

Ageing selected for its own sake

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ABSTRACT

Ageing has a negative impact on individual fitness. From this, it has been inferred that ageing could not have arisen as an adaptation. Two alternative hypotheses were proposed more than 40 years ago: (1) that ageing has been selected as a side-effect of fertility maximization ('antagonistic pleiotropy') and (2) that ageing is a manifestation of mutational load ('mutation accumulation'). There was good theoretical support for these hypotheses at the time. But in the intervening years, a body of experimental data has accumulated that is surprisingly distant from theoretical expectations. Indeed, some results may be interpreted as a direct refutation of each of the two theories. The evidence reviewed here is adduced in support of an adaptive theory, in which ageing has been selected for its own sake. This possibility has been dismissed historically because it requires strong group selection. In a companion paper, I intend to address this objection and describe a computational model in which ageing is affirmatively selected for its contribution to demographic homeostasis.

Keywords: ageing, group selection, hormesis, senescence.

INTRODUCTION

Modern theories of ageing are linked historically to the group selection controversy. In the 1950s and 1960s, mathematically minded theorists held evolutionary scientists to a new standard of rigour, insisting on mechanistic accounts of natural selection to supplant teleological references that had sometimes served in the past (Williams, 1966; Maynard Smith, 1976). Population genetic theory was established as the canonical quantitative realization of Darwinian dynamics. Despite a few dissenting voices (Gilpin, 1975; E.O. Wilson, 1975; D.S. Wilson, 1980), a theoretical consensus emerged that the dominant operation of natural selection was individual-by-individual and gene-by-gene.

Within this framework, it would be impossible for ageing to evolve as an adaptation. Senescence is, by definition, a decline with age that affects both survival and reproductive ability. Its impact on individual fitness can only be negative; hence it must have arisen as a side-effect rather than a direct result of natural selection. Several variant hypotheses were put forward concerning the evolutionary origin of ageing, all based on the declining force of natural selection with age, and none ascribing to senescence any adaptive value.

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Alleles associated with senescence may be adopted into the genome as a side-effect of strategies that enhance fertility or survival early in life. This idea was first codified as the theory of antagonistic pleiotropy by George Williams (1957): there are genes that have survival value to the young individual, or that enhance fecundity directly, but incur a cost by contributing to the rate of senescence. A second variety of pleiotropy, called the disposable soma theory (Kirkwood, 1977), derives from the thesis that metabolism may be optimized under genetic control to allocate scarce resources in a way that maximizes fitness. If fitness is assumed to depend both on present reproduction and somatic repair, then optimization will produce a compromise entailing imperfect performance of the latter task. Accumulation of damage over the lifetime of the organism is identified with the constellation of symptoms we identify as senescence.

These two varieties of antagonistic pleiotropy have the support of leading theorists in the field. An earlier alternative theory derives similarly from the principle that the force of natural selection decreases over the life of an individual. Without postulating any pleiotropic trade-offs, it is still possible to imagine that genes that adversely affect fitness late in life accumulate in the genome as part of the mutational load. This is the theory of mutation accumulation, based on the ideas of Medawar (1952) and formalized by Edney and Gill (1968). The theory of mutation accumulation has lost favour in recent years, as evolutionarily conserved mechanisms of ageing have been described by genetic researchers (Guarente and Kenyon, 2000). Such phenomena are difficult to reconcile with the hypothesis that ageing derives from chance mutations, recently acquired, which selection has had insufficient time to eliminate.

In the decades since these three theories were proposed, a great deal has been learned about the physiology and genetics of ageing. A lot of the data do not support the theories, and some contradict them outright.

My position herein will be that the weight of the evidence is now sufficient to overthrow the pleiotropic theories, and that new theories for the evolutionary origin of senescence must be considered. Laboratory evidence indicates that ageing has emerged under independent genetic control and must be considered an adaptation, selected for its own sake. In support of this thesis, I will review three classes of animal studies:

1. The long-term studies by Michael Rose, who has been breeding flies for longevity since 1980. In early data from his experiments, fertility of the long-lived strain declined. This result was reasonably reported as confirmation of pleiotropic theories of ageing. But when fertility of the long-lived strain subsequently rose, surpassing that of the controls, the new result was reported not as evidence contradicting the pleiotropic hypothesis, but as an experimental artifact.
2. The suppression of ageing associated with caloric restriction is *prima facie* evidence for the plasticity (under genetic control) of those ageing processes. This and other hormetic* phenomena lend the impression that the metabolism could slow the ageing process, were it only programmed to do so. Theorists have sought refuge in the hypothesis that life extension in calorically restricted animals is mediated by fertility suppression, but the correlation between increased life span and depressed fertility is inconsistent.

* The word 'hormetic' refers to low-dose toxins and other environmental challenges that strengthen the body and, paradoxically, induce overall health benefits.

