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Advice to an aging scientist

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Abstract

Fifty years ago, Peter Medawar and George Williams developed two now-classic theories for the evolution of senescence. In the past 20 years, evolutionary biologists studying aging have developed explicit mathematical models of these theories, used these models to derive explicit predictions, and tested these predictions using a variety of approaches. But, we argue here, our singular focus on these models may have hindered progress in evolutionary studies of aging. Research in this area has not kept pace with dramatic advances in evolutionary theory and molecular genetics. Progress in evolutionary studies of aging will depend on a bold, integrative approach, incorporating evolutionary and molecular advances from other fields, along with the powerful statistical and mathematical tools now available. We discuss several specific examples where we may gain new insight into the causes of aging by looking to other evolutionary phenomena, including sexual conflict and the evolution of social behavior. In addition, we present new results which suggest that the analysis of gene networks may lend particular insight into the genetic underpinnings of the aging process.

Keywords: Senescence; Evolutionary theory; Networks; Demography; Gene expression

> I cannot give any scientist of any age better advice than this: the intensity of the conviction that a hypothesis is true has no bearing on whether it is true or not (Medawar, 1979, p. 39).

1. Introduction

Bernard Strehler, to whom this issue is dedicated, recognized the value of looking back to his predecessors, while at the same time creating enormously forward-looking ideas. But of his many significant discoveries, Strehler complained that today's scientists did not look back enough and were ignorant of what he had discovered[1]. However, for evolutionary biologists working on aging, we feel that just the opposite problem might exist. Here we argue that evolutionary biogerontologists look back to classic theories of aging with an intensity that has hindered their

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[1] 'Most of my ca. 250 scientific publications are unknown to the present generation of scientists…'—B. Strehler, writing on http://www.fihg.org/founder1.htm.

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progress. Casting caution to the wind, we describe how one might transcend the standard theories of aging, using recent advances in molecular and evolutionary biology to develop fundamentally new ideas about how and why organisms age.

The two standard evolutionary theories of aging were introduced by Sir Peter Medawar (the 'mutation accumulation' model) (Medawar, 1946, 1952), and George Williams ('antagonistic pleiotropy') (Williams, 1957) some five decades ago. Following Haldane (1941) and Fisher (1930), Medawar noted that while selection could easily weed out deleterious mutations that affected fitness early in life, selection would be much less effective at eliminating mutations from the gene pool whose effects were confined to later ages. Thus, late-acting deleterious mutations would accumulate over many generations, leading to an age-related increase in mortality. Williams thought that trade-offs might lie at the heart of aging. As a corollary to Medawar's model, Williams suggested that these deleterious genes could actually be favored by natural selection if they had early-acting beneficial effects.

The evolutionary literature on aging is dominated by detailed mathematical treatments of these models (Rose, 1982; Charlesworth, 1990; Charlesworth and Hughes, 1996; Charlesworth, 2001), and tests of their predictions (e.g. Rose and Charlesworth, 1980, 1981a,b; Luckinbill et al., 1984; Arking, 1987; Tucic et al., 1988, 1990; Zwaan et al., 1995; Partridge et al., 1999), including studies by the two of us (Promislow, 1991; Promislow and Tatar, 1994; Promislow, 1995; Promislow et al., 1996; Pletcher et al., 1997, 1998; Pletcher, 1999). While Kirkwood's (1977) 'disposable soma' theory provides a widely-cited alternative, the trade-offs that underlie his model are conceptually similar to Williams' model.

All of these mathematical and experimental studies suggest that the ultimate explanation for aging probably has something to do with an interaction between the declining intensity of natural selection with age and the age-dependence of genetic effects. Unfortunately, progress seems to have stagnated here, and biologists have failed to develop a deeper understanding of what these evolutionary concepts imply. Important factors that may well affect the decline in selection intensity (e.g. sexual conflict, phenotypic plasticity, kin selection, etc.) have been broadly ignored, and experiments looking for tell-tale signs of whether genes with deleterious effects late in life also have beneficial effects early are, for the most part, opaque and inconclusive (Curtsinger et al., 1995).

