Senescence and the Genome or, Change and Decay in All Except Lobsters I See

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The number of biologists working on the fundamental problems of ageing may seem surprisingly small, considering the importance of the subject; but the problems are so intricate, and our present ignorance so extensive, that it is more remarkable that anyone will spend their time labouring against such difficulties, when they could opt for an easier life and ask fewer questions. The dominant insight into ageing was achieved over a century ago by August Weismann, the originator of (among other things) the concept that somatic cells have a finite lifespan which determines the lifespan of the organism. Obviously, there is a major genetic component that regulates this process, which varies enormously between different species. The three sets of authors considered here[1-3] approach this problem of ageing from different angles, but all have considerable merit.

Of the three, Finch[1] is the heavyweight champion; wide-ranging, formidably erudite, honest to the point of putting [not read] beside a very few of the entries in his massive bibliography, and astonishing value for money. He has set out to consider two major questions: is senescence inevitable in somatic cells? And when it is, how is the genome involved in regulating it? In meditating on these problems, he has created a book which is literally marvellous, full of wonders, of fascinating details that are almost all relevant to the main theme. For instance: a queen bee carries and preserves (without benefit of liquid nitrogen) the sperm from her mating flight for eight years or so. When at last she finds her powers failing, her workers (genetically identical, but with a fraction of her lifespan) surround her and lick her to death; this goes on for three or four days, her body shrinking until little more than the shrivelled skin is left. For another: when the breeding cycles of the 13-year and 17-year American cicadas coincided in 1868, they hybridized. Or, horrifyingly: judicially enforced adult castration in the United States, during the first half of the 20th century, was sufficiently frequent to allow for detailed studies of the increased lifespan of eunuchs (though that was not the original purpose of the program). Perhaps explicably, widowers have an increased death rate in the three years after their bereavement, but widows don't. Lastly, as a consolation, if you aren't schizophrenic by 40, you almost certainly won't be; and human paternity has definitely been proved at the age of 94.

But there is far more than detail in Finch's book. In as far as one can summarise such a complex treatise, his argument is that senescence is not obligatory, but is instead a biological strategy that has evolved, probably several times, with different patterns and mechanisms.

The avoidance of senescence is well documented. The most extensive lifespans, as recounted by Finch, are in plants; apart from the sempiternal bristlecone pine, many lesser plants have grown clonally for ages. All of the noble Cabernet Sauvignon vines have been propagated by grafting for at least 800 years, and saffron crocuses for three times as long; and the groves of quaking aspens in the Rockies have not set seed since the end of the last ice age. (Fungi rival plants in longevity; but the recent discoveries of massive underground fungal clones, of millennial age[4], are too recent for this edition.) More surprisingly, many animals also show no signs of ageing as we know it, no increase in rate of mortality after reaching maturity. Lobsters are one of the best-established cases of an animal that just keeps on living and growing, more slowly perhaps as they get older, but with no diminution of fertility (and with rare cancers that do not increase in frequency with age). Other examples include many gastropods, flatfish and sturgeons.

To some extent, this avoidance of aging fits in with the theory that G. P. Bidder[5] advanced sixty years ago: that organisms which show senescence are those with developmental programs that cause them to stop growing. But Finch has counter-examples. Consider the interesting ocean perch of the genus *Sebastes*, which do not show continual growth (they are never more than half a metre long) but can live to at least 140, with a steady attrition of the population but with no detectable increase in mortality with age. Or the fulmars of Orkney, studied since 1950 by George Dunnet: these gull-sized birds start breeding from ages 9 to 19 years, and continue for at least 22 years more, with a low annual mortality (about 3%) apparently unaffected by age.

Conversely, there are a range of examples of semelparity, in which the developmental program compels the organism to reach maturity, breed and die shortly afterwards. Finch stresses that semelparity has evolved repeatedly in many different phyla, often in some members of an order but not in others. Sometimes it is ecologically intelligible, as when the young hatchlings of chinook salmon feed in the spring upon blowflies, hatched from the carcasses of the hatchlings' parents that died after spawning the previous autumn. Other cases are less explicable; among vertebrates, semelparity is oddly common (though not invariable) among migratory fish. There are even semelparous mammals: marsupial mice of the genus *Antechinus*, where the males (of some but not all species) mate in their first year, with repeated bouts of copulation lasting 12 hours or more, during which they produce massive levels of corticosteroids and waste away.