3. Genetic studies of *Caenorhabditis elegans*, many conducted and interpreted by Cynthia Kenyon, support the hypothesis that senescence is regulated by genes independently of fertility. Several point mutations have been identified that extend life span in a way that suggests this is happening without countervailing cost.

LABORATORY EVOLUTION OF *DROSOPHILA*

Rose's *Drosophila* lab hosts a long-running selection experiment in which two strains of fly have been bred. Control strain B is maintained on a fixed two-week generation cycle, with eggs collected early in the adult life span for each successive generation. In strain O, eggs are collected only from the longest-lived individuals of each generation, creating an ever-increasing selection pressure for longevity. The two strains started from the same stock, but diverged in the laboratory. The life span of strain O has increased steadily over time, attesting to a substantial genetic variance for longevity.

During the first two years of the experiment, early fecundity was observed to decline in the O strain. Rose hailed the result as direct confirmation for pleiotropic theories of senescence: 'The scientific significance of these conclusions is that senescence in this population of *Drosophila melanogaster* appears to be due to antagonistic pleiotropy, such that genes which postpone senescence appear to depress early fitness components. Put another way, these results corroborate the hypotheses of a cost of reproduction (Williams, 1957, 1966), since prolonged life seems to require reduced early reproductive output' (Rose, 1984, p. 1008).

But in later results, longevity continued to increase, uncoupled from fertility decline. After 12 years of laboratory evolution, Leroi *et al.* (1994) reported that mean life span of strain O had grown 81% longer than that of strain B (74 vs 41 days). But, at the same time, average fecundity of strain O had grown steadily, and had surpassed that of strain B (63 vs 52 eggs in a 24-hour period). In the early data (based on 1982 assays), the fecundity difference O-B was strongly negative during early life, and weakly positive from 10 days after eclosion. But in the new data (1991 assays), the fecundity difference was found to be positive at every age.

This result baldly contradicts the prediction of pleiotropic theory. Leroi *et al.* (1994) explain the rise in O fecundity in terms of a genotype-by-environment interaction. Specifically, they write that 'the disappearance of the tradeoff is probably caused by evolution of increased acquisitive ability in the O population in the cage environment'. They explain that pleiotropic theories concern themselves with the division of fixed resources between immediate fecundity and somatic maintenance towards delayed fecundity. If the total of available resources is not fixed, then it is possible for both allocations to rise together. The O strain, in an environment with population density thinned by attrition and in the presence of abundant nutritional resources, evolved the ability to appropriate those resources towards both greater fertility and greater somatic maintenance; while the B strain, evolving in a crowded environment with relatively scarce nutrition, experienced no selection pressure for the efficient conversion of abundant resources towards enhanced fecundity.

This explanation seems to be motivated by allegiance to the theory. No reason is presented why the O strain should evolve greater efficiency of resource acquisition. It would be equally compelling to argue that the B strain, which had to reproduce in a more competitive environment with nutrition that was less abundant, would evolve an acquisition capacity that was more aggressive. Leroi *et al.* (1994) emphasize that in the 'B environment',

the O flies lay fewer eggs; but they mean only that the B flies have been genetically ‘trained’ to lay eggs quickly after they are anaesthetized with carbon dioxide and transferred to a new cage. The long-term B regimen involved (for convenience and handling efficiency) collecting only eggs laid during the first hour after transfer, and adaptation to this inadvertent selection had produced B flies that were able to respond directly to this stimulus.

During every 24-hour period, the O flies laid more eggs than the B flies. Despite this, the observed positive correlation between fecundity and longevity was not considered to detract from the credibility of pleiotropy. Rather, the pleiotropic theory was treated as a fixed framework within which the new data must be accommodated.

The logic of the situation should have supported the opposite attitude: theory holds that pleiotropy is the underlying reason for the evolution of senescence. The existence of trade-offs is a necessary but not a sufficient condition for the theory to be correct. If trade-offs between early fecundity and longevity are observed in the laboratory, these may be connected to the underlying cause of senescence or they may be incidental. But Rose’s results indicate the existence of genes for longevity that do not reduce fertility, contradicting a basic premise of the pleiotropic theories.

At the back of Stearns’s (1992, pp. 214–218) textbook is a table of experiments that were designed to look for evidence of trade-offs between fertility and longevity in diverse animal species. About half the studies find some relationship and half find none. On its face, this would indicate that trade-offs between fertility and longevity are secondary modifiers of ageing genes rather than their *raison d’être*.

