

# Pharmacology, Genomics, and the Evolutionary Biology of Ageing\*

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Aging is a multifold process affected by many genes and thus many biochemical pathways. This conclusion is underscored by the failure to find simple central controls for the aging process during the 20th Century. This situation poses a fundamental challenge to anti-aging medicine: how to develop effective therapies for a genomically complex pathology. We propose such a strategy. As a first step, we recommend the use of model systems in which significant genetic intervention is not proscribed or impractical. Second, we propose that work with such model systems begin with selected lines that have genetic enhancements that allow increased lifespan. Third, genomic methods should be used to identify a number of biochemical pathways for increasing lifespan. Fourth, biochemical pathways that have been identified in model systems would then be available for pharmaceutical development, first in rodents, eventually in a clinical human population. This may seem to be a cumbersome R&D strategy, but starting with human populations or inadequately pre-screened compounds would be unlikely to succeed because of the complexity of the aging problem.

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## INTRODUCTION: AGING IS TOUGH

Aging is one of the hardest problems in biology. The 20th Century saw a parade of theories of aging based on the gambit that aging would be controlled by simple mechanisms. Among these theories were Metchnikoff's auto-toxification theory, Bidder's

limited growth theory, somatic mutation, error catastrophe theory, and limited cell replication, among other proposals, conjectures, and speculations. None of these ideas worked out.

This was a portentous fact of 20th Century biology, because medicine has developed few tools for the alleviation of aging, though it has made progress with some of the quaternary consequences of aging, like heart disease, impotence, and cancer. However, such restorative treatments as surgery and chemotherapy are often drastic and unappealing. The need is instead for therapies that prevent, or greatly forestall, the occurrence of the life-threatening diseases of advanced age. The biology of aging still needs to supply medicine with the foundations for progress with human aging. Why did that biology make so little progress in the past?

There was a specific reason for the failure of so many ingenious biological theories of aging: aging is not an organismal function. Development, sexual maturation, locomotion in animals, photosynthesis in plants, and mitosis in cells are all functions that have been established and refined by natural selection. As functions, they can be analyzed using the common tools of experimental biology, from knock-out mutagenesis to surgical ablation. A large part of biology is based on the discovery and unraveling of function. The temptation to use the conventional tools of functional biology in the analysis of aging was strong, and many succumbed. But it is a mistake to approach aging in this way. It is different *in kind* from other problems of biology.

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## AN EVOLUTIONARY PROBLEMATIQUE FOR AGING

One source of confusion about aging for biologists is the regular, predictable acceleration in mortality rates during most of adulthood. In organisms for which we have good data, like humans, fruit flies, and nematodes, the acceleration in mortality seems like a programmed process. Such a programmed process in turn seems to call out for an underlying regulatory mechanism to establish and control aging. But all attempts to find such regulatory mechanisms have failed.

In 1966, William Hamilton derived a function that qualitatively predicted the acceleration of mortality. That function is known as “the force of natural selection”. This function is not itself “functional”. It is not there to benefit the organism in some way. Instead, Hamilton’s force of natural selection gives the quantitative fading out of natural selection.

Before adulthood, the force of natural selection predicts, natural selection will be at full strength. Under such conditions, we expect that the biochemical machinery of life will be finely tuned to keep organisms alive. This does not mean that there will not be death. Genetic diseases and environmental accidents will still kill juveniles even under good conditions. But the majority of young animals are expected to be healthy. Natural selection acts on mortality during pre-reproductive ages with the same intensity, because any death prior to reproduction has the same effect on the dead organism’s fitness.

But shortly after the start of reproduction, the force of natural selection is expected to fall,<sup>[4]</sup> at least in organisms that do not reproduce by fission,<sup>[13]</sup> an unusual case for most animals. This fall in natural selection will continue until it reaches zero, after which it will never rise again. This fall in the force of natural selection establishes, and controls, the aging process. This has been repeatedly shown experimentally with *Drosophila*<sup>[7,14]</sup> and other organisms, like *Tribolium*.<sup>[16]</sup>

But this control by the force of natural selection should not be misconstrued. The force of natural selection does not operate by one or a few efficient controlling mechanisms, unlike some physiological processes. As natural selection fades out, many loci can impact the aging process, as secondary and incidental effects of the evolutionary process. There are two main types of evolutionary mechanism that can occur. The simplest in the accumulation of alleles that have differential, and deleterious, effects confined to late ages. Most of these effects are expected to be deleterious because most random genetic effects are neutral or deleterious, rather than beneficial. Neutral alleles will not matter, since they have no effect. There is some evidence for this

genetic mechanism of aging.<sup>[5,15]</sup> The other type of evolutionary mechanism involves natural selection favoring alleles that have beneficial effects at early ages, along with pleiotropic deleterious effects at late ages. In this case, the action of natural selection in fostering early health and reproduction is coupled with a genetic cost later in life. This makes aging a by-product of natural selection. There is a considerable body of evidence in favor of this genetic mechanism of aging.<sup>[13]</sup> In either case, there is no direct selection for aging *per se*. Aging is just an accidental side-effect of the deteriorating force of natural selection. It should also be noted that these two evolutionary mechanisms are not mutually exclusive; evidence for one is not evidence against the other.

