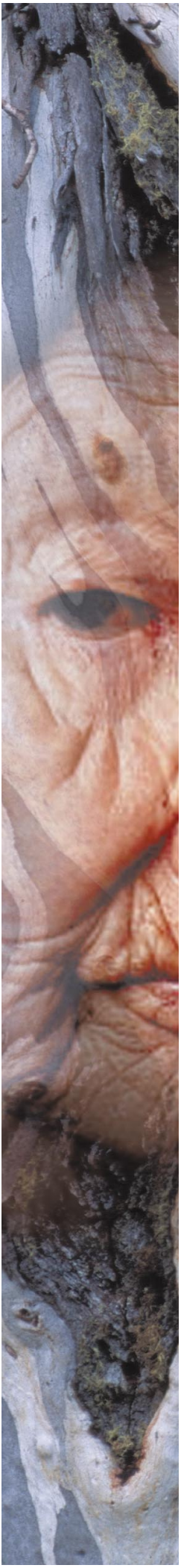

Why do we age?

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The evolutionary theory of ageing explains why ageing occurs, giving valuable insight into the mechanisms underlying the complex cellular and molecular changes that contribute to senescence. Such understanding also helps to clarify how the genome shapes the ageing process, thereby aiding the study of the genetic factors that influence longevity and age-associated diseases.

Ageing is usually defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age. Such a trait, which impairs survival and fertility, is clearly bad for the individual, raising intriguing questions about why and how it has evolved[1–4]. Ageing shows a broad phylogenetic distribution but is not universal, as some species show no age-associated increase in mortality or decline in fertility[5]. Thus, ageing cannot be explained simply as the inevitable result of biological wear-and-tear. So, why does it occur?

An early explanation for evolution of ageing was the idea that senescence is programmed in order to limit population size or accelerate the turnover of generations, thereby aiding the adaptation of organisms to changing environments. One essential flaw in this argument is that for most species, other than those like Pacific salmon where death coincides directly with the end of a semelparous (once-only) reproductive cycle, there is scant evidence that senescence contributes significantly to mortality in the wild. Natural mortality, as opposed to that seen in protected populations, is mostly due to extrinsic hazards, such as infection, predation, starvation or cold, and occurs mainly in young individuals. As a rule, wild animals simply do not live long enough to grow old (Fig. 1a). Therefore, natural selection has limited opportunity to exert a direct influence over the process of senescence. Even in species where senescence does make some contribution to mortality in the wild (for example, larger mammals and some long-lived birds), any hypothetical 'accelerated ageing gene' would be disadvantageous to the individual. It is therefore difficult to see how genes for accelerated ageing could be maintained in stable equilibrium, as individuals in whom the genes were inactivated by mutation would enjoy a selection advantage.

The rarity of aged animals in the wild in fact gives the clue to an important principle underlying all of the current evolutionary theories of ageing. As a result of extrinsic mortality, there is a progressive weakening in the force of selection with increasing age[6]. By an age when wild survivorship has declined to very low levels, the force of selection is too weak to oppose the accumulation of germ-line mutations with late-acting deleterious effects[7] (Fig. 1b). This 'selection shadow' allows a wide range of alleles with late deleterious effects to accumulate over the generations with little or no check. This is the 'mutation accumulation' theory, and because the deleterious alleles are essentially unselected, we might expect considerable heterogeneity in the distribution of such alleles among individuals within the population[8].

A second theory is that of 'pleiotropy', also sometimes called 'antagonistic pleiotropy'. Williams[9] suggested that pleiotropic genes with good early effects would be favoured by selection even if these genes had bad effects at later ages (Fig. 1c). Because the contribution to fitness is a composite of both the size of the effect and the probability of surviving to be affected by it, a small beneficial effect early in life can outweigh a late deleterious effect even if the latter results in senescence and death. This introduces the important idea of a life-history trade-off, which is also a central feature in the third theory, the 'disposable soma' theory[10,11], which is based on optimal allocation of metabolic resources between somatic maintenance and reproduction.