I would argue further that observation of an artificially evolved fly that is fitter in every aspect of its life history than the wild type calls into question a postulate of the evolutionary theory of life histories (Stearns, 1992; Charlesworth, 1994). A great deal of this mathematical science derives from the premise that natural selection optimizes life histories for reproductive potential, which is a weighted integral of fertility over an individual lifetime. If this quantity is under strong directional selection in nature, it should be very difficult to increase it further by laboratory selection. Rose’s O strain, evolved in just a few hundred generations, appears to have both a longer life span and a higher fertility than natural selection has been able to evolve over a vastly longer period in the wild. This suggests that reproductive potential might not be as closely associated with nature’s definition of fitness as life-history theory has assumed.

Spitze (1991) documented a similar paradox from laboratory studies of *Daphnia pulex*. A variety isolated in the laboratory appears to be superior to the wild type in its ability to command resources, in its growth rate, and in its fertility and longevity. Reznick *et al.* (2000) highlight the challenge this poses to the theory of life-history evolution: why has this ‘superflea’ failed to dominate its intraspecific competitors in the wild? Like Leroi *et al.* (1994), they present detailed models of environmental interactions that might explain the disparity, but never call into doubt the premise that natural selection optimizes individual life histories for reproductive potential.

CALORIC RESTRICTION AND HORMESIS

A large body of laboratory data attests to a smooth, inverse relationship between caloric intake and life span (Weindruch and Walford, 1986; Yu, 1993). This phenomenon has been observed in a broad variety of species, suggesting that it is an evolutionary adaptation with a substantial and general purpose. The explanation (Holliday, 1989; Masoro and Austad,

1996) is that in times of famine, the immediate prospects for successful reproduction and for survival of vulnerable offspring are diminished, while the reward for surviving through to the end of the famine is an opportunity to deliver offspring into the newly abundant but depopulated world that emerges. The fitness value of immediate reproduction is therefore reduced, while the value of long-term survival is enhanced.

Caloric restriction comprises both a suppression of fertility and an extension of life span. Pleiotropic evolutionary theory demands that these two responses must be tightly linked. In fact, the accepted theoretical explanation is that suppression of fertility provides the means by which life extension is achieved. Laboratory results, however, suggest that the two responses may be separate.

The curve of life extension with caloric restriction is similar in males and females despite the fact that the reproductive metabolisms of the two are so different. In male mice, fertility suppression is modest (Merry and Holehan, 1981), yet caloric restriction enhances longevity in males about the same as in females (increases of 50% or more).

The life span curve for females also poses a challenge to the idea that life extension is achieved by means of fertility suppression. In mice, female reproductive capacity is shut down altogether beyond 40% restriction. But female life span continues to increase linearly right up to the threshold of starvation – around 70% restriction (Ross and Bras, 1975; Weindruch *et al.*, 1986). In this regime of extreme caloric restriction where the mouse is no longer expending any resources on reproduction, what reservoir does it draw upon to continue increasing its longevity as calories are withdrawn?

This experimental profile is the qualitative signature we might expect if fertility suppression and life extension were independent adaptive responses to food shortage. But it is not at all what we would expect if life extension were achieved only as a secondary result of suppressing reproduction. The adaptive value of extended life span is equal in males and females, and that value rises smoothly with the severity of famine, as measured by its surrogate, caloric restriction. The adaptive value to females for temporary suppression of reproduction arises from substantial direct savings in *prospective* energy expenditure associated with pregnancy and lactation. It is for this reason that motivation for females to suppress fertility is far higher than for males.

If we assume that ageing rates evolve as a result of a compromise between individual selection for longevity and group selection for limited life span, it is easy to imagine that the balance between these two forces shifts smoothly in times of scarcity. An adaptive theory of senescence may thus be consistent with the observation that life span and feeding have a smooth, inverse relationship, while female fertility is curtailed to zero below a threshold feeding level. On the other hand, the standard hypothesis that life extension is an indirect benefit deriving from suppression of fertility cannot reconcile the smooth life extension curve with the step-function for female fertility.

In the disposable soma theory, this mismatch with experiment is compounded by the hypothesis that conservation of caloric energy itself is the driving force behind the body's failure to protect against senescence. The disposable soma theory holds that the capacity of the soma to repair itself and forestall senescence is related directly to the caloric energy available for the task. To explain the caloric restriction response within this theory, one must postulate that with less total energy, there is more energy available for repair and maintenance. This assumption violates our intuition that re-optimizing allocation with less total energy available is best accomplished by sharing the reduction among each of the competing demands.

The challenge to the disposable soma theory is the more formidable because of other ways in which calorically restricted animals are more robust than *ad libitum* controls. They are more active, and they enjoy heightened immune function, enhanced resistance to malignant lesions and ability to withstand toxins. Disposable soma theory must therefore insist that energy apportionment for each of these functions is upregulated as total caloric intake is reduced.

