

EVOLUTIONARY AND MECHANISTIC THEORIES OF AGING

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■ **Abstract** Senescence (aging) is defined as a decline in performance and fitness with advancing age. Senescence is a nearly universal feature of multicellular organisms, and understanding why it occurs is a long-standing problem in biology. Here we present a concise review of both evolutionary and mechanistic theories of aging. We describe the development of the general evolutionary theory, along with the mutation accumulation, antagonistic pleiotropy, and disposable soma versions of the evolutionary model. The review of the mechanistic theories focuses on the oxidative stress resistance, cellular signaling, and dietary control mechanisms of life span extension. We close with a discussion of how an approach that makes use of both evolutionary and molecular analyses can address a critical question: Which of the mechanisms that can cause variation in aging actually do cause variation in natural populations?

INTRODUCTION

Senescence is defined as a decline in performance and fitness with advancing age (34, 117, 123). Often referred to simply as “aging,” senescence is a nearly universal feature of multicellular organisms, and understanding why it occurs is a long-standing problem in biology. Both proximate (mechanistic) and ultimate (evolutionary) causes of aging have been debated since the early twentieth century. Evolutionary biologists have been drawn to the question because of the seemingly maladaptive features of aging and because the evolution of life span has become a paradigm for the evolution of complex phenotypes. Molecular and cellular biologists have become increasingly interested in the problem of aging as age-related disorders and life span itself have proven amenable to study using standard tools of these disciplines. Although strides have been made in understanding both mechanistic and evolutionary causes of aging, a general consensus has not been reached on the relative importance of the many proposed

molecular mechanisms or on the relative contributions of different evolutionary processes.

Our goal here is to provide an integrated view of the ultimate and proximate causes of aging. Several recent reviews focus on genetic and physiological processes that affect life span and rates of senescence (48, 49, 62, 84, 110, 143, 147). There are also several sources of information on evolutionary theories of aging, although most of these are now more than a decade old (15, 34, 70, 117, 123). We therefore present a concise review of the relevant literature from both fields, with an emphasis on studies using insects and nematodes. We indicate questions and areas that we believe have been understudied and that could make substantial contributions to future aging research.

EVOLUTIONARY THEORIES OF AGING

Development of the Theory

The general evolutionary theory of senescence posits that senescence occurs because the force of natural selection declines with age in populations that have age structure. Populations with age structure are those that consist of individuals of different ages, and therefore have overlapping generations. In such populations, a gene that affects survival or reproduction at a young age has a greater effect on the Darwinian fitness of an individual than does a gene with the same magnitude of effect expressed later in life.

To understand this concept, it is easiest to imagine a nonsenescent population with age measured on a convenient timescale (years for human or days for *Drosophila*). In such a population, mortality and reproductive rates are constant at each age (Figure 1). Death occurs for reasons unrelated to age, e.g., accident, predation, or non-age-specific disease. In the evolutionary literature, mortality from such sources is called *extrinsic mortality*. Starting with this nonsenescent population, assume that a new mutation arises that affects survival during a specific adult age class. Hamilton (43a) reasoned that the fate of this mutation would be determined by its effect on the fitness of the organism bearing it. He proposed that the fitness of a genotype is equivalent to its intrinsic rate of increase (r), which is the long-term population growth rate associated with a specific schedule of survival and reproduction.

Using this measure of fitness, it is possible to calculate the strength of natural selection on any mutation that causes a change in reproduction or survival during a specific time in the life of the organism (Figure 1). Derivation of the appropriate equations is outlined in the Appendix. The general conclusions arising from these calculations can be summarized as follows. The force of selection on survival is constant until the age of first reproduction, after which it always declines with age. For reproduction, the force of selection declines with age in a stable or growing population, but it can increase with age if the population is rapidly decreasing

