

Optimality, mutation and the evolution of ageing

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Evolutionary explanations of ageing fall into two classes. Organisms might have evolved the optimal life history, in which survival and fertility late in life are sacrificed for the sake of early reproduction and survival. Alternatively, the life history might be depressed below this optimal compromise by deleterious mutations: because selection against late-acting mutations is weaker, these will impose a greater load on late life. Evidence for the importance of both is emerging, and unravelling their relative importance presents experimentalists with a major challenge.

DESPITE all human efforts to the contrary, death is one of life's only two certainties. It is often preceded by intimations of mortality, in the form of a decline in fertility and most aspects of biological performance, characteristic of 'senescence' or 'ageing'¹⁻⁴. Ageing presents not only a medical problem, but also an evolutionary paradox: if organisms can function well in youth, why can they not continue to do so in old age? Recent theoretical work has clarified two possible causes of ageing. It could evolve as the necessary cost of processes beneficial to youth, or could instead be purely maladaptive, and evolve because of the pressure of deleterious mutations on populations^{2,4}. Evidence for the importance of both causes of ageing is emerging, and unravelling their relative importance presents experimentalists with a major challenge. The view that mutation contributes to the senescence of individuals parallels recent emphasis on its role as an evolutionary force capable both of maintaining sexual reproduction and of producing decline and extinction of evolutionary lineages^{5,6}.

We confine the term 'ageing' or 'senescence' solely to the drop in survival probability and/or fertility later in the life of individuals, to distinguish it from other changes during the life history, such as early development or the onset of reproduction. Ageing reduces the contribution that older individuals make to future generations. It is often seen in nature⁷⁻⁹, but becomes much more obvious when natural hazards are removed in captive and human populations^{1,10}. The rate of ageing is highly variable. In semelparous forms, which breed only once, there is often a sudden loss of function after reproduction; adult mayflies live for less than a week, and male Pacific salmon show catastrophic senescence after their single breeding attempt. In iteroparous organisms, which can breed repeatedly, the decline is usually more gradual, as in many beetles and in fish such as haddock and rockfish.

Mechanistic accounts of ageing invoke various forms of damage, to DNA, cells, tissues and organs^{3,11-14}. From this perspective, the rate of ageing could be determined solely by level of exposure to damaging influences: the process would be inevitable, and in need of no evolutionary explanation. But even under optimal conditions in captivity, we see very different potential lifespans in organisms that do not seem to differ in their risk of damage, showing that the degree of ageing has evolved. For instance, most birds considerably outlive mammals of comparable size. Among more closely related organisms, bats have very much longer maximum lifespans than do similar-sized rodents, and the flightless emu and ostrich are notably short-lived among birds^{1,10}. The thick-shelled bivalves have greater lifespans than do other molluscs, and turtles and tortoises outlive other reptiles¹⁰. The proximate cause of ageing may well be various kinds of damage; however, these comparisons show that organisms vary in the extent to which they avoid or combat it^{10,15}. It is this variation that any evolutionary theory of ageing must explain.

Evolutionary theories of ageing

Because ageing reduces the genetic contribution of individuals to future generations, it is opposed by natural selection. But as Medawar first pointed out^{16,17}, the natural selection that maintains survival and fertility becomes weaker through the life history. Even without ageing, organisms are at risk of death and impaired fertility from disease, predation and accidents. If the genes affecting survival and fertility are to some extent age-specific in their effects, then those that influence later life will be subject to weaker selection because, by the time they take effect, more of the original carriers will already have died or become infertile for other reasons^{10,16,17}. (Note that what matters is when a gene affects survival and fertility, not when it is expressed; for example, a gene which is expressed early in the development of the heart might increase the chance of heart disease much later in life.) The sensitivities of fitness to changes in survival and fertility at different ages are explained in Box 1, and illustrated by Fig. 1, which is based on data from lines of *Drosophila* selected for early or late reproduction^{18,19}.

We shall contrast two kinds of explanation for the evolution of ageing. First, constraints on the combinations of survival and fertility that the organism can achieve at each age may mean that a single optimal genotype evolves, which shows senescence because fitness is maximized by increasing early performance at the expense of late (the optimality explanation). Second, senescence might evolve because of a greater mutation load on the later, and less strongly selected, part of the life history (the mutational explanation).

The arguments over the relative importance of optimality versus mutational explanations are relevant to a wide range of evolutionary questions⁶, and the problem of senescence is one instance of a general question: to what extent does the degree of adaptation reflect the strength of selection? For example, does the precision of mimetic patterns in butterflies reflect the degree of protection they confer²⁰. Can very weak selection maintain adaptations? Perhaps the best example of an adaptation maintained by weak selection ($s \approx 10^{-5}$) is the bias towards use of the most efficiently translated triplet codon, seen for some loci in *Escherichia coli*, yeast and *Drosophila*²¹. Yet, we are still ignorant of the general magnitude of the selection that produces and maintains adaptations.