One published model (Shanley and Kirkwood, 2000) attempts a quantitative reconciliation of the disposable soma theory with caloric restriction phenomena. However, this model was substantially refuted by Mitteldorf (2001). The model compares energy requirements of pregnant females with that of calorically restricted females that have become temporarily infertile. But in most laboratory rodent studies, control animals do not breed. The difference in energy intake is as much as a factor of two (Bronson, 1989), and this is the reservoir tapped in the Shanley-Kirkwood model to enhance energy expenditure on somatic repair in calorically restricted animals. Energy invested in activity and immune function is also ignored by the model, and these must be presumed higher in the calorically restricted animals, which are more robust. Still, most versions of the model stubbornly predict what intuition tells us is reasonable: more food intake corresponds to more calories available for repair and maintenance. Only in the context of their model's least credible variant is the observed sign for the relationship between food intake and longevity correctly reproduced, and this is so only in a narrow range of caloric restriction where reproduction is presumed to shut down abruptly. Outside this parameter range, even this version of the model predicts perversely that longevity must decline as feeding is restricted.

Generality of the caloric restriction effect attests to a physiological capacity under genetic control to extend life even when resources are limited. This is one example of the phenomenon known as *hormesis*: modest environmental stresses frequently *enhance* the average life span in a population. That aerobic exercise increases life span is so ordinary a fact that it is never posed as an evolutionary dilemma. Only because it is less familiar do we find it curious that life span can also be increased by low doses of toxins or ionizing radiation, by electric shocks, by infection, by physical injury, by heat and by cold. Small doses of chloroform have been found to extend the lives of mice (Roe *et al.*, 1979), rats (Palmer *et al.*, 1979) and dogs (Heywood *et al.*, 1979). A steady, low exposure to gamma radiation extends the life spans of rats (Carlson *et al.*, 1957) and flies (Sacher, 1963). Human as well as animal evidence concerning radiation hormesis is reviewed by Luckey (1994). Mice exposed to daily electric shocks enjoy enhanced longevity (Ordy *et al.*, 1967). Immersion in cold water extends the lives of rats (Holloszy and Smith, 1986). Heat shock induces life extension in roundworms (Butov *et al.*, 2001; Cypser and Johnson, 2002).

Hormetic phenomena suggest that life span is plastic under genetic control, that it can be increased without cost or side-effects in response to a more challenging and competitive environment. Forbes (2000) reviews a wide range of hormetic phenomena, and concludes that fitness hormesis is surprising in the context of evolutionary theory based on individual selection. The essence of the paradox is this: Why is the life extension program not implemented in less challenging times? If genes are available for extending life and, thereby, enhancing fitness, then why is this program ever shut down? Why should animals that have plenty to eat, that are not poisoned or heat-shocked or compelled to exercise vigorously live shorter lives?

Hormetic phenomena with no associated fertility cost cannot be squared with a pleiotropic theory of senescence. They fit comfortably, however, within a *demographic theory of*

ageing, in which senescence is seen as a group adaptation associated with modulation of population volatility. Reserving the highest level of survivability and endurance for circumstances in which the population is experiencing elevated levels of mortality tends to damp population cycles and prevent extinctions. (I am preparing a companion paper to outline this theory in detail.)

GENETIC STUDIES OF *CAENORHABDITIS ELEGANS*

Biochemical pathways that regulate ageing have been studied extensively in the roundworm *C. elegans* (reviewed by Guarente and Kenyon, 2000). Because the system is so well understood, a detailed comparison can be made with the predictions of antagonistic pleiotropy. Four major disparities emerge:

1. Many point mutations have been identified that extend life without apparent cost.
2. Pleiotropic theory demands that life span extension be mediated through curtailed fertility, while experiments indicate that life span and fertility are regulated through separate pathways.
3. The life span of *C. elegans* responds dramatically to chemical cues in the environment, indicating a metabolic ability to avoid ageing when external circumstances indicate that this would likely benefit the local population.
4. *Caenorhabditis elegans* has a substantial post-reproductive life span, defying theories (like disposable soma theory) based on optimal resource allocation.

1. Single genes extend life span without apparent cost

A large number of point mutations have been discovered that extend life span. For many of these, no offsetting fitness cost has yet been identified (Arantes-Oliveira *et al.*, 2003). If even one of these genes incurs no compensating cost, then it would qualify as a true ‘ageing gene’. It would contradict antagonistic pleiotropy, and established theory would not be able to explain its maintenance in the genome. In deference to existing theory, the reporting of these results has emphasized the few trade-offs that have been found, rather than the many life-extending genes for which no cost has been identified. When a cost has been identified, authors have been quick to report it as positive evidence for pleiotropic theory. This kind of reporting masks the larger picture, in which pleiotropy appears to play a secondary role in the regulation of senescence, rather than a primary role as its root cause.