Effective somatic maintenance is required only to keep the organism in sound physiological condition for as long as it has a reasonable chance of survival in the wild (Fig. 1d). For example, because more than 90% of wild mice die in their first year[12], any investment in mechanisms for survival beyond this age benefits at most 10% of the population. Nearly all of the mechanisms required to combat intrinsic deterioration (such as DNA repair or antioxidant systems) require metabolic resources. Resources are scarce, as shown by the fact that the main cause of mortality for wild mice is cold, owing to failure to maintain thermogenesis[13]. The disposable-soma theory therefore suggests that the mouse will benefit by investing any spare resource into thermogenesis or reproduction, rather than into better repair capacity, even though this means that damage will eventually accumulate to cause ageing. Although the distinction between the pleiotropy and disposable-soma concepts is sometimes blurred, the latter can be viewed as focusing specifically on mechanisms, particularly the role of somatic maintenance and repair, whereas the former is formulated in terms of a general pattern of gene action and may involve pleiotropic genes of various kinds.

The three theories provide complementary explanations for why ageing occurs. Each also addresses the question: why do species have the life spans they do? The principal determinant in the evolution of longevity is predicted to be the level of extrinsic mortality. If this level is high, life expectancy in the wild is short, the force of selection attenuates fast, deleterious gene effects accumulate at earlier ages, and there is little selection for a high level of somatic maintenance. Consequently, the organism is predicted to be short lived even when studied in a protected environment. Conversely, if the level of extrinsic mortality is low, selection is predicted to postpone deleterious gene effects and to direct greater investment in building and maintaining a durable soma.

Evolutionary theories tested

The evolutionary ageing theories implicitly assume an age-structured population[6,9], that is, a population in which

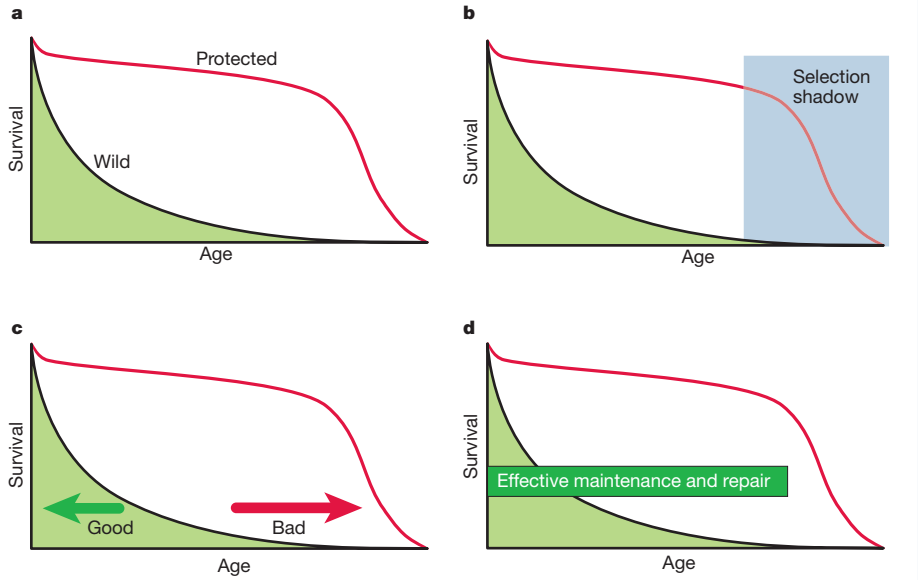

Figure 1 Evolutionary theories of ageing. **a**, Extrinsic mortality in wild environments occurs to an extent that senescence-associated mortality is rare, undermining any idea that genes specifically for ageing have evolved. **b**, The 'selection shadow' at older ages may permit an accumulation of late-acting deleterious mutations (mutation-accumulation theory). **c**, Pleiotropic genes that benefit organisms early in life will be favoured by selection even if they have bad effects at later ages (pleiotropy theory). **d**, Selection pressure to invest metabolic resources in somatic maintenance and repair is limited; all that is required is to keep the organism in sound condition for as long as it might survive in the wild (disposable-soma theory).