Optimality theories of ageing

Optimality theory^{22,23} has been successful in explaining diversity in life-history traits such as age of first breeding, number of breeding attempts and number of offspring produced at each^{2,24-27}. Optimality models do not contain specific genetic parameters, and instead specify the life history that gives the greatest fitness, within the intrinsic constraints of physiology and the extrinsic constraints imposed by the environment. Optimality arguments are consistent at least with simple genetic

models²⁸⁻³⁰: the optimal life history is an evolutionarily stable strategy, which cannot be displaced by any feasible genotype.

Ageing could evolve as part of an optimal life history. On this view, senescence arises from the deleterious side-effects late in life of processes that are favourable early on (see Fig. 2). An antagonism between early performance and resistance to ageing can occur if prolonged growth during the pre-adult period elevates juvenile mortality but yields a longer-lived adult^{18,19,31}. Similarly, early reproduction may impair survival or future fertility, by consuming resources, causing somatic damage or exposing the organism to environmental injury: there is a 'cost of reproduction'^{16,17,32-38}. The 'disposable soma' theory is an optimality account of ageing, in which allocation of resources to reproduction jeopardizes somatic repair mechanisms and hence longevity^{15,39}. Selection intensity on early, beneficial effects will be stronger than on later, deleterious ones and so the optimal life history will include ageing.

Of course, as for any other trait, it may be impossible for any one homozygous genotype to achieve the optimum. In diploids, it may instead be best approximated by a heterozygote, if each homozygote is better for a different life history trait, and the alleles are dominant for their advantageous trait (sometimes

known as 'antagonistic pleiotropy'⁴⁰⁻⁴²). If such balancing selection maintains a substantial fraction of life-history variation, it will generate negative genetic correlations among life-history traits. However, such polymorphism does not account for senescence itself, which depends on the mean life history, determined by maximization of fitness subject to what is possible.

The optimality account of ageing has come to be known as the 'pleiotropy theory of senescence', because it is often developed in terms of genes with effects on more than one aspect of the phenotype, in this case on survival and fertility at different ages. But mutational theories of ageing can also involve pleiotropy (for example see Box 2). Moreover, under the optimality theory the outcome does not depend on patterns of pleiotropy, and could in theory be reached by fixation of alleles each with an effect on fitness at only one age. (On Fig. 2, this could be represented by evolution up to the optimum life history by a series of horizontal or vertical steps.) Such evolution would imply that the constraints on optimization occur only on the trade-off curve itself, and not inside it, and so is hardly plausible: our point is that the optimality argument is independent of genetic details such as pleiotropy. Provided that the population is not trapped at a local optimum, and provided there is at least

