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Integrating Evolutionary and Molecular Genetics of Aging

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Abstract

Aging or senescence is an age-dependent decline in physiological function, demographically manifest as decreased survival and fecundity with increasing age. Since aging is disadvantageous it should not evolve by natural selection. So why do organisms age and die? In the 1940's and 1950's evolutionary geneticists resolved this paradox by positing that aging evolves because selection is inefficient at maintaining function late in life. By the 1980's and 1990's this evolutionary theory of aging had received firm empirical support, but little was known about the mechanisms of aging. Around the same time biologists began to apply the tools of molecular genetics to aging and successfully identified mutations that affect longevity. Today, the molecular genetics of aging is a burgeoning field, but progress in evolutionary genetics of aging has largely stalled. Here we argue that some of the most exciting and unresolved questions about aging require an integration of molecular and evolutionary approaches. Is aging a universal process? Why do species age at different rates? Are the mechanisms of aging conserved or lineage-specific? Are longevity genes identified in the laboratory under selection in natural populations? What is the genetic basis of plasticity in aging in response to environmental cues and is this plasticity adaptive? What are the mechanisms underlying trade-offs between early fitness traits and life span? To answer these questions evolutionary biologists must adopt the tools of molecular biology, while molecular biologists must put their experiments into an evolutionary framework. The time is ripe for a synthesis of molecular biogerontology and the evolutionary biology of aging.

Keywords

Aging; Senescence; Longevity; Evolution; Genetics; Genetic Variation

1. Introduction

Aging or senescence is a progressive decline in physiological function, leading to decreased rates of survival and reproduction with increasing age and ultimately to death [1-7]. We can ask two fundamental questions about aging, one evolutionary, the other mechanistic: why do organisms age, and how do they age? On the evolutionary level, the puzzle is to understand why such an apparently maladaptive trait harbors genetic variation and evolves despite natural selection acting to increase Darwinian fitness. On the mechanistic level, the challenge is to understand the molecular basis of aging. In the last 70 years evolutionary and molecular biologists have made enormous progress in answering these questions, yet largely independently of each other [3, 5-7].

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Why do organisms age? In the 1940's and 1950's J.B.S. Haldane, P.B. Medawar, and G.C. Williams realized that aging might evolve because the force of natural selection declines with age and might thus be inefficient at maintaining function at old age [1-3, 7-9]. Subsequent theoretical work in the 1960's and 1970's, chiefly by W.D. Hamilton and B. Charlesworth, put the evolutionary theory of aging on a firm population genetic basis [3, 10-11], and by the 1980's and 1990's the theory had received major empirical support [3, 11-13]. The major lessons from this work were that (1) aging is not "programmed", but an inevitable, maladaptive byproduct of the strength of selection declining with age; (2) life span is a polygenic and genetically variable trait which responds readily to selection; and (3) evolutionary changes in life span often trade off with changes in early-life fitness traits [3, 5-7, 9, 11]. Evolutionary biologists also speculated that aging should not be affected by mutations of large effect and that different species are unlikely to share the same mechanisms of aging [2, 5-7]. However, by traditionally treating the genetics of aging as a black box, progress in the evolutionary genetics of aging has to a large extent stalled and been overshadowed by advances in molecular biogerontology.

How do organisms age? In the 1980's and 1990's several geneticists decided to apply the powerful tools of molecular genetics to the problem of aging [6-7, 14-17]. They reasoned that, if one can understand the sophisticated process of development from a fertilized egg to a complex adult by mutation analysis, one might be able to use the same approach to elucidate the mechanisms whereby organisms age. Using the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the yeast *Saccharomyces cerevisiae* as models, they had remarkable success at identifying mutations that can extend life span, in some cases more than ten-fold [18-23]. At least three major lessons emerged from these experiments, several of which contradicted evolutionary predictions or intuitions. First, although many genes affect life span, mutations in major signaling pathways can have large effects on life span. Second, several longevity mechanisms seem to be conserved among species, from invertebrates to mammals. Third, mutations influencing life span can have antagonistic pleiotropic effects on reproduction or other fitness traits; however, not all of them display such effects, and trade-offs between life span and reproduction or other fitness components are either not ubiquitous or can be uncoupled [6, 14-17, 24]. However, by focusing on mutants in laboratory models, molecular biologists have traditionally neglected to ask questions about the genetic basis of variation in aging within and among species.