But we, of course, fit neither of these extreme patterns. Finch is inclined to think that our inescapable, accelerating senescence is a consequence of our evolutionary history. We are descended from the very small mammals of the Mesozoic, which were in all probability short-lived, and which had lost some of the developmental features that in various other vertebrates permit continuous viability. Replaceable teeth, for instance. The specialisation of mammalian teeth into molars, premolars, canines and incisors, seen in the cynodonts of the Upper Triassic, seems to have coincided with

the loss of the primitive ability to renew teeth indefinitely. For short-lived mammals, two sets are enough. But when, after the providential disappearance of the dinosaurs, mammals were given the opportunity to fill the niches now vacant, for large terrestrial organisms, those who occupied such niches had to evolve developmental strategies compatible with an extended lifespan. Tooth replacement is one of the obvious problems, solved in different ways.

Continuous growth is one solution (as in elephant tusks, horse molars, and the incisors of rodents which have acquired this capacity so as to deal with intense abrasion rather than prolonged age). Another, which can be traced in the elephant lineage back to the Miocene, and which Finch regards as a crucial aspect in their acquirement of longevity, is the renewal of molars by sequential replacement. In an adult elephant, there are six (or sometimes seven) molars in each jaw quadrant, but they come into use one at a time. The front molars erupt first; as they are worn down by the prodigious task of grinding the elephant's daily consumption of 100-200 kg of greenstuff, they are replaced from behind by the next in the series. The sixth set do not appear till the age of 40. Human wisdom teeth are a trivial adaptation by comparison.

Remarkably, some mammals have solved the problem in a third way, by unlimited continuous replacement. The manatees of the genus *Trichetus* can produce at least 30 molars in each quadrant; and a solitary marsupial, the rock wallaby *Petrogale*, has evolved something similar. In a sense, balleen whales have done the same by evolving a substitute for teeth. Toothed whales, instead, adopt a feeding strategy in which teeth are not used for grinding, and are either vestigial or unimportant stumps. (Either way, whales have acquired surprising longevity; it is remarkable that in some species, a quarter of adult females are post-menopausal.) Reversal to the ancestral vertebrate pattern of complete replacement of entire sets of teeth does not seem to have happened, though there are sporadic cases in the medical literature of three sets of human dentition, and even one overlooked by Finch – Lison's Case, in France in 1896[6] – of a fourth set.

Dentition is a long way from the genome: but there may be parallels. If mammals, for the greater part of their evolutionary history, have been small and rapidly ageing creatures, then in reacquiring longevity they may be supposed to have changed their senescence-determining molecular strategies, as well as their dental development, by various independent routes. And it may be that some of the factors associated with mammalian ageing are unique inheritances from their small, short-lived, nocturnal reptilifuge ancestors of the Mesozoic, with no necessary parallels in other vertebrates. (Finch mentions, in passing, that sauropod skeletons show no sign of arthritis; though condors, among the longest-lived of birds, do suffer from cataracts which make their landings hazardous).

The Gavrilovs[2], however, maintain that there is a fundamental unity to the aging process. They have attempted something rather different from Finch: a rigorous study of the published data of species-dependent longevity, and an analysis of the various mathematical models for ageing, with digressions on mechanisms. This, too, is an excellent book. The basic mathematical model is derived from the work of the actuaries Gompertz and Makeham in the last century. It relates the force of mortality, $\mu(x)$ at age x (a derivative of the instantaneous mortality rate of a population in which $l(x)$ is the proportion surviving to age x) to an age-independent and an age-dependent component:

$$\mu(x) = -\frac{dl(x)}{l(x)dx} = A + Re^{(\alpha x)},$$

where A, α and R are constants.