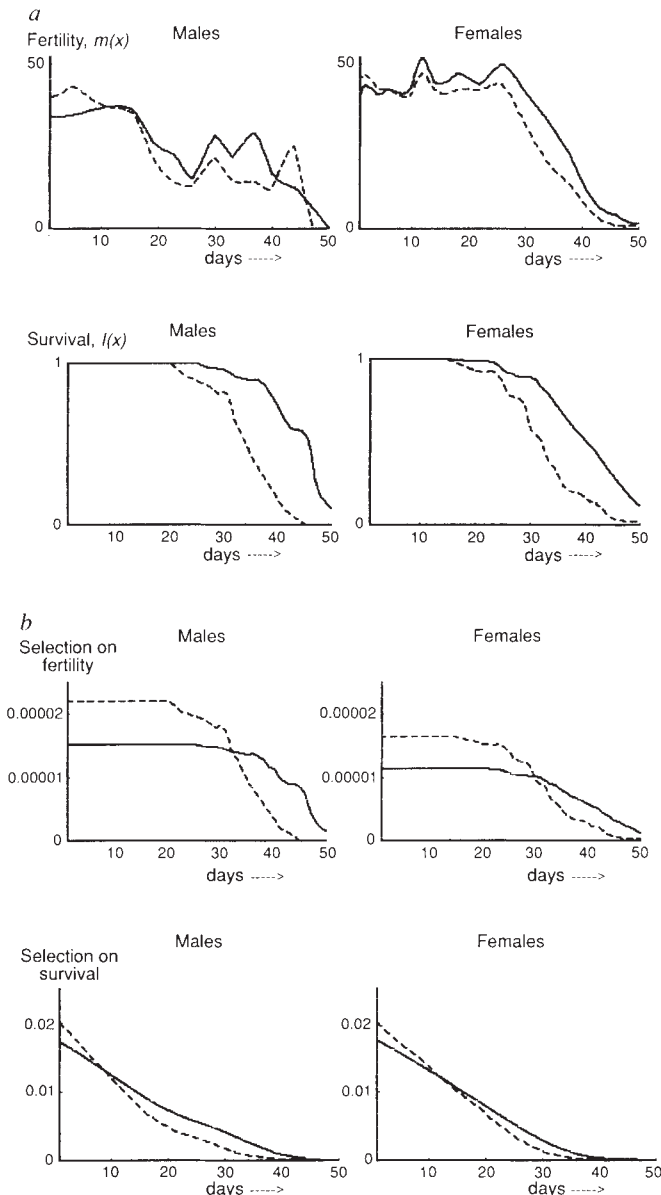


FIG. 1 An illustration of how selection on fertility and survival for the two sexes decreases with age. *a*, Survival, $l(x)$, and fertility, $m(x)$, in the two sexes, as a function of time since eclosion, for lines of *Drosophila melanogaster* which had been selected for early or late reproduction^{18,19}. Female fertility is measured in eggs per day, and male fertility is measured as the percentage of progeny relative to a standard competitor. Dashed lines correspond to 'early' lines, solid lines to 'late' lines. Data are from crosses between replicate selected lines, to avoid inbreeding effects¹⁹. *b*, Sensitivity of fitness (r) to survival and fertility in the two sexes; this is calculated from $l(x)$, $m(x)$ using equation (2) (modified to allow for separate sexes). In all four figures, selection is stronger in early life on the lines selected for early reproduction, (dashed lines) and stronger in late life for the 'late' lines. The difference is greater for selection on fertility (top row) than on survival (bottom row). The top pair of figures shows the sensitivity of fitness to an increase in fertility in the two sexes ($\partial r/\partial m(x)$). We assume that larvae take 10 days to develop (ignoring differences between lines), and that larval viability is adjusted by density-dependence to maintain constant population size ($r=0$). The units for females are rate of increase per day per extra egg; to translate these into selection coefficients per generation per extra egg, multiply by the generation time ($T=25$ days for 'early' lines, 24 days for 'late'). Units for relative male mating success are as in *a*. The second pair of figures shows the sensitivity of fitness to a decrease in death rate, as a function of adult age ($-\partial r/\partial \mu(x)$; equation (2b)). Units here are rate of increase per day per unit decrease in death rate. (Note that these figures are for a hypothetical population with the life history given by *a*, not for the life history as it was while the lines were selected.)

some genetic variation in the appropriate direction, the optimal life history will eventually be reached.

Senescence as a consequence of mutation pressure

Mutation pressure could lead to ageing^{2,17,43}. Because the intensity of selection on later-acting mutants declines with age, alleles with deleterious effects will reach a higher frequency in a mutation-selection balance the later the age at which they reduce fitness. In a small population, or with asexual reproduction, random sampling drift may combine with mutation to overwhelm selection, so that alleles with late, deleterious effects become common. Even in a large sexual population, senescence can be caused by the cumulative effects of many rare deleterious alleles held in mutation-selection balance.

With asexual reproduction of diploids, mutation reduces mean fitness by a factor $\exp(-2U)$, where U is the total number of deleterious mutations per haploid genome per generation, and mutations are assumed not to be completely recessive^{44,45}.

BOX 1 Fitness in age-structured populations

THE dynamics of an age-structured population depend on the probability $l(x)$ of surviving from birth to age x , and the expected number of offspring at that age, $m(x)$. The dynamics depend only on the product of survival and fertility, which we denote by $k(x) = l(x)m(x)$. A homogeneous population will eventually grow at a steady rate r , which is given by the Euler-Lotka equation,

$$1 = \int_0^{\infty} \exp(-rx)k(x) dx \quad (1)$$

In an asexual population, or a population of sexually reproducing haploids that vary only at a single locus, the outcome of natural selection depends simply on the long-term growth rates associated with each genotype, in the absence of density- or frequency-dependent interactions, each genotype will eventually grow exponentially at a rate that depends on its own life history, given by equation (1). With certain simplifying assumptions (that selection is weak, and mating is random with respect to age and genotype) the theory can be extended to diploids and to variation at multiple loci².

The intensity of selection on different stages of the life history can be found from the effect on fitness, r , as defined above, of small changes in fertility or mortality at age x (refs 2, 32)

$$\frac{\partial r}{\partial m(x)} = \frac{\exp(-rx)l(x)}{T} \quad (2a)$$

$$\frac{\partial r}{\partial \mu(x)} = \frac{-\int_x^{\infty} \exp(-ry)l(y)m(y) dy}{T} \quad (2b)$$

where the death rate $\mu(x)$ is defined by $l(x) = \exp(-\int_0^x \mu(y) dy)$, and $T = \int_0^{\infty} x \exp(-rx)k(x) dx$ is the generation time. These expressions give the effect on fitness of small changes confined to a specific age, x ; they are applied to *Drosophila* data in Fig. 1. Similar expressions can be found for the effect of changes in age of onset of alleles which have permanent effects once first expressed³².

These formulae involve the rate of population growth, r ; this is because the value of an offspring depends on the size of the population into which it is born. In an increasing population, offspring produced early will themselves start to reproduce, and so are more valuable than those produced late. But in the long term, density-dependent factors must regulate population size by decreasing survival and fertility such that $r=0$. Then (for a given total reproductive output $\int_0^{\infty} l(x)m(x) dx$), the timing of offspring production is selectively neutral.

