focused on a variety of physiological, cellular, and molecular mechanisms which detune during the aging phase (Rose, 1991; Graves, 1997). Much of this work was described before modern whole genomic approaches and computational methods. At that time, we proposed that there must be suites of genes with age-associated expression related to organismal fitness undergoing age-specific decline. Subsequent work supports our original suppositions, even if this work has been carried out in *Drosophila* stocks of compromised quality with regards to elucidating generalizable patterns of aging (e.g. inbred and mutant strains; Girardot et al., 2006; Zhan et al. 2007) For example, Zhan et al. (2007) utilized microarray experiments to study gene expression

in a variety of tissues (muscle, accessory gland, brain, testes, Malphigian tubules, fat body, and gut) in the *D. melanogaster* w^{1118} mutant strain. They found that approximately 4 – 9% of all genes had an age-specific profile and different levels of up- and down-regulated genes with age in various tissues (**Table 1**):

Table 1. Up- and down- regulated genes with age in various tissues of *D. melanogaster*.

Tissue	Up-regulated	Down-regulated	Total
Accessory Gland	477	635	1255
Brain	380	452	832
Testes	429	394	823
Malphigian Tubules	387	432	819
Fat Body	339	323	662
Muscle	613	612	1255
Gut	305	282	587

This study also elucidated a number of genes that were age-associated and shared between different tissue types. An examination of the numbers of age-associated genes in this study suggests that many genes show age-independent expression profiles. For example, data from FlyAtlas suggests that about half the fly genome is expressed in all tissue types (Chintapalli et al. 2007) If this is so, then with an estimated *Drosophila* genome size of 14,000, we expect about 7,000 genes to be operational in all tissues. Indeed, Cherbas et al. (2011) examined the transcriptional diversity of 25 *Drosophila melanogaster* cell lines. They probed 14,807 genes and found that 64% were expressed at a detectable level in at least one cell line. On average 5885 genes were detected (range 5398 – 6221.) If we can rely on the Zhan et al. (2007) and Cherbas et al. (2011) studies to provide ball-park age-associated and tissue-specific gene expression profiles, then we can conclude that a very high fraction of (> 75%) *Drosophila*'s genes show age-independent expression. Clearly there are methodological issues which will need to be addressed to determine more exact figures of age-associated gene expression in particular stocks living in specific environmental conditions. For example, it is also known that gene expression profiles differ between *Drosophila* males and females (Muller et al., 2011) and that evolutionary histories impact these profiles as well (Huter et al., 2008). However, with all these sophistication aside, the existence of a genomic basis for a plateau in late-life survivorship is not too surprising. Clearly not all gene-expression must shut down at later age, and if enough remains to sustain crucial gene-network function under sufficiently benign conditions, survival could go on for quite a long time.

These calculations suggest an immediate set of studies related to the genomics of the aging and late-life phases. It would be useful to use microarray studies to examine exactly how flies living in sufficiently benign environments transition at the genomic level into late-life. It is my own suspicion that an important aspect of this transitioning will be found among the control of transposable genetic element (TGE) expression (e.g. Murray, 1990) This will be particularly important in helping to apply the results of late-life research in *Drosophila* to humans, since there are documented patterns of TGE replication with age impacting human disease (Biemont and Vieira, 2006; Collier and Largaespada, 2007; Lowe et al., 2007; Fontana, 2010; Pornrutsami and Mutirangura, 2010).

J.L.G.

The surprising discovery that age-specific mortality rates (and fecundity rates) plateau in late life, in contrast to the traditional Gompertz prediction of the acceleration of age-specific mortality rates, has motivated Rose and his collaborators to seek explanations grounded in evolutionary theory, such as the decline of natural selection with age predicted by Hamilton in 1966 (Mueller et al., 2011). From this approach has emerged Rose's stated idea that aging is the deterioration or detuning of adaptation with age. To push the musical metaphor, as the forces of natural selection attenuate, the coherent tuned signal of adapted allele frequencies fades until lost in the static noise of random genetic drift. The long-lived fly experiments in the Rose laboratory which indicate that adjusting the timing of the antagonistic pleiotropy between reproduction and survival adjusts the timing of the late life plateaus comprise a most striking confirmation of the evolutionary approach.

Despite these predictive successes, aging continues to be almost reflexively thought of by many as the inexorable accumulation of cellular and physiological damage and wear with age. Even researchers who know of the decline of Hamilton's forces have often assumed, as Rose points out, that this decline fits with the inexorable damage accumulation thesis. This old thinking casts a shadow not only on much aging research but its application in traditional pharmaceutical approaches to the "diseases of aging" and in clinical gerontology.

Now in the age of genomics, we can test aspects of the model with rich new data sets. In comparing the genomes of long-lived flies with flies of ordinary life span, it would be fascinating to search for altered expression (*via* microarray studies as J.L.G. suggests) specifically in the so-called genes with age-associated expression differences between long-lived and ordinary fly lines. Another potentially illuminating test would be to utilize traditional phylogenetic methods (Suzuki, 2010) to detect differences between lineages in the strength of natural selection versus nearly neutral genetic drift in those specific genes and across regions of the genomes of long-lived and ordinary flies.

L.F.G.