The field is clearly indebted to Medawar and Williams, but we should not be too much in awe of them. The time has come to stand on the shoulders of these giants, and reach farther than they might have imagined possible. In the following article, with presumption that perhaps is fitting of young scientists who have yet to learn their lessons, we suggest ways in which we might reach the next level of evolutionary gerontology, theoretically, empirically and conceptually. In many cases, while we have few answers, the first step is just to raise questions that have not been raised before. The move forward will depend on a firmly integrative approach uniting three areas of study—biodemography, modern evolutionary theory, and molecular genetics and genomics.

2. Demography

Gompertz first developed a mathematical model of age-related increases in mortality almost two centuries ago (Gompertz, 1825). But it was not until Strehler and Mildvan's work in the 1960's (Strehler and Mildvan, 1960) that biologists began to think seriously about ways to integrate the age-related decline in biological function with demographic consequences.

Since then, and especially in the last decade, we have developed much greater insight into the process of aging through the use of mathematical demography. These contributions have helped both evolutionary and molecular gerontologists understand the causes of aging in many ways. First, we now have a rigorous and well-developed mathematical machinery that allows us to study various environmental and genetic influences on growth rate in age-structured populations (Charlesworth, 1994; Lynch and Walsh, 1998). Changes in growth rate directly affect population

fitness, which in turn influences the strength of selection on senescence-causing mutations. Second, demographers have shifted the focus of biogerontology away from average longevity, and towards age-specific mortality rate as the relevant phenotype when it comes to measuring aging (e.g. Curtsinger et al., 1995; Vaupel et al., 1998). This has enabled us to come up with quantifiable measures of senescence and to compare these estimates within species and even between them (Finch et al., 1990; Promislow, 1991). Third, with this focus on age-specific mortality, we have now begun to examine transient, age-specific effects of genes that may be important in the aging process, but may have previously been overlooked (e.g. Pletcher et al., 1999; Yampolsky et al., 2001). We are also able to better understand how life span altering interventions, whether genetic or environmental, actually work. Do they decrease underlying mortality rates, or actually slow down the aging process (Promislow et al., 1999)? Finally, with a focus on age-specific mortality, we have been able to observe phenomena such as late-age mortality deceleration, which turns out to be common (Vaupel et al., 1998) but was unknown until just a few years ago. These new observations have led, in turn, to a new body of mortality-based theory (Mueller and Rose, 1996; Pletcher and Curtsinger, 1998).

3. Evolutionary theory

Medawar's and Williams's theories of aging stand up, even 50 years later, as great conceptual advances in evolutionary biology. But since then, enormous theoretical advances have been made in other areas of evolution. We now have a large body of theory to explain how and why sex evolved (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Michod and Levin, 1987; Bernstein and Bernstein, 1991). Seminal work by W.D. Hamilton and John Maynard Smith led to fundamental advances in our understanding of the evolution of social behavior (Hamilton, 1964; Maynard Smith, 1982). Experimental studies have taught us a great deal about how organisms adapt to novel environments (Mousseau et al., 2000). And of course, in the past 20 years we have seen unprecedented advances in our understanding of the evolution of development, in molecular evolution, and so on.

But relative to these impressive advances in evolutionary theory, theories of aging have remained relatively stagnant. An infusion of the conceptual advances realized in other evolutionary areas is required to stimulate the current state of evolutionary gerontology. While there are numerous areas in which we could illustrate the point, we focus on just three points discussed above. For want of space, discussions of the evolutionary relationship between aging and development, molecular evolution, host–parasite interactions, and other factors, are notably absent. In particular, we address two specific and evolutionarily important issues as they may relate to aging—sex and social behavior. A third evolutionary factor, adaptation in response to lab environments, offers a good example of the importance of cross-talk between evolutionary and molecular biogerontologists. We explore this in a later section.

Evolutionary biologists have spent enormous time (and more than a few grant dollars) working on the following questions: Why are there two sexes? Why do these sexes usually appear in approximately equal frequency, but not always (Charnov, 1982)? Why do the sexes often look so different (Andersson, 1994)? And is evolutionary change dictated more by conflict than cooperation between the sexes (Rice, 1992; Gowaty, 1997)? These are major evolutionary questions, and ones that may relate to factors that could play a very important role in the evolution of senescence.