individuals can be segregated by age, and therefore predict that ageing should not arise in populations where age classes cannot be assigned. At a unicellular level, it is therefore unsurprising that ageing is generally not seen in bacterial populations. Some unicellular organisms show asymmetry of cell division, such as the budding yeast, *Schizosaccharomyces cerevisiae*. Mother yeast cells age in that they show an increasing probability of cell death with successive divisions. In multicellular organisms, the need for age structure also applies and ageing is generally predicted to require a clear separation between germ line and soma[1,9]. The presence or absence of ageing is sometimes attributed to the presence or absence of sexual reproduction, but this is erroneous. It is the distinction between soma and germ line (a common but not universal correlate of sex) that holds the key. In accord with these predictions, two oligochaete species that reproduce by symmetrical fission were found to show no increase in age-specific mortality, whereas four species (two rotifers, one ostracod and one cladoceran crustacean) which reproduce by asexual egg production all showed highly significant increases[14]. *Hydra*, which can reproduce sexually but usually reproduces by asexual budding, and which can regenerate a new individual from almost any part of the organism, lacks clear separation of germ line and soma and shows no obvious signs of intrinsic senescence[15].

An important prediction of the evolutionary theories is that altering the rate of decline in the force of natural selection will lead to the evolution of a concomitantly altered rate of ageing. This has been tested by applying artificial selection on life-history variables or by intra- and interspecies comparisons of populations that are subject to different levels of extrinsic mortality.

Most selection experiments have used the fruitfly *Drosophila melanogaster*. By restricting reproduction to later ages, the intensity of selection on the later portions of the life span was increased. This consistently extended the longevity of the selected populations[16–19]. Furthermore, a general correlate of delayed senescence has been reduced fecundity in the long-lived flies (Table 1), which supports the idea of a trade-off between fertility and survival, as suggested by the disposable-soma and pleiotropy theories. A similar trade-off was observed in an experiment selecting directly for longevity[20]. Lastly, when selection operates through the level of extrinsic mortality, populations subjected to low mortality showed increased longevity, longer development times and decreased early fecundity[21].

The general finding from selection experiments in *Drosophila* is therefore that retarded ageing is associated with depression of fitness components in early life, although there is variation in which fitness components are affected. Some studies found that body size and development time were increased in the longer-lived lines[21] whereas other studies fail to observe these effects. Sgró and Partridge[22] demonstrated that reduced early fecundity — the most consistently observed feature — was causally involved in the retarded ageing of their long-lived lines; after abolishing reproduction through either irradiation or genetic manipulation they found that the differences in ageing rate between their controls and long-lived selected lines disappeared.

Trade-offs have also been reported in the nematode *Caenorhabditis elegans* where a host of long-lived mutants has been identified. However, although some studies report the same sort of trade-offs between longevity and early-life fitness components as found in *Drosophila*[23], this is not invariably true even for the same genotypes in other laboratories[24]. A recent study of the long-lived *age-1* mutant in *C. elegans* showed that the relative fitness effects of mutations can be strongly affected by environment[25]. When mutants were reared together with wild-type individuals under standard culture conditions, neither genotype exhibited a competitive advantage. However, when cultures were alternatively fed and starved — mimicking conditions in nature — the wild type quickly outcompeted the mutant.

Although abundant data support the existence of life-history trade-offs, evidence for the mutation-accumulation theory remains more controversial[26]. An early *Drosophila* selection experiment indicated a role for mutation accumulation acting alongside trade-offs[27], although probably in a minor capacity. Other studies have directly tested the prediction that mutation accumulation should be revealed by an increase in additive genetic variance in mortality rate at later ages. Initial studies in *Drosophila*[28,29] seemed to support mutation accumulation but later experiments and further analysis have led to doubts about this conclusion[30,31].