In the development of a "new evolutionary genetics of aging" there is one issue that, in my opinion, requires additional attention: negligible senescence. Trees with longevities of hundreds of years or small invertebrates, like the hydra, that appear immortal seem to defy traditional theories of aging. Can we imagine a scenario in which Hamiltonian forces of natural selection never decline? Can some species maintain an age-independent adaptive tuning? Are particular physiologies more "permissive" than others to the evolution of better adaptive tuning? The acknowledgement that late-life plateaus in mortality and fecundity are real phenomena rather than artifacts fostered important progress, both theoretical and experimental. At first sight negligible aging, like cessation of aging, does not seem to fit neatly under Hamilton's theory of a decline of the force of natural selection with increasing age. Unless we do not believe that negligible senescence is real, it seems that we should seek a better explanation for it.

Understandably, the experimental aging field has been mainly focused on the study of short-lived animal and plant models. Given our own limited lifespan, the study of species with negligible aging is likely to demand very creative approaches. Evolutionary biology should provide the framework to guide that research.

D.E.M.

One of the neglected issues in the discussion of the main thesis is the role of genetic variation with age-independent genetic effects. Genetic variation affecting aging and other life-history characters can, in theory, be maintained by mutation-selection balance for genes that are age-specific in expression, where the expectation is a higher additive genetic variance of fitness-components at more advanced ages. However, this mechanism – accumulation of mutations – though leading to the maintenance of genetic variation, does not involve a consistent performance across ages, and thus doesn't produce a plateau late in life characterized by the type of positive correlations assumed by lifelong heterogeneity theories. But in any case, both age-independent and age-dependent mechanisms will play a role in defining the timing and state at which aging stops.

Given the present knowledge that aging may stop at an advanced age, do we require a re-definition of aging?

Before going into that 'new' definition, we need to distinguish between: 1) Proximal and ultimate causality; thus aging may be due to a deterioration of physiological functions as *proximate* cause, and due to a decline of the forces of natural selection as *ultimate* cause. This decoupling leads to different levels of understanding and predictions; e.g. only the latter leads to both the prediction that aging is multifactorial and *also* that it can stop at advanced ages. Moreover, even considering just the first level of understanding, a common confusion occurs between correlation and causality. This can even lead to such claims as that aging is due to a reduction of the size of telomeres with age, a clear confusion between association and causality, with no power to add understanding to the deep, general, evolutionary causes of aging.

In light of this, there is a potential problem with the definition of what is 'internal physiological deterioration', used in the 'Evolutionary Biology of aging' book (Rose, 1991). In fact, the term may be interpreted wrongly as if 'some' physiological processes can be disentangled from the environments with which they interact. All processes do in fact depend on interactions between genotypes and the environment, with the latter being both 'internal' and 'external' factors. As an illustration, once a population starts aging, the subsequent unfolding of aging processes will be affected by the cumulative effects of deterioration also, both by the 'external' and 'internal' environment (since natural selection will decline even faster when mortality at late ages rises, due to both 'internal' and 'external' deterioration of functions with age, in an exponential way). The evolution of aging is thus an integrative process, involving all kinds of factors, and the statement 'internal physiological deterioration' may convey a wrong message. In other words, speaking about 'internal' processes may give a wrong idea that *before* aging evolves there is already the potential for the deterioration of particular physiological mechanisms. A better definition, taking this into account, as well as the fact that the *ultimate causes* of aging lead to a prediction that from a certain point on aging stops, might be the decline of *age-dependent* physiological functions. This leaves aside physiological functions (whichever

they may be) that affect the performance across all life (i.e. with positive correlation across ages), as well as factors such as damage, diseases etc inflicting the same degree of deterioration independent of age.

How much can we shape the change of performance with age?

At a first glance, it is intuitive that an environment with 'benign conditions' leads to a lowering of age independent mortality, rendering more likely that a plateau is expressed, as it increases the probability that an individual survives past the age where aging stops (the 'breakday') and thus enters the 'plateau phase'. But the classification of an environment as 'benign' or 'detrimental' depends on the evolutionary history of the population: e.g. can new environments, even if 'stress free' be considered benign? Can environments where the population has been long adapting, even if 'stressful', be considered 'detrimental'? This is not superficial rhetoric, since we have seen recurrent arguments in the literature that defend contrasting expectations for the evolution of aging, relative to the general theory of aging, as a function of the environment/ history of the populations. This was the case with the old 'Giesel' argument that a stressful long-term laboratory environment leads to an evolutionary trade-off between early and late performance in terms of fitness, that is not the 'true' pattern under mild conditions (Giesel et al., 1982; but see Rose ,1984). It has also been invoked more recently by several researchers (e.g. Promislow and Tatar, 1998; Sgrò and Partridge, 2000; Linnen et al., 2001; Hoffmann et al., 2001) who defend the idea that a 'relaxed' environment such as the lab leads to the accumulation of mutations that inflate the aging pattern, arguing again that the lab is not a 'real' scenario, and that the evolution of aging (or related traits, such as resistance to a stress) should be analyzed under more 'natural' conditions. What is correct is to consider the differences between populations under different age-dependent selection regimes in an environment where populations have already adapted, whatever the specificities of that environment. Given this, we may say that by 'benign' is meant an environment where populations have had the opportunity to adapt.

An illustration may be changes in nutrients: does an increase in the concentration of yeast improve a *Drosophila* environment? Not necessarily: it may lead to higher reproduction at the expense of survival, and thus to a quicker aging while the populations are still adapting to these new conditions. This in turn will render it less likely that an individual survives past the 'decline' (aging) phase, reaching the phase where aging stops (the plateau). But given enough time, maybe the populations do increase their capacity to assimilate the new concentration of nutrients, improving all fitness components and thus reducing the aging rate and increasing the chances of reaching a lower plateau (in terms of mortality). On the other hand, it is possible that a constraint such as lower nutrients leads to a reduction of early fecundity and an improvement in longevity, again far from equilibrium, affecting the probability that an individual reaches the age where the plateau is expressed. Again, only after evolution in that environment can we see whether or not such a change allows a higher likelihood that a plateau is expressed.

