MECHANISMS OF AGEING: PUBLIC OR PRIVATE?

Linda Partridge and David Gems

Ageing — the decline in survival and fecundity with advancing age — is caused by damage to macromolecules and tissues. Ageing is not a programmed process, in the sense that no genes are known to have evolved specifically to cause damage and ageing. Mechanisms of ageing might therefore not be expected to be as highly conserved between distantly related organisms as are mechanisms of development and metabolism. However, evidence is mounting that modulators of the rate of ageing are conserved over large evolutionary distances. As we discuss in this review, this conservation might stem from mechanisms that match reproductive rate to nutrient supply.

PEROXIDATION
A type of oxidation that involves the addition of oxygen to, for example, unsaturated fatty acids.

PROTEIN CARBONYL
A protein that contains oxygen that is linked by a double bond to carbon, resulting from protein oxidation.

Department of Biology, University College London, Gower Street, London WC1E 6BT, UK.
Correspondence to L.P.
e-mail: l.partridge@ucl.ac.uk
DOI: 10.1038/nrg753

Ageing is a process of intrinsic deterioration that is reflected at the population level as an increase in the likelihood of death and a decline in the production of offspring[1–3]. During ageing, macromolecules accumulate damage, including the PEROXIDATION of lipids, PROTEIN CARBONYLS and various forms of damage to DNA[4,5]. In turn, macromolecular damage can cause organelles, particularly the mitochondria, to malfunction[6–10]. It can also lead to the necrosis and apoptosis of cells, and tissues can suffer additional damage from changes in the extracellular matrix[11,12]. One of the principal aims of research into ageing is to identify the processes that lead to the generation of ageing-related damage, and to understand exactly how and where that damage occurs.

Much of the experimental work on ageing is on model organisms, such as the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*. These organisms have many advantages for ageing studies that have allowed research to progress rapidly, including their relatively short lifespans, completely sequenced genomes and well-characterized biology. They are also simple and inexpensive to culture. However, for work on these organisms to be relevant to research in humans, their mechanisms of ageing need to be in common with those in mammals. We therefore need to know which mechanisms of ageing are 'public' — those shared across distantly related evolutionary lineages — and which are 'private' — those peculiar to particular evolutionary lineages[13]. The evolutionary conservation of genes, their products, and their structures and functions, generally occurs by descent from a common ancestor. One leading candidate for a public (shared) mechanism of ageing is the accumulation of oxidative damage, a topic that has been well covered elsewhere[13–15]. In this review, we focus on the upstream mechanisms that might lead to the generation of ageing-related damage.

Mechanisms that control development and metabolism show a strong degree of conservation across the animal kingdom. There is every reason to believe, therefore, that similar findings will apply to ageing. However, ageing is a very different kind of process from development. Whereas development is characterized by precise timing, a well-defined set of genetic control mechanisms and events that occur repeatedly in different individuals, ageing often occurs at different rates in different tissues and in different individuals, and seems to have a stochastic element[16]. Furthermore, no genes seem to have evolved specifically because they cause damage and, therefore, ageing. Ageing, and changes in its rate by genes and the environment, can be understood only as a side effect of something else (BOX 1).

Even though there is no development-like programme for ageing itself, two key interventions have been shown to reduce the rate of ageing throughout the

Box 1 | **Is ageing a programmed process?**

Whether the ageing process represents a wear-and-tear type process of damage accumulation or whether it is, like development, an ordered, programmed process that is determined by changes in gene regulation has been a long-standing debate within BIOGERONTOLOGY**. We outline the arguments for both cases below.**

The case for no programme

The evolutionary theory of ageing predicts that ageing is a non-adaptive process. There is much evidence of damage accumulation (for example, oxidative damage) during ageing, and because no genes are known to function specifically to cause damage, ageing is believed not to be programmed.

The case for programmed ageing

The large differences in lifespan between species must be genetically determined. Moreover, single-gene mutations can lead to marked increases in lifespan, therefore ageing must be genetically determined — that is, it must be a programmed process.

Resolving the paradox

This apparent paradox can be resolved by taking into account two points. The first is to draw a distinction between 'programmed', as in involving an ordered series of regulatory changes, such as in programmed cell death, and 'programmed', meaning simply 'affected by genetic variation'. The second is to recognize that genetic programmes have evolved to control processes such as reproduction that inflict ageing-related damage, and processes such as repair and resistance that contribute to survival[95]. On current evidence, although ageing itself is not a programmed process, variation in the genes that affect the rate at which damage is generated or repaired will, as a side-effect, contribute to the time of onset and the rate of ageing.

animal kingdom: calorific restriction and reduced reproductive rate. Calorific restriction, in which nutrient intake is restricted to 60–70% that of voluntary levels, slows ageing in many organisms, which range from yeast to mammals[17–21]. A lower rate of reproduction also slows down ageing[21,22]; this so-called 'cost of reproduction' also occurs across a range of organisms, from yeast to higher plants and animals. Interestingly, calorific restriction reduces both daily and lifetime fecundity[23,24]. Its effects on the rate of ageing might, therefore, be related to the cost of reproduction. The precise mechanisms by which calorific restriction and a reduced reproductive rate slow down ageing are not understood. However, their widespread effects indicate that common mechanisms might be at work in different evolutionary lineages. So, the rate of ageing could be dependent on the rate of reproduction, which itself could be modulated by mechanisms that link reproductive rate to nutrition. Indeed, the evolutionary processes that have led to the existence of ageing, and of variation in its rate between different species, point to a link with reproduction.

Evolutionary theories of ageing

Ageing might seem to be a paradox from an evolutionary perspective because it is a deleterious trait. However, despite its deleterious nature, it occurs throughout the animal kingdom and is seen in natural populations in the wild[25,26]. So, it is not an artefact of captivity or, in humans, of life in industrialized societies. Ageing could be viewed as inevitable, if organisms do indeed wear out like cars or washing machines. However, it occurs at very different rates in different kinds of animals. For instance, birds are generally much longer lived than are comparably sized mammals[27], indicating that the rate of ageing might evolve and is not simply an inevitable consequence of wear and tear. Interestingly, among mammals, bats are long lived for their size[27], which indicates that something about flight might lead to the evolution of a slower rate of ageing.

Most biological features — such as eyes, wings and digestive systems — are there for a reason. Surely ageing must also serve its purpose, especially as it happens at such different rates in different species? So, what is ageing for? In the nineteenth century, Alfred Russel Wallace proposed that ageing occurs for the good of the species. Old and worn-out individuals would, as it were, bow out gracefully, freeing up resources for subsequent generations and for the good of the species. This comforting scenario of ageing as a natural virtue was, unfortunately, shown to be untenable by evolutionary biologists in the latter half of the twentieth century. They showed that natural selection very rarely acts for the good of the species if this conflicts with selection on individuals[28]. Selection on individuals to produce more offspring is, in general, far more powerful than the selection on them to refrain from doing so for the good of their species. The idea that ageing evolved as a device to prevent old individuals from further reproduction so as to make way for the young is therefore theoretically unsound.

The key to the evolution of ageing lies in an observation by J. B. S. Haldane in the 1940s (REF. 29). Haldane was puzzled by Huntington disease — a genetic disease that causes severe mental illness and death. Huntington disease is unusual in two respects: it strikes its victims in later life, usually in their 30s or 40s, and the mutation that causes the disease is dominant rather than recessive. Generally, dominant mutations that cause fatal diseases are expected to disappear quickly from the population. But, as Haldane saw, by the time Huntington disease strikes, most people have already had their children, half of whom will have inherited the mutation. So, dominant lethal mutations can be maintained in a population by mutation if their effects are delayed until after reproduction.

Haldane made a further, clever observation. If natural selection is unable to purge the Huntington mutation from the general population, how does it act against mutations that take effect even later? The power of natural selection to remove such mutations must be very weak. Is it possible that ageing itself is the result of mutations that strike very late in life, at ages beyond the control of natural selection? The evolutionary theory of ageing crystallized around this idea. As with Mendel's laws of genetics, it took many years before the significance of Haldane's observation was fully appreciated but, by 1980, a mature evolutionary theory of ageing was in place, based soundly on theoretical population genetics. Two key theories had emerged: the mutation-accumulation theory and the PLEIOTROPY or trade-off theory (BOX 2). In the light of these evolutionary theories, we can see that ageing has evolved as a late-onset genetic disease that affects us all. The evolutionary theories of ageing give us a clear, but stark, picture of the biological function of ageing: there is none. It is merely a non-adaptive epiphenomenon.