For example, Walker *et al.* (2000), working with one of the *age-1* alleles (*hx546*), determined that worms with this mutation live longer but are less resistant to starvation than wild types. The reproductive value of the mutant is higher than that of the wild type in standard laboratory culture; but the wild-type allele is better in an environment where the worms are exposed to periodic food shortages. Whether the mutant would outperform the wild type in natural settings remains a matter of speculation. Thus the authors overstate their conclusion when they report ‘that the extension of life span, by mutation of a single gene, is associated with reduced fitness . . . [and that] . . . The observed fitness cost associated with field-like conditions provides an example of a single gene that acts in early life, ageing and Darwinian fitness, as predicted by the pleiotropy theory of ageing’ (p. 297).

Pleiotropic theories demand that *all* genes that extend life span must involve trade-offs, and that the associated costs must outweigh the life extension benefits for each independent

gene. But many other mutations in genes labelled *age-1*, *daf-2*, *daf-4*, *daf-7*, *daf-16*, *fer-15*, *spe-26* have been identified that extend life span without any known cost, and in fact most of these mutations are associated with additional benefits, such as enhanced resistance to heat and chemical stressors in the environment (Lithgow *et al.*, 1995).

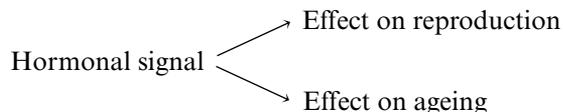
There are good reasons to expect trade-offs between fertility and longevity to appear even if one rejects the hypothesis of the primacy of pleiotropy in the evolution of ageing. Trade-offs may be part of a population-level system for regulating demographic homeostasis. They may be an evolved mechanism for maintaining senescence in the face of individual selection pressure. Or they may be incidental to the root causes of ageing. While pleiotropic theories demand trade-offs, the converse is not true: the existence of trade-offs does not compel the conclusion that trade-offs are responsible for the evolution of senescence.

2. Separate pathways for the regulation of ageing and fertility

According to the theory of antagonistic pleiotropy, genetic extension of life span must be tightly associated with fertility depression; in fact, it is predicted that the fertility effect must be primary, and that extension of life span must be a downstream effect:

Hormonal signal → Effect on reproduction → Effect on ageing

But the experiments imply that reproduction and ageing are separately regulated:



Two worm experiments have clarified this separation of pathways. In the first (Dillin *et al.*, 2002), the timing of *daf-2* signals was manipulated via RNA interference to separate effects on life span from effects on fertility. Animals in which *daf-2* expression was curtailed only after maturation experienced full life span extension without incurring the fertility cost. In the second experiment, destruction of the germ line (via laser ablation) was found to lengthen life span (Hsin and Kenyon, 1999). If the surrounding gonads were removed with the worm's germ cells, the effect was negated, and no life extension was observed. This observation rules out a simple explanation in terms of conserved resources (disposable soma theory). Rather, two signals must have been involved: a life-lengthening signal from the gonads and a life-shortening signal from the germ line (as Kenyon has emphasized).

3. Extended life span in response to chemical signals

Disabling a worm's chemical sensors by genetic modifications, by laser ablation or by other physical interventions that interfere with sense organs can increase life span up to 50% (Apfeld and Kenyon, 1999). In some of these interventions, the number of offspring is simultaneously increased, indicating a positive effect on individual reproductive value.

A straightforward interpretation of this remarkable finding is that individual worms accelerate their ageing in response to chemical signals that they receive from their surroundings. No theoretical account of this phenomenon has been attempted within the context of the pleiotropic theories, perhaps because the result seems, on its face, to be incompatible with the notion of ageing as a side-effect of fertility genes.

The metabolism of the wild-type worm appears to be programmed to shorten its life span and, possibly, to suppress fertility in response to chemical signals from the environment. Although this phenomenon is difficult to comprehend in the context of individual selection, it has a straightforward interpretation in terms of population regulation as an adaptation: the signals could be the 'scent' of available food, or pheromones indicating the presence of nearby worms, or a combination of the two. Confirmation of the former possibility would imply that the life extension response to caloric restriction is not mediated through fertility suppression but derives instead from an independent regulatory signal, a signal that works to *shorten* life span in the presence of adequate food resources. In the latter case, the individual worm may be reducing its lifetime reproductive output in response to crowding, a variant of the much-maligned 'lemming hypothesis'. This possibility also demands strong group selection.

The ageing mechanism associated with chemical sensation may be related to the *daf-2* signalling pathway studied by Wolkow *et al.* (2000). In their experiments, mosaic animals were created with *daf-2* mutations in some of the body's tissues but not others. Life extension was noted when the mutant allele was substituted for the wild type in nervous tissue, but not in other tissues. This finding strengthens the inference that wild-type worms are programmed to senesce in response to a signal from the nervous system.

4. Post-reproductive life span in *Caenorhabditis elegans*

It is well known to geneticists but seldom appreciated by evolutionists that the roundworm *C. elegans* has a substantial post-reproductive life span. The finding that the worm's post-reproductive life span responds to hormonal and environmental signals indicates that it is not an evolutionary spandrel (*sensu* Gould and Lewontin, 1979) but an adaptation.