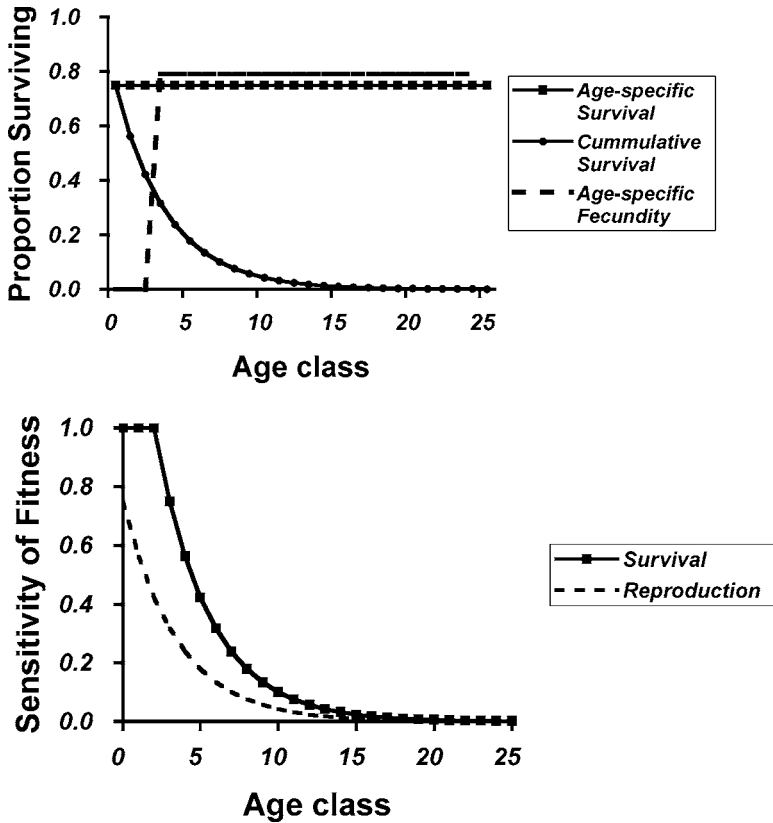


Figure 1 The top panel shows the demographic parameters for a nonsenescent population. Age-specific survival is constant and cumulative survival declines geometrically. Age-specific reproductive rates are constant after the age of first reproduction, which in this case is age class 3. The bottom panel illustrates that the force of natural selection declines with age in the population described in the top panel. The solid curve shows the force of selection acting on age-specific changes in survival; the dashed curve shows the force of selection acting on age-specific fecundity.

in size. Because rapid population decline cannot be sustained for long without extinction, the generalization normally holds.

These conclusions are valid even for populations that do not senesce. Therefore, in an initially nonsenescent population, a mutation that increases mortality or decreases reproduction early in life will be more strongly selected against than a mutation that has the same effect delayed until later in life. Conversely, a mutation that increases survival or reproduction early in life is more strongly favored than a similar mutation with a delayed age of effect. Over the course of many generations, deleterious mutations with late acting effects can increase to substantial frequencies

in the population, because they are not opposed by natural selection. As the number of these mutations increases, an initially nonsenescent population will become senescent with lower survival and reproduction at late ages than at early ones. The process can be self-reinforcing, because low survival and reproduction late in life will cause the strength of selection to decline even more rapidly with age, so that earlier-acting mutations become effectively neutral with respect to selection.

On the basis of this population genetic theory, the fundamental evolutionary cause of aging is extrinsic mortality. Because extrinsic mortality is unavoidable, the force of natural selection declines with age. A hypothetical nonsenescent, age-structured population eventually evolves a senescent life history, as long as some mutations have some degree of age-specificity in their effects on fitness.

The original theoretical analyses made several simplifying assumptions, but later work has shown that the general conclusions do not depend greatly on those assumptions (18). For example, early models assumed that demographic parameters are independent of density, but similar conclusions hold for populations with density-dependent fecundity and survival (15). The theory has also been extended to include nonrandom mating, inequality of demographic parameters in males and females, and environmentally caused fluctuations of demographic parameters. Even with these complications, the above conclusions are generally valid for predicting the genetic composition of populations at evolutionary equilibrium (15). Hamilton derived his original models assuming that mutations affect only a single age class, but he noted that the analysis could be extended to multiple age classes by calculating the net effect on fitness of mutations affecting many ages; this approach was used in later theoretical treatments (1a, 15, 16). The general evolutionary model has also been extended to include fitness effects of parental and even grand-parental care (78).

Assuming the general evolutionary model is valid, there are multiple routes by which senescence can evolve. Mutation accumulation (MA), antagonistic pleiotropy (AP), and disposable soma are special cases of the general model that make different assumptions about patterns of age-specific effects of mutations. For example, if there are alleles that have purely detrimental effects, and these effects are confined to late life, over evolutionary time these mutations rise to substantial frequencies within populations and contribute to senescence. This scenario describes the MA theory of senescence (123). This theory suggests that age-specific deleterious alleles may be highly polymorphic and distributed throughout the genome. Because the effectiveness of natural selection depends on the severity of deleterious effects as well as the age of onset of effects, MA predicts that many genes contributing to senescence may have individually small effects on late-life survival and health. There may also be individual alleles with severe effects, but these are less numerous than mildly detrimental alleles. A major-effect mutation that has often been cited as an example of MA is the dominant mutation in the *HD* gene, which causes Huntington disease in humans (15).

An alternative scenario involves mutations with pleiotropic effects on different age classes. If there are some mutations that have beneficial effects at young ages,

but deleterious effects at late ages, these mutations will be strongly favored by selection. Mutations with the opposite pattern of effects (beneficial late, deleterious early) will be strongly disfavored. For example, mutations that lead to increased reproduction at early ages might have generally deleterious effects at later ages. Increased reproduction in *Drosophila melanogaster* females causes a delayed pulse of increased mortality, which suggests that evolutionary changes that increase early reproduction lead to decreased survival at later ages (132). This scenario is known as the AP theory of senescence (123). Specific mutations that appear to fall in this category are discussed below.

The disposable soma theory (68) is a special case of AP. It is based on the premise that somatic maintenance and repair are metabolically costly and that metabolic resources devoted to reproduction are not available for maintenance and repair. Therefore, an allele that increases the allocation of energy to reproduction has the pleiotropic effect of decreasing allocation to maintenance and repair. This theory posits a selective advantage for organisms that allocate most of their resources to development and reproduction and that allocate just enough to somatic maintenance to keep the organism in good condition for the expected duration of life. Any additional resources would increase fitness more if they were devoted to reproduction rather than to somatic maintenance. Under this model, the progressive accumulation of unrepaired somatic damage results in senescence and eventual death.