From the comparative perspective, numerous opportunities exist to test the prediction that in safe environments (those with low extrinsic mortality) ageing will evolve to be retarded, whereas ageing should evolve to be more rapid in hazardous environments. Adaptations that reduce extrinsic mortality (for example, wings, protective shells or large brain) are generally linked with increased longevity (in bats, birds, turtles and humans). Field observations comparing a mainland population of opossums subject to significant predation by mammals, with an island population not subject to mammalian predation, found the predicted slower ageing in the island population[32]. Among social insect species, those with the most

Table 1 **Life-history traits and selection for longevity in *D. melanogaster***

Mode of selection	Traits affected
Delayed reproduction[16]	↑Longevity; ↓early fecundity
Delayed reproduction[19]	↑Longevity; ↓larval viability
Delayed reproduction[18]	↑Longevity; ↓early fecundity
Reduced extrinsic mortality[21]	↑Longevity; ↓early fecundity; ↑development time
Increased longevity[20]	↑Longevity; ↓fecundity

Shown are the life-history traits associated with successful laboratory evolution of increased longevity in *Drosophila*.

protected nests contain reproductive females with by far the longest life spans[33]. A comparative analysis of mortality patterns among birds found that the rate of mortality increase with age was directly correlated with the magnitude of presenescent mortality[34].

At the molecular and cellular levels, the disposable-soma theory predicts that the proportional effort devoted to cellular maintenance and repair processes will vary directly with longevity. Numerous studies support this idea. For instance, the long-lived rodent species *Peromyscus leucopus* exhibits lower generation of the reactive oxygen species (ROS), which are widely seen as an important contributor to the ageing process, higher cellular concentrations of some antioxidant enzymes, and overall lower levels of protein oxidative damage than the shorter-lived species *Mus musculus*[35]. A direct relation between species longevity and rate of mitochondrial ROS production in captive mammals has also been found[36,37], as has a similar relationship between mammals and similar-sized but much longer-lived birds[38]. Markers of glycoxidation, the non-enzymatic modification of reducing sugars, are also found to accumulate more slowly in long-lived, as opposed to short-lived, mammals[39]. DNA repair capacity has been shown to correlate with mammalian life span in numerous comparative studies[40], as has the level of poly(ADP-ribose) polymerase[41], an enzyme that is important in the maintenance of genomic integrity. The quality of maintenance and repair mechanisms may be revealed by the capacity to cope with external stress. It is notable, therefore, that environmental or genetic manipulations that confer increased longevity on a range of animals also confer increased resistance to environmentally imposed stressors (Table 2). Likewise, comparisons of the functional capacity of cultured cells to withstand a variety of imposed stressors have shown that cells taken from long-lived species have superior stress resistance to that of cells from shorter lived-species[8,42,43]. All of these studies support the idea that it is the evolved capacity of somatic cells to carry out effective maintenance and repair that governs the time taken for damage to accumulate to levels where it interferes with the organism's viability, and hence regulates longevity.

Specialized life histories

Many organisms live their lives in highly variable environments. In such circumstances we can expect the 'genetic architecture' of the life history, that is, the co-adapted set of traits influencing survival and fecundity, to possess a degree of evolved plasticity that permits a range of optimal responses suited to different circumstances. In poikilotherms, for instance, environmental temperature often influences longevity. This is generally a straightforward consequence of altering metabolic rate, as indicated by the fact that longevity can be similarly altered by manipulating activity[44], although the capacity to make such adjustments and retain viability will reflect the range of temperatures to which organisms have been exposed in their evolutionary past. More revealing are cases of plasticity induced by conditions such as lack of food, to which many organisms seem to have evolved a non-reproductive, highly stress-resistant state. Periods of famine often trigger what appear to be metabolic switches that paradoxically extend the normal life span, without sacrificing subsequent reproduction and survival when favourable conditions return[45].