BIOGERONTOLOGY
The study of biological processes that give rise to ageing.

PLEIOTROPY
The capacity of different alleles of a gene to affect more than one aspect of a phenotype.

Box 2 | **Theories for the evolution of ageing**

Mutations that have deleterious effects on older organisms have a greater chance of escaping removal from the population by natural selection (see text for more). This leads to two theoretical predictions.

The mutation-accumulation theory
This theory predicts that mutations with a later age of onset will be able to reach a higher frequency in the population under mutation–selection balance. They will enter the population in accordance with their mutation rate, and selection will remove them with decreasing efficacy the later their age of onset. Ageing can then evolve as a result of the greater accumulation of mutations with deleterious effects later in life[1,2,96–98].

The pleiotropy or trade-off theory
Mutations that produce beneficial effects early in life, but that later increase the rate of ageing, can be incorporated into a population because natural selection will act more strongly on the early beneficial effect than on the later deleterious one[1,3,28]. For example, a hypothetical mutation that promotes calcium deposition might accelerate bone growth and increase fitness early in life, but then lead to hardening of the arteries later in life.

Both the mutation-accumulation and trade-off theories predict that the intrinsic rate of ageing will evolve in response to the level of extrinsic hazard. Species that lead a hazardous lifestyle or live in environments with high levels of external hazard from predators or disease will have high death rates, even in the absence of any intrinsic ageing process. These high death rates will, in turn, lead to the evolution of more rapid ageing under either the mutation-accumulation or the trade-off mechanism, because the force of natural selection that maintains survival and fertility will decline more rapidly with age. Late-acting deleterious mutations will therefore reach a higher frequency under the mutation–selection balance, and the late-life deleterious effects of pleiotropic mutations will weigh less heavily against their early benefits. Comparative studies have provided support for this association between high extrinsic hazard and a high intrinsic rate of ageing in social insects[30], birds and mammals[31]. In conclusion, species-specific lifespans are the product of the varying ability of natural selection to counter the entry into a population of either late-acting deleterious mutations or pleiotropic mutations, which produce early-life benefits but cause ageing later in life.

These two evolutionary theories have different implications for the conservation of the mechanisms of ageing over large evolutionary distances, and therefore for whether they will be public, that is shared, between different evolutionary lineages[13]. Mutation accumulation might be expected to lead to lineage-specific, private mechanisms, because there is no reason for new mutations or their phenotypic effects to be shared across different evolutionary lineages. Chance will have a role in determining which late-acting mutations will occur and reach appreciable frequencies[32,33]. In addition, mechanisms that generate new mutations, such as the transposition of mobile genetic elements, will differ in their contribution to the total mutation rate in different organisms[34], and the genes that are available to be mutated will differ between different evolutionary lineages. By contrast, the re-setting of the balance of a life-history trade-off, such as that between early reproductive rate and the subsequent rate of ageing, could occur by similar mechanisms in different evolutionary lineages. It is therefore important to assess the relative contribution of these two processes to the evolution of ageing.

INBREEDING DEPRESSION
This occurs when deleterious, recessive alleles become homozygous in the progeny of matings between relatives, causing reduced fitness among these individuals.

Mutation accumulation vs trade-off

Both the mutation-accumulation and the trade-off theories for the evolution of ageing have been subjected to extensive empirical testing, mainly in experiments with *Drosophila melanogaster*. These tests have all relied on either examining the properties of naturally existing genetic variation in fly populations, or the properties of new mutations as they occur. We discuss the results of these experiments below and how they support one theory — the pleiotropy/trade-off theory — more than the other.

Mutation-accumulation theory. Under the theory of mutation accumulation, the heritable (additive) genetic contribution to death rates should increase in magnitude with age[35]. This is because mutations with late-onset deleterious effects should be more common in the population the later the age at which they take effect. Each individual should therefore contain more of these mutations, and therefore pass more of them to their offspring. However, additive genetic variation for mortality rate has been shown to decrease at advanced ages in *Drosophila*[36,37]. The mutation-accumulation theory also predicts that the effect of INBREEDING DEPRESSION in the progeny of inbred matings should increase at later ages. According to the mutation-accumulation theory, late-onset deleterious mutations will be more common in the population, and the likelihood that they will become homozygous on inbreeding increases with the age at which they take effect. Although evidence to support this idea has been published[38], the authors of this study pointed out that older individuals might be more susceptible to the effects of homozygous, deleterious alleles, because they are already impaired by their age, and so this issue remains unresolved. This theory also predicts that new mutations with late ages of onset should occur at a measurable frequency, a prediction that has been experimentally tested in *Drosophila* by allowing new mutations to accumulate. These experiments have yielded little evidence that mutations with only late-age effects occur[39,40]. The experimental support for a role of mutation accumulation in the evolution of ageing is therefore weak. Furthermore, if mutations with late-onset effects are responsible for ageing, then we would expect to see an abrupt increase in death rates at the age at which selection intensity declines to zero, particularly post-reproductively[3,39,41]. However, these sudden increases in post-reproductive death rates are only rarely observed in the animal kingdom, and only when a single, suicidal burst of reproduction has occurred[1,4].

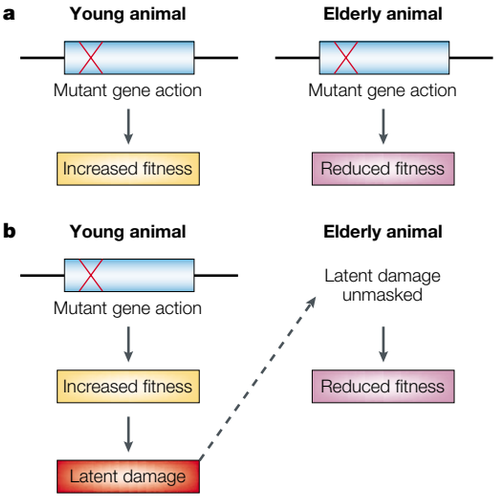

Figure 1 | **Two models of antagonistic pleiotropy. a** | Late action model. In this model, the early and late actions of a pleiotropic gene have distinct effects on fitness, and the timing of gene action and its effect on phenotype are the same. A probable example of such a gene is the wild-type allele of the *Caenorhabditis elegans* gene *daf-2*, which reduces DAUER LARVA formation (therefore promoting reproduction) but accelerates ageing. **b** | Latent damage model. In this model, both the early and late effects of a mutation occur as a consequence of its action in early life, with the later effect occurring long after the period of action of the mutant gene. Mutations that lead to reduced early fertility and increased lifespan in *Drosophila* are examples of such mutations, as described in REF. 50.

Trade-off theory. The trade-off/pleiotropy theory has been tested mainly in *Drosophila*[42,43] by using artificial selection experiments. In these experiments, so-called 'young' lines of flies are generated by collecting progeny from only young adults. These flies are compared with those from 'old' lines, which are generated by collecting progeny from only older adults that have already been reproducing for some time[44,45]. Because more of the adults that give rise to the 'old' lines die before their offspring are collected, longevity has been more strongly selected for in the adult flies of this line. A direct response to this selection is usually seen, with the longevity of the 'old' lines increasing relative to that of the 'young' lines, and their mortality rate increasing less rapidly with age (for example, REFS 44–49). If the pleiotropy theory of ageing applies, then the more rapid increase in mortality rate with age in the 'young' line adults, as compared with the 'old' line flies, should be associated with an earlier beneficial effect. And, indeed, higher fecundity early in adulthood has been consistently observed in the 'young' line flies relative to those from the 'old' lines in these experiments[44,45,48,49]. Furthermore, the quicker ageing of the 'young' line flies can be abolished by genetic- or X-ray-induced sterilization[50]. Therefore, this more rapid ageing is a direct consequence of the higher early fecundity of these flies rather than being a consequence of an independent, late-age effect of the genes that caused it (FIG. 1). Also, there was a substantial time lag between high fecundity in the early life of the 'young' lines and the increase in mortality that it caused, supporting the pleiotropy theory of the evolution of ageing. In addition, mutation accumulation had no role in the more rapid ageing of the 'young' selection lines; if it had done so, then the difference in the rate of ageing between the 'young' and 'old' lines would not have been abolished by sterilization.