Tests of the Theory

Because the evolutionary theory posits that the fundamental cause of aging is extrinsic mortality, it has been argued that the general evolutionary theory can be tested by comparing populations of the same species that differ in the level of extrinsic mortality, because changing the survival values should cause changes in the rate at which sensitivity functions change with age (69, 115, 149). However, this simple prediction ignores the fundamental requirement for age specificity. For example, if two populations differ only in the survival values, and the difference is constant with respect to age, the populations will experience the same rate of change of sensitivity functions with age, because the difference in survival is exactly offset by the resulting change in r (1a, 15, 23).

In contrast, if demographic changes have some age specificity (for example, being confined to adult ages), the sensitivity functions can change. For example, if fecundity is increased (a change that affects only adults) in a density-independent population, there will be an increase in r , and the fitness sensitivities decline faster with age. Under density dependence, relationships between demographic changes and the sensitivity functions can be complex and depend on the age specificity of demographic changes and on whether density dependence is mediated through changes in survival or through changes in reproduction (1a, 15).

The general evolutionary theory has been tested in laboratory and field experiments, and by comparative analysis (15, 34, 123). A great deal of comparative

evidence supports the general evolutionary theory, even accounting for the above caveats (15, 123). The theory predicts that species with low extrinsic adult mortality (e.g., low predation rates) should evolve delayed senescence relative to species with high adult extrinsic mortality. It has been argued that birds tend to senesce much more slowly than equivalently sized mammals, despite having higher metabolic rates, because birds have lower rates of extrinsic mortality owing to their ability to fly (51, 52). One would then predict that flying mammals also senesce more slowly than equivalently sized nonflying mammals. Indeed, bats live about three times as long as nonflying mammals with similar basal metabolic rates, even when differences in hibernation patterns are accounted for (7). A similar prediction has been confirmed by comparing senescence patterns in freshwater zooplankton from high- and low-risk environments (28).

Taxa, such as insects, characterized by high extrinsic mortality usually exhibit rapid senescence and short life spans even when reared in protected environments. A striking exception to this pattern is the extreme longevity of eusocial insect queens (61). This pattern supports the evolutionary theory because, in many species, established queens are nearly immune to predation pressure and are thus subject to low extrinsic mortality (50). Colony reproductive success also tends to increase with colony size, which means that queens experience an age-dependent increase in reproductive success (61), thereby slowing the rate of decline of $s(x)$ with age (see above).

Experimental data also support the theory. Several artificial selection experiments have been conducted in which the force of natural selection was increased at late ages by selectively breeding from older individuals (86, 111, 122). In each of these experiments, conducted in different laboratories using different source populations, the mean and maximum life spans increased after relatively few generations of selection.

Perhaps the most direct experimental test of the general theory is that reported by Stearns et al. (138). Replicate populations of *D. melanogaster* were subjected to different adult mortality rates while keeping the larval environment constant. The populations evolved life span differences in the predicted direction: Populations with elevated adult mortality evolved life spans that were shorter than those of low-mortality controls.

In light of the empirical results supporting the general evolutionary model, evolutionary biologists have been interested in determining the relative importance of the MA and AP mechanisms. Although the two processes are not mutually exclusive and may contribute to senescence in any population, they assume different patterns of allelic variation, and unique predictions can be made for each. Nevertheless, evolutionary biologists have been interested in determining if both mechanisms do contribute, or if one or the other is the primary evolutionary cause of aging.

Antagonistic pleiotropy has received considerable support from artificial selection experiments and from analysis of longevity-enhancing mutations in *D. melanogaster* (22, 86, 111, 122, 124, 145). The artificial selection experiments

used selective breeding from relatively old individuals. In one of these experiments, in which five replicate control and selection lines were maintained, the mean longevity of females increased by 25% after 15 generations, but the early-life fecundity of females was depressed (122). Similar experiments conducted in other laboratories indicated that life span could be increased by selecting on late-age reproduction and that life span extension was often (but not always) accompanied by decreased early fecundity (86, 87, 122, 125), increased development time (109), and increased resistance to oxidative stress (3, 86, 109, 122). This pattern has been interpreted as evidence for a negative genetic correlation (or “trade-off”) between early- and late-life fitness caused by antagonistic pleiotropy.

Recent discovery of single-gene mutations that confer extended longevity also provide support for the AP theory. Null or hypomorphic mutations in several genes can dramatically increase life span in *D. melanogaster* (22, 81, 120, 145) and in *Caenorhabditis elegans* (62). Each of the *Drosophila* mutations causes reduced fertility or sterility, at least under some conditions. Fertility decrements or developmental delays are also seen in mutations conferring increased life span in *C. elegans*, although there are few reports of longevity-enhancing mutations that do not affect fertility under standard laboratory conditions (62). Because most of these longevity-enhancing mutations are potentially involved in regulation of growth and metabolism, they also provide support for the disposable soma version of the AP theory.

The MA theory has received more limited support (17, 57, 73, 121, 131). A unique prediction of the theory is that MA should lead to age-related increases in inbreeding depression and in the genetic variance of fitness components. Although AP is also consistent with an increase in additive genetic variance with age, only MA predicts that dominance variance, the variance among homozygous lines, and the inbreeding load increase with age (17). To our knowledge, these predictions have only been tested in *D. melanogaster*. Increasing inbreeding depression with age seems to be a general pattern, as it has been reported in several independent experiments (17, 58, 59, 134). Increasing dominance variance and increasing variation among homozygous lines has also been reported (59, 72).