Consider sperm competition and aging in fruit flies as just one example. In flies and many other species, females mate multiply in a short span of time, and store sperm from multiple males. Each male is favored by selection to do whatever he can to ensure that his sperm fertilize all the eggs that a female carries. This has led males to evolve mechanisms that may increase their own fitness (by maximizing the ability of their sperm to compete with sperm from other males), but at a cost to the female's fitness. For example, the male fruit

fly ejaculate includes proteins that reduce the receptivity of the female to mate again and that cause her to lay more eggs than she would normally (Chapman et al., 1995). But there may also be proteins that increase her mortality. One protein in the male ejaculate has been shown to be similar to a spider neurotoxin (Wolfner et al., 1997)!

At the same time that males are evolving mechanisms that increase their own fitness (and coincidentally, but not deliberately, decrease female fitness), the female is evolving ways to stop this biochemical manipulation (Rice, 1996). Genes that cause these 'male benefit/female detriment' effects are known as 'sexually antagonistic' alleles. It is not a far stretch to imagine that these sex-specific 'antagonistically pleiotropic' genes could play a role in the aging process. Recent studies by Bill Rice and colleagues (Chippindale et al., 2001) have shown that certain alleles increase competitive ability when found in males but decrease competitive ability when found in females. It would be of obvious interest to know if these same genes show sex-specific effects in rates of aging. If so, they may help to explain why we see so much variation in aging, and may also serve as likely targets for single-gene analysis of the aging process.

Our second example considers the role of social evolution in aging. Darwin struggled to explain behaviors that were apparently altruistic. Why, for example, should whole cohorts of ants forego reproduction, allowing a single queen to produce all the progeny. Hamilton argued convincingly that such 'eusocial' organization may arise when benefits gained by helping relatives outweigh the losses due to foregoing reproduction (Hamilton, 1964). What does this have to do with aging? It turns out that queens in eusocial colonies tend to have extraordinarily long life spans. Keller and Genoud (1997) describe ant species in which queens live for almost 30 years. In contrast, solitary insects never live for more than a year or two.

Keller and Genoud argue that reduced aging evolves in eusocial species because these species build colonies that are sheltered from many extrinsic causes of mortality. According to standard theory, reduced levels of extrinsic mortality should lead to reduced rates of aging. But might there be another, yet to be developed theory that could explain this result? For example, recent genetic models of social interaction show that interaction between individuals can lead to dramatic shifts in the way that selection acts on traits (Wolf et al., 1998). These models have not yet incorporated age-structure. If interactions between conspecifics change the selective landscape, then the forces acting on long-term changes in senescence could change dramatically. Perhaps the reason that eusocial queens show no evidence of aging has little to do with the protected environment in which they live, and much more to do with the intense social interactions that take place within the colony. Here is a case ripe for theoretical exploration, uniting classical age-structured models with much more recent theories of social interaction.

4. Molecular genetics, genomics, and evolution

There has been tremendous excitement in the aging community with the discovery of numerous genes that extend longevity in various model systems, including the nematode worm (*Caenorhabditis elegans*), the fruit fly (*Drosophila melanogaster*), Brewer's yeast (*Saccharomyces cerevisae*) and lab mice. And for good reason; the elucidation of key metabolic pathways involved in age-dependent physiological deterioration will have great medical benefit.

Leaving a discussion of the benefits of the single-gene approach to our colleagues (see other articles in Strehler Special Section), we note that none of these genes acts alone. When we find a mutant gene that extends life span, we can be certain that the gene, or the protein derived from that gene, interacts with many other genes or proteins in the organism. Thus, the effect of a mutant gene may depend on the specific genotype of the organism in which it is found. Here we discuss two ways that epistatic interactions may be important in our understanding of the evolutionary genetics of aging.

First, consider the role of the entire genetic background in which a mutant gene is identified. At least in fruit flies, the strains used to identify mutants have typically been under lab domestication for many hundreds of generations. In fact, in a rather influential paper, Service and Rose (1985) argued that in genetic studies of aging in the lab, it was critical to use organisms that had been in the lab long enough to reach genetic equilibrium.