But the question remains: can we manipulate the environment in order to render it more likely that an individual reaches the plateau? Unfortunately there is no simple 'recipe' at present. The important message is that we cannot neglect the history of a population, and a key factor may be considering the ancestral environment where populations have evolved, particularly for populations that are at present under novel conditions. For example, in humans recent drastic

changes of life-style may have led to faster senescence and a rise in the value and age where a plateau may be expressed.

One important issue is how independent is the 'breakday' from the rate of senescence and the plateau value. Can we disentangle the three?

It is fairly intuitive that populations that have a slower senescence rate will present at advanced ages a lower plateau than populations that age faster. But, as the plateau derives from the evolution 'after' selection ends, i.e. at ages under the 'selection shadow', it is expected that a quicker decline of selection with age will lead to a more 'precocious' age when the plateau is reached. In other words, faster senescence is associated with a younger age where the plateau is expressed (smaller break day). The question is: can we disentangle the three parameters: rate of senescence, level of plateau, and age at which it is reached? It is interesting to suppose that we could by genetic changes or environmental manipulation slow aging in a population so that a 50 year old human could be as vigorous as a 30 year old in the unmanipulated group; but wouldn't it be better if we could 'manipulate' aging so that aging stopped at 30? Is this possible, in genetic and environmental terms?

Can aging be reversed at the individual level?

If aging is not a progressive deterioration with age, could the effects of aging be reversible? Could we target the physiological functions that have a genetic basis that is age-specific, or that have a genetic trade-off across ages – which cause aging – and which have a universally positive effect across ages – which are the ones responsible for the plateau? In fact, there is empirical evidence that late-life physiology is distinct from the physiology of aging (Shahrestani et al. 2012). As there is redundancy of functions in our genome, could it be possible to compensate for the loss of functions of those genetic mechanisms which cause aging by activating the over-expression of other genes that confer stable performance throughout life?

Furthermore, we need to distinguish two levels of definition of a phenomenon, as *state* and as *process*; taking the example of the concept of adaptation. It is fundamental to be clear whether we consider the concept as capacity to respond to the environment – as *state*, and, in the latter case, whether directly or as a by-product of selection - and as the *process* of becoming adapted, that is, natural selection per se. This is not an irrelevant issue, as it is at the core of critics such as the old argument of Popper in the 1970's that natural selection is a tautological theory. The 'solution' came from Dunbar (1982), as well as Sober (1984), with the proposition that a distinction between adaptation – as state – and fitness is essential to uncouple the two, rendering the outcomes of selection not inevitably as a rise in adaptation (=fitness). Taking this difference into account, we see how it may affect our perception of such complex phenomena as aging. In particular, there is evidently a substantive difference between considering aging as 'ultimate process' or as 'immediate state'. Such different perspectives and limitations, as well as the important distinction between correlation and causality (see above), are essential for progress on the connection between evolutionary and genetic causes of aging, particularly how environmental and/ or genetic manipulation may affect the physiological changes that occur with age.

M.M.M.

The main thesis part of this article points to the problems of using experimental material that are not likely to reveal genetic adaptations, like mutant stocks and inbred lines. This is not simply a theoretical argument; there are now good experimental results demonstrating these problems. So, for instance, free radical scavengers like superoxide dismutase, SOD, (Orr and Sohal, 1994) are believed to prolong life. However, the effects of SOD are affected by both genetic background and sex (Tyler et al, 1994; Spencer et al., 2003). In a novel laboratory environment, the *p*-insertion mutant *I'm not dead yet* (*Indy*) and the single gene mutant *methusaleh* (*mth*) were found to live no longer than their controls (Kaezaeli et al., 2005). Zwaan et al. (2006) then showed that a longevity effect of *methusaleh* was dependent on environment and mating status. These are not examples of specific failures to find longevity enhancing genes, they are rather the failure of a general research paradigm.

L.D.M.

If aging is "not a process," then what is it?

My response would be that it is an age-dependent trajectory of interacting system states – the sum of all molecular and physiological states and their interaction networks, many but not all of which shift in a consistent direction over time. This definition broadens our focus to include components that do not themselves depend on age, but which cohabit networks containing components that do. Gene-environment interactions are a case in point, wherein environmental variation can help to shape the age-structure of a population despite being quite obviously independent of age.

Perhaps the best-established genetic pathway to influence lifespan is insulin-like signaling, believed to have evolved at least in part for its ability to maximize reproduction under favorable environments while postponing both reproduction and individual mortality under conditions of crowding or insufficient food (Kenyon, 2005; Kim, 2007; Magwire et al., 2010; Hanover et al., 2010). Since natural populations are polymorphic for ostensibly rate-limiting components of this pathway (Bonafe and Olivieri, 2009), it is likely that individuals genetically predisposed to low insulinlike signaling should survive famine better than those geared for higher signaling and shorter lifespan. This is a conclusion of some import for population biologists, since the age-composition of any population must then be modified by the availability of food. A particularly instructive example is the near-ubiquitous evolutionary requirement for species or their constituent populations to survive extended periods of famine (de Grey, 2005). Groups experiencing more prolonged famines (or just over-wintering, if their lifespans are measured in weeks) will have more diverse age structures, including many individuals for whom reproduction has been delayed.