So, the balance of experimental evidence is not strongly in support of mutation accumulation as a significant mechanism for the evolution of ageing. By contrast, pleiotropy/trade-offs and, in particular, a time-lagged cost of reproduction, is implicated as an important general mechanism for the kind of delayed genetic effect that will lead to the evolution of ageing. That mutation accumulation does not occur as predicted also tells us something more general about the nature of gene action in later life; it would seem that genes, as a rule, do not have effects in late adulthood that can be affected in isolation by mutation. So, Haldane's generalization from Huntington disease might have been misleading.

Linking evolution and mechanism

Evolutionary explanations in biology can tell us why a trait, such as the age at which organisms first reproduce or the shape of a wing, takes the particular value or form that it does, in terms both of its evolutionary history and of the way that natural selection acts on it. The evolutionary theories of ageing and the empirical tests of them indicate that differences in the rate of ageing between animal species might be partly due to a re-balancing of the trade-off between early reproductive rate and the subsequent rate of ageing in response to differing levels of extrinsic hazard. Evolutionary explanations, however, tell us little about the mechanisms that link early reproduction to the subsequent rate of ageing.

The evolutionary data imply that the early fitness-enhancing effects of a pleiotropic mutation result in latent damage that takes time to emerge as pathology and mortality. The key to understanding how the length of an organism's life is specified is therefore to understand the mechanisms that generate latent damage and that determine the length of time between its occurrence and its expression as later mortality. On this subject, the evolutionary theory of ageing says nothing. How, then, can we discover the processes that underlie ageing and its timing?

A different type of approach to these questions is to identify genetic modifiers of the rate of ageing, by generating mutants with increased lifespan. This classical FORWARD GENETICS approach can provide a way to understand the determinants of ageing and, during the past decade, has allowed rapid progress in the field of ageing research to be made. Long-lived mutants have been isolated in *S. cerevisiae*, *C. elegans*, *D. melanogaster* and mice (reviewed in REFS 51–53). Recent findings from these model organisms, although somewhat different, hint at a possible mechanistic link between ageing and reproductive rates, through the response of both to variations in nutrient supply.

DAUER LARVA
A developmentally arrested, immature, long-lived and non-feeding form of *Caenorhabditis elegans* that forms under conditions of food scarcity and high population density, and that resumes development if food levels increase.

FORWARD GENETICS
A genetic analysis that proceeds from phenotype to genotype: for example, by positional cloning or candidate-gene analysis.

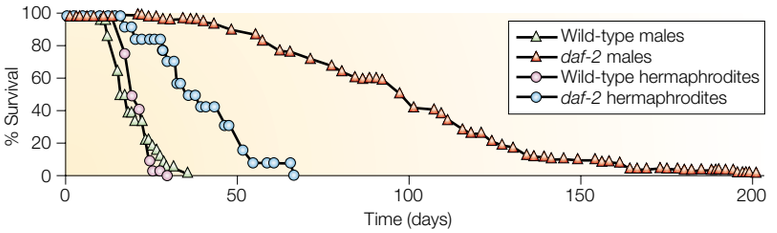

Figure 2 | **Extended lifespan in *daf-2* mutant *Caenorhabditis elegans*.** The percentage survival over time of wild-type or *daf-2* mutant *C. elegans* males and hermaphrodites (in *C. elegans*, the sexes consist of self-fertilizing hermaphrodites and males). The effect of this *daf-2* mutation (allele *e1391*) is much greater in males[80], resulting in a 6.4-fold increase in maximum lifespan, and is the result of a single base change in the *daf-2* gene[56] (D. Gems, unpublished data). *daf*, dauer larva formation abnormal.

Insulin/IGF signalling and ageing in the worm

The shortest lived of the multicellular model organisms is *C. elegans*. It is in this organism that the most rapid advances in the genetics of ageing have been made, and many dozens of genes have been identified with mutations that increase lifespan. The most striking examples involve genes that encode the elements of a signalling pathway that is similar to that which responds to insulin and insulin-like growth factor 1 (IGF1) in mammals[54–58]. Some mutants in this pathway have lifespans that are increased by threefold or more, relative to wild type (FIG. 2) (reviewed in REF. 59). Below, we outline some of the key discoveries in the genetics of ageing that have been made in *C. elegans* and consider their significance in the light of evolutionary theories of ageing.

A neuroendocrine system that modulates ageing. The first-identified, long-lived *C. elegans* mutant carried a mutation in *age-1* (REF. 54). This gene encodes part of a lipid kinase enzyme (phosphatidylinositol-3 kinase)[57] that transmits signals from DAF-2 (dauer formation constitutive) — a receptor that is thought to respond to insulin-like ligands — into the cell[56] (FIG. 3a). Although the completely sequenced *C. elegans* genome contains only a single insulin/IGF receptor gene, it contains an astonishing 37 genes that encode insulin-like proteins[60]. Of these, INS-1 most closely resembles human insulin. Genetic studies have indicated that both INS-1 and human insulin modulate the action of DAF-2, not by activating it, however, but by antagonizing it (REF. 60).

Reduced insulin/IGF signalling in *C. elegans* also leads to increased dauer formation. The analysis of *C. elegans* that contain both normal and mutant *daf-2* cells has shown that the expression of wild-type *daf-2* in only a few neuronal cells can be sufficient to prevent dauer formation[61]. So, the *daf-2* gene product acts not only in the cells that express it, but also at a distance.

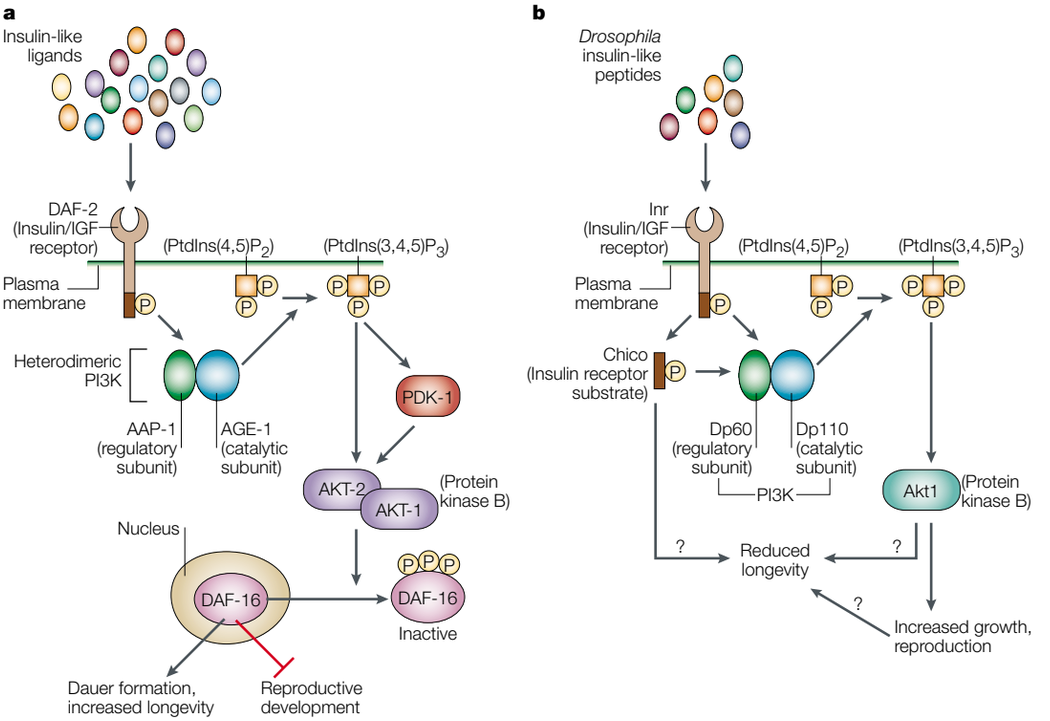

Figure 3 | **Insulin/IGF signalling pathways in *Caenorhabditis elegans* and *Drosophila*. a** | The pathway in *C. elegans*. The principal effect of signalling from DAF-2 to AKT-1 and AKT-2 is to inactivate the DAF-16 transcription factor by phosphorylation (P). **b** | The pathway in *Drosophila*. Although mutations in *Inr*, *chico* and *Akt1* each result in dwarf flies, only *Inr* and *chico* dwarves are long lived, indicating that the signalling downstream of *chico* that affects ageing does not occur through *Akt1* (REF. 79). It is not yet known whether insulin/IGF signalling in *Drosophila* acts through a forkhead transcription factor that is homologous to DAF-16. AAP-1, *age-1*-associated protein; DAF, dauer larva formation abnormal; IGF, insulin-like growth factor; Inr, insulin receptor; PDK-1, 3-phosphoinositide-dependent kinase 1; PI3K, phosphatidylinositol-3-kinase; PtdIns(4,5)P_2, phosphatidylinositol 4,5-biphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-triphosphate.