This may turn out to be very problematic. Consider the life cycle of the fruit fly. For convenience, most laboratories keep fly stocks on a 2-week generation time. Newly emerged flies are allowed to lay eggs for 2–3 days, after which time the adults are discarded. Eight or nine days later, the next generation of adult flies begins to emerge. By day 14, all adults are collected, placed into new bottles to lay eggs for 2–3 days, and the cycle starts anew. Under this regime, no flies older than 6 or 7 days will ever survive. Now, imagine that a deleterious mutation arises with effects confined to flies older than 7 days. Under this culture regime, any mutations with effects occurring later than 7 days will be hidden from the purging effects of natural selection. Considering that wild-caught flies can easily live for 2 months, 7 days is relatively young. In effect, over time cultures become sick from their high load of late-acting germ-line mutations (Promislow and Tatar, 1998; Harshman and Hoffman, 2000; Sgrò and Partridge, 2000; Linnen et al., 2001). At the same time, selection favors high early reproduction, which can also reduce life span (Sgrò and Partridge, 2000).

Successful attempts to artificially select on extended longevity (e.g. Rose and Charlesworth, 1980) may simply be purging the genome of recently accumulated deleterious mutations, rather than selecting on *natural* variation for alleles that confer longevity. A recent study found that a wild-caught fly population had survival rates as high as laboratory strains that had been under selection for nearly 20 years (Linnen et al., 2001).

This raises the important possibility that so-called 'longevity assurance genes' isolated in lab-adapted genetic backgrounds may simply be extending life span of unusually short-lived flies. Whether these genes are effective in natural backgrounds remains to be seen.

We can also think about gene interactions not just in terms of these very general 'mutation × genotype' interactions, but rather in terms of single-genes interacting with one another within a complex network. Such thinking may yield great insight into the genetics of aging.

The study of complex, interactive entities has a long history in pure mathematics (Bollobás, 2001), physics (Erdos and Renyi, 1960), computer engineering (Claffy and Monk, 1997), and even sociology (Wasserman and Faust, 1994). Researchers are just beginning to take the experience they have gained from efforts to understand and characterize such diverse entities as social networks, the topology of the US power grid, and the structure of the world-wide-web, and to apply that experience to unravel the intricacies of metabolic networks (Jeong et al., 2000). Where once our research of biochemical networks focussed on analytical models of the only the simplest of networks (Fell, 1997), it has now grown to encompass simulation and numerical based inference regarding global properties of realistic and highly complex metabolic systems.

Work by Kacser and Burns (1981) provides a nice example of how the study of global and emergent properties of interacting gene networks can illuminate biological questions at both the proximate (i.e. genetic or physiological) and ultimate (i.e. evolutionary) level. In their study on the molecular basis of dominance, they provided a simple and elegant network-based explanation for why most loss of function mutations in genes with large effect are recessive. At the time, this problem had stymied evolutionary biologists and geneticists alike. We are confident that we might stimulate similar progress, both in molecular and evolutionary studies of the biology of aging, by incorporating the study of gene networks into a field dominated by a focus on single-gene effects.

As an example, we provide a network model to explain why we do not see a 'wall of mortality' among very old organisms. From a standard evolutionary approach, the fact that aging is observed at all requires that a subset of genes, and mutations in those genes, have effects that are confined to a narrow range of ages (Charlesworth, 1990; Promislow and Tatar, 1998). There is em-

pirical evidence to support this assumption (Pletcher et al., 1999; Yampolsky et al., 2001). However, evolutionary genetic models of aging that explicitly incorporate age-specific effects invariably predict a 'wall' of mortality at post-reproductive ages (Charlesworth, 1990; Pletcher and Curtsinger, 1998; Wachter, 1999). And indeed, we do see this pattern in some animals (e.g. pacific salmon, the 'marsupial mouse' *Antechinus stuartii*) and plants (e.g. bamboo, agave, yucca). But why are not there more organisms that show a catastrophic die-off after reproduction? One possible explanation is that our models of aging fail to account for genetic interactions.