Classical evolutionary theory attributes post-reproductive life span in higher animals to inclusive fitness effects. Many mammals actively nurture and train their offspring into the third generation, and some live in groups where post-reproductive members can continue to contribute to communal life ('the grandmother effect'). Thus it is plausible that some mammals can continue to add to their own inclusive fitness even after their reproductive capacity has ended.

But worms do not nurture their grandchildren. They do not cooperate in groups. They do not gather food for their young or teach their children to fly. What, then, is the evolutionary provenance of a life cycle that continues two or three times the length of its fertile life span?

The portion of the life span that is post-reproductive does not contribute to an organism's individual fitness; but enhanced repair and maintenance of the soma during early life is a necessary preparation for post-reproductive life. From a pleiotropic perspective, resources are being squandered on a useless life extension, resources which could have been profitably deployed to increase present reproduction. The post-reproductive phenomenon is itself a refutation of the fundamental premise of the disposable soma theory: that resource allocation is determined by an optimization process with the goal of increasing total reproductive output and acceleration of its schedule. From the perspective of antagonistic pleiotropy, post-reproductive life span is an unexplained error in genetic programming.

But the worm's post-reproductive life span is not an accident or error. The post-reproductive life span of *C. elegans* appears to be the target of a well-developed genetic regulatory system (Gems *et al.*, 1998; Kenyon, 2001). Three families of genes (*daf*, *clk* and *sir*) all regulate post-reproductive life span, proclaiming that the response of life span to environmental cues is an adaptation.

Although neither individual nor inclusive fitness is enhanced by post-reproductive life span, post-reproductive worms may possibly contribute to a system of population regulation. The worm's life cycle is known to be unusually plastic, in response to availability of food and other chemical signals from the environment. Perhaps post-reproductive worms provide a pheromonal signal that aids in the suppression of population fluctuations. Exploration of this idea represents an opportunity for theorists in conjunction with experimenters.

OTHER ANIMAL EVIDENCE

Heritability of mortality rates

Promislow *et al.* (1996) report on a cleanly designed study of heritability for mortality in *Drosophila*. The authors' own interpretation of their results (Curtsinger *et al.*, 1995) focuses on the mathematical concept of additive genetic variance, but the data have a wider significance. The fly's mortality rate becomes less and less responsive to selection at advanced age. This contradicts the mutation accumulation theory, which flows from the premise that ageing is caused by mutations that affect survival only at advanced ages when selection is too weak to weed them out.

But it is not only the theory of mutation accumulation that is demolished by this finding. Low additive genetic variance is the signature of a trait that has been optimized by natural selection. In this case, what has been 'optimized' is death. This study is a direct demographic test for programmed death and, remarkably, it produced a clear, positive result.

From a pleiotropic perspective, one would like to protest that what we are seeing is really the optimization of fertility, and that the reason for the low additive genetic variance in late mortality rates is that these rates are tightly linked by presumed metabolic constraints to early fertility. But the results of the study by Leroi *et al.* (1994) exclude this explanation: in long-term laboratory selection, fertility and mortality are handily separable. Consideration of the results of Leroi *et al.* alongside those of Curtsinger *et al.* makes a solid statistical case for programmed death in *Drosophila*.

Late mortality plateaus

A common premise of pleiotropic theories as well as the theory of mutation accumulation is that senescence derives from the decline of selection pressure with age, something that may be expected of any population that is exposed to a finite risk of incidental mortality (Medawar, 1952). It is a robust prediction of these theories that mortality should increase monotonically with age (Demetrius, 2001). However, Carey *et al.* (1992) working with medflies and Curtsinger *et al.* (1992) with fruitflies both observed a clear levelling of mortality values between 0.15 and 0.20 per day at advanced ages, and the data even hint that mortality may actually decline. Pletcher and Curtsinger (1998) expose flaws in the claim of

Mueller and Rose (1996) that pleiotropic theories can predict these results, and then go on to present a detailed argument that mortality plateaus are difficult to reconcile with any of the three standard theories.

Point mutations that extend life span

In his original paper proposing antagonistic pleiotropy, Williams (1957, p. 104) deduced that it was a 'logical impossibility' that changes in a *single system* (let alone a single gene) could substantially extend life span. His observation was astute. In ageing animals, many metabolic functions decline concurrently. Williams reasoned that this could be the result of a global optimization of resource allocation, since little value is to be had by investing resources to preserve one bodily function after others are expected to fail. But if a single gene were found to be capable of affecting the lifetime of all systems simultaneously, this could only be the signature of an overall 'ageing program'. Such a gene would provide a deep contradiction to his premise of pleiotropy.