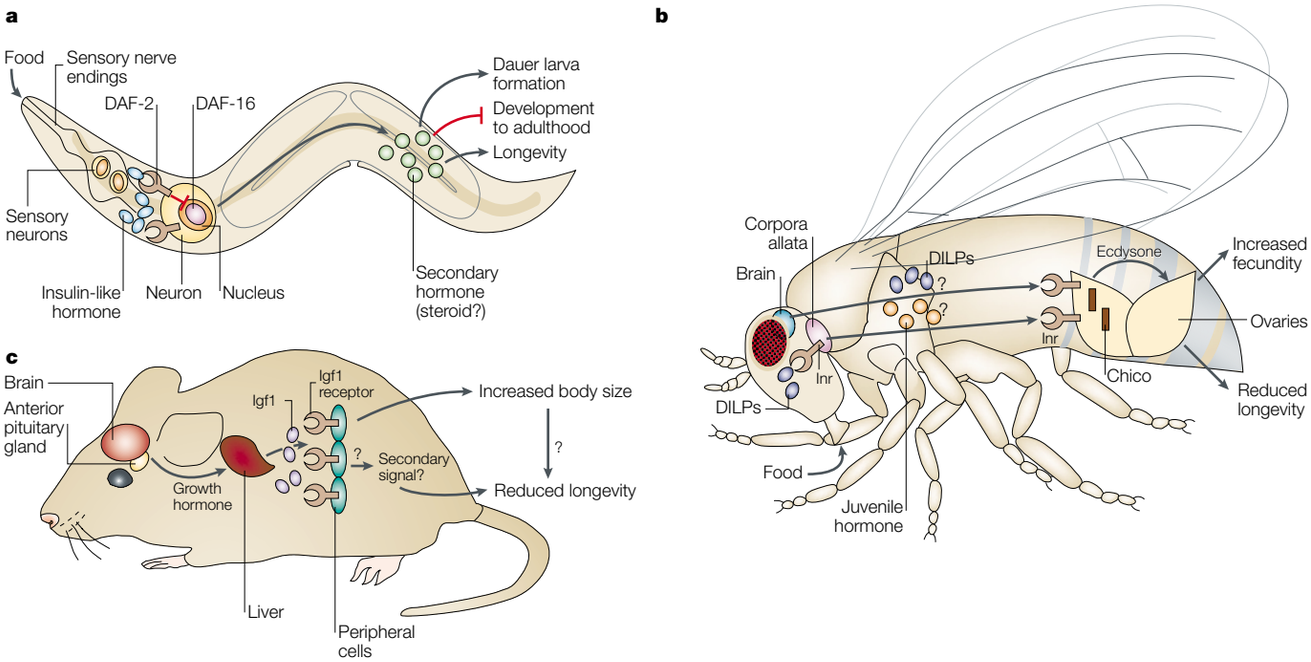

Figure 4 | **Neuroendocrine regulation of ageing. a** | Insulin/IGF signalling in a two-step hormone signalling system in *Caenorhabditis elegans*. In this model, food modulates the production of an insulin-like peptide hormone (INS) by chemosensory neurons. This acts on DAF-2, which is also expressed in the nervous system, to cause the production of a second hormone signal, which modulates development and ageing throughout the organism. Elements of this model are speculative, and the following remain to be determined: whether environmental stimuli regulate INS production and what these stimuli are; the role of DAF-16 in regulating secondary hormone production; and whether this hormone regulates longevity. **b** | Two hypotheses for the role of insulin/IGF signalling in ageing in *Drosophila*. In both models, *Drosophila* insulin-like peptides (DILPs)[99] are produced by the brain in response to environmental or internal nutritional stimuli. How the production of DILPs is regulated is unknown. In one version of this model, DILPs act directly on the ovaries, stimulating the production of the steroid hormone ecdysone[81]; in the other, DILPs stimulate the production of the isoprenoid hormone juvenile hormone[78] by the CORPORA ALLATA. **c** | Insulin/IGF signalling in mice. This model proposes that Igf1, rather than insulin, acts as a modulator of ageing in mammals; this role of Igf1 in ageing remains to be shown directly. DAF, dauer larva formation abnormal; Igf1, insulin-like growth factor 1; Inr, insulin-like receptor.

This indicates that a second hormone signal exists downstream of *daf-2*, which is received by cells throughout the animal and which regulates development and ageing (FIG. 4a). The role of DAF-2 in the nervous system in the regulation of ageing has been recently confirmed by the rescue of *daf-2* and *age-1* mutant phenotypes in specific tissues[62]. In these experiments, wild-type *daf-2* or *age-1* was expressed under the control of cell-type-specific promoters. The life-extension phenotype was blocked when insulin/IGF signalling was restored in the nervous system, but not when it was restored in the muscle or intestine[62].

What might insulin/IGF signalling be responding to? One possibility is the presence of food, as revealed by its taste or smell. Mutant worms with defects in the chemosensory neurons that innervate the AMPHIDS show increases in mean lifespan of up to 121% (REF. 63). Studies of compound mutants have shown that these increases in lifespan result from reduced insulin/IGF signalling[63]. Furthermore, *ins* genes are mainly expressed in neurons, including chemosensory neurons[60]; the sensing of food might therefore result in the secretion of INS ligands from chemosensory neurons (FIG. 4a). So, if the DAF-2 receptor has a function similar to that of the insulin receptor, it would be as if the worm had its pancreas in its nose.

What does *daf-2* regulate? The main target of signalling through *daf-2* is *daf-16*, which encodes a forkhead transcription factor that presumably acts to regulate downstream target genes[55,58] (FIG. 3a), which, so far, remain unidentified. But *daf-16* might regulate the genes that are involved in the biosynthesis of a secondary signal that emanates from *daf-2*-expressing cells (FIG. 4a). One possible target for such a signal is the orphan nuclear hormone receptor, DAF-12 (REFS 64,65). The DAF-12 protein mediates the regulation of dauer formation by transforming growth factor (TGF)-β signalling, the timing of larval development[65] and, perhaps, ageing[66–68]. Its ligand is unknown, but recent findings indicate that it might be a steroid[69]. However, gene interaction studies do not support the view that the *daf-2*-regulated lifespan signal acts principally through *daf-12* because, unlike mutations in *daf-16*, *daf-12* mutations do not block the extended lifespan phenotype of *daf-2* mutants. In some *daf-2* mutants, *daf-12* mutations partly block lifespan extension, whereas in others they actually enhance it[66,67].

Although the genetics of insulin/IGF signalling and *daf-12* remain unclear, these studies have shown that the insulin/IGF signalling pathway is part of a central neuroendocrine axis (FIG. 4a) that regulates development and ageing, perhaps through two secretory steps. First,

CORPORA ALLATA
Endocrine glands located in the head of insects.

AMPHIDS
Paired openings in the nose of nematodes that act as smell and taste organs.

environmental stimuli — perhaps food levels — modulate the secretion of INS ligands. These are then received by neuronal DAF-2, resulting in the production of a second, global signal, possibly a steroid, which is received throughout the organism by receptors such as DAF-12. This type of two-step endocrine signalling system, involving a polypeptide hormone and then a steroid, is found throughout invertebrates and vertebrates.