Table 2 **Increased stress resistance in long-lived populations**

Organism	Population	Nature of stressor				
		ROS	Heat	UV	Trauma	Chemical toxins
C. elegans	Dauer larvae[48]	↑	↑	↑	?	?
C. elegans	Various mutants[48]	↑	↑	↑	?	?
D. melanogaster	Artificial selection[72,73]	↑	↑	↑	?	?
D. melanogaster	*methuselah* mutant[74]	↑	↑	?	?	?
M. musculus	Calorically restricted[51]	↑	↑	?	↑	↑
M. musculus	p66^{shc} mutant[75]	↑*	?	↑†	?	?

*Resistance to apoptosis or growth arrest of cultured embryonic fibroblasts.
†Both *in vitro* and *in vivo* resistance.

The most thoroughly investigated instance of this type of life-history plasticity is seen in the nematode *C. elegans*. At 20 °C, wild-type *C. elegans* hermaphrodites live for an average of about 17 days with a maximum of about 25 days. However, under conditions of high larval density and low food availability, larvae develop into the alternative, non-feeding, non-reproducing third instar called the dauer, which can survive for at least 60 days[46]. If conditions improve, dauers moult to adulthood and exhibit normal adult longevity and reproduction. Although dauers exhibit reduced activity and metabolic rate compared with non-dauer larvae[46,47], the preservation of full adult longevity after even an extended dauer period indicates that the effect is not entirely due to reduced metabolism. Like longer-lived strains and species of other animals, dauers are also resistant to a variety of environmental stresses including ROS, temperature extremes and ionizing radiation[48]. Increased longevity of adult worms results from mutations in several of the genes involved in the dauer pathway, indicating that some activation of dauer physiology may be involved in these mutants, even in the absence of a triggering shortage of food. Exactly what fraction of the longevity-enhancing effect of dauer formation or long-lived genetic mutants is due to reduced metabolic rate is the subject of current controversy[23,49].

It is well known that reduced calorie intake slows ageing in laboratory rodents[50], an effect that may have parallels with the invertebrate phenomenon. That is, the rodent caloric-restriction response may be a dauer-like adaptive physiological state to 'wait out' periods of food shortage and is generally associated with a partial or complete interruption of fertility. Like many invertebrates, laboratory rodents under caloric restriction show enhanced resistance to a range of stresses[51], but unlike invertebrates, no general reduction in mass-specific metabolic rate is required for the effect. Despite the lack of reduced metabolic rate under caloric restriction, Walford and Spindler[52] have pointed out a number of biochemical similarities between the caloric-restriction state and that of mammalian hibernation, which can also extend life span. A recent evolutionary model[53] has shown that rodents may well have evolved a response to temporary fluctuations in resource availability, in which energy is diverted from reproduction to maintenance functions in periods of food shortage, thereby enhancing survival and retaining reproductive potential for when conditions improve.

Perhaps the most pronounced impact of environment on ageing rate is illustrated by differences between longevity of queens and workers among species of the eusocial insects[5]. Life spans of workers are typically measured in weeks, those of queens in years. Differences in longevity as great as 100-fold have been reported, even though both queens and workers develop from eggs laid by the same mother and fertilized by the same father. The divergence in their relative longevity is mediated by pheromone exposure or the rate and composition of food supplied to the larvae. Although workers are more physically active, queens produce hundreds of thousands of eggs per year, so the difference in ageing rate is unlikely to be due to differences in metabolic expenditure. Indirect evidence indicates that neuroendocrine factors are involved[5], although underlying these factors must be differences in gene expression. A question as yet

unanswered is the extent to which such marked differences in mortality schedules result from purely extrinsic factors (danger to the workers foraging in the environment compared with the relative safety of the queen ensconced in a protected, climate-controlled nest surrounded by self-sacrificing defenders) or from differences in the rate of internal decay. This is an area ripe for research into how differential expression in the same configuration of genes might profoundly affect senescence.