The Gavrilovs consider this relationship in its various avatars and modifications. Finch considers the Gompertz-Makeham law more briefly; I think the Gavrilovs would approve of the data he quotes, showing that the privations of Australian prisoners-of-war in the hands of the Japanese, or of Dutch civilians during the last war of European unity, dramatically increased death rates without affecting the age-dependence of death. The Gavrilovs trace its applicability to a wide range of species: man, horses, mice, rats, sheep, *Drosophila*, mosquitoes and confused flour beetles. They show that the great improvement in human longevity in recent decades is due to a reduction in A, not R or α – that is, to a decrease in background, not age-dependent mortality – and they argue eloquently against the view that there is a predetermined limit to the lifespan of any species. Semelparous cases are, of course omitted from their calculations.

One item extensively treated by the Gavrilovs is the "senescence" of mammalian cells cultured in vitro. Here, they have an interesting and important story to tell, which is not as widely known as it should be. It starts with articles published over 30 years ago, concerning the proliferation of human diploid fibroblasts in culture[7,8]. Such cells were shown to proliferate rapidly, after an initial lag; but after a certain number of serial passages, rates of proliferation decline, and eventually multiplication ceases and the cells degenerate. This work contradicted the dogma of the time, which held that fibroblast cultures are effectively immortal; but the dogma was wrong. The three phases of fibroblast culture – initial lag, proliferative phase, and eventual senescence – are now familiar to most mammalian cell biologists. What they may not find familiar is the name of the author of the seminal papers, H. Earle Swim. For the concepts of cellular senescence in vitro, and of the finite lifespan of fibroblasts, are generally credited to Leonard Hayflick[9], who from 1961 onwards described his findings (essentially identical with Swim's) and gained general acceptance for them, while Swim was largely ignored. A quick trawl through the Science Citation Index database reveals 20 citations of Swim's work, three of them erroneous, during the last decade; while Hayflick's first 1961 paper has 469.

The Gavrilovs are moderately scathing about Hayflick, attributing his success to "vigorous propaganda". Swim was indeed unlucky in his choice of media; in 1957, he had so little influence with the editors of biological journals that he had to publish in the American Journal of Hygiene. (Hayflick, nevertheless, read and appreciated that paper; at least, he cited it in 1961, though never again). But Hayflick's errors, in the eyes of the Gavrilovs, are not limited to sup-

planting Swim. His publications likewise ignore Weismann. And, to judge by the quotations acerbically provided by the Gavrilovs, Hayflick seems undecided whether to ignore or to incorporate as his own a crucial revision of Swim's pattern of senescence. The Gavrilovs themselves, and independently E. Bell and co-workers, have observed that fibroblasts in the final "senescent" phase do not in fact die, but simply cease growing; attempts to subculture them, by detaching them from their culture vessels with trypsin, will kill them, but left undisturbed they will live happily for months. Thus, "mammalian cellular senescence" is really a form of differentiation. In appropriate media, indeed, it can be prevented altogether[10].

To be fair to Hayflick, he had to make a considerable effort to get his revolutionary findings accepted; and unlike Swim, he went on to develop a human diploid cell strain, WI-38, which has been very widely used for the preparation of human virus vaccines. Again, he had to battle against the received opinion, this time in the Division of Biologics Standards who preferred vaccines to be grown in primary monkey kidney cells, home of the lethal Marburg virus. And the WI-38 story ended sadly for Hayflick; when he tried to patent his cell line, he became engaged in a ruinous law-suit with the DBS, who broke into his laboratory, confiscated his samples, and only admitted defeat when the money paid to Hayflick in settlement was far less than his lawyers' fees[11].

There is far more to the Gavrilov's book than a quarrel with Hayflick; but the overall effect is, intentionally, one of creative destruction rather than resynthesis. They state, memorably, that:

> "The biology of life span at present resembles a building for which a strong and powerful foundation has been constructed. However, the completion of the upper section of the building has been delayed because the majority of investigators turned in their time to other objects, mostly of a molecular genetic nature, which seemed to them more promising. As a result, what we see on the foundation is a number of temporary barns, and quite a large amount of builders' rubble in the form of various scientific myths and prejudices."

They have cleared away a good deal of rubble, but have no definite blueprint for the structure that should replace it.