Since many alleles will fit the two patterns just described, it follows that we expect many genetic and biochemical mechanisms of aging. There are some experiments that have attempted to estimate the number of genes involved in aging, particularly in *Drosophila*. Quantitative genetic estimates of gene number have probably been subject to artifacts,<sup>[6,8]</sup> and are highly imprecise. Molecular genetic estimates using 2-D gels<sup>[3]</sup> and high-density gene-expression arrays<sup>[12]</sup> indicate the involvement of at least 300 genetic loci in *Drosophila* aging, and that estimate is highly conservative. For now, the best conclusion is probably that *many* genes are involved in aging in fruit flies. Vertebrates are unlikely to have fewer genes involved in aging, in view of their larger genomes.

The evolutionary genetic theory of aging and its subsidiary implications, together with the experiments that support them, reveal that aging is fundamentally harder than many other problems that biologists study. It will not be solved in molecular detail or in medical application as easily. It requires the development of new strategies of experimental research and medical application.

Here we propose one such strategy. We do not claim that it is the only one having any promise. Our ambition is simply to find at least one R&D strategy that offers the prospect of developing new therapeutics for aging. We are motivated partly by the intellectual challenge of this problem, but also by the great importance of aging for life and death in our times. Most people in OECD countries will suffer mortality and morbidity that is related to aging. To take a defeatist attitude to this situation strikes us as faint-hearted, if not callous.

## FIRST STEP: WORK WITH GOOD MODEL SYSTEMS

There are numerous reasons to begin medical research on aging with well-known, tractable,

model organisms. Humans are not ethically appropriate subjects for some experiments, from experimental mutagenesis to stress tests that proceed to death. On the practical side, it is also difficult to perform experiments on such large, long-lived organisms. Even a ruthless experimenter would grow impatient with experimental genetic research on humans.

As to appropriate model systems, there is room for a diversity of choices. But some systems seem unlikely prospects, among them extremely long-lived vertebrates, such as some large tortoises and some fish species. Most vertebrate species will be slow-going experimentally, even rodents, although they will still be useful in the later stages of pharmaceutical testing, as discussed below.

The obvious model systems in which to try developing therapeutics for human aging are the two premier animal genetic systems. *Drosophila melanogaster* and *Caenorhabditis elegans*. Mutants are available that show increased longevity for both species. *Drosophila* have also been bred for postponed aging,<sup>[13]</sup> potentially affording hundreds of genetic differences to work with. Recently there has been a controversy concerning the status of the *C. elegans* mutants with increased lifespan. In some laboratories, these mutants show increased longevity according to the degree of reduction in metabolism.<sup>[17]</sup> If so, then these mutants modulate lifespan by tuning metabolic activity, a discovery well-known in research with poikilotherms since 1917, and of limited interest. By contrast, the *Drosophila* bred for postponed aging are known to have no reduction in metabolic rate, and a substantial increase in their lifelong metabolism.<sup>[2]</sup> For these reasons, our initial model system recommendation is *Drosophila*. This does not mean that we reject the later use of other model systems, as they are refined for the study of aging.

## SECOND STEP: WORK WITH LINES THAT HAVE POSTPONED AGING

One of the biggest problems with past research on aging was that there was no control. In almost all the experimental research on aging performed in the 20th Century, all the animals under study aged, usually at the same underlying rate. Thus, there were hundreds, if not thousands, of papers published that merely documented longitudinal changes with age in some biological variable(s). Such research could do little more than establish the *temporal* involvement of a biological process in aging, not the *causal* involvement of this process. It is the latter that is the crucial issue.

Another strategy in the study of aging was the use of groups that exhibited earlier death or

deterioration, such as mutant fruit flies<sup>[11]</sup> or humans with progeric disorders.<sup>[1]</sup> As Maynard Smith<sup>[9]</sup> pointed out in an eloquent critique, this method leaves unsolved the problem of whether the aberrant early-death group is dying of a novel pathology or the acceleration of pathologies involved in normal aging. In many ways, the study of such groups adds a second set of problems to those of normal aging itself. Therefore, we do not recommend their use.