Reproduction and ageing

Most discussion of the evolution of ageing focuses on its effects on mortality, rather than reproduction, in spite of the fact that in terms of an impact on fitness, the form of the reproductive schedule is as important as that of the mortality curve[54,55]. A good reason for this is that many aspects of reproductive senescence can be explained in the same general terms as physiological senescence. Nevertheless, in addition to the important issues of the trade-offs between survival and fecundity, considered earlier, there are intriguing evolutionary questions about the links between reproduction and ageing, notably the significance of post-reproductive survival (where it occurs) and the effects of damage and selection on the germ line.

The existence of a distinct post-reproductive phase is characteristic of certain semelparous species, in which individuals reproduce only once. On the other hand, many semelparous species undergo extremely rapid senescence on completion of reproduction, often as a direct consequence of the massive physiological changes associated with an explosive reproductive burst[5]. The evolutionary basis of semelparity is well understood[54], and from the perspective of the evolutionary theories of ageing, semelparity represents an extreme version of the decline in the force of natural selection with age[1]. In a semelparous species, the force of natural selection approximates a step function, being uniformly high until reproduction begins, and declining abruptly as reproduction is completed, because the chance of surviving to breed again is effectively zero. This explains the sudden collapse of any pressure to invest in somatic maintenance and repair. Whether or not there is significant post-reproductive survival may be governed chiefly by whether or not the post-reproductive adult contributes actively to the survival chances of the offspring.

A very different example of post-reproductive survival is the human menopause, where fertility in iteroparous human females comes to a relatively abrupt halt at around the age of 45–50 years, when the impact of senescence on most other functions is still small. Although the proximate cause of menopause seems to be oocyte depletion (linked also with neuroendocrine changes), this begs the question why natural selection has not produced a store of oocytes which lasts for longer. One possibility is that during most of humanity's evolutionary history, women rarely survived beyond 45–50 years, so selection simply produced about as many oocytes as would be required. But evidence from hunter–gatherer communities indicates that even though average life expectancy is short, women who avoid the hazards of early life and reach childbearing age have a reasonable chance of surviving to the age of menopause and beyond[56]. This indicates that the menopause may have a deeper evolutionary significance.

Early female reproductive senescence has been reported in other species (for example, chimpanzees, macaques and toothed whales) but is generally less clear-cut, indicating that if the menopause has an evolutionary basis, this may be found in the special circumstances of the human life history. In particular, menopause could be linked with the evolution of human longevity, notably, through the effects of increased brain size and sociality[9,56–61]. Increased neonatal brain size coupled with the constraint on the birth canal imposed by the mechanics of a bipedal gait has made giving birth unusually difficult for human females. The risks of childbearing, particularly in the absence of modern obstetric care, would increase even more steeply with age if fertility were to persist during the later period of the life span. The problem of a large brain size is also reflected in the fact that human infants are born unusually early, relative to other species, with respect to the completion of brain growth and development. Infants remain highly dependent for extended periods and, in the ancestral environment, their survival will have been unlikely if their mother died in childbirth. There may thus be a fitness advantage in limiting reproduction to ages when it is comparatively safe, thereby increasing the likelihood of the mother surviving to raise her existing offspring to independence. In addition, post-menopausal women may contribute to the successful rearing of their grandchildren, by providing assistance to their own adult offspring and thereby increasing their inclusive fitness, that is, their overall genetic contribution to future generations. It is likely that a combination of all of these factors is required to explain the human menopause[62], which may account for the lack of evolutionary support for menopause in other species[63].