This 'wall of mortality' conundrum is easily resolved by thinking about aging as caused not by single-genes, but rather by a network of interacting genes. The following illustration is the first example, to our knowledge, of a gene network model to examine the evolution of aging.

Consider a simple network of 50 interacting binary genes (with expression either 'on' or 'off'), a subset of which are chosen to represent the phenotype. The state of each gene at age $x+1$ is determined by its interaction with 4 other genes in the network at time x. We subjected one such network to simulated natural selection to evolve a specified age-dependent phenotype (Fig. 1A). We then asked whether changes (i.e. germ-line mutations) in the structure of the network could lead to age-specific changes in the phenotype. Indeed, we did find mutations that affected the phenotype over a narrow range of ages, and in some cases these effects were seen only at old ages (Fig. 1B). Since in reality mortality rates are affected by multiple mutations, we asked what would happen if we combined two independent mutations, each of which had age-specific effects. Strikingly, in most cases if two of these age-dependent mutations were combined, the epistatic interactions were such that phenotypic deviations were seen over nearly all ages: a much greater range than expected if the mutations acted independently (Fig. 1C).

Can this finding of *age-dependent epistasis* help to explain the lack of a 'wall of mortality' in most organisms? In the classical evolutionary models of aging, mutations will accumulate only if their deleterious effects are confined to late ages. Here we show that significant age-dependent epistasis can reduce the age-specificity of effects. This

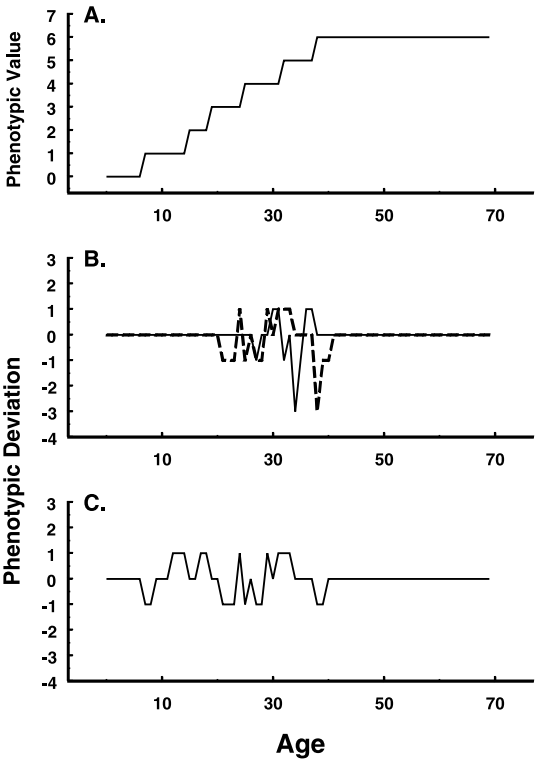

Fig. 1. Age-dependent phenotypes for a network model of aging. See text for details. The wild type network shown here was subjected to 50 000 generations of simulated evolution where fitness was proportional to the network's ability to exhibit a step-wise increasing phenotype from ages 1–50. (A) The adapted network shows a phenotype identical to the specified optimum. Following age 50, selection is absent, but the network maintains a constant phenotypic value. (B) The deviation from wild type phenotype (which is shown in panel A) for two networks that differ from the wild type by a single mutational event. Both mutations have age-specific effects. The mutant networks are identical to the wild-type network before age 20 and after age 45, differing only at intermediate ages. (C) The deviation from wild type phenotype for a network that was constructed by combining the two single mutations shown in panel B into the wild type network. In this case the two mutations show age-dependent epistasis. The single mutations, each with effects confined to a narrow range of old ages, generate large effects early in life when placed in the same genetic background. Given that the effect of the combined mutations is seen at a much earlier age, selection would be much stronger to remove these age-dependent mutations than it would be on each of these mutations individually.

could, in turn, slow the rate of accumulation of late-acting mutations. Whether or not this would be sufficient to eliminate the late-life 'wall of mortality' has yet to be determined.