Why does insulin/IGF signalling accelerate ageing? We have seen that in wild-type *C. elegans*, ageing is accelerated by insulin/IGF signalling — at least, under standard laboratory conditions (that is, on a Petri plate with ample food supplies). This signalling seems to act as a component of a neuroendocrine system that is responsive to environmental cues, possibly food levels. However, it seems strange that a system that accelerates ageing in this way should have evolved. How could it possibly enhance fitness? The evolutionary theory predicts that the accelerated ageing is likely to be the downside of a trade-off with a fitness-enhancing trait, such as increased early reproductive output. As in other organisms, calorific restriction in *C. elegans* reduces fecundity and increases lifespan[18]. It has been suggested that the capacity to respond to calorific restriction in this way enhances fitness by helping to delay reproduction until a period of limited food availability has ended[21,22]. One possibility is that insulin/IGF signalling mediates the effect of calorific restriction on fertility and ageing — as would be consistent with the role of mammalian insulin signalling in mediating responses to nutritional changes — and might also modulate trade-offs between fecundity and longevity in response to changes in nutrition.

However, there is little evidence for these interpretations. If calorific restriction increases lifespan by reducing insulin/IGF signalling, one might expect that it would increase lifespan to a lesser extent in insulin/IGF-pathway mutants, but this is not the case, which indicates that insulin/IGF signalling does not mediate the effects of calorific restriction[70,71]. The trade-off model is also particularly implausible in this instance because in *C. elegans*, unlike in *Drosophila*, egg-production levels have no discernible effect on lifespan[54,55,72]. So, there is no evidence of a trade-off between fecundity and ageing in *C. elegans*. Moreover, although some *daf-2* mutants show reduced hermaphrodite fertility, some mutants have a greatly increased lifespan but normal fertility[66,67], and fertility among *daf-2* mutant males is increased in many cases[67].

Nonetheless, questions remain about the interpretation of these findings. It is possible, for example, that the fertility–lifespan trade-off tests and calorific-restriction–insulin-pathway interaction tests will have different outcomes depending on the level of nutrition at which they are carried out. A further possibility is that whereas insulin/IGF signalling regulates fecundity and longevity, reducing fecundity does not cause increased longevity (discussed in REF. 73). Also, if the sensation of food regulates INS production, it would be surprising if food levels did not affect ageing through insulin/IGF signalling. The *C. elegans* gonad certainly does have a role in regulating ageing and body size: if the germ line is removed by laser microsurgery, there are increases in lifespan[68] and body size[74]. This indicates the presence of a system that coordinates reproduction, lifespan and body size in this organism. Ultimately, the adaptive significance of the modulation of ageing by insulin/IGF signalling in *C. elegans* will only be resolved by further investigation.

Do mutants have increased fitness? The finding that the mutation of a single gene, such as *daf-2*, could double lifespan was initially greeted with some consternation by evolutionary biologists. It begged the question: Why do mutations of this sort not already exist in the population? After all, a worm with a doubled lifespan might potentially be around for twice as long to reproduce. It was assumed that such mutations must, therefore, have deleterious pleiotropic effects, which would reduce overall fitness in nature. To worm geneticists, the identity of this defect was obvious: insulin/IGF mutants more readily form dauer larvae and are therefore at a reproductive disadvantage. *age-1* mutant dauer larvae also take longer to resume development[75]. So, insulin/IGF signalling does regulate a trade-off between fertility and longevity, although of an unusual sort[66]. This trade-off, plus the fact that most insulin pathway mutants are less fertile than the wild type, means that these mutations lower net fitness.

This is an interesting example of antagonistic pleiotropy as it would seem that mutations affecting insulin/IGF signalling have separate effects on gene action in larvae and adults (FIG. 1a). This is in contrast to the type of antagonistic pleiotropy that is seen in short-lived *Drosophila* with an increased early-adult fecundity, which causes deleterious effects in later life[50] (FIG. 1b). One possibility is that the late-action type of antagonistic pleiotropy (see FIG. 1a) occurs when an allele has distinct pleiotropic effects on juveniles and adults, whereas the latent-damage type occurs when both early and late effects occur in adulthood (see FIG. 1b).

Insulin signalling and ageing in *Drosophila*

In *C. elegans*, *daf-2* regulates the formation of dauer larvae, which can survive more than five times longer than the adult[76]. It was originally thought that *daf-2* mutant adults might live longer because of the re-expression in the adult of the dauer longevity trait[55]. If correct, this predicts that such extensions of lifespan by single-gene mutations would not occur in species that do not have a long-lived diapausal form. A simple test of whether the role of insulin/IGF signalling in ageing is specific to nematodes is to look at the equivalent mutant in a different organism.

The *Drosophila* equivalents of *daf-2* (*Inr*, *Insulin-like receptor*) and *age-1* (*Dp110*, also known as *Pi3K92E*), and also *chico*, a fruitfly insulin receptor substrate (FIG. 3b), have a role in determining body and organ size (reviewed in REF. 77). As in *C. elegans*, they probably function within a neuroendocrine system (FIG. 4b). Strikingly, a mild reduction of *Inr* function increases mean female lifespan by up to 85% (REF. 78) and loss of function of *chico* by up to 52% (REF. 79).

Table 1 | **Long-lived mouse mutants**

Strain	Affected gene	Phenotype	Increase in lifespan	References
Ames dwarf	*Prop1*	Dwarf, CPHD, obese, infertile	Male, 49%; female, 64%	86
Snell dwarf	*Pit1*	Dwarf, CPHD, obese, infertile	Similar to Ames dwarf	86,87
Laron dwarf	*Ghr*	Dwarf, IGHR, delayed fertility	Male, 55%; female, 38%	92
Little mouse	*Ghrhr*	Dwarf	Male, 23%; female, 25%	87
Overexpresses plasminogen activator	*Plau*	Small, reduced feeding, self-imposed CR?*	~20%	100
Shc1 mutant	*Shc1*	Stress resistant	30%	101

*This mutant eats less, and it has been proposed that its extended lifespan results from self-imposed caloric restriction. CPHD, combined pituitary hormone deficiency; CR, caloric restriction; *Ghr*, growth hormone receptor; *Ghrhr*, growth-hormone-releasing hormone receptor; IGHR, isolated growth hormone resistance; *Plau*, urokinase-type plasminogen activator; *Prop1*, prophet of *Pit1*; *Pit1*, pituitary-specific transcription factor 1; *Shc1*, src homology 2 domain-containing transforming protein C1.

These mutations also cause the flies to be dwarves. Null alleles of *Inr* and *Dp110* do not produce viable adults; the effect of weak *Dp110* alleles on lifespan is, at present, unknown.

There are some differences between worms and flies in the role of insulin/IGF signalling in ageing. For example, only certain insulin/IGF pathway mutations increase lifespan in the fly, and mainly in females[78,79]. By contrast, all *daf-2* and *age-1* mutant adult worms show increased lifespans[54,55,66,67], and in *daf-2* mutants, males are the longer-lived sex[80]. So, although there is a simple relationship between lifespan and insulin/IGF signalling levels in worms, in flies it seems that reductions in this pathway increase lifespan only over a narrow range of gene expression levels or in particular contexts. Understanding these subtleties is a priority for future research.

Why does insulin/IGF signalling accelerate ageing? In contrast to studies in *C. elegans*, evidence from *Drosophila* studies provides some support for the hypothesis that the insulin/IGF pathway has a role in modulating a trade-off between fertility and longevity in response to changes in nutrition. As in *C. elegans*, calorific restriction reduces fecundity and increases lifespan in *Drosophila*[23,81]. Unlike in *C. elegans*, there are striking trade-offs between fecundity and longevity, as discussed earlier. However, it is not yet clear whether reduced fecundity actually causes the increase in lifespan in calorie-restricted flies, or whether the effects of calorific restriction on lifespan involve reduced insulin/IGF signalling.