Human reproductive ageing also highlights the interesting puzzle that although the germ line must, in a fundamental sense, be immortal (as damage cannot be permitted to accumulate across generations without immediate risk of extinction), there is clear evidence that individual germ cells do accumulate faults. Indeed, it would be surprising if the germ line was immune to accumulation of damage, because germ cells are subject to the same kinds of molecular damage as somatic cells. From a statistical perspective it is clear that the germ-cell population does undergo significant ageing. In the case of the human ovary the rate of follicular loss accelerates from around age 35 years, and male fertility begins to decline from around age 30. There is also an increase in the frequency of chromosomal abnormalities in newborn children as a function of maternal and, to a lesser extent, paternal age[64,65]. Nevertheless, healthy children born to older parents are not prematurely aged, although there is some suggestion that daughters' (but not sons') longevity is adversely affected by advanced paternal age[65]. Thus, either germ cells are endowed with special maintenance and repair systems[10] — the enzyme telomerase being a good example — or selection at the cell or embryo level during gametogenesis, conception and pregnancy serves to screen out most faults. It may be relevant that during each human menstrual cycle about 20 ovarian follicles are triggered to start the process of maturation, although usually only one completes its development and is ovulated. A mechanism of probable significance in the evolution of female germ-line immortality is the stringent bottleneck in the size of the cellular mitochondrial population in early embryogenesis. A healthy complement of mitochondria is essential for subsequent viability of the offspring, and mutations in mitochondrial DNA (mtDNA) tend to accumulate with age[66]. If the mitochondrial population in the oocyte contains a fraction of organelles bearing mtDNA mutations, a bottleneck

Box 1
Evolutionary genetics of ageing

The evolutionary theories of ageing predict:
1. Specific genes selected to promote ageing are unlikely to exist.
2. Ageing is not programmed but results largely from accumulation of somatic damage, owing to limited investments in maintenance and repair. Longevity is thus regulated by genes controlling levels of activities such as DNA repair and antioxidant defence.
3. In addition, there may be adverse gene actions at older ages arising either from purely deleterious genes that escape the force of natural selection or from pleiotropic genes that trade benefit at an early age against harm at older ages.

It is clear that multiple genes contribute to the ageing phenotype, some being particular to individuals ('private'), others being shared across populations and species ('public')[8]. The principal challenge is to identify how many of each category exist, and which are the most important.

coupled with an effective quality screen might select embryos that carry only intact mitochondria[67].

Implications

Understanding the forces that have sculpted our genetic makeup is likely to provide important insights that can not only guide our investigation of the molecular and cellular basis of ageing, but may also help to identify new routes to positive interventions in the ageing process.

There is a clear prediction that multiple genes influence the ageing process and that these are of several kinds (Box 1). The basis of genetic variation for longevity has begun to be studied in *Drosophila* by estimating quantitative genetic parameters and mapping quantitative trait loci[68]. Gene-expression profiling of ageing rodent tissues is beginning to reveal genes that alter their expression with advancing age or whose expression is altered by interventions, such as caloric restriction, which affect rate of ageing[69]. Unsurprisingly, several of these genes are being identified as those involved in damage/stress-response pathways. Just possibly, such techniques might also reveal late-acting deleterious alleles, as suggested by the mutation-accumulation theory. In general, however, the evolutionary theories caution against interpreting genes whose expression alters in old age as genes that accelerate ageing, and such changes in gene expression are likely to be secondary consequences rather than primary causes of the ageing process.

An important corollary of the prediction (and emerging evidence) that key genes regulating rate of ageing are those that control somatic maintenance and repair, is that at the level of the individual there is considerable scope for the action of stochastic chance[70]. Not only will individual cells within tissues experience different random accumulations of faults, but there may also be important stochastic variations in developmental processes resulting, for example, in different numbers of cells being formed in key organs, such as the hippocampus. Variation in initial cell number and damage rate will in turn affect the time taken before a threshold for dysfunction is crossed during the progressive neurodegeneration that occurs later in life. This means that genetically identical individuals, maintained in uniform environments, may nevertheless exhibit considerable variation in aspects of the senescent phenotype, as has been frequently observed in ageing studies on inbred laboratory organisms[70]. Heterogeneity in the senescent phenotype, arising from intrinsic stochasticity as well as genetic and environmental variations, may also help to explain the intriguing phenomenon that in several species age-specific mortality rate eventually slows its rate of increase and may even decline[71]. Heterogeneity explains such an effect if we assume that the frailer individuals die first, leaving a residual population that, as time goes by, represents a dwindling sub-population made up of those individuals that were always the most resilient.

In conjunction with exciting advances in genome analysis, there is clearly much scope for further development and testing of the evolutionary theories of why we age. □

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