Sensitivities of fitness to changes in both fertility and mortality are related to the probability of reaching the age of gene expression, and are inversely proportional to the generation time. For a gene affecting survival, the proportion of total reproduction expected to occur after that age (which depends on the fertility schedule) is also important (hence the integral in equation (2b))³². For mortality, the intensity of selection in a stable population ($r=0$) remains constant during the pre-reproductive period because the consequences of death for reproductive success remain constant; selection intensity starts to decline only at the time of first breeding. □

Surprisingly, minor mutations reduce fitness just as much as those with larger effects, because they rise to higher frequency in a mutation selection balance⁴⁶. This is why the mutation load (that is, the reduction in mean fitness) depends on the total mutation rate, U , and not on the strength of selection. If the effects of mutations on fitness are multiplicative, similar relations hold in sexual populations: fitness is reduced by $\exp(-U)$ if mutations are recessive (or almost so), and $\exp(-2U)$ otherwise⁴⁷. Suppose now that deleterious mutations are age-specific. Then, the reduction in fitness caused by a loss in performance at various ages reflects the mutation rates to genes affecting those ages. But because a given change in performance has less effect on fitness later in life, we expect deleterious mutations to cause a greater drop in fertility and survival probability later in life, assuming the same mutation rate for each age. This increased load on later life can be accentuated by a positive feedback: as late-life performance declines, selection to maintain it becomes weaker, accelerating the decline. If mutations that specifically damage survival or fertility of the old are common enough, mutation may overwhelm selection^{2,10,16,30}, causing catastrophic senescence (Box 2 and Fig. 2). The same argument can explain why different functions often decline at a similar age: synchronous collapse does not imply a single mechanism of senescence (J. Maynard Smith, personal communication).

Other evolutionary theories of ageing have been proposed. Arguing from the example of Huntington's chorea, Medawar¹⁷ suggested that there could be selection for genetic modifiers delaying the age of onset of the effects of deleterious alleles. The argument was not that mutation itself caused senescence, but rather that, given some mutation load, the adaptive evolution of modifiers would shift the effects of that load to later life. This

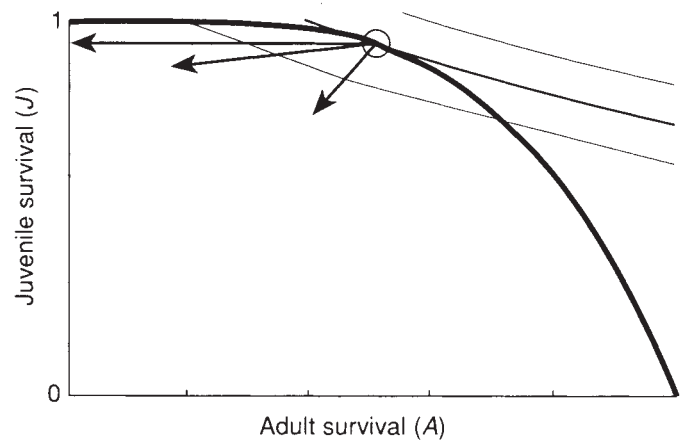


FIG. 2 A simple model of an organism with two age classes (see Box 2). Each female produces one daughter at each of two ages ($m=1$); the chance of survival from birth to age 1 is J and from age 1 to age 2 is A . The heavy curve shows the maximum possible survival probabilities, and represents the trade-off between adult and juvenile survival ($J + A^2 \leq 1$). The light curve show contours of fitness, spaced at intervals $\Delta r=0.1$. The optimal life history is where the furthest contour from the origin just touches the trade-off curve (open circle, $J=0.935$, $A=0.505$). The arrows show the reduction of survival below the optimal value caused by deleterious mutations at a rate $U=0.1$. The horizontal arrow shows the effect of mutations that reduce only adult survival ($\delta J=0$; $v=(\delta A/A)/(\delta J/J)=\infty$); when the net rate of mutation exceeds a threshold ($U>0.067$), these accumulate indefinitely, and the life history becomes semelparous. The lower diagonal arrow shows the effects of mutations that reduce juvenile and adult survival equally ($\delta A = \delta J$; $v=1$). There is now no threshold, as the net mutation rate, U , increases, there is a steady decrease in survival, to 0.765, 0.414 when $U=0.1$. The intermediate arrow shows the effects of mutations with a slight pleiotropic effect ($v=16$); these reduce survival to 0.866, 0.155 when $U=0.1$.

is closer to the optimality theory than to one based on straightforward mutation accumulation. As for Fisher's analogous explanation of the evolution of dominance⁴⁸, the argument is implausible, because selection on such specific modifiers is extremely weak (of the order of the mutation rate per locus) and likely to be overwhelmed by their pleiotropic effects^{2,49}.

Hamilton³² argued that, because selection is stronger on earlier ages, alleles with advantageous effects early in life would be more likely to be fixed by selection than those with a later age of action, an argument that implies that populations are continually adapting, and so are not in equilibrium. The late part of the lifespan would then lag behind the current optimum more than would the early part, and senescence would be caused by the extra 'lag load'⁵⁰. This idea can be seen as the converse of the mutational argument. For it to be plausible, continual adaptation must be necessary for the maintenance of the life history, just as the Red Queen had to keep running to stay in the same place⁵¹. It is then the failure to keep up with the requirements of changing conditions that leads to a substantial 'lag load' on late life. The idea is perhaps implausible because it does not explain why ageing is associated with pathological

changes, which would have been the ancestral state under Hamilton's theory.