Although these results support MA, there is one caveat to this interpretation. Assuming that only AP causes senescence, declining vigor at late ages may cause old individuals to be more susceptible to the deleterious effects of inbreeding and of pleiotropic alleles. This means that the average effect of deleterious alleles increases with age. An increase in the effects of alleles leads to increasing inbreeding depression and increasing genetic variance, even in the absence of MA. Perhaps the only way to conclusively demonstrate that an MA contributes to aging is to conduct molecular tests of selection on a candidate gene. If patterns of variation were consistent with neutral evolution rather than with positive selection, then MA would be strongly supported.

On the basis of current evidence, it appears nearly certain that AP is a cause of senescence and that MA likely contributes. In a fundamental sense, the evolutionary approach to the problem of aging has been successful. Although there are still

important questions to answer, the basic evolutionary cause of aging is well understood. The theory is codified mathematically, and predictions and assumptions of the theory have been repeatably supported by experiment.

But does understanding the ultimate cause of the phenomenon provide any insight into proximate mechanisms? Linda Partridge (108) has argued that it does. She reasons that the AP model implies that there should be conserved aging mechanisms across broad taxonomic ranges, because trade-offs between fitness components are likely based on similar physiological processes. This reasoning implies that model organisms are more likely to be relevant to human aging if aging has evolved via pleiotropy. In fact, an insulin-signaling pathway that regulates life span appears to be highly conserved in yeast, nematodes, fruit flies, and mammals (62). However, highly conserved pathways might be more subject to selective constraint and thus not contribute greatly to naturally occurring within-species variation in longevity. We return to this issue below.

MECHANISTIC THEORIES OF AGING

The number of different mechanistic explanations for aging has been estimated to be in the hundreds (93), but recent discoveries have focused attention on only a handful of these. In particular, the discovery of single-gene mutations and transgenes that substantially increase longevity has implicated stress-resistance mechanisms, cellular-signaling pathways, and dietary restriction as proximate mechanisms that regulate life span. Other theories, such as “rate of living” and telomere shortening, have not been as well supported by recent experiments (147). We concentrate here on the evidence and possible connections among stress resistance, cellular signaling, and dietary restriction.

Oxidative Stress Resistance

Harman (45, 46) first proposed that aging is a consequence of cellular damage caused by reactive oxygen species (ROS). ROS generation in animals occurs mainly in the mitochondria, where more than 90% of the oxygen used by cells is consumed (112). About 1% of the electrons transported to oxygen in a cell result in the production of superoxide radicals (O_2^-) (11, 12, 26). O_2^- itself is toxic, especially through inactivation of proteins with iron-sulfur centers (38), and it interacts with nitric oxide to form peroxynitrite, which may damage cells by promoting membrane lipid peroxidation and nitration of protein (10). O_2^- and hydrogen peroxide (H_2O_2 , another ROS) cause the production of hydroxyl radicals (OH^-), which damage proteins, lipids, and DNA (116, 133b).

Several enzymes are thought to mitigate oxidative damage in cells; as a class, these are known as antioxidants. Superoxide dismutase (SOD) occurs in two forms in insects, all of which convert O_2^- to H_2O_2 . Cu/Zn SOD/SOD1 exists in the cytoplasm and outer mitochondrial space; MnSOD/SOD2 is produced only in the

mitochondria. Catalase (CAT) is also an important antioxidant that converts H_2O_2 to O_2 and H_2O .

D. melanogaster has been used extensively in tests of the oxidative stress theory of aging. Aging in *Drosophila* is correlated with increased levels of protein carbonyls and 8-oxo-guanine (105, 136), which are the result of oxidative damage (35, 38a). Artificial selection for late-life reproduction in *Drosophila* results in populations with increased life span and, often, increased resistance to oxidative stress (86, 109, 122). For example, increased life span in two such selected strains was correlated with increased oxidative stress resistance and increased expression of antioxidant genes, including *CuZnSOD*, *MnSOD*, and *Cat* (4–6, 27). Interestingly, in one long-lived strain there was an increase in the expression of SOD, and in the other it was CAT; it appears that selection achieved high longevity by increasing levels of antioxidants, but different enzymes were selected in the two strains. In another selection experiment (96) there was increased longevity, increased resistance to oxidative stress (paraquat test), but decreased CAT activity. This long-lived strain also had higher metabolic rates, higher metabolic potential, and higher average walking speed than controls did.

Transgenic studies of *Drosophila* also provide support for the oxidative stress theory (Table 1). Tissue-specific (GAL4/UAS) or conditional (FLP-out) gene expression systems have been used to overexpress *Cu/ZnSOD* (107, 140) and *MnSOD* (139, 141). Conditional overexpression of *Cu/ZnSOD* or *MnSOD* under the control of a heat shock promoter produced life span increases of up to 48% and 33%, respectively, without decreasing metabolic rate (140, 141). Conditional overexpression of both *Cu/ZnSOD* and *MnSOD* in the same flies had partially additive effects

TABLE 1 Single-gene manipulations that increase life span in *Drosophila melanogaster*

Gene	Manipulation	Specificity	Life span increase ^a	Reference
CuZnSOD	Transgene	Adult tissues	48%	(140)
CuZnSOD	Transgene (human)	Motor neurons	40%	(107)
<i>EF1α</i>	Transgene	Adult tissues	41% ^b	(133a)
Hsp70	Transgene	Adult tissues	8% ^c	(144)
MnSOD	Transgene	Adult tissues	75%	(141)
MsrA	Transgene	Nervous system	70%	(127)
Chico	Hypomorphic mutant		52%	(22)
Indy	Hypomorphic mutant		80%	(120)
InR	Hypomorphic mutant		85%	(145)
Methuselah	Hypomorphic mutant		35%	(81)

^aMaximum life span extension reported in the cited study.