An appreciation of the richness and complexity inherent in the age-dependent dynamics of gene networks may provide insight into a host of important questions in the biology of aging. What types of dynamics are seen at old ages where selection has not been effective? How does network structure relate to age-dependent epistasis? Does network structure predict the rate of decline in function during aging? And can we use network models to predict which genes should show stable expression levels over time, and which should be more likely to change (Pletcher et al., 2002)?

5. Tools for the new generation

The successful molecular biogerontologist of the future will likely be a good mathematician. It is clear that genomics and proteomics, although powerful new technologies, will be useful techniques for the aging researcher only if new methods in statistics and mathematical demography are brought to bear on the genomic data.

It is becoming increasingly recognized that statistical analysis of microarray expression data is fraught with pitfalls. When examining complex phenotypes such as aging, treatment effects on expression levels are rarely clear patterns of presence vs. absence or 100-fold changes (Pletcher et al., 2002). In most cases, we need complex statistical methods to discern patterns. But statisticians and mathematicians have been generally unwilling to spend the time to make their methods accessible and easy to use, and even at the best of times, many molecular biologists are suspicious of conclusions that depend on statistical inference. The result is that many recent genomic analyses, expression studies in particular, have been statistically soft, with significance based on reaching threshold x-fold changes rather than statistical significance (Zou et al., 2000). Fortunately, workers are becoming aware of the challenges inherent in microarray analyses, including the need for extensive replication and rigorous methods for protecting against false identification of significant treatment differences (Miller, 2001).

But added to these challenges is the fact that senescence can be a very difficult phenotype to accurately measure. Fortunately, we have seen recent advances in the genetic analysis of traits that change with age (that is, 'function-valued traits'), such as gene expression, reproductive output, or mortality rate (Pletcher and Geyer, 1999; Jaffrezic and Pletcher, 2000). These methods provide a statistically powerful machinery to examine age-dependent changes both in the mean of the trait and the variation (both genetically and environmentally derived) around the mean. Moreover, powerful demographic models that quantify how life span differences between any two cohorts or populations depend on differences in their underlying age-pattern of mortality are currently available (Pletcher et al., 2000). We predict that user-friendly versions of these methods will soon be in standard use among molecular biodemographers.

For experimental systems in which longitudinal measures within individuals are not possible, studies of aging are consistently hampered by the problem of demographic selection. Imagine that the level of expression of a particular gene does not change with age, and that higher levels of expression lead to higher mortality risk. If same-aged individuals vary in their expression levels, individuals with higher levels will tend to die young, leaving only individual with lower levels of expression at older ages. This could lead to an apparent age-related decrease in expression levels where none actually exists. It is imperative that we use demographic models to account for this sort of change and to avoid drawing false conclusions.

Finally, along with these new mathematical and statistical tools come new and powerful experimental tools. In particular, we would like to manipulate gene expression in an age-dependent manner. Age-dependent gene-inducible systems are becoming readily available to molecular biologists studying a variety of organisms. The tetracycline-inducible systems (Stebbins et al., 2001), which have been available in rodents for some time, are now reliable in flies, and newer systems

are coming online (e.g. the P{switch} system, Roman et al., 2001). These will no doubt become important components of the biogerontologist's toolbox.

6. Conclusion

In his lifetime, Bernard Strehler's intellectual output was notable for both size and breadth. His roughly 250 scientific publications included work on many different questions, from light production in plants and animals, to information theory, to his vast contributions in aging. Strehler was very forward-looking in his ideas, and in his enthusiasm was never afraid to challenge orthodoxy. Here, in our attempts to emulate Strehler, we have presented a broad array of ideas, some controversial and some less so, which we hope will help motivate evolutionary biologists to look beyond established models and molecular biologists to learn from their evolutionary colleagues.

Rather than acting as reviewers, discussing what we already know from evolutionary gerontology (which would focus mainly on mutation accumulation and antagonistic pleiotropy), we have tried to be prognosticators, focussing on what we *need* to know in the future. We believe that we are on the cusp of a great integrative leap forward in biogerontology. As evolutionary theorists, molecular and quantitative geneticists, statisticians and demographers begin to work together on the problem of aging, the potential for new discovery is enormous.

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