The same potential also exists for gene-gene interactions (including genes that dictate dietary preferences) to affect long-term survival. For example, only one component of a gene network may actually be age-dependent, while other genes create the background context of homeostatic states and their oscillations within which age-dependent genes must function. An increased probability of death with age could then arise from components undergoing essentially monotonic age-dependent declines, confronting extreme-value system states (in variable but age-

independent parameters) to which they cannot respond adequately, in any essential tissue or organ. Alternatively, an age-dependent increase in the variance of system oscillations may exceed the response range of one or more age-independent gene functions. In either case, the precise cause of death or debility will vary in a stochastic way, appearing as the "weakest link" in any one tissue or organism, although the underlying age-associated changes may be common to many or all individuals (Shmookler Reis, 1989).

Live smarter, live longer?

There remains, in my view, one last "trivial" explanation for the plateau in mortality, which I believe should be dealt with. I will term it "the perseverance of acquired characteristics", but I really just mean *learning* in its several forms. If we posit that individuals are to some degree capable, as a function of time, of developing and improving their ability to avoid evitable causes of mortality, then those individuals who managed to survive until late age could *ipso facto* have reduced their late-life risk of death – although aging *per se* might continue unabated. Examples of such learning would include immune memory, strategies to avoid situations and behaviors that place one at increased risk of injury or death, and a reduction in speed of movement or action in recognition of slower response times. My suggestion that immune memory might be involved, in organisms with an adaptive immune system, agrees with the observed age-dependent increase in memory T cells but appears contradicted by the decline with age in recruitment to this niche (Nikolich-Zugich and Rudd, 2010). However, if individuals exist who retain sufficient naïve T cell reserves to augment immune memory at late age, and if those are among the longest-lived in a population, then that subset of the population should see a reduction in their force of mortality. Of course, the strongest evidence for a cessation of aging comes from insects, which lack an adaptive immune system and may be thought incapable of learning. Experimental evidence clearly supports learning by *Drosophila* (Shuai et al., 2010; van Swinderin, 2010), however, and in the rather simple and uniform environments in which they are maintained, the last-surviving individuals might only need to have learned to avoid activities that place them at greatest risk of a collision or loss of balance leading to entrapment on a sticky surface (a major life-hazard for laboratory insects).

Addressing the gap between inbred model systems and the complexity of natural populations.

It is noteworthy that many gerontologists with broader vision have for some time been "adding back" complexities of genotypic (and more rarely, environmental) variation to aging studies – in keeping with the theoretical advances discussed here, but probably quite independent of them. A recent manifestation of this is the utilization of genetically heterogeneous mouse populations (e.g., 4- and 8-way cross progeny (Harrison et al., 2009; Klebanov et al., 2001)). Yet another has been the gradual realization that population studies of humans are not only invaluable for initial, weak-inference "discovery" of putative genetic mechanisms underlying diseases and predisposing traits, but are also ultimately needed to validate functional conclusions that arose from experiments with controlled and highly-inbred animal populations (Parsons et al., 2005; Szumska et al., 2007).

Human populations of course provide the ultimate in "realism" for both genetic and environmental complexity, but the anticipated harvest of clinically meaningful findings has been delayed and frustrated by the very large numbers of subjects required even for relatively simple traits, and the unforeseen degree of complexity of most quantitative traits (most certainly

including longevity and age-dependent diseases) has further diluted the inferential power of such studies (Terwilliger and Weiss, 2003). Nevertheless, with larger and larger cohorts being drafted into studies which interrelate either high-density SNP maps or whole-genome sequencing, with accurate and complete medical and family histories, this type of post-hoc "experimentation" will soon be pushed to its limits. These studies either have multiple proposed end-points, or else blanket consent forms to permit unforeseeable future applications. Such volumes of data require improved computer algorithms for data analysis, and rigorous statistical evaluation to compensate for multiple-end-point inflation of observed, superficially significant results (Lai et al., 2007; Lam et al., 2009; Erbe et al., 2011).

The worst (and most underappreciated) deficiency of these approaches is the very high level of confounding among the "independent variables" being considered. An obvious example is afforded by genotype-diet interactions, since dietary preferences tend to vary systematically by ethnic group. Another subtle danger that accompanies this particular brave new world is that routine statistical handling of multiple testing ("Bonferroni correction") can easily be overlooked or ignored. The problem is exacerbated because few investigators (and even fewer reviewers) have the breadth of training to understand both the fundamental biology addressed by a study, and also the arcane "cyber-discipline" of complex-trait analysis along with its own peculiar modes of statistical interpretation, usually trumping model-dependent statistics with permutation analyses. Even among scientists who do appreciate the necessity of multiple end-point compensation, there are many who conveniently forget them when their own data would, if properly adjusted, miss the conventional threshold for significance.

R.J.S.R.

My framework for aging research has developed over the last eight years, at a time when many of the articles that make the above thesis inevitable were already available. Not having been involved in the aging field prior to 1992, I never saw aging as an inevitable accumulation of damage to cells common to all organisms, and did not think in terms of physiological processes of aging. Instead, I have viewed aging as an evolutionarily derived condition, dependent entirely on the pattern of the force of natural selection. Aging is very much tied in to our evolutionary histories. My own experimental results, which I summarize below, support the thesis presented by Michael Rose in this paper.