Here we argue that some of the most interesting questions in the biology of aging today are directly at the interface between evolution and molecular mechanisms [6-7, 9]. Evolutionary biologists can use the extensive knowledge about mechanisms of aging to assess whether the assumptions and predictions of the evolutionary theory have been met or whether they need refinement [6-7, 9]. At the same time, molecular biologists have become interested in asking questions about aging that are inspired by evolutionary concepts. Are candidate genes affecting life span in the laboratory genetically variable and under selection in natural populations [25-26]? Are the mechanisms of aging evolutionarily conserved or lineage-specific [6-7, 27-28]? What is the genetic basis of the remarkable variation in life span among species [3, 6, 9]? How many genes affecting longevity have antagonistic pleiotropic effects [9, 24]? Are trade-offs between life span and reproduction (or other fitness components) due to differential resource allocation or signaling processes independent of metabolism [29-31]? What are the age-dependent effects of mutations on aging [7]? What is the molecular basis of plasticity in life span and is this response to the environment adaptive [6-7, 32]? Answering these and other fundamental questions about aging will require a synthesis of molecular and evolutionary approaches.

2. The Evolutionary Genetics of Aging

2.1. The Evolutionary Theory of Aging

As far as we know, most organisms probably age, from bacteria to humans [3, 33-34]. But why we must age and die has puzzled scientists for centuries. Since aging affects survival and reproduction deleteriously, it was difficult to envision how natural selection would favor it. Two thousand years ago, the Greek poet and philosopher Lucretius argued that aging and death existed for the good of society, noting that death ensured that there would always be room for the next generation [35]. Similarly, Darwin's contemporary Weissmann suggested that, in a world of limited resources, death of the elderly ensures the permanence of species by making space for more youthful individuals and their offspring [36]. Weissmann even postulated that there must exist a specific death mechanism, designed by selection to eliminate the old. However, the cost of deterioration and death to individuals likely exceeds any benefits to the group: since a long-lived organism would leave more offspring than a short-lived individual (assuming equivalent rates of reproduction), selection would not be predicted to favor such a death mechanism [2].

The evolutionary paradox of aging was not resolved until the 1940's and 1950's, when J.B.S. Haldane, P.B. Medawar, and G.C. Williams came up with three key insights [1-3, 8]. First, the world is a dangerous place. Organisms in natural populations rarely grow old: infections, predators, or accidents kill most individuals long before they would undergo intrinsic decline of old age. Second, the force of selection declines with age (Figure 1). In a dangerous world, old individuals have a higher cumulative risk of death than young individuals, and the chances of being alive and reproductive at old age are so slim that selection is weak at advanced age. Third, since the strength of selection declines with age, selection is unable to counteract deleterious effects that are expressed during old age. An early-acting mutation with negative effects on survival and reproduction will be rapidly eliminated by strong selection early in life. By contrast, if the effects of a deleterious mutation are confined to a late age, when reproduction has ceased and survival is increasingly unlikely, carriers of the mutation can pass it on to the next generation before any negative late-life effects become apparent. Natural selection will be weak and thus relatively ineffective at eliminating such mutations; over evolutionary time they should slowly accumulate in the population by genetic drift, leading to the emergence of aging.