What about the effect of insulin/IGF signalling on reproduction? The modulation of egg production by nutrition levels has been recently shown to be mediated by *chico*[81]. So, in fruitflies, insulin/IGF signalling is regulated by food levels. Moreover, both *chico*^{1} and *Inr* mutant adults are sterile. Therefore, can the increased longevity of *chico*^{1} females be attributed to their sterility? This issue is unresolved, but evidence so far indicates that the later stages of egg production do not have a significant role in *chico*^{1} mutant longevity. *ovo*^{D1} mutant females, which are sterile due to a block early in oogenesis (stage 4), are long lived. However, they are not as long lived as *chico*^{1} females[79], which indicates that most of the increased longevity of *chico*^{1} mutant flies cannot be attributed to the absence of egg production beyond stage 4, which includes the provisioning (yolking) of the egg. However, it is possible that parallel processes, such as yolk protein synthesis or earlier endocrinological processes, do have a role in lifespan determination.

Overall, these findings are consistent with the hypothesis that trade-offs between fertility and lifespan are modulated by insulin/IGF signalling in response to nutrition, although more research is needed to confirm this hypothesis. These findings indicate that the reason why insulin/IGF signalling shortens lifespan under replete nutritional conditions is that it might be a pleiotropic, deleterious side effect of the role of the insulin/IGF pathway in maximizing reproductive output under these conditions.

So, insulin/IGF signalling modulates the rate of ageing in two very distantly related animal species, and therefore represents a public rather than a private mechanism of ageing. But, is this mechanism also shared by mammals? Molecular phylogenetics has indicated that nematodes and arthropods are part of a single clade, the Ecdysozoa (creatures that moult)[82]. It therefore remains possible that the role of insulin/IGF signalling in regulating ageing evolved after the divergence of the ecdysozoan ancestor from that of the vertebrates more than 500 million years ago; in which case, this role could be confined to moulting animals. However, recent findings indicate that insulin/IGF signalling might also have a role in modulating ageing in mammals.

Insulin/IGF signalling, ageing and dwarf mice

In worms and flies, there is a single insulin/IGF receptor, whereas in higher animals there are three related receptors: the insulin receptor (Insr), the Igf1 receptor and the insulin receptor-related receptor (Insrr). Is there evidence that these receptors are involved in the regulation of ageing?

Insulin is involved in cell growth, glucose homeostasis and in promoting glucose uptake by the cell and its conversion to lipid. Reduced functioning of Insr results in type II (non-insulin-dependent) diabetes, which shortens lifespan. Homozygous loss of Insr function in humans results in leprechaunism, a syndrome that is characterized by low birth weight, hyperinsulinism, mental retardation and early death[83]. In mice, loss-of-

function mutations of *Insr* lead to neonatal lethality[84]. However, complete loss of function of the insulin/IGF receptor also results in lethality in flies[85] and worms[67], so a slight reduction in insulin signalling might still reduce the rate of ageing in mammals.

Igf1 promotes growth, protein synthesis and cell survival. Circulating Igf1 is produced mostly by the liver in response to pituitary growth hormone (FIG. 4c); Igf1 is also produced locally. Longevity has recently been examined in three dwarf mouse mutant strains with reduced levels of circulating Igf1 (TABLE 1). The Ames and Snell dwarf mice both result from mutations that affect the transcription factors that control pituitary development, resulting in deficiencies in several pituitary hormones (reviewed in REF. 53). In the Ames dwarf mutant, a mutation in *Prop1* (prophet of *Pit1*) results in a 64% and 49% increase in mean lifespan in females and males, respectively[86]. Mutation of the Snell dwarf gene, *Pit1* (pituitary-specific transcription factor 1) results in a more than 40% increase in mean lifespan in both sexes[87].

There are many potential explanations for the increased lifespan of these dwarf mice: they are deficient in thyroid-stimulating hormone, growth hormone and prolactin, and have reduced fertility. However, several lines of evidence indicate that reduced growth hormone levels, rather than reduced thyroid-stimulating hormone or prolactin levels, might be a cause of their increased longevity. First, the overproduction of growth hormone in over-sized transgenic mice results in increased plasma levels of Igf1 and accelerated ageing[88,89]. Second, it has recently been shown that several mouse mutants with defects that selectively reduce growth hormone and Igf1 levels are long lived. The first is the growth hormone receptor knockout mouse[90,91]. These mice show a 90% reduction in plasma Igf1 and, although litter size is reduced from a control level of 6.6 pups to 2.7 pups, this could be because the mice are so tiny — less than half the mass of normal mice[90]. In other respects, they seem vigorous and healthy, and their mean lifespans are increased by 38% and 55% in females and males, respectively[92]. The second is the so-called 'little mouse', which has a defect (*Ghrhr*^{lit}) in the growth-hormone-releasing hormone-receptor gene, and an increased mean lifespan of 23% and 25% in males and females, respectively[87]. Given that the main action of growth hormone is to stimulate Igf1 production, this implicates Igf1 as a modulator of mammalian ageing. However, its role in ageing remains to be tested directly.

These findings indicate that in mammals, as in nematodes and probably insects, there exist powerful neuroendocrine modulators of ageing (FIG. 4a–c), which shorten lifespan in wild-type animals. As in nematodes and fruitflies, an attractive explanation for this apparently maladaptive trait in mammals is that it is the downside of a trade-off against other fitness-enhancing traits. Pertinent clues might also be provided by calorific restriction, which reduces fertility and increases lifespan in rodents; perhaps significantly, it also results in reduced circulating insulin and Igf1, and in enhanced insulin sensitivity (reviewed in REFS 53,93). However, it was recently shown that calorific restriction causes similar increases in Ames dwarf and wild-type mouse lifespans[94], from which it was inferred that different mechanisms of retarded ageing are involved. Moreover, whereas calorie-restricted mice are infertile, long-lived growth hormone receptor knockout mice have almost normal fertility[90].

Conclusions and future directions

Several distinct sub-disciplines have emerged in the nascent field of biogerontology, including the testing of evolutionary theories of ageing, the classical genetics of ageing and BIODEMOGRAPHY, which is not discussed here. Arguably, it will only be possible to understand the significance of the data that are generated by each sub-discipline once a synthesis of the knowledge generated by each has been achieved.

A central aim of this discussion has been to explain some recent findings from the classical genetics of ageing in terms of the evolutionary theory of ageing. The former has identified an evolutionarily conserved neuroendocrine system that apparently functions to accelerate ageing, which in evolutionary terms is paradoxical. We have attempted to explain this by means of a hypothesis: that this effect on ageing is part of a trade-off with fitness-enhancing pleiotropic effects, which are subject to neuroendocrine (insulin/IGF-pathway) modulation in response to changes in nutrition. In the case of both *C. elegans* and *Drosophila*, the shoe fits the foot quite well, although the detailed mechanisms at work seem to be somewhat different. The challenges now are to unravel the mechanisms in these two invertebrate model organisms, and to determine if they are public (shared) with those in evolutionary lineages outside the Ecdysozoa, particularly the mammals.

Update — added in proof

Arantes-Oliveira *et al.* have recently shown that life-shortening signals from the *C. elegans* germ line result from germ-line stem-cell proliferation[102].

BIODEMOGRAPHY
The study of age-specific mortality and fecundity rates and their biological determinants.

1. Charlesworth, B. *Evolution in Age-Structured Populations* (Cambridge Univ. Press, Cambridge, UK, 1994).
2. Hamilton, W. D. The moulding of senescence by natural selection. *J. Theor. Biol.* **12**, 12–45 (1966).
3. Partridge, L. & Barton, N. H. Optimality, mutation and the evolution of ageing. *Nature* **362**, 305–311 (1993).
4. Finch, C. E. *Longevity, Senescence and the Genome* (Univ. Chicago Press, Chicago, 1990).
5. Halliwell, B. & Gutteridge, J. M. C. *Free Radicals in Biology and Medicine* (Oxford Science, Oxford, UK, 1999).
6. Attardi, G. & Schatz, G. Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* **4**, 289–333 (1988).
7. Lal, S., Ramsey, J., Monemdjou, S., Weindruch, R. & Harper, M. Effects of caloric restriction on skeletal muscle mitochondrial proton leak in aging rats. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**, B116–B122 (2001).
8. Pamplona, R. *et al.* Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse. *Aging (Milano)* **11**, 44–49 (1999).
9. Schwarze, S. R., Weindruch, R. & Aiken, J. M. Decreased mitochondrial RNA levels without accumulation of mitochondrial DNA deletions in aging *Drosophila melanogaster*. *Mutat. Res.* **382**, 99–107 (1998).
10. Wei, Y. H., Lu, C. Y., Lee, H. C., Pang, C. Y. & Ma, Y. S. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. *Ann. NY Acad. Sci.* **854**, 155–170 (1998).