There is much evidence suggesting that aging is conditional on the life cycle and that the existence and nonexistence of aging conforms to the expectations of evolutionary theory (Hamilton, 1966; Charlesworth, 1980; Rose, 1991; Charlesworth, 1994). Specifically, there appears to be no aging in the absence of a steady decline in the forces of natural selection acting on mortality and reproduction. This is the case during development, when natural selection acts at full force. This does not preclude fluctuations in mortality rates during the developmental period, but it does imply the absence of a strong, persistent, and predictable deterioration in survival rates of the type seen in biological aging. Some organisms experience natural selection at full force their entire lives and are therefore expected not to age. For example, organisms with strictly symmetrical fission do not apparently exhibit aging (Bell, 1984; Martinez, 1998). In these cases, if aging were to occur, it would extinguish all the descendant lineages, wiping out any such aging species, because senescent deterioration would then accumulate from cell division to cell division. This outcome would be opposed by natural selection acting with full force, which

would halt such aging among surviving species. The same is not true for asexually reproducing organisms in which reproduction is asymmetrical.

In late adult life there is no effective force of natural selection. This leads to an absence of differentiation between age classes and an absence of consistent changes in fecundity and mortality. One prediction of the evolutionary theories is that other fitness characters should also plateau in late life. An experiment in which we followed individual virility of 1000 males, revealed that virility also plateaus in late life (unpublished results). This result conforms to the predictions of the evolutionary theories of late life. We also found evidence against a lifelong heterogeneity explanation for these late life virility plateaus (unpublished results).

Late adult life is therefore a period in which mortality, fecundity, and virility all plateau. This raises an obvious question about what happens to the constituent physiological mechanism of individuals as they transition from a period of deteriorating fitness characters to a period of stable fitness characters. In a large-scale study of more than 57,000 *D. melanogaster* from six replicate populations, we looked for changes in the patterns of physiological deterioration in the transition from aging to late life (Shahrestani et al., 2012). As the cohorts demographically transitioned from aging to late life, the qualitative patterns of change in physiology were different for the characters tested. Specifically, time in motion and desiccation resistance approached stabilization, much like fitness characters do. Starvation resistance declined at the same rate in late life as it did during aging. But negative geotaxis declined at a much faster rate in late life compared to its rate of decline during aging. These results suggest that late life physiology is distinct from that of aging.

We have also tested whether the physiological transitions between aging and late life will shift in accordance with the age of onset of late life mortality plateaus. Preliminary results show that populations with earlier onsets of the mortality plateau also have correspondingly earlier onsets of physiological transitions from aging to late life (unpublished results). Overall, it is clear from these results that late life is governed by very different rules than aging. In late life, chronological ages are not differentiated by natural selection, so we cannot make specific predictions about physiological changes. This is a scenario similar to what happens during development in which the force of natural selection does not differentiate between age classes and physiological characteristics vary with respect to their improvement, deterioration, or stabilization with increasing chronological age.

Understanding aging in terms of a detuning of adaptation has obvious advantages. In principle what can be produced by forces of natural selection can be manipulated with the use of medications or lifestyle choices. It may also be possible to alter the age of onset of the mortality plateau, leading to earlier ages for the cessation of aging. Without a pre-existing framework for aging research, for me viewing aging as a consequence of a fall in forces of natural selection seems obvious. It may be time to revisit older frameworks for thinking about aging.

P.S.

Further Commentaries

M.M.M., concerning the initial commentary of T.F.: I think clarification is needed concerning the common features of previous definitions of aging versus those definitions that

clearly state that aging can eventually stop at advanced ages. In this sense, I have to disagree with T. F., both with his statement about what Rose's present definition of aging is (a decline or loss of adaptation with increasing age, caused by a time-progressive decline of Hamilton's forces of natural selection) and about T. F.'s discrimination between *progressive* and *persistent* decline. I think that M.R.R.'s phrase *aging might be best conceived as a facet of adaptation, specifically its de-tuning during the first part of adulthood* illustrates that a new definition should incorporate the idea that aging is a *phase* which occurs during the first part of adulthood, and not a process that will go on till death. In that sense my modest proposal, which is nothing fundamentally new but may be useful for its simplicity and for forstalling misleading conclusions, is that aging is a decline of *age-dependent* physiological functions (see below).

M.M.M., concerning the initial commentary of J.L.G.: I confess that I strongly mistrust results of genetic analysis involving mutant strains when addressing the evolutionary genetics of sexual random mating populations, as such analysis may inflate the effect of mutations that may be irrelevant for the evolution of outbred populations. Having said this, I think that such new techniques as NGS may allow us to tackle the most relevant issue of how genes with age-dependent versus age-independent affect fitness-related traits. My bet is that the data obtained by Chintapalli et al. (2007) cited by J.L.G. are substantial over-estimates of the number of genes that have effects which are independent of age. Though it seems to me that the simple observation of the patterns of aging imply such results, only future research can tell whether this conjecture is right or wrong.

M.M.M. comment on D.E.M.'s initial commentary: I agree that it seems counterintuitive that such a general explanation for aging –the decline of the forces of natural selection with age – allows some organisms to live for very long time without clear signs of senescence, or even at least apparently not aging. While for some time one simple explanation was that populations where there is no separation between soma and germline do not senesce – and this is clearly not a contradiction but a corollary of the Hamiltonian theory of aging – it is now becoming frequent to find that aging is a much more universal phenomenon. In fact, bacteria, which were a model example of the absence of aging, do age, and they also 'obey' the general condition that they have a distinction between the 'mother' and 'daughter' line, in other words of germ and soma (though not in separate tissues, obviously, as this is not required, as has already been seen in yeasts). As it is always easier to prove that a phenomenon occurs than that it doesn't, the apparent mystery of the absence of aging in some organisms may be just a result of a diversity of rates of aging due to different age-independent mortalities, mortality patterns that in turn lead to very slow declines in the forces of natural selection, and thus of aging, in the paradigmatic organisms that exhibit very long lifespans.