Empirical tests of evolutionary theories of ageing

We must distinguish between predictions that are common to all evolutionary theories, and those that can distinguish between optimality and mutation-accumulation. Some support for evolutionary theories comes from the comparative findings, which show that high maximum lifespans in optimal conditions (cited in the introduction) occur in creatures with a low risk of mortality in nature, and hence with strong selection maintaining late life. For example, birds have higher maximum lifespans than mammals and are less prone to death in the wild. The ability to fly and so escape predators may be important: among mammals, bats survive well both in nature and under optimal conditions, whereas among birds flightless species survive poorly in both situations^{1,7,9,10,52,53}. Bivalves and tortoises have longer maximum lives than their relatives, perhaps because of the protection conferred by their thick shells^{1,10}. High maximum lifespans are also found in organisms whose fertility increases with age, such as many fish and trees, as the evolutionary theory

BOX 2 Optimality and mutation

HERE we illustrate optimality arguments, and also show how mutation pressure can lead to catastrophic senescence. Consider a simple model of an organism that reproduces at just two ages. The probability of survival from birth to age 1 is J , and from age 1 to age 2 is A . For simplicity, we assume that the expected fertility is the same at both ages ($m(1)=m(2)=m$; hence, $k(1)=mJ$, $k(2)=mJA$). The growth rate is then given by the discrete version of equation (1),

$$1 = mJ e^{-r} + mJA e^{-2r} \tag{3a}$$

This has solution:

$$e^r = \frac{mJ}{2} \left(1 + \sqrt{1 + \frac{4A}{mJ}} \right) \tag{3b}$$

First, consider the optimal life history, given some constraint on the rates of juvenile and adult survival that can be achieved. We suppose that it is impossible to sustain both high juvenile and adult survival, and choose the arbitrary constraint that $J + A^4 \leq 1$. Then there are progressively large decreases in adult survival for each increment in juvenile survival, and hence a convex trade-off curve (heavy line in Fig. 2). Other constraints give qualitatively similar conclusions, provided that the curve remains convex. For fertility $m=1$, the life history that maximizes fitness (r) involves substantial senescence, in the sense that adult survival is much lower than juvenile, $J=0.945$, $A=0.505$ (open circle in Fig. 2).

Now, suppose that deleterious mutations reduce survival below this optimal value. Consider a class of mutations that reduce juvenile survival by a factor $(1 - \epsilon)$, and adult survival by a factor $(1 - v\epsilon)$, where $\epsilon \ll 1$; v describes the degree of pleiotropy. These will reduce fitness by,

$$\begin{aligned} \delta r &= \epsilon \frac{\partial r}{\partial \mu(1)} + v\epsilon \frac{\partial r}{\partial \mu(2)} \\ &= \frac{-\epsilon(1 + 2(v+1)\gamma + \sqrt{1+4\gamma})}{\sqrt{1+4\gamma}(1 + \sqrt{1+4\gamma})} \end{aligned} \tag{4}$$

where $\gamma = A/(mJ)$. (δr is negative because these mutations decrease fitness). Selection on deleterious alleles depends on the current life history through γ . When fertility and adult mortality are high ($m, J \gg A$; $\gamma \approx 0$), $\delta r \approx -\epsilon$, so that selection depends on the effects on juvenile survival. When fertility is low and juvenile mortality is high ($m, J \ll A$; $\gamma \gg 1$), $\delta r \approx -(v+1)\epsilon/2$, and selection depends on the average effect on juvenile and adult survival.

If we just consider a single locus, the equilibrium frequency is $p = \mu/\delta r$. For small μ , homozygotes are rare, and the frequency of heterozygotes is approximately $2p$. J and A are therefore reduced by factors

$$\begin{aligned} (1 - 2\mu\epsilon/(-\delta r)) &\approx \exp(-2\mu\epsilon/(-\delta r)) \\ (1 - 2\mu\epsilon v/(-\delta r)) &\approx \exp(-2\mu\epsilon v/(-\delta r)) \end{aligned}$$

respectively. The next step is to combine these factors across all loci. For simplicity, we assume that the effects of different loci multiply, and we neglect linkage disequilibrium and genetic variation⁸, to derive approximations to the survival probabilities,

$$\begin{aligned} J &= \bar{J} \exp\left(\frac{2U}{\partial r/\partial \mu(1) + v \partial r/\partial \mu(2)}\right) \\ &= \bar{J} \exp\left(\frac{-2U\sqrt{1+4\gamma}(1 + \sqrt{1+4\gamma})}{(1 + 2(v+1)\gamma + \sqrt{1+4\gamma})}\right) \end{aligned} \tag{4a}$$

$$\begin{aligned} A &= \bar{A} \exp\left(\frac{2vU}{\partial r/\partial \mu(1) + v \partial r/\partial \mu(2)}\right) \\ &= \bar{A} \exp\left(\frac{-2Uv\sqrt{1+4\gamma}(1 + \sqrt{1+4\gamma})}{(1 + 2(v+1)\gamma + \sqrt{1+4\gamma})}\right) \end{aligned} \tag{4b}$$

where $\gamma = A/(mJ)$, and \bar{J} , \bar{A} give the optimal life history in the absence of mutation. These two equations can be solved numerically to find the equilibrium load, (J/\bar{J}) , (A/\bar{A}) .

Two key points emerge. First, consider mutations that only affect adult survival ($v = \infty$). As these increase in frequency, adult survival drops, and so selection against the mutations decreases. Above a critical threshold, mutation overwhelms selection, and the population becomes semelparous. Then, the only solution to equations (4) is at $A=0$, $J = \bar{J} \exp(-2U)$. For the present example, the threshold is quite low, $U = 0.067$. This quantifies the feedback effect postulated by Williams and Medawar^{10,20}.