Single-site mutations that extend life span have been studied in *C. elegans* for more than 15 years (Friedman and Johnson, 1988). More recently, such alleles have been discovered in yeast, in *Drosophila* and in mice.

Migliaccio *et al.* (1999) studied a gene in mice ($p66^{\text{shc}}$) that controls apoptosis in cells that have suffered oxidative damage. To rid the body of such cells once they have become useless (and potentially cancerous) promotes individual fitness. However, the wild-type allele invokes apoptosis too quickly, so that healthy, functional cells are also lost. When $p66^{\text{shc}}$ is knocked out in the laboratory, life span is extended 40% and the mice have enhanced resistance to oxidative stress. There is no reason to expect $p66^{\text{shc}}$ to affect early fertility. The only conceivable benefit to fitness from this gene would come if cancer rates in the wild are so high that loss of some healthy cells might be worth the cost. If this is not the case, then $p66^{\text{shc}}$ is a candidate for a mammalian ageing gene, an allele that has been selected only for its role in limiting life span.

Holzenberger *et al.* (2003) studied a mouse gene affecting insulin-like growth factor (IGF-1). The gene can be excised from the genome to induce extended life and enhanced stress resistance. Deletion of both copies of the gene negatively affects the animal's size and development, but heterozygous individuals seem to enjoy the life extension benefits without paying the developmental cost. Lithgow and Gill (2003) make the case that this is an example of cost-free life extension: 'It seems that a mouse does not have to be sub-fertile, metabolically compromised, or small to age slowly' (p. 126).

Lin *et al.* (1998) document a single gene in fruitflies (dubbed *methuselah*) that extends life span while improving stress resistance and survival in conditions of starvation. This gene seems to encode a guanosine triphosphate-binding regulatory protein, and may operate in a separate pathway from the IGF regulatory system (whose relevance to ageing has been demonstrated in worms and mice).

In yeast, the gene-silencing function has been linked to regulation of ageing. Point mutations of the gene *sir2* have been shown to extend life span (reviewed by Tissenbaum and Guarente, 2002).

It may never be possible to prove absolutely that a given life-extending allele carries no corresponding pleiotropic cost to animals in the environment in which they evolved. However, enough is known about the domain of action of some of these genes to support the judgement that substantial pleiotropic costs are unlikely.

Evolutionary conservation of senescence controls

Another remarkable result from ageing genetics is that there are common biochemical pathways that regulate ageing in yeast, flies, worms and mammals (Kenyon, 2001). The best-studied of these is the biochemistry of the IGF-1 system. However, the response of life span to caloric restriction is common to a broad range of taxa. Thus it should not be surprising that insulin metabolism, which responds to available food energy, would be adopted as a trigger to control the rate of ageing. What is surprising is the fact that some genes and proteins in this pathway have been conserved over the huge evolutionary distance between yeast and mice, indicating that the ageing systems on which they act are also a conserved biological function. This is entirely inconsistent with the mutation accumulation premise that ageing is a phenomenon of mutational load, or recently acquired mutations that have yet to be weeded out by natural selection. It is also affirmative evidence for the notion of ageing as an adaptation, since it is difficult to imagine why a biochemical function lacking any adaptive benefit would be preserved intact over such a time span.

Does apoptosis itself play an important role in senescence? Migliaccio's results certainly indicate that this is the case (Skulachev, 2000). Yet apoptosis is, by all accounts, a well-orchestrated scheme for cellular suicide. Its genetics and biochemistry are among the most ancient and highly conserved of eukaryotic cell functions. It is universally described as adaptive. The co-option of apoptosis into the ageing function would constitute incontrovertible proof that ageing is an adaptation (Skulachev, 1997, 2002).

Replicative senescence

The oldest of all senescence mechanisms is replicative senescence, present in protists, conserved over hundreds of millions of years, and active today in every somatic eukaryotic cell. Its operation is so transparently deliberate that there can be no doubt of its origin as an adaptation shaped by selection. The mechanism comprises:

- a replication counter;
- machinery that kills the cell when the counter reaches a pre-programmed level;
- an enzyme that resets the counter to zero.

Telomeres are the protective 'tails' of repetitive base sequences, found at the end of every chromosome. They are not fully reproduced during mitosis and so become shorter with every cell division. Protists with depleted telomeres languish and die. The enzyme telomerase is able to restore telomeres to their full length, and telomerase is in the genome of every eukaryotic cell. But in protists, telomerase is only expressed during conjugation (when one organism undergoes a sexual exchange of genetic material with another). During mitotic reproduction, the telomere is permitted to shorten, even as it threatens death to the individual.