11. Campisi, J. Aging and cancer: the double-edged sword of replicative senescence. *J. Am. Geriatr. Soc.* **45**, 482–488 (1997).
12. Wallace, D. & Melov, S. Radicals r'aging. *Nature Genet.* **19**, 105–106 (1998).
13. Martin, G. M., Austad, S. N. & Johnson, T. E. Genetic analysis of ageing: role of oxidative damage and environmental stresses. *Nature Genet.* **13**, 25–34 (1996).
14. Beckman, K. B. & Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **78**, 547–581 (1998).
15. Sohal, R. S., Ku, H.-H., Agarwal, S., Forster, M. J. & Lal, H. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech. Ageing Dev.* **74**, 121–133 (1994).
16. Finch, C. E. & Kirkwood, T. *Chance, Development and Ageing* (Oxford Univ. Press, Oxford, UK, 1999).
17. Carey, J., Liedo, P., Muller, H., Wang, J. & Chiou, J. Mortality oscillations induced by periodic starvation alter sex-mortality differentials in Mediterranean fruit flies. *J. Gerontol. A Biol. Sci. Med. Sci.* **54**, B424–B431 (1999).
18. Klass, M. R. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech. Ageing Dev.* **6**, 413–429 (1977).
19. Lane, M. A. *et al.* Short-term calorie restriction improves disease-related markers in older male rhesus monkeys (*Macaca mulatta*). *Mech. Ageing Dev.* **112**, 185–196 (2000).
20. Lin, S., Defossez, P. & Guarente, L. Requirement of NAD and *SIR2* for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* **289**, 2126–2128 (2000).
21. Masoro, E. & Austad, S. The evolution of the antiaging action of dietary restriction: a hypothesis. *J. Gerontol. A Biol. Sci. Med. Sci.* **51**, B387–B391 (1996).
22. Holliday, R. Food, reproduction and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? *Bioessays* **10**, 125–127 (1989).
23. Chapman, T. & Partridge, L. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proc. R. Soc. Lond.* B **263**, 755–759 (1996).
24. Holehan, A. E. & Merry, B. J. The experimental manipulation of ageing by diet. *Biol. Rev.* **61**, 329–368 (1986).
25. Loison, A., FestaBianchet, M., Gaillard, J. M., Jorgenson, J. T. & Jullien, J. M. Age-specific survival in five populations of ungulates: evidence of senescence. *Ecology* **8**, 2539–2554 (1999).
26. Newton, I. in *Ecology: Achievement and Challenge* 67–92 (Blackwell Scientific, Oxford, UK, 2001).
27. Austad, S. N. & Fischer, K. E. Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. *J. Gerontol. A Biol. Sci. Med. Sci.* **46**, B47–B53 (1991).
28. Williams, G. C. Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11**, 398–411 (1957).
29. Haldane, J. B. S. *New Paths in Genetics* (Allen & Unwin, London, 1941).
30. Keller, L. & Genoud, M. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature* **389**, 958–960 (1997).
31. Ricklefs, R. E. Evolutionary theories of aging: confirmation of a fundamental prediction, with implications for the genetic basis and evolution of life span. *Am. Nat.* **152**, 24–44 (1998).
32. Mani, G. & Clarke, B. C. Mutational order — a major stochastic process in evolution. *Proc. R. Soc. Lond.* B **240**, 29–37 (1990).
33. Korona, R. Genetic divergence and fitness convergence under uniform selection in experimental populations of bacteria. *Genetics* **143**, 637–644 (1996).
34. Hartl, D. L. & Clark, A. G. *Principles of Population Genetics* (Sinauer, Sunderland, Massachusetts, 2000).
35. Hughes, K. A. & Charlesworth, B. A genetic analysis of senescence in *Drosophila*. *Nature* **367**, 64–66 (1994).
36. Promislow, D., Tatar, M., Khazaeli, A. A. & Curtsinger, J. W. Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. *Genetics* **143**, 839–848 (1996).
37. Shaw, F., Promislow, D. E., Tatar, M., Hughes, K. A. & Geyer, C. J. Toward reconciling inferences concerning genetic variation in senescence in *Drosophila melanogaster*. *Genetics* **152**, 553–566 (1999).
38. Charlesworth, B. & Hughes, K. A. Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. *Proc. Natl Acad. Sci. USA* **93**, 6140–6145 (1996).
39. Pletcher, S., Houle, D. & Curtsinger, J. W. Age-specific properties of spontaneous mutations affecting mortality in *Drosophila melanogaster*. *Genetics* **148**, 287–303 (1998).
40. Pletcher, S., Houle, D. & Curtsinger, J. W. The evolution of age-specific mortality rates in *Drosophila melanogaster*: genetic divergence among unselected lines. *Genetics* **153**, 813–823 (1999).
41. Charlesworth, B. & Partridge, L. Ageing: levelling of the grim reaper. *Curr. Biol.* **7**, R440–R442 (1997).
42. Rose, M. & Charlesworth, B. Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics* **97**, 187–196 (1981).
43. Wattiaux, J. M. Cumulative parental age effects in *D. subobscura*. *Evolution* **22**, 406–421 (1968).
44. Luckinbill, L. S., Arking, M. J., Clare, M. J., Cirocco, W. C. & Muck, S. A. Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* **38**, 996–1003 (1984).
45. Rose, M. R. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**, 1004–1010 (1984).
46. Partridge, L. & Fowler, K. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **46**, 76–91 (1992).
47. Roper, C., Pignatelli, P. & Partridge, L. Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster*. *Evolution* **47**, 445–455 (1993).
48. Partridge, L., Prowse, N. & Pignatelli, P. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Proc. R. Soc. Lond.* B **266**, 255–261 (1999).
49. Zwaan, B., Bijlsma, R. & Hoekstra, R. F. Direct selection on life span in *Drosophila melanogaster*. *Evolution* **49**, 649–659 (1995).
50. Sgrò, C. & Partridge, L. A delayed wave of death from reproduction in *Drosophila*. *Science* **286**, 2521–2524 (1999).
This study shows that the differences in longevity between selected *Drosophila* lines are due to the delayed effects of differences in fertility, supporting a delayed damage/antagonistic pleiotropy model of ageing.
51. Kenyon, C. A conserved regulatory system for aging. *Cell* **105**, 165–168 (2001).
52. Gems, D. & Partridge, L. Insulin/IGF signalling and ageing: seeing the bigger picture. *Curr. Opin. Genet. Dev.* **11**, 287–292 (2001).
53. Bartke, A. in *The Molecular Genetics of Aging* (ed. Hekimi, S.) 181–202 (Springer, Berlin and Heidelberg, 2000).
54. Friedman, D. B. & Johnson, T. E. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75–86 (1988).
55. Kenyon, C., Chang, J., Gensch, E., Rudener, A. & Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464 (1993).
56. Kimura, K. D., Tissenbaum, H. A., Liu, Y. & Ruvkun, G. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**, 942–946 (1997).
57. Morris, J. Z., Tissenbaum, H. A. & Ruvkun, G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**, 536–538 (1996).
58. Ogg, S. *et al.* The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**, 994–999 (1997).
59. Gems, D. in *The Biology of Nematodes* (ed. Lee, D. L.) 413–455 (Taylor & Francis, London, 2002).
60. Pierce, S. *et al.* Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev.* **15**, 672–686 (2001).
This paper revealed that the *C. elegans* genome contains 37 putative insulin-encoding genes.
61. Apfeld, J. & Kenyon, C. Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. *Cell* **95**, 199–210 (1998).
62. Wolkow, C., Kimura, K., Lee, M. & Ruvkun, G. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* **290**, 147–150 (2000).
63. Apfeld, J. & Kenyon, C. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* **402**, 804–809 (1999).
This study describes the retarded ageing that is seen in *C. elegans* mutants with chemosensory/olfactory defects.
64. Antebi, A., Yeh, W., Tait, D., Hedgecock, E. & Riddle, D. *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. *Genes Dev.* **14**, 1512–1527 (2000).
This paper reported the existence of *C. elegans* DAF-12 nuclear hormone receptor.
65. Antebi, A., Culotti, J. G. & Hedgecock, E. M. *daf-12* regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. *Development* **125**, 1191–1205 (1998).
66. Larsen, P. L., Albert, P. S. & Riddle, D. L. Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* **139**, 1567–1583 (1995).
67. Gems, D. *et al.* Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* **150**, 129–155 (1998).
68. Hsin, H. & Kenyon, C. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* **399**, 362–366 (1999).
In this paper, how signals from the germ line and somatic gonad modulate ageing in *C. elegans* is described.
69. Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V. & Antebi, A. A hormonal signaling pathway influencing *C. elegans* metabolism, reproductive development, and life span. *Dev. Cell* **1**, 841–851 (2001).
This paper describes evidence that the *C. elegans* DAF-9 cytochrome P450 enzyme is involved in the synthesis of a steroid that controls dauer formation and, possibly, ageing.
70. Vanfleteren, J. R. & De Vreese, A. The gerontogenes *age-1* and *daf-2* determine metabolic rate potential in aging *Caenorhabditis elegans*. *FASEB J.* **9**, 1355–1361 (1995).
71. Lakowski, B. & Hekimi, S. The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **95**, 13091–13096 (1998).
72. Gems, D. & Riddle, D. L. Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production. *Nature* **379**, 723–725 (1996).
73. Leroi, A. Molecular signals versus the Loi de Balancement. *Trends Ecol. Evol.* **16**, 24–29 (2001).
74. Patel, M., Knight, C., Karageorgi, C. & Leroi, A. Evolution of germ-line signals that regulate growth and ageing in nematodes. *Proc. Natl Acad. Sci. USA* **99**, 769–774 (2002).
This study shows that the *C. elegans* germ line controls body size, as well as longevity.
75. Walker, D., McColl, G., Jenkins, N. L., Harris, J. & Lithgow, G. J. Evolution of lifespan in *C. elegans*. *Nature* **405**, 296–297 (2000).
76. Klass, M. R. & Hirsh, D. I. Nonaging developmental variant of *C. elegans*. *Nature* **260**, 523–525 (1976).
77. Weinkove, D. & Leevers, S. The genetic control of organ growth: insights from *Drosophila*. *Curr. Opin. Genet. Dev.* **10**, 75–80 (2000).
78. Tatar, M. *et al.* Mutations in the *Drosophila* insulin receptor homologue retard senescence and impair neuroendocrine function. *Science* **292**, 107–110 (2001).
Here, it is shown that the *Drosophila* insulin/IGF receptor gene *Inr* regulates ageing, possibly through the regulation of juvenile hormone production.
79. Clancy, D. *et al.* Extension of lifespan by loss of *chico*, a *Drosophila* insulin receptor substrate protein. *Science* **292**, 104–106 (2001).
This describes the increased longevity of the *Drosophila* insulin receptor substrate mutant *chico*^{1}, as well as its other phenotypic characteristics.
80. Gems, D. & Riddle, D. L. Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* **154**, 1597–1610 (2000).
81. Drummond-Barbosa, D. & Spradling, A. C. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* **231**, 265–278 (2001).
This study includes evidence for a role of the chico insulin receptor substrate in mediating the regulation of oogenesis by nutritional status in *Drosophila*.
82. Aguinaldo, A. M. A. *et al.* Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* **387**, 489–493 (1997).
83. Krook, A., Breuton, L. & O'Rahilly, S. Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. *Lancet* **342**, 277–278 (1993).
84. Accili, D., Drago, J., Lee, E. J., Johnson, M. D. & Cool, M. H. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nature Genet.* **12**, 106–109 (1996).
85. Fernandez, R., Tabarini, D., Azpiazu, N., Frasch, M. & Schlessinger, J. The *Drosophila* insulin receptor homolog: a gene essential for embryonic development encodes two receptor isoforms with different signaling potential. *EMBO J.* **14**, 3373–3384 (1995).
86. Brown-Borg, H. M., Borg, K. E., Meliska, C. J. & Bartke, A. Dwarf mice and the ageing process. *Nature* **384**, 33 (1996).