References

Baldal, E. A., Baktawar, W., Brakefield, P. M., and Zwaan, B. J. 2006. *Methuselah* life history in a variety of conditions, implications for the use of mutants in longevity research. *Exp. Geront*. 41, 1126–1135.

Bell, G. (1984). Evolutionary and non-evolutionary theories of senescence. *Amer. Nat.* 124, 600–603.

Biémont, C., and Vieira, C. (2006). Genetics: junk dna as an evolutionary force. *Nature* 443, 521-524.

Bonafe, M., and Olivieri, F. (2009). Genetic polymorphism in long-lived people: cues for the presence of an insulin/IGF-pathway-dependent network affecting human longevity. *Mol. Cell. Endocrinol*. 299, 118-123.

Bronikowski, A. M., and Flatt, T. (2010). Aging and its demographic measurement. *Nature Educ. Knowl.* 1, 3.

Brooks, A., Lithgow, G. J., and Johnson, T. E. (1994). Mortality-rates in a genetically heterogeneous population of *Caenorhabditis elegans. Science* 263, 668-671.

Carey, J. R., Liedo, P., Orozco, D., and Vaupel, J. W. (1992). Slowing of mortality rates at older ages in large medfly cohorts. *Science* 258, 457-461.

Charlesworth, B. (1980). *Evolution in Age-Structured Populations*. London: Cambridge University Press.

Charlesworth, B. (1994). *Evolution in Age-Structured Populations. 2nd ed*. London: Cambridge University Press.

Charlesworth, B. (2011). Patterns of age-specific means and genetic variances of mortality rates predicted by the mutation-accumulation theory of ageing. *J. Theor. Biol.* 210: 47-65.

Cherbas, L., Willingham, A., Zhang, D., et al, (2011). The transcriptional diversity of 25 *Drosophila* cell lines. *Genome Res*. 21, 301-314.

Chintapalli, V. R, Wang, J., and Dow, J. A. T. (2007). Using FlyAtlas to identify *Drosophila melanogaster* models for human disease. *Nature Genet.* 39, 715-720.

Collier, L., and Largaespada, D. (2007). Transposable elements and the dynamic somatic genome. *Genome Biol. 8, S5.*

Comfort, A. (1979). *The Biology of Senescence*, 3rd ed. Edinburgh and London: Churchill Livingstone

Curtsinger, J. W., Fukui, H. H., Townsend, D. R., and Vaupel, J. W. (1992). Demography of genotypes: Failure of the limited life span paradigm in *Drosophila melanogaster. Science* 258, 461-463.

de Grey, A.D. (2005). The unfortunate influence of the weather on the rate of ageing: why human caloric restriction or its emulation may only extend life expectancy by 2-3 years. *Gerontology* 51, 73-82.

Drapeau, M. D., Gass, E. K., Simison, M. D., Mueller, L. D., and Rose, M. R. (2000). Testing the heterogeneity theory of late-life mortality plateaus by using cohorts of *Drosophila melanogaster. Exp. Geront.* 35, 71-84.

Dunbar, R. I. M. (1982). "Adaptation, fitness and the evolutionary tautology," in: *Current Problems in Sociobiology*, ed. King's College Sociobiology Group, Cambridge University Press.

Erbe, M., Ytournel, F., Pimentel, E.C., Sharifi, A.R., and Simianer, H. (2011). Power and robustness of three whole genome association mapping approaches in selected populations. *J. Anim. Breed. Genet*. 128, 3-14.

Fabian, D., and Flatt, T. (2011). The evolution of aging. *Nature Ed. Knowl.* 2, 9.

Flatt, T., and Schmidt, P. S. (2009). Integrating evolutionary and molecular genetics of aging. *Biochim. Biophys. Acta* 1790, 951-962.

Fontana, A. A. (2010). A hypothesis on the role of transposons, *Biosystems* 101, 187-193.

Giesel, J. T., Murphy, P. A., and Manlove, M. N. (1982). The influence of temperature on genetic interrelationships of life history traits in a population of *Drosophila melanogaster*: what tangled data sets we weave. *Am. Nat.* 119, 464-479.

Girardot, F., Lasbleiz, C., Monnier, V., and Tricoire, H. (2006). Specific age-related signatures in *Drosophila* body parts transcriptome. *BMC Genomics* 4, 7-69.

Graves, J. L. (1997). "General Theories of Aging: Unification and Synthesis," in: *Principles of Neural Aging*, ed. F. Sergio, M.D. Dani, M.D. Akira Hoi, G.F. Walter, Elsevier Press, pp. 35-55.

Greenwood, M., and Irwin, J. O. (1939). Biostatistics of senility. *Hum. Biol.* 11, 1-23.

Hamilton, W. D. (1966). The moulding of senescence by natural selection. *J. Theor. Biol.* 12, 12-45.

Hanover, J.A., Krause, M.W., and Love, D.C. (2010). The hexosamine signaling pathway: O-GlcNAc cycling in feast or famine. Biochim. Biophys. Acta 1800, 80-95.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., Pahor, M., Javors, M.A., Fernandez, E., and Miller, R.A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392-395.

Hellmann-Blumberg, U., Hintz, M.F., Gatewood, J.M., and Schmid, C.W. (1993). Developmental differences in methylation of human alu repeats. Mol. Cell. Biol. 13, 4523-4530.