In 1941 Haldane argued that such a scenario explains the relatively high prevalence of the dominant allele causing Huntington's disease [8]. In 1951 Medawar expanded upon Haldane's idea and suggested that the declining force of selection allows late-acting deleterious mutations in a population to accumulate over evolutionary time, an idea known as mutation accumulation (MA) [1]. In 1957 Williams built on this hypothesis by proposing that selection can favor such late-acting deleterious mutations if they have beneficial pleiotropic effects early in life when selection is strong, a concept called antagonistic pleiotropy (AP) [2, 9]. Because the advantages of such positive effects outweigh the costs at advanced age, such genetic variants would be favored and enriched in a population, thereby allowing aging to evolve. In 1977 T.B.L. Kirkwood developed an extension of the AP hypothesis by proposing that organisms face resource allocation trade-offs between energy invested into reproduction versus somatic maintenance, repair, and survival [37]. Under this "disposable soma" (DS) hypothesis, aging evolves because selection favors alleles that increase investment into reproduction at the expense of the energy required to support survival.

These basic ideas form the cornerstones of the evolutionary theory of aging [1-11]. Given that the force of selection declines with age, and assuming mutations with deleterious effects late in life, this theory explains the evolution of aging from an initially non-aging state [5].

The theory was later mathematically formalized and put on a population genetic basis by W.D. Hamilton, B. Charlesworth, and others [5, 10-11, 38-40].

More recently theoreticians have refined several aspects of the theory [6-7]. In contrast to Hamilton's classical analysis [10, 38], recent work by A. Baudisch shows that the strength of selection does not necessarily always increase with age; it can remain constant or even increase during adulthood [41], which might lead to either no, negligible, or "negative" aging [33]. Although the evidence for negligible or negative senescence is scant and aging might be ubiquitous [34], Baudisch's work suggests that the evolution of aging might be more complex than expected under the basic Haldane-Medawar-Williams model. Classical theory also predicts that the evolution of aging is driven by changes in extrinsic mortality because such changes affect how rapidly the force of selection declines with age [2, 5, 10]. Species or populations facing high extrinsic mortality should age more rapidly than those experiencing low levels of extrinsic mortality because the strength of selection declines more rapidly in the former than in the latter, a prediction experimentally confirmed in fruit flies by S.C. Stearns and collaborators [42]. However, theory developed by P. Abrams shows that in density-independent populations extrinsic mortality does not alter the rate of aging, whereas under density-dependence the effect of extrinsic mortality depends on the age-specificity of demographic changes and on whether density-dependence is mediated by changes in survival or reproduction [43-44]. Other work suggests that in social species resource transfer across generations might select for extended postreproductive survival (e.g., menopause) and shape the evolution of aging [45]. Finally, a model by Ackermann and colleagues has addressed the origin of aging in the history of life. The results demonstrate that asymmetric cell division among unicellular organisms can evolve as a strategy to limit cellular damage by distributing damage unequally and that this asymmetry causes the evolution of aging; aging might thus be a fundamental and inevitable property of cellular life [34].

Recent progress on theoretical aspects of the evolution of aging has been slow, however, and much remains to be done [6-7]. For example, more realistic models need to be developed which study the effects of different types of age-dependent mutations on aging and how such mutations interact with the environment and other genes to influence the evolution of aging [7, 46-47]. Mutations that affect aging might have more complex age-dependent effects than hypothesized by classical theory, with MA and AP/DS representing extremes along a continuum of possible mutational effects [7].

2.2. Experimental Tests of the Evolutionary Theory of Aging

The evolutionary theory of aging makes several assumptions and predictions that have been tested empirically in the laboratory. By the 1980's and 1990's the basic tenets of the theory had received solid support, mainly from laboratory selection, mutation accumulation, and quantitative genetic experiments [3-7, 9, 11-13]. While the fundamental insights of Haldane, Medawar, Williams, and others have turned out to be correct [3, 5, 9, 11], several concepts need revision today [6-7, 29, 34].