87. Flurkey, K., Papaconstantinou, J., Miller, R. & Harrison, D. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl Acad. Sci. USA* **98**, 6736–6741 (2001).
This paper reports the increased longevity of two mouse mutants: the Snell dwarf and the 'little' mouse.
88. Naar, E. *et al.* Fertility of transgenic female mice expressing bovine growth hormone or human growth hormone variant genes. *Biol. Reprod.* **45**, 178–187 (1991).
89. Wolf, E. *et al.* Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: lessons from transgenic animal models. *Mech. Ageing Dev.* **68**, 71–87 (1993).
90. Zhou, Y. *et al.* A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc. Natl Acad. Sci. USA* **94**, 13215–13220 (1997).
91. Kopchick, J. & Laron, Z. Is the Laron mouse an accurate model of Laron syndrome? *Mol. Genet. Metab.* **68**, 232–236 (1999).
92. Coschigano, K., Clemmons, D., Bellush, L. & Kopchick, J. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* **141**, 2608–2613 (2000).
This paper describes the increased longevity of the growth-hormone receptor knockout mouse.
93. Sonntag, W. *et al.* Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. *J. Gerontol. A Biol. Sci. Med. Sci.* **54**, B521–B538 (1999).
94. Bartke, A. *et al.* Extending the lifespan of long-lived mice. *Nature* **414**, 412 (2001).
95. Kirkwood, T. B. L. & Rose, M. R. Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. Lond.* B **332**, 15–24 (1991).
96. Medawar, P. B. Old age and natural death. *Mod. Q.* **2**, 30–49 (1946).
97. Medawar, P. B. *An Unsolved Problem of Biology* (H. K. Lewis, London, 1952).
98. Charlesworth, B. Mutation–selection balance and the evolutionary advantage of sex and recombination. *Genet. Res.* **55**, 199–221 (1990).
99. Brogiolo, W. *et al.* An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* **11**, 213–221 (2001).
100. Miskin, R. & Masos, T. Transgenic mice overexpressing urokinase-type plasminogen activator in the brain exhibit reduced food consumption, body weight and size, and increased longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **52**, B118–B124 (1997).
101. Migliaccio, E. *et al.* The $p66^{shc}$ adaptor protein controls oxidative stress response and life span in mammals. *Nature* **402**, 309–313 (1999).
102. Arantes-Oliveira, N., Apfeld, J., Dillin, A. & Kenyon, C. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* **295**, 502–505 (2002).

Acknowledgements
The authors acknowledge support for their work from the Biotechnology and Biological Sciences Research Council, the Natural Environment Research Council, the European Union FP5 and the Royal Society.

Online links

DATABASES
The following terms in this article are linked online to:
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
Akt1 | *chico* | *Dp110* | growth hormone | growth hormone receptor | Igf1 | *Inr* | Insr | Insrr | *ovo* | *Pit1* | prolactin | *Prop1* | thyroid-stimulating hormone
Mouse Genome Informatics: http://www.informatics.jax.org/
Ames | *Ghrhr*^{lit} | Snell
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Huntington disease | leprechaunism | type II (non-insulin-dependent) diabetes
WormBase: http://www.wormbase.org/
AAP-1 | *age-1* | AKT-1 | AKT-2 | DAF-2 | DAF-12 | *daf-16* | INS-1 | PDK-1

FURTHER INFORMATION
Alfred Russel Wallace: http://www.wku.edu/~smithch/home.htm
David Gems' laboratory: http://www.gene.ucl.ac.uk/nematode/
Encyclopedia of Life Sciences: http://www.els.net/
Ageing as a global challenge in the new millennium | Ageing genes: gerontogenes
Linda Partridge's laboratory:
http://www.gene.ucl.ac.uk/flies/partridge.html
Access to this interactive links box is free online.