Hoffmann, A. A., Hallas, R., Sinclair, C., and Partridge, L. (2001). Rapid loss of stress resistance in Drosophila melanogaster under adaptation to laboratory culture. *Evolution* 55, 436–438.

Hutter, S., Saminadin, P., Stephan, W., and Parsch, J. (2008). Gene expression variation in African and European populations of *Drosophila melanogaster*. *Genome Biol.* 9, R12.

Kenyon, C. (2005). The plasticity of aging: insights from long-lived mutants. *Cell* 120, 449-460.

Khazaeli, A. A., Pletcher, S. D., and Curtsinger, J. W. (1998). The fractionation experiment: Reducing heterogeneity to investigate age-specific mortality in *Drosophila. Mech. Ageing Dev.* 105, 301-317.

Khazaeli, A. A., Van Voorhies, W. and Curtsinger, J. W. (2005). The relationship between life span and adult body size is highly strain-specific in *Drosophila melanogaster*. *Exp. Geront*. 40, 377–385.

Khazaeli, A. A., Xiu, L., and Curtsinger, J. W. (1995). Effect of adult cohort density on age-specific mortality in *Drosophila melanogaster. J. Gerontol. A* 50, 262-269.

Khazaeli, A. A., Xiu, L., and Curtsinger, J. W. (1998). Effect of density on age-specific mortality in *Drosophila*: A density supplementation experiment. *Genetica* 98, 21-31.

Kim, S.K. (2007). Common aging pathways in worms, flies, mice and humans. *J. Exp. Biol.* 210, 1607-1612.

Klebanov, S., Astle, C.M., Roderick, T.H., Flurkey, K., Archer, J.R., Chen, J., and Harrison, D.E. (2001). Maximum life spans in mice are extended by wild strain alleles. *Exp. Biol. Med. (Maywood)* 226, 854-859.

Lai, C.Q., Parnell, L.D., Lyman, R.F., Ordovas, J.M., and Mackay, T.F. (2007). Candidate genes affecting Drosophila life span identified by integrating microarray gene expression analysis and QTL mapping. *Mech. Ageing Dev.* 128, 237-249.

Lam, A.C., Powell, J., Wei, W.H., de Koning, D.J., and Haley, C.S. (2009). A combined strategy for quantitative trait loci detection by genome-wide association. *BMC Proc* 3, S6.

Linnen, C. M., Tatar, M., and Promislow, D. (2001). Cultural artifacts: A comparison of senescence in natural, laboratory-adapted and artificially selected lines of *Drosophila melanogaster*. *Evol. Ecol. Res*. 3, 877-888.

Lowe, C., Bejerano, G., and Haussler, D. (2007). Thousands of human mobile element fragments undergo strong purifying selection near developmental genes. *Proc. Natl. Acad. Sci.* 104, 8005-10.

Magwire, M.M., Yamamoto, A., Carbone, M.A., Roshina, N.V., Symonenko, A.V., Pasyukova, E.G., Morozova, T.V., and Mackay, T.F. (2010). Quantitative and molecular genetic analyses of mutations increasing Drosophila life span. *PLoS Genet* 6, e1001037.

Martinez, D.E. (1998). Mortality patterns suggest lack of senescence in hydra. *Exp. Geront.* 33, 217–225.

Medawar, P. B. (1952). *An Unsolved Problem in Biology*. London: H.K. Lewis.

Mueller, L. D., and Rose, M. R. (1996). Evolutionary theory predicts late-life mortality plateaus. *Proc. Natl. Acad. Sci. USA* 93, 15249-15253.

Mueller, L. D., Drapeau, M. D., Adams, C. S., Hammerle, C. W., Doyal, K. M., Jazayeri, A. J., Ly, T., Beguwala, S. A., Mamidi, A. R., and Rose, M. R. (2003). Statistical tests of demographic heterogeneity theories. *Exp. Geront.* 38, 373-386.

Mueller, L. D., Rauser, C. L., and Rose, M. R. (2011). Does Aging Stop? New York: Oxford University Press.

Muller, L., Hutter, S., Stamboliyska, R. et al. (2011). Population transcriptomics of *Drosophila melanogaster* females. *BMC Genomics* 12, 81.

Murray, V. (1990). Are transposons a cause of ageing? *Mutat. Res.* 237, 59-63.

Nikolich-Zugich, J., and Rudd, B.D. (2010). Immune memory and aging: an infinite or finite resource? *Curr. Opin. Immunol*. 22, 535-540.

Nusbaum, T. J., Graves, J. L., Mueller, L. D., and Rose, M. R. (1993). Letter to the editor. *Science* 260, 1567.

Orr, W.C., Sohal, R.S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science*, 263, 1128-1130.

Parsons, C.A., Mroczkowski, H.J., McGuigan, F.E., Albagha, O.M., Manolagas, S., Reid, D.M., Ralston, S.H., and Shmookler Reis, R.J. (2005). Interspecies synteny mapping identifies a quantitative trait locus for bone mineral density on human chromosome Xp22. *Hum Mol Genet* 14, 3141-3148.

Partridge, L., and Barton, N. H. (1993). Optimality, mutation and the evolution of ageing. *Nature* 362, 305-311.

Popper, K. (1976). *Unended Quest: An Intellectual Autobiography*. Glasgow: Fontana Press.

Pornrutsami, J. and Mutirangura, A. (2010). Distinctive patterns of age-dependent hypomethylation in interspersed repetitive sequences, *Physiol. Genomics* 41, 194-200.