Instead, we recommend the use of organisms that have had their aging slowed or postponed, but that do not merely have life “stretching”, unlike cooled poikilotherms. That is, we propose that aging studies normally be based on the comparison of normal healthy animals with an experimental group that lives even longer, with increased total biological activity, from reproduction to locomotion to metabolic work. Such organisms exist among *Drosophila* stocks, including some of the mutants with increased lifespan and the selectively bred populations.

Mutants with increased lifespan have great analytical interest, not least because the site of genetic modification is already known. Such mutants deserve close study. However, they sometimes have drastic pleiotropic effects, such as sterilization.

Populations that have been bred for postponed aging are a very different sort of research problem. The genetic loci involved in the postponement of aging will not be known in most cases, and there are many of them. One advantage to the use of such populations is that they have undergone genetic changes that postpone aging, but do not have drastically deleterious side-effects. A mutant that increases lifespan but knocks out fertility would not be favored in selectively bred populations, making selected lines a better source of medically useful genetic variants.

From a medical R&D standpoint, there is an additional advantage to the use of a model system that is differentiated at hundreds of loci. Many of the loci that control aging in *Drosophila* will not have the same effect on human aging. On the other hand, we expect that other loci will work in a parallel manner in humans. We have no way of knowing *a priori* which group any particular locus will belong in. Thus, the individual mutants that increase *Drosophila* lifespan may or may not come from loci that have effects on human lifespan. If one were to pick one or a few such mutants to work with for medical R&D, there is the possibility that none of them will work out at the level of developing medical therapies. But if one were to study dozens or hundreds of loci that increase lifespan in bred stocks, then success with only 5–15% of these loci might translate into a large therapeutic benefit at the other end of the pipeline. A critic might point out that this assumes that the experimenter has access to methods that can handle loci by the hundreds.

Now of course we do have such methods: genomics.

### THIRD STEP: GENOMIC METHODS FOR STUDYING AGING

With the sequencing of the complete *Drosophila* genome, it is now routine to monitor the expression of every gene using high-density arrays. An obvious application of this technology is to characterize the adult age-dependence of gene expression, and this has been done by Pletcher *et al.*<sup>[12]</sup> More than 1000 loci exhibit age-dependent changes in gene expression (1264 genes). This is a substantial problem, because not all of these loci will be causally involved in aging, and there are so many to sort out. An additional application of gene “chip” technology is to compare flies with and without a lifespan modulating physiological treatment. Pletcher *et al.* have performed this experiment using dietary restriction. They found 2188 genes that responded to a restricted diet which also increased lifespan. Again, this poses a considerable problem of causal dissection. On the other hand, it suggests that there are likely to be many loci involved in aging, particularly relative to the total number of genes in the fruit fly, about 14,000.

With modern genomic technologies and large-scale data analysis methods, it is possible to sift through the genes of populations to find the loci that act to postpone aging.<sup>[3]</sup> There are uncertainties with the comparison of populations with different rates of aging. However, it is superior to experimental designs that only consider age-dependence or dietary-response, without determining causal mechanisms.

### FOURTH STEP: MEDICAL TESTING OF CANDIDATE DRUGS

Many genes are common between fruit flies and mammals, but by no means all. Therefore, it is important to test biochemical pathways that work in fruit flies with mammals. Mice are the system of choice, as they have relatively short lifespans (2–3 years) and a great deal is known of their genetics. Mortality rate measurements, like those studied in fruit flies,<sup>[10]</sup> might speed up mouse trials to just 6–12 months. Mouse trials would also help address issues of safety, such as liver and kidney toxicity, before going on to human trials.

Drugs that ameliorate mouse aging and are safe become prime candidates for clinical trials. Since these drugs will not be intended to cure any particular disease, it may be appropriate to use

volunteers, with a double-blind design to prevent subjective biases.

### CONCLUSION: AGING DOES NOT HAVE TO BE UNSTOPPABLE

Thirty years ago, the genetic or biochemical postponement of aging was regarded as impossible in any organism. But the last few decades have seen aging become an easily ameliorated condition in model organisms, especially *Drosophila*. The toy electrical machines of Michael Faraday pointed to the future electrification of industry. The rockets of Robert Godard pointed toward space travel. Likewise, tiny Methuselahs show that aging can be substantially postponed. There is no biological necessity to any particular rate of aging, only the practical difficulty of changing that rate.

The postponement of human aging will be far harder than the postponement of aging in fruit flies and other laboratory organisms. But there are no absolute barriers to be overcome, only technical barriers. We have proposed one strategy for overcoming these technical barriers. No doubt there are other strategies worth trying. But it seems obvious that at least one of them should be tried in the immediate future, unless aging is to be accepted fatalistically.

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