If, however, mutations affect both juvenile and adult survival equally, selection against their early effects keeps them at low frequency, and prevents the collapse of late survival. Surprisingly little pleiotropy is needed to reduce the degree of senescence substantially. For example, if mutations reduce adult survival 16 times more than they reduce juvenile survival (that is, $v=16$), a net mutation rate $U=0.10$ reduces juvenile and adult survival from their optimal values (0.945, 0.505) to (0.866, 0.155); if the mutations only affected adult survival, a mutation rate $U > 0.067$ would reduce adult survival to zero. Thus, the plausibility of mutation accumulation as an explanation of senescence depends on whether mutations with very age-specific effects are sufficiently frequent. □

⁸ Combining the effects of many loci is nontrivial. First, even though the effects of mutations on J and A are assumed multiplicative, their effects on fitness are not. Hence linkage disequilibrium may build up, and may substantially affect the equilibrium mutational load⁹. Second, mutation will generate variation in life history around the mean. A full treatment, following Charlesworth³⁰, would be valuable.

of ageing would again predict. More data of this kind would be valuable.

It is very much harder to evaluate different evolutionary theories. Because we know that there is a substantial input of deleterious mutations to populations^{6,54}, and that it is impossible for individuals to combine indefinitely high survival and fertility, both the optimality and the mutation-accumulation explanations of senescence must apply. The aim is to discover how far mutation has depressed survival and fertility below their optimal values. But determining the optimal life history of any organism is difficult because the trade-off curves that reflect constraints on survival and fertility at different ages have never been measured³⁷. Furthermore, the total rate of production of deleterious mutations and their pattern of age-specificity are unknown^{6,55}.

The amount of standing genetic variation for ageing within populations, and the number of genes involved, are not informative. Both theories are consistent with the absence of any genetic variance because deleterious mutations could become fixed by drift, and the optimal life history could in theory be achieved by fixation of a single optimal genotype by selection. By contrast, both theories are consistent with the presence of genetic variation for ageing, which is the norm for quantitative characters including other life-history traits⁵⁶. At least in *Drosophila*, ageing is in general genetically variable and polygenic⁵⁷⁻⁵⁹. Genetic variation could result from a balance between mutation and selection, or from balancing selection alone; given the general difficulties of finding the causes and genetic basis of polygenic variation⁶⁰, it is hard to take this line of evidence further.

The pattern of standing genetic variance has been exploited in investigations of the evolution of ageing. If senescence is primarily due to late-acting deleterious mutations, then (other things being equal) one would expect the additive genetic variance for survival and fertility to increase with age. One study⁶¹ found no increase in additive genetic variance for female fertility with age in *Drosophila*. A problem is that mean values for fertility and survival probability decline with age, which may not necessarily be accounted for by a logarithmic transform. Also, some genotypes are lost from experiments through death, which will cause an inevitable decrease in variance if fecundity and survival are correlated.

Standing genetic variance has also been used to study the sign and magnitude of genetic correlations between ages, for survival probability and fertility. Results of chromosome extractions and breeding designs have been mixed, with some showing negative correlations between early and late fitness and others showing no significant associations⁶²⁻⁶⁵. These tests are not particularly sensitive; very large sample sizes are needed to generate reasonable confidence limits on genetic correlations⁶⁶ and biases can be introduced by deaths before late fitness is measured. In addition, some studies used inbred lines of *Drosophila*, which are liable to give artefactual positive correlations between fitness components, and are therefore unsuitable for testing evolutionary theories of ageing⁶⁷.

Artificial selection has also been used to investigate the evolution of ageing. Restriction of breeding to older adults would be expected to reduce ageing because there is selection for higher longevity and high fertility at old ages in these 'old' lines. If a response to selection occurs, and if ageing in the original base population were attributable entirely to the presence of more or less age-specific deleterious mutations, then no immediate drop in survival or fertility would be expected to occur earlier in life. If trade-offs were important, then an immediate drop (a correlated response) would be predicted. This type of experiment in *Drosophila* has in general produced evidence for trade-offs, with lifespan and fertility late in life increasing in lines propagated from old adults, and either pre-adult survival or fertility of young adults showing a correlated decline^{18,19,68,69}. Although the timescale of artificial selection experiments means that new mutations can contribute to the responses, in the above

experiments accumulation of partially recessive mutations could not account for the decline in early performance, because the drop persisted in crosses between 'old' lines^{19,58,59}. In any case, the effect occurred too rapidly to be accounted for by mutation accumulation^{70,71}. At least some of the increase in late life fitness in 'old' lines could have been caused by reduction in the frequency of predominantly late-acting mutations, and a study of the effects of reverse selection suggested that this was the case⁷², and that mutation accumulation had therefore also contributed to ageing in the base stock.