^bBut see Reference 142a.

^cIncrease in life expectancy calculated from change in two-week survival rate.

(139). In contrast, conditional overexpression of *CAT* had neutral or slightly negative effects on life span, even when combined with overexpression of *Cu/ZnSOD* or *MnSOD* (140, 141). Longevity may critically depend on SOD expression in particular tissues, as overexpression of SOD enzyme only in adult motor neurons (using the GAL4/UAS system to drive human *Cu/ZnSod*) caused a 40% increase in life span (107).

It has been argued that the longevity extension achieved in these transgenic studies was due to the artificially short life spans of the laboratory stocks that were used (104, 137). This hypothesis was recently tested by Spencer et al. (137), who placed the transgenic *SOD* construct of Parkes et al. (107) on several different long-lived genetic backgrounds. The construct increased life span overall, although there were sex- and genotype-specific effects. The longevity-enhancing effect was more pronounced in females, in which it significantly extended life span in 6 of 10 different long-lived backgrounds (life span was also extended in the other 4 backgrounds, but not significantly). In males, life span was extended in 5 of 10 backgrounds, but the difference was significant in only 1 case. These results show that the longevity-enhancing effect of *SOD* overexpression is not limited to short-lived laboratory strains of flies.

Pharmacological treatment with antioxidants can also increase longevity. Treatment of *C. elegans* with a synthetic SOD-CAT mimic increased the life span of wild-type worms by 44%, while treatment of prematurely aging worms rescued normal life span (~67% increase) (94). When Melov et al. (94) used the same technique, treatment with an MnSOD mimic resulted in life span extension in MnSOD nullizygous mice, although no extension was observed in wild-type mice.

Despite strong evidence that overexpression of antioxidant genes can increase *Drosophila* life span, a recent study suggests that naturally long-lived insects (ant queens) do not achieve their longevity via upregulation of *SOD* (106). Queens of *Lasius niger* can live up to 28 years, whereas workers live only 1 to 2 years and males live only a few weeks. In this species, queens have CuZnSOD activity levels similar to those of *D. melanogaster*. However, they have lower expression and lower activity of this enzyme than do workers or males, indicating that high *SOD* activity is not required for the evolution of extreme life span.

In addition to scavenging ROS, an organism can reduce oxidative damage by reducing the amount of ROS generated in the first place (11, 12, 37, 135). In support of this hypothesis, comparative studies in vertebrates indicate that ROS production varies inversely with maximal life span and that long-lived species produce fewer ROS than do short-lived species (8, 9, 74, 112). This relationship is apparently not due to a general decrease in metabolism with increasing life span, because long-lived species (35) and long-lived artificially selected strains (96) consume more oxygen per unit mass than do short-lived organisms.

One mechanism for reducing ROS production is by increasing respiration efficiency. *Drosophila* studies have shown a general age-dependent decrease in abundance of mRNAs involved in respiratory function (13, 150) and reduced activity of different respiratory complexes (98, 135). Comparative analysis of five fly species

that vary more than twofold in life span indicated a positive correlation between life span and cytochrome oxidase activity, and negative correlations between life span and H₂O₂ production and oxidative damage (136). In *C. elegans*, dietary restriction of ubiquinone (coenzyme Q, a carrier of electrons and protons in complexes I, II, and III) results in a 50% increase in life span (77). The *clk-1* long-lived mutant is deficient in the function of a gene involved in ubiquinone synthesis (33, 76, 95, but see 77).

Repair of oxidative damage is a means of life span extension that has received less attention than ROS scavenging or respiration efficiency. Recent studies suggest that methionine sulfoxide reductase (MsrA) may be important in aging because of its ability to repair oxidized proteins. Overexpression of this gene in the nervous system of *D. melanogaster* showed that overexpressing flies lived up to 70% longer than controls (127) (Table 1). In yeast, MsrA expression is induced by oxidative stress (44) and deletion of the gene results in extensive oxidative damage and cell death (101). The gene also shows an age-related decrease in expression in rats (113), and knock-out mice lacking the gene have short life spans (101).

Other Types of Stress Resistance

Mild heat stress can cause a small but significant increase in the life span of *Drosophila* and *C. elegans* (64, 82, 91, 92). Larger heat-induced increases in life span were achieved in *Drosophila* transgenic for extra copies of the *hsp70* gene (Table 1), demonstrating that a heat shock protein can have positive effects on longevity (144). Aging itself induces hsp70 protein in flight and leg muscle, even without heat shock (148). Aging also induces other heat shock proteins such as hsp22 (67). In *C. elegans*, overexpression of *heat-shock factor* (*hsf-1*), a master transcriptional regulator of stress response and protein-folding homeostasis, increases life span (56).