The Bernsteins[3], by contrast, have a very clear hypothesis about what causes ageing; one considered, but not finally accepted, by Finch and the Gavrilovs. Hedgehogs to Finch's fox, the Bernsteins know One Big Thing. Ageing is caused by damage to DNA. It is therefore seen by them as a nonadaptive consequence of DNA damage, though the rate of ageing in different species may be under genetic control; variations in ageing can be explained by intrinsic variations in damage rates, or in protection against damaging agents, or in the extent to which damage is repaired. And one of the fundamental mysteries of ageing – how it happens that aged parents produce young offspring – can be explained if sexual meiotic recombination is considered as a form of DNA repair. Semelparous species (the lethal side of sex) are not considered as significant exceptions. Apparently immortal, asexual lineages can be explained as cases where cells are replicating faster than they acquire DNA damage, and thus continuously dilute the genetic poison they carry.

Parts of this thesis would be accepted everywhere. Recombination is certainly related to repair; indeed, some kinds of damage (double-strand DNA breaks, or interstrand DNA crosslinks) can only be repaired through recombination with homologous undamaged DNA. And even repair that does not require recombination, such as excision repair where the undamaged strand opposite a lesion is used as a template for synthesis of new, undamaged DNA, involves enzymes that are also used for recombination. Both processes, after all, involve mechanisms for cutting and splicing DNA, and for performing non-replicative DNA synthesis. In both *Drosophila* and yeast, mutants selected for repair deficiency often turn out to be deficient also in meiotic recombination, and vice versa.

The rejuvenating effect of sexual recombination in unicellular lineages, once controversial, is now everywhere accepted[12]. Usually, the benefits of sexual recombination are attributed to its outcrossing effects, converting homozygous mutations to harmless heterozygotes. The Bernsteins are inclined to think that it also has a direct repair function; that the non-replicative, repair-like DNA synthesis seen during meiosis is in fact an enhanced form of repair. Direct evidence for this is hard to seek. It is striking that a large number of plant species have become self-pollinating and effectively abandoned outcrossing, but keep meiotic recombination; but is this really evidence for a repair function in recombination, or just an indication that recombination is a part of the pollen-forming pathway that is rather hard to dispose of?

When it comes to DNA damage and repair in somatic cells, the Bernsteins have at least an attractive argument; but I am not persuaded that it is necessarily correct. They assemble a very valuable body of information on the correlations between damage and ageing, or repair deficiencies and ageing, that have been reported by diverse workers; and, being thoroughly honest, they include the reports of failure to observe such correlations. (The latter are less numerous, but this may be due in part to editors' reluctance to publish negative results.)

But there is a fundamental problem in any theory relating DNA damage to other phenomena. Nobody really knows which of the very diverse sorts of damage should be considered significant. Consider the spectrum of molecular lesions in DNA. By far the greater number are inflicted by the two inescapable carcinogens, air and water; in a human cell, about 10,000 bases are oxidised every day, and removal of purines by spontaneous hydrolysis of the glycosidic bond is the next most common form of damage. Both of these types of lesion are repaired by excision systems which have something in common, but there are very many kinds of base damage and each is repaired through a separate key enzyme, a DNA glycosylase. Other less common forms of damage, for instance alkylation products, are repaired through other glycosylases. All this repair machinery is entirely different from those systems used to repair relatively rare, but perhaps very important, forms of damage: the helix-distorting lesions inflicted by UV radiation and UV-mimetic chemicals, interstrand crosslinks, and the DNA strand breaks caused by ion-

izing radiation. Each of these classes of lesion is dealt with by repair mechanisms with some unique features. As a further complication, the same lesion may be subject to repair by different pathways; UV-induced damage, for instance, may be removed either by excision or by photolysis, and alkyl groups on bases may be removed by excision or by kamikaze proteins which transfer the damage to themselves.

It is no easy matter, then, to say what "the DNA repair capacity" of a particular cell type, let alone an organism, may be. The Bernsteins are aware of this problem; but their response is to plough doggedly on, accumulating more and more examples and counter-examples of age-dependence of repair. One would like to know (but cannot yet say) which kind of repair is most relevant. And nothing is known of any kind of repair in animals like lobsters, *Sebastes* or fulmars, which (to follow Finch) should be particularly relevant.