In protists, the telomeric mechanism of replicative senescence serves only to enforce genetic exchange by limiting the number of reproductions between conjugations. Because it involves a counting mechanism and the withholding of an available enzyme (telomerase), it gives every appearance of being an adaptation. What could be the purpose of such an adaptation? Its contribution to individual fitness is purely negative. Any adaptive value it

has must accrue to the population. The most plausible interpretation is that replicative senescence evolved to increase the frequency of conjugation, and thereby enhance the genetic diversity of protist populations (Clark, 1996, 1998, 1999).

In higher organisms, it has been hypothesized that replicative senescence defends against the runaway reproduction characteristic of tumour growth. But it may be that telomeric ageing remains an effective senescence mechanism even in higher organisms. In a recent demographic study (Cawthorn *et al.*, 2003), telomere length was measured from archival samples of blood drawn from 60-year-old individuals. In the ensuing 15 years, people with the shortest telomeres in their blood cells were more than twice as likely to die as those with the longest telomeres.

Even if telomeric ageing does not substantially affect mortality in higher organisms, the example of protists is instructive. Having observed the operation of affirmative selection for senescence in protists, we would be imprudent to dismiss the possibility of an analogous mechanism having evolved in higher organisms.

Semelparity

Some life histories are organized around a single burst of reproduction. Such organisms generally experience accelerated senescence and die promptly when reproduction is complete. This is one of nature's most dramatic demonstrations of senescence. Is it an extreme example of reproductive effort that destroys the soma? Or is it programmed death?

Every gardener knows that flowering annuals wither and die shortly after their flowers go to seed. However, if the flowers are removed before they form pods, the plant can be induced to flower repeatedly over an extended time. If it were the burst of reproductive effort that killed the plant, one would not expect the plant to be capable of replacing its flowers so handily. It is more fitting to regard this phenomenon as a form of programmed death, triggered by the final stages of seeding.

After laying her eggs, the female octopus stops eating and starves to death (Wodinsky, 1977). Lest we doubt that this is an example of programmed death, the animal's behaviour can be altered by surgical removal of the optic gland, which evidently asserts control over a genetic program. Without the optic gland, the animal resumes feeding and can survive to breed another season.

The semelparous Pacific salmon is thought to have evolved fairly recently from iteroparous cousins in the Atlantic (Crespi and Teo, 2002). The usual hypothesis is that semelparity evolved as a result of the attrition attendant upon the animal's long and difficult migration out to the open ocean and back. But some species of Atlantic salmon undertake comparable migrations and presumably endure a comparable attrition rate for second-time breeding. They have evolved a reproductive burst similar to that of the Pacific species but do not always die afterwards. This suggests that reproductive bursts and accelerated senescence may be separate adaptations.

Many ecologists accept as a matter of course that 'genetically programmed, irreversible degeneration subsequent to breeding' is a group-level adaptation (Crespi and Teo, 2002). Northcote (1978) has proposed that the carcasses of salmon that have completed their breeding help to fertilize small ponds, sustaining food stocks for the hatchlings. If this explanation is correct, then semelparity in salmon is a case of individual death programmed for communal benefit.

CONCLUSIONS

The theory of life-history evolution is a well-developed body of machinery, the mathematical validity of which is self-evident. We can reliably compute the dependence of individual reproductive value on various life-history traits. But has individual reproductive value indeed been optimized in nature's laboratory? This is a question that can be answered only by observations.

The idea that natural selection may not be optimizing individual reproductive value casts a shadow on a great body of evolutionary theory. But much of this theory remains untested and therefore vulnerable, because complex real-world ecosystems so seldom provide opportunities for clean tests of simple hypotheses.

Experimental efforts to validate pleiotropic theories of ageing have focused on their assumption of trade-offs between fecundity and longevity. Although evidence has been inconsistent on the existence of such trade-offs, authors have frequently cited evidence of a particular trade-off as positive validation for the theory. This is a logical error. Although pleiotropic theory requires trade-offs, the existence of the trade-offs does not require that the theory be correct.

If we ask only whether trade-offs can be detected, we are setting the bar too low. We need to ask whether the trade-offs we see are sufficiently robust and sufficiently ubiquitous to provide firm grounding for the thesis that pleiotropy is the deep cause of senescence. To this question, the answer is clearly negative.

In addition to evidence for failure of the pleiotropic theories, I have summarized a diverse body of data indicating that senescence is an adaptation selected for its own sake. Genetic systems that limit life span have been conserved over vast evolutionary time-scales. Additive genetic variance for mortality rates that decrease with age are the signature of an adaptation. And nature's two known mechanisms of programmed death appear to be implicated in the metabolism of senescence: apoptosis and telomeric ageing.

It has been a century since Weismann disavowed his idea of 'making room' for the younger generation, but despite all its problems, this theory has never been completely laid to rest. The experimental data demand a theory of senescence as an adaptation, and there has been a paucity of alternatives to Weismann. In a companion paper, I will present a computational model in which senescence evolves as an adaptation based on its benefit to demographic homeostasis.

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