If *hsp* expression is a response to protein damage that can increase or accumulate with age, then its effect on life span might be due partly or wholly to the repair of oxidative damage. Indeed, mutations in *Cat* and *CuZnSOD* caused induction of *hsp70* in young flies, which suggests that aging-related *hsp* induction may be a response to oxidative damage (148). However, there is little other evidence relating to this hypothesis, and hsp expression may have longevity-enhancing effects independent of the repair of oxidative damage.

Responses of aging flies to starvation, desiccation, and temperature extremes have been investigated in several artificial selection experiments (96, 103, 118, 130). Although these studies have generally found correlations between life span and stress resistance, the details have been inconsistent across studies. For example, Rose's (130) extended longevity lines had increased starvation and desiccation resistance, and additional selection for resistance to these stressors led to further increases in longevity (126). However, lines that were independently selected for starvation resistance did not show a correlated increase in longevity (47). Luckinbill's (36, 86) selection lines showed no relation between extended longevity and

starvation resistance, an inconsistent relationship between longevity and desiccation resistance (36), and a positive association between life span and cold tolerance (85).

Later experiments with Luckinbill's lines indicated that heat stress caused increased longevity in normal-lived animals but decreased longevity in flies from the long-lived selection strains (75). However, a subsequent study reported contradictory results in lines derived by inbreeding from Luckinbill's lines: Long-lived strains were relatively resistant to heat stress and controls were resistant to cold stress (96). These long-lived lines were also more starvation and desiccation resistant than controls. However, there was substantial variation in resistance to stressors among the different long- and short-lived strains used in this experiment, which suggests again that different mechanisms of longevity extension had been exploited in the different long-lived lines.

Signal Transduction Pathways and Aging

During the past 15 years, many longevity-enhancing mutations in *C. elegans* and *D. melanogaster* have been discovered (Table 1). The first such mutations were discovered in nematodes (25, 40, 60, 63, 66, 100) and can extend life span two- to threefold. Single mutations in fruit flies extend life span by 35% to 100% (22, 81, 120, 145). Many of the mutations in worms and flies involve signal transduction pathways (22, 25, 63, 66, 81, 100, 145). Signal transduction mutations also extend the life span of nondividing yeast (*Saccharomyces cerevisiae*) cells and the replicative life span of budding yeast mother cells (19, 30, 31, 80, 83, 142).

INSULIN SIGNALING A conserved signal transduction pathway involving insulin and insulin-like molecules appears to negatively regulate life span in *S. cerevisiae*, *C. elegans*, *D. melanogaster*, and possibly also in mammals (62, 84). This pathway can affect life span by several proposed mechanisms. Mutations in the pathway that increase longevity normally result in sterility, thereby reducing metabolic costs associated with reproduction (22, 62, 145). This pathway might also interact with oxidative-stress resistance mechanisms, because many of the longevity-enhancing mutations also cause upregulation of antioxidant genes, resistance to oxidative stress, or both (31, 43, 53, 81, 146).

Single mutations in *C. elegans* can extend life span up to threefold compared with wild-type controls (39, 40, 60, 63). These mutations occur in a cell-signaling pathway that is similar to the insulin and insulin-like growth factor pathway in mammals. In this pathway, a cell surface receptor (*daf-2*) is activated by insulin-like ligands, and the resulting signaling cascade causes deactivation of a gene encoding a forkhead transcription factor (*daf-16*). Mutations that decrease signaling (weak mutations) through this pathway led to expression of *daf-16* and increased longevity. Strong mutations in the same genes led to arrested development as a dauer larva. Dauers are a stress-resistant larval stage that occurs in wild-type worms under conditions of food limitation or crowding. These

environmental stressors reduce signaling through the pathway and produce a long-lived but nonreproductive individual. Once signaling is restored, the dauer can resume development to become reproductive adults. Olfactory and gustatory sensory systems are involved in dauer formation (128), and ablation or mutation of sensory cells also extends life span (2). Ablation of germline tissue also increases life span, but this requires *daf-16* expression (55).

A similar signaling pathway modulates life span in *D. melanogaster*. The pathway is less well characterized in flies, but a direct effect on life span has been established. The fly equivalent of *daf-2* is *Insulin-like receptor (InR)*. Weak mutations in *InR* produce dwarf flies that live up to 85% longer than controls; null alleles are lethal (145). Null mutations in *chico* (*chico¹*), a substrate for *InR*, produce dwarf flies that live up to 52% longer than wild-type flies (22). Both kinds of mutants are sterile, and life span extension is greater in females than in males. *InR* dwarf mutants are also deficient for juvenile hormone production; treatment of mutant females with a juvenile hormone analog produces near-normal longevity and promotes vitellogenesis (145).

Insulin-signaling and stress-resistance mechanisms seem to be intimately connected. Mutations that increase longevity in worms and flies typically increase the expression of antioxidant genes, increase resistance to oxidative stress, or both (31, 41, 43, 79, 81, 146). A notable exception is the *Drosophila chico¹* mutant, which is not consistently associated with increased oxidative stress resistance (22). Homozygotes and heterozygotes for this mutation do show increased starvation resistance, and homozygotes have increased expression of total SOD. This result raises the possibility that SOD modulates life span via some mechanism other than its antioxidant activity.

Life span-extending mutation in the insulin-signaling pathway can also induce increased expression of heat shock proteins and increased thermotolerance in worms and flies (22, 43, 81). In *C. elegans*, expression of *hsf-1* is required for the longevity-enhancing effects of *age-1* and *daf-2* mutations (56, 99). *Hsf-1* is also required for temperature-induced dauer formation in *age-1* mutants (99). Extra copies of a heat shock protein (*hsp-16*) increase life span, but this effect is abolished by a *daf-16* mutation that reduces the expression of *hsp-16*.