A fundamental difficulty with any approach based on standing genetic variance is that there need be no direct connection between the pattern of genetic covariances, and either the trade-off curve (which reflects the set of possible phenotypes), or the effects of spontaneous mutations. When variation in a set of quantitative traits is maintained by a balance between mutation and selection, the genetic covariances depend in a complex way on the covariances of effects of new mutations on the traits and on fitness, and on the pattern of selection on the traits^{30,73-76}. Similar arguments apply when variation is a pleiotropic side-effect of balancing selection⁷⁷. Artificial selection may circumvent some of these difficulties. Genetic variation within a base population can be magnified as variation between selected lines, giving greater statistical power. More important, selection over tens of generations can take traits well beyond their original values, and new mutations can contribute to the response, so that it may reflect the fundamental limits on the character, rather than just the initial covariances.

Artificial selection is not free of problems. It is possible inadvertently to apply direct selection to the characters whose correlated responses are of interest. For instance, in many experiments there may have been unintended selection pressure for rapid development and reproductive maturation in the control line flies with which the 'old' lines were compared, potentially leading to artefactual effects on early fertility¹⁹. In addition, all estimates of quantitative genetic variables by any method are sensitive to the environment in which they are measured; misleading gene-environment interactions can occur in an environment other than that in which the life history evolved⁷⁸⁻⁸⁰. This objection may apply to some extent to all published studies. Unless the base stock was kept for many years in the same conditions as those in which estimates were made, problems could have arisen.

An alternative to genetic approaches is to use environmental manipulations of the rate of ageing to study its mechanisms. For instance, in *Drosophila* females, reduction of egg production by X-ray irradiation, lowering of protein intake or denial of oviposition sites, has been shown to extend lifespan⁸¹⁻⁸³. Some aspects of mating itself also shorten female lifespan⁸⁴. These data suggest that both egg production and mating could accelerate ageing. Environmental manipulations have the considerable advantage that they can be used in wild populations, and they are also relatively quick. In addition, reversal experiments can be done, allowing the time course of the effects of the manipulation to be observed⁸⁵. They can be especially powerful when used in conjunction with genetic manipulation^{86,87}, and the two techniques are at their most convincing when they yield similar results.

In principle, one could find the contribution of deleterious mutations to ageing by turning off mutation, and allowing the population to recover to its optimal life history. Unfortunately, this is hardly feasible. One could instead increase mutation rates artificially: if doubling mutation rates doubled senescence, or if a slight increase in the mutation rate produced catastrophic senescence (Box 2), then this would suggest a substantial effect of mutation in the original population. This is a worthwhile approach, though one should bear in mind that different mutagens give qualitatively different kinds of mutation. Another promising way forward would be to use mutation-accumulation experiments⁵⁴ to make better estimates of the total mutation

load and its pattern of age-specificity. These experiments somewhat underestimate mutation rates because, even where new mutations are held heterozygous against balancer chromosomes, it is impossible to eliminate natural selection altogether if mutations are not completely recessive; to take the extreme example, dominant lethals can never be accumulated. But because only a small part of the mutation load is likely to be due to alleles with major effects^{6,54,71}, this gives little error.

Long-inbred populations might be useful for assessing the effects of new mutations. In outbred populations, selection acts on new mutations mainly through their heterozygous effects. In contrast, under persistent inbreeding, the mutation load will mainly be due to homozygotes, and can be largely eliminated by outcrossing. This would allow direct measurement of how far senescence in the original population had been caused by accumulation of partially recessive mutations. Selfing populations of plants could be valuable material^{88,89}, although the retention of unused yet presumably expensive floral features in some populations raised doubts as to whether they have been selfing for long enough to reach evolutionary equilibrium. An alternative might be to investigate the effects on life history of making a diploid from two independent strains of a habitually haploid organism⁹⁰.

Additional evidence for the importance of mutation accumulation has come from experiments where artificial selection has proceeded by restricting breeding to young adults, releasing the late part of the life history from natural selection, an approach originally proposed by Edney and Gill⁴³. For instance, restriction of the opportunity for reproduction in *Drosophila* to 3–6-day-old flies for 120 generations resulted in a fall in the late but not the early-life fecundity of females in these 'r' lines relative to that of females of 'K' lines where adults of any age could breed⁹¹. The fall in late-life fecundity was much smaller in crosses between 'r' lines, showing that the genetic basis of selection response differed between them, and that senescence had evolved by accumulation of partially recessive deleterious alleles, which would not be expected on the optimization theory. Further support for mutation accumulation came from the slow appearance of the drop in late fertility. But the r and K lines were cultured at different densities, so that selection on life-history traits other than late fecundity may also have differed between them and may have accounted for the decline in late fertility of the r-line females. In addition, population size was smaller in the r lines, so that inbreeding depression may have contributed to the difference from the K lines.

The experimental results suggest that ageing in *Drosophila* has evolved in part as a consequence of selection for an optimal life history, and in part as a result of accumulation of predominantly late-acting deleterious mutations. But the magnitude of the contribution from mutation is unknown. Quantification of these effects presents a major challenge for the future.

Decline and extinction of lineages

If some of the germ lines that led from the primaevial soup had not been, to a first approximation, immortal then extant organisms would not exist. But germ lines can deteriorate and cause extinction of lineages. Long-term cultures of unicellular organisms in small, asexual populations have shown a steady decline in the rate of division, and cultures sometimes went extinct, no matter how carefully they were kept. Senescence could be prevented or reduced either by retaining binary fission but keeping the population size large, or by allowing sexual reproduction⁵. This kind of clonal ageing has also been found in multicellular organisms reproducing asexually by apomixis, fission or budding⁵, although the data for vegetative reproduction are mixed.