Worse, the overall repair capacity for a particular kind of lesion may not be the appropriate measure of repair competence. For some lesions, at least, repair is precisely targeted. Unfortunately, this was not understood when some of the most influential studies on the relationship between DNA repair capacity and ageing were done. A fair number of the correlations between longevity and repair are based on measurements of overall excision repair of UV damage, which is technically undemanding. The universal finding is that repair of UV damage is far lower in cells from short-lived rodents than from long-lived humans. But it turns out that rodents employ a different strategy. (It is an intriguing question whether rodents, still small, nocturnal and short-lived, have in this case kept to the original repair mechanisms of the ancestral Mesozoic mammals. Does anyone know about UV-repair strategies in tree-shrews or tenrecs, the most primitive placentals? Or in monotremes or marsupials?) For whatever reason, rodents use excision to repair only the transcribed genes, a minor fraction of the genome, whereas humans excise everything. However, residual damage in non-transcribed genes might not be relevant to ageing. And it is hard to say whether such differential targeting of repair might also explain reported differences in repair of damage caused by agents other than UV radiation.

There is one final complexity. Differences in developmental strategies are important. Some adult tissues (such as the human central nervous system), and sometimes entire adult somatic assemblies (as in such favourite models as *Drosophila* or *Caenorhabditis*), are composed of post-mitotic, non-proliferating cells. Other tissues depend on continuous proliferation. Unrepaired damage in non-transcribed regions of a non-proliferating genome may well be totally irrelevant to the continued performance of cellular functions. In proliferating cells, this is not necessarily so: if an attempt to replicate a damaged template leads to a chromosome break, genes very far from the damage may be affected. Therefore, a very low overall repair rate in a non-proliferating tissue may be entirely adequate, if it is targeted to the right genes. Whether it will be adequate or not in a proliferating tissue will depend on the cells' strategies for replicating damaged templates; these are known to be complex, and possibly variable, but are far more obscure than direct repair.

Thus the case that DNA damage determines senescence is inevitably full of holes; though not enough to make it a draughty uninhabitable barn (as the Gavrilovs imply), yet enough to render it no more than a possibility (as Finch concludes). But the entire DNA repair community is indebted to the Bernsteins for their meticulous compilation of case studies. No laboratory interested in DNA repair can afford to be without their book; nobody interested in ageing should fail to read all three.

References

1 Finch, C. E. (1991). *Longevity, Senescence and the Genome.* University of Chicago Press. ISBN 0-226-24888-7. Pp xv + 922. $49.95.

2 Gavrilov, L. A. and Gavrilova, N. S. (1986). *The Biology of Life Span: a Quantitative Approach.* Nauka, Moscow; revised and updated English translation (by John and Liliya Payne) Harwood Academic Publishers GMBH, Chur, Switzerland, 1991. ISBN 3-7186-4983-7. Pp vi + 385. $120/£62/ECU 88.

3 Bernstein, C. and Bernstein, H. (1991). *Aging, Sex and DNA Repair.* Academic Press, San Diego. ISBN 0-12-092860-4. Pp xii + 382. £32.

4 Smith, M. L., Bruhn, J. N. and Anderson, J. B. (1992). The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* **356**, 428-431.

5 Bidder, G. P. (1932). Senescence. *Brit. Med. J.* **2**, 583-585.

6 Guinness Book of Records (1981). 28th edition, p. 21.

7 Swim, H. E. and Parker, R. F. (1957). Culture characteristics of human fibroblasts propagated serially. *Am. J. Hygiene* **66**, 235-243.

8 Swim, H. E. (1959). Microbiological aspects of tissue culture. *Ann. Rev. Microbiol.* **13**, 141-176.

9 Hayflick, L. and Moorhead, P. (1961). The serial culture of human diploid cell strains. *Exp. Cell Res.* **25**, 585-621.

10 Loo, D. T., Fuquay, J. I., Rawson, C. L. and Barnes, D. W. (1987). Extended culture of mouse embryo cells without senescence: inhibition by serum. *Science* **236**, 200-202.

11 Hayflick, L. (1989). History of cell substrates used for human biologicals. In *Developments in Biological Standardization*, **vol. 70**: *Continuous Cell Lines as Substrates for Biologicals* (ed. L. Hayflick and W. Hennessen), pp 11-26. S. Karger AG, Basel.

12 Bell, G. (1988). *Sex and Death in Protozoa; the History of an Obsession.* Cambridge University Press.

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