OTHER SIGNALING PATHWAYS The *D. melanogaster* mutant *methuselah* extends longevity by 35% relative to that extended by wild-type controls (81). It also confers resistance to a variety of stressors. Little is known of its specific function, but the gene product of *methuselah* is a seven-transmembrane G protein, presumably involved in cellular signaling. No homologs of this gene have yet been identified in *C. elegans* or vertebrates. Schmidt et al. (129) reported natural polymorphism at this locus in populations of *D. melanogaster* and *D. simulans* on the east coast of North America. Patterns of molecular variation were consistent with strong positive selection and rapid evolution. However, there is no published evidence of direct correlation between variation at this loci and variation in life span.

Mutations in the gene *Indy* (*I'm not dead yet*) also dramatically increase life span. Flies heterozygous for P-element insertions in this gene live up to 80% longer than controls; insertion homozygotes live 10% to 20% longer (120). Sequence analysis suggests that *Indy* is a dicarboxylate cotransporter: a membrane protein responsible for the uptake or reuptake of di- and tricarboxylic acid Krebs cycle intermediates such as succinate, citrate, and alpha-ketoglutarate. *Indy* is thought to mimic the life extension effects of caloric restriction (see below) by decreased efficiency of energy metabolism (32, 71, 89, 120).

Dietary Restriction

Dietary restriction (DR) increases life span in yeast, nematodes, flies, and mammals (reviewed in Reference 84). The mechanism by which DR increases life span is not yet known, but investigators have proposed that DR may work via increasing metabolic efficiency (and thereby decreasing ROS production) or by creating a nonreproductive dauer-like state in which energy may be invested in somatic repair rather than production of gametes.

In *Drosophila*, dilution of nutrients leads to increased life span (14, 20) and reduced egg laying in females (89). DR seems to mediate life span by reducing immediate risk of death, not by slowing accumulation of irreversible damage, because adult flies placed on DR at intermediate ages adopt the same mortality schedule as flies reared on DR since emergence (88). This result argues against the hypothesis that DR mediates life span by reducing the accumulation of oxidative damage.

It has been postulated that the insulin/IGF pathway regulates a trade-off between fecundity and longevity that is mediated by nutrition levels. Direct evidence supporting this hypothesis comes from experiments on DR in *Drosophila* carrying the insulin-signaling mutation *chico*¹. DR increased the longevity of wild-type flies, not of *chico*¹ mutants (21), indicating that DR and *chico*¹ operate in the same pathway to influence life span.

DR also operates in the same pathway as a histone deacetylase gene, *rpd3*. DR downregulates *rpd3* in flies (114), and flies heterozygous for null or hypomorphic mutations in this gene live up to 52% longer than controls do. DR does not extend life span of *rpd3* mutants, indicating that *rpd3* operates in the pathway through which DR mediates life span (119).

Sir2 is another histone deacetylase that operates in the same pathway. In yeast, increased expression of *Sir2* or decreased expression of *rpd3* extends life span (42, 65), and extension of life span by DR depends on *Sir2* expression (80). In *Drosophila*, both DR and mutation of *rpd3* cause *Sir2* to be upregulated (119). It thus appears that DR, *rpd3*, and *Sir2* act within an evolutionarily conserved pathway that affects life span.

In summary, evidence from selection experiments, transgenic manipulations, and mutant screens provide strong support that pathways related to oxidative stress resistance, insulin signaling, and DR can regulate longevity in laboratory organisms. However, the interactions between these mechanisms have not been

resolved. For example, insulin-signaling mutations can increase life span without increasing oxidative stress resistance in *Drosophila*, but not in *C. elegans*. DR seems intimately related to insulin signaling, but the mechanistic details are not known. Investigation of these relationships will be a critical focus of aging research in the coming decade. However, there are crucial questions about aging that this experimental approach has not yet addressed. In particular, the question of which mechanisms cause naturally occurring variation in aging has not been addressed. Strategies to address this question are described in the final section of this review.

DISCUSSION

Experiments using transgenics and life span mutants have been the principal means of investigating mechanistic theories of aging, and they have been enormously successful. They will likely be the tools of choice for unraveling details of currently known mechanisms and for discovering new ones. However, these experiments have not addressed two related questions: (a) Which of the mechanisms that can increase life span have been exploited by evolution to create naturally long-lived forms; and (b) which genes and pathways are responsible for the life span variation existing within natural populations? These issues are critical to understanding the causes of variation in human life span, and for devising strategies to intervene in human senescence.

It is possible that genes identified by mutant screens and transgenic studies do not account for much of the life span variation existing in natural populations. Because these genes have large phenotypic effects, and because most seem to have deleterious effects on fertility, they might be subject to strong natural selection that tends to remove variation. Thus, the aging genes that are easiest to detect may be those that are most highly constrained in the variation they exhibit both within and between species.