Senescence of clones is probably caused by the accumulation of deleterious mutations. The simplest case is where the clone is propagated through a single, randomly chosen cell. There is then no selection, and an inevitable decline in viability and

fertility. Even if a moderately large number (*N*) of individuals is used, mutations are likely to be fixed by chance if they reduce fitness by less than $s \approx 1/N$. Moreover, with strictly asexual reproduction, mutations will inevitably accumulate. Even in a large population, very few individuals may be free of any deleterious mutations; if this fittest class fails to leave descendants, it can never be recovered, and the mean fitness of the population will decline irreversibly, in a process known as 'Muller's ratchet'^{92,93}. In multicellular organisms that reproduce asexually through a single-cell stage, the effects of Muller's ratchet will be exacerbated, both because population sizes tend to be smaller than for protozoans, and because genomes tend to be large and hence to accumulate more mutations per genome. Sex may therefore have been a necessary prelude to the evolution of multicellular organisms^{5,6}. Immortal germ lines require, first, efficient systems for the endogenous repair of biochemical damage and, second, large population sizes and sexual reproduction, which allow elimination of the unfit by natural selection (a kind of exogenous repair⁵). Persistent asexual lineages of organisms with large genomes would therefore present something of a paradox, and they do not seem to occur, at least in animals. Ostensibly asexual lineages of fish and salamanders have been shown by analysis of their mitochondrial DNA to be of some antiquity (4–5 million and 100,000 years, respectively). But neither is truly asexual. Analysis of the nuclear DNA of the mole salamander *Ambystoma* has shown that there has been crossing with males of related sexual species^{94,95}. In the fish *Poeciliopsis* males of a sexual species contribute to every 'unisexual' offspring an expressed haploid genome that is then discarded at meiosis⁹⁶. The effects of Muller's ratchet in the maternal complement in these hybrid offspring may therefore be masked to some extent by wild-type alleles in the paternal complement.

Clonal senescence has been thought to bear on the mechanisms of ageing in Metazoan individuals. But there is little direct connection. To the extent that a multicellular soma is not maintained by selection between cells, accumulation of somatic mutations is inevitable. Nevertheless, the empirical evidence is that these contribute little to individual ageing⁴. The loss of division potential with age in cultures of mammalian cells is caused by specific mechanisms preventing proliferation in differentiated cells^{97,98}, rather than by accumulation of mutations.

Soma and germ line

Several authors have suggested that ageing should be confined to organisms with "... a clear distinction between soma and germ plasm"^{4,10}. But the logic of this point of view is not compelling, and it is not supported by the data⁹⁹. Consider a multicellular organism in which randomly chosen cells dedifferentiate and divide to produce germ cells until the organism has completely turned into gametes, and where there is therefore no distinction between soma and germ line. Will this creature, as an individual, age? As gamete-production proceeds, it will have a non-zero probability of death or of becoming unable to produce gametes. The intensity of selection on survival and rate of gamete-production will therefore decline with age and ageing will evolve. The same argument would apply to a unicellular organism that produces, by asymmetrical cell division, a series of daughter cells that become gametes, before itself dividing to produce gametes. The critical requirement for the evolution of ageing is that there be a distinction between a parent individual and the smaller offspring for which it provides. If the organism breeds by dividing equally into identical offspring, then the distinction between parent and offspring disappears, the intensity of selection on survival and reproduction will remain constant and individual ageing is not expected to evolve.

Prospects

The polygenic nature of ageing contains a baleful message for gerontologists; it is most unlikely that engineering of a few genes

or intervention in a handful of physiological pathways will prevent the process from occurring. But an evolutionary decrease in the rate of ageing in humans is likely to occur over the next few generations. Senescence in industrialized human societies has become so apparent because of the removal of most extrinsic causes of death and lowered fertility; in the circumstances in which the life history evolved, these would have predominated, and few individuals would have lived long enough to show evidence of ageing. Industrialization has not only uncovered ageing, but has also been accompanied by an increase in the average age of reproduction, and economic factors may mean that this trend continues. Increasing the age at breeding leads to selection for reduced senescence, and both evolutionary theories specify that survival and fertility later in the lifespan will in consequence increase over the generations to come. But the two theories carry very different implications for the nature of the correlated responses. If mutation-accumulation is the

predominant evolutionary cause of ageing in humans, then reduced ageing could evolve by purging of late-acting mutations, with little immediate cost to survival and fertility in earlier life. If, by contrast, ageing has evolved as part of an optimal life history, then there may be a direct cost, for instance in the form of a reduction in survival to maturity, delayed maturity or lower fertility in early adulthood. These predictions assume a constant environment. But as well as removing hazard, industrialization has been accompanied by an improvement in nutrition which may, if it persists, reduce or abolish any early-life costs of the late-life improvement. Unfortunately the authors and contemporary readers of this review are unlikely to see the results of this experiment. Work with other organisms should yield some insight into the likely outcome. □

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