Promislow, D. E. L., and Bronikowski, A.M. (2006). "The Evolutionary Genetics of Senescence," in: *Evolutionary Genetics: Concepts and Case Studies*, ed. J. B. Wolf and C. Fox, New York: Oxford University Press, pp. 464-481.

Promislow, D., and Tatar, M. (1998). Mutation and senescence: where genetics and demography meet. *Genetics* 103, 299-314.

Rauser, C. L., Abdel-Aal, Y., Shieh, J. A., Suen, C. W., Mueller, L. D., and Rose, M. R. (2005). Lifelong heterogeneity in fecundity is insufficient to explain late-life fecundity plateaus in *Drosophila melanogaster*. *Exp. Geront*. 40, 660-670.

Rauser, C. L., Tierney, J. J., Gunion, S. M., Covarrubias, G. M., Mueller, L. D., and Rose, M. R. (2006). Evolution of late-life fecundity in *Drosophila melanogaster*. *J. Evol. Biol.* 19, 289-301.

Reynolds, R.M, Temiyasathit, S, Reedy, M.M. et al. (2007). Age specificity of inbreeding load in *Drosophila melanogaster* and implications for the evolution of late-life mortality plateaus. *Genetics* 177, 587-595.

Rose, M. R. (1984). Genetic covariation in *Drosophila* life-history: untangling the data. *Am. Nat*. 123, 565-569.

Rose, M. R. (1985). Life-history evolution with antagonistic pleiotropy and overlapping generations. *Theor. Pop. Biol.* 28, 342-358.

Rose, M. R. (1991). *Evolutionary Biology of Aging*. New York: Oxford University Press.

Rose, M. R. (2009). Adaptation, aging, and genomic information. *Aging* 1, 444-50.

Rose, M. R., and Burke, M. K. (2011). Genomic Croesus: Experimental evolutionary genetics of aging. *Exp. Geront.* 46, 397-403.

Rose, M. R., Drapeau, M. D., Yazdi, P. G., Shah, K. H., Moise, D. B., Thakar, R. R., Rauser, C. L., and Mueller, L. D. (2002). Evolution of late-life mortality in *Drosophila melanogaster*. *Evolution* 56, 1982-1991.

Rose, M. R., Mueller, L. D., and Burke, M. K. (2011). New experiments for an undivided genetics. *Genetics* 188, 1-10.

Rose, M. R., Rauser, C. L., Benford, G., Matos, M., and Mueller, L. D. (2007). Hamilton's Forces of Natural Selection after forty years. *Evolution* 61, 1265-1276.

Sgrò, C. M., and Partridge, L. (2000). Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am. Nat*. 156, 341-452.

Shahrestani, P., Quach, J., Mueller, L. D., and Rose, M. R. (2012). Paradoxical physiological transitions from aging to late life in *Drosophila. Rejuv. Res.* PMID: 22233126

Shmookler Reis, R.J. (1989). Model systems for aging research: syncretic concepts and diversity of mechanisms. *Genome* 31, 406-412.

Shuai, Y., Hu, Y., Qin, H., Campbell, R.A., and Zhong, Y. (2011) Distinct molecular underpinnings of *Drosophila* olfactory trace conditioning. *Proc. Natl. Acad. Sci*. 108, 20201-20206.

Sober, E. (1984). *The Nature of Selection: Evolutionary Theory in Philosophical Focus*. Cambridge: Bradford Books/MIT Press.

Spencer, C. C., Howell, C. E., Wright A. R., and Promislow, D. E. L. (2003). Testing an "aging gene" in long-lived *Drosophila* strains: increased longevity depends on sex and genetic background. *Aging Cell* 2, 123-130.

Stearns, S. C. (1992). *The Evolution of Life Histories*. New York: Oxford University Press.

Suzuki, Y. (2010). Statistical methods for detecting natural selection from genomic data. *Genes Genet Syst*. 85, 359-76.

Szumska, D., Benes, H., Kang, P., Weinstein, R.S., Jilka, R.L., Manolagas, S.C., Shmookler Reis, R.J. (2007). A novel locus on the X chromosome regulates post-maturity bone density changes in mice. *Bone* 40, 758-766.

Tatar, M. (2001). "Senescence," in: *Evolutionary Ecology - Concepts and Case Studies*, ed. C. W. Fox, D. A. Roff, and D. J. Fairbairn. New York: Oxford University Press, pp.128-141.

Terwilliger, J.D., and Weiss, K.M. (2003). Confounding, ascertainment bias, and the blind quest for a genetic 'fountain of youth'. *Ann Med* 35, 532-544.

Tyler, R. H., Brar, H., Singh, M., LaTorre, A., Graves, L.L., Mueller, L.D., Rose, M.R., and Ayala, F.J. (1993). The effects of superoxide dismutase alleles on aging in *Drosophila*. *Genetica* 91, 143-149.

van Swinderin, B. (2011). Conditioning to colors: a population assay for visual learning in *Drosophila*. *Cold Spring Harb. Protoc*. 2011, 1334-1336.

Vaupel, J. W. (1988). Inherited frailty and longevity. *Demography* 25, 277-287.

Vaupel, J. W., Manton, K. G., and Stallard, E. (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography* 16, 439-454.

Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. Evolution 11, 398-411.

Zhan, M, Yamaza, H, Sun, Y, et al. (2007). Temporal and spatial transcriptional profiles of aging in *Drosophila melanogaster*, *Genome Res.* 17, 1236-43.