In contrast to the mechanistic approach, the evolutionary approach does address the questions of within- and between-species variation, and the evolutionary framework does a good job of explaining patterns of variation. However, the evolutionary paradigm has not yet identified genes or pathways causing this variation. Combining evolutionary and mechanistic tools holds great promise for finding the causes of naturally occurring variation. Genetic polymorphisms causing within-population variation in life span can be discovered using QTL and linkage-disequilibrium procedures. Until recently, most mapping studies have investigated between-population variation. However, Mackay and colleagues (24) reported that three single nucleotide polymorphism variants in the *Ddc* (*Dopa decarboxylase*) gene are significantly associated with life span variation within a natural population of *D. melanogaster* and that these variants account for a large fraction of the genetic variation in the trait. This study shows that causes of within-population variation cannot necessarily be deduced from the experiments described above. *Ddc* catalyzes the final step in the synthesis of dopamine and serotonin; it is not a candidate gene that would have been readily predicted from the well-supported mechanistic theories.

Molecular evolutionary analysis of candidate aging genes is another promising approach to finding polymorphisms responsible for within-population variation. The analysis of *methuselah* variation performed by Schmidt et al. (129) is an excellent start in this direction. As sequence data become available for more species, similar analyses, combined with phenotypic assays of life span variation, can be applied more broadly.

Sequence variation is not the only source of naturally occurring variation in life span, as is obvious when comparing the adult longevity of queens and workers of eusocial insects (61). Queens and workers develop from the same genotypes, so life span differences are purely the result of differential gene expression. These species therefore provide an opportunity to measure gene expression differences corresponding to extreme differences in life span (106), and to investigate how these differences arise. The first draft of the genome sequence of the honey bee (*Apis mellifera*) was released in January 2004 (1). This resource should promote the development of new tools for understanding the extreme longevity of social insect queens.

Other insects with unusual life histories provide a rich resource for aging research. The class includes organisms with extraordinarily short adult life spans and others with extremely juvenile periods (13a). The mayfly *Dolania americana* has been described as the insect with the shortest adult life span, with adult females typically living less than five minutes (147a). Several groups have very prolonged juvenile periods, including the periodic cicadas (Cicadidae), which require up to 17 years for nymphal development, and some wood-boring beetles (Cerambycidae and Buprestidae) that can survive over 50 years as larvae (149a). The prolonged development time of these insects is associated with a nutrient-poor juvenile diet. Data on the expression of insulin-signaling genes in juveniles and adults could provide an interesting test of the DR hypothesis.

Aging research has a long history of incorporating diverse research tools, organisms, and methodology. Progress in understanding aging in natural contexts is likely to emerge from studies using new genomic tools and in adopting techniques that combine molecular and evolutionary analysis. *Drosophila* species will continue to provide novel insights because of their genetic tractability and the rich evolutionary literature on these species. However, new model systems such as eusocial insects can provide opportunities to understand the evolutionary and mechanistic basis of life spans that are much longer than those observed in *Drosophila*. We believe that critical advances in aging research will come from pursuing diverse questions on diverse organisms.

APPENDIX

W.D. Hamilton proposed that the fate of the mutation would be determined by its effect on fitness and that the fitness of a genotype is equivalent to the intrinsic rate of increase (r) of the genotype (43a). In this context, r is the long-term

population growth rate associated with a genotype-specific schedule of survival and reproduction, defined by the Euler-Lotka equation:

$$\sum_0^{\infty} e^{-rx} l(x) m(x) dx = 1, \quad 1.$$

In this equation, x is the age class, $l(x)$ is the probability of survival from birth to age x , and $m(x)$ is the number of offspring produced by an individual of age x (where only offspring of the same sex as the individual being considered are counted) (29, 133). r is the solution to this equation and is usually calculated by an iterative process. $l(x)$ is equal to the product of the probability of surviving each age class, $P(y)$, where y varies from age 0 to age x :

$$l(x) = \prod_{y=0}^x P(y). \quad 2.$$

Following Hamilton's argument, the fate of a mutation that affects survival at age x is determined by the effect on r of the change in survival, or in other words by the partial derivative of r with respect to the change in $P(x)$:

$$\frac{\partial r}{\partial \ln P(x)} = \frac{\sum_{y=x+1}^d e^{-ry} l(y) m(y) dy}{P(x) \sum_{y=b}^d y e^{-ry} l(y) m(y)}, \quad 3.$$

where b is the age of first reproduction, and d is the age of the oldest reproductive individuals in the population. The denominator of this function is simply a measure of generation time, T , and so can be considered constant over short time periods. The sensitivity of fitness to a change in survival at age x is therefore given by the numerator of Equation 3:

$$s(x) = \sum_{y=x+1}^d e^{-ry} l(y) m(y). \quad 4.$$

Note that $s(x)$ is not affected by changes in juvenile age classes because $m(x)$ is zero at those ages. For adult age classes, the value of $s(x)$ invariably declines with age, reflecting the fact that old individuals contribute fewer offspring to future generations than do young individuals because $l(y)$ decreases with age. The rate of this decline with age can be slowed, but not eliminated, if $m(y)$ increases with age (15).

A similar calculation gives the sensitivity of r to a change in age-specific fecundity:

$$s'(x) = e^{-rx} l(x). \quad 5.$$

This function always decreases with age if $r \geq 0$. If r is negative enough to offset the decline with age in $l(x)$, $s'(x)$ can increase with age. However, a highly negative r is unlikely to persist for many generations, because a population with a negative growth rate would soon go extinct. Consequently, under biologically realistic scenarios, both sensitivity functions decrease monotonically with adult age.

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