One of the strongest assertions is Williams' expectation that only organisms with a germline-soma separation should age: the germline is maintained indefinitely, but the aging soma is "disposable" after having fulfilled its reproductive role [2-4]. Thus, organisms without a soma-germline distinction (e.g., prokaryotes, many protozoans and algae, and asexual, symmetrically dividing organisms) should not age. However, the observation that asexual metazoans can undergo senescence, for example, is inconsistent with this notion [48], and Williams' germline-soma requirement turned out to be too stringent [4, 34].

Partridge and Barton hypothesized that what matters is not the germline-soma distinction, but the phenotypic distinction, or asymmetry, between parents and offspring [4]. Since organisms without parent-offspring asymmetry do not have clearly delineated age classes, the intensity of selection should remain constant, and individual aging is not expected to evolve [4]. Symmetrically dividing unicellulars should not age because parents and offspring are indistinguishable: since there is no clear age structure, selection cannot distinguish among age classes, and aging should not evolve. Aging should therefore only exist in asymmetrically reproducing organisms where aging parents are phenotypically distinct from offspring [4]. Indeed, an asymmetrically dividing bacterium has recently been found to exhibit aging, confirming this prediction [49]. Similarly, aging also occurs in *E. coli* which divides symmetrically in terms of morphology but which distributes subcellular structures unequally at cell division [50]. Cellular or subcellular asymmetry might thus be a fundamental aspect of cellular life that inevitably leads to the evolution of aging [34]. Future work in cellular biology promises to yield important insights into the origin and mechanisms of aging among prokaryotes.

Another major tenet of the evolutionary theory of aging is that aging is a heritable, genetically variable trait and that age-specific mutations exist which cause age-progressive functional decline, as predicted by the AP and MA hypotheses. A large body of work in evolutionary genetics shows that life span is a complex quantitative trait determined by many loci, with heritabilities ranging between 10 and 50% [5, 11, 13, 51-52]. Since the 1980's several artificial selection and experimental evolution experiments, mostly in fruit flies (*D. melanogaster*), have found that outbred populations contain ample amounts of genetic variation affecting aging, that this variation can readily respond to selection in the predicted direction, and that the evolution of increased longevity is often accompanied by trade-offs with early life history traits such as fecundity [12-13, 41, 53-60]. These experiments, chiefly by M.R. Rose, L.S. Luckinbill, L. Partridge, B.J. Zwaan, and others, represent some of the strongest empirical tests of the evolutionary theory of aging. In the future it will be important to combine such selection experiments with genomic and genetic analyses (e.g., microarrays, massively parallel sequencing, functional genetics) to determine the genetic basis of evolutionary change.

Evolutionary quantitative genetics has also addressed whether longevity mutations exhibit the kind of age-specific effects predicted by theory. While mutation accumulation experiments in fruit flies suggest that mutations with age-specific effects on mortality early in life might be more common than those with effects late in life [61-62], some studies have found limited support for MA [5, 11, 13, 63-66]. Several experiments have found an age-dependent increase in inbreeding depression and dominance variance in fitness components, as expected under MA [63, 66]. Thus, the relative importance of MA for the evolution of aging remains somewhat unclear [5, 7, 11, 13]. Assessing the significance of MA is complicated by the fact that the types of mutations expected under MA represent an extreme case, whereas in reality mutations might exhibit a continuum of age-specific effects [7]. Unfortunately, we do not know much about the spectrum of such age-specific effects, and future work in evolutionary genetics is required to address this problem.

The AP hypothesis has overall received better empirical support than MA, mainly from selection experiments and mutant analyses [3, 5, 9, 11, 13]. By selecting on breeding at late ages, or directly on increased life span, several experiments have found that the evolution of increased longevity is correlated with decreases in fitness traits such as early fecundity or egg-to-adult survival (viability), suggesting the existence of a negative genetic correlation, or trade-off, between adult survival and other fitness components [55-59]. While such trade-offs with life span are in principle also consistent with linkage disequilibrium, they are most

readily explained by selection on alleles exhibiting antagonistic pleiotropy, as originally predicted by Williams [2, 9, 13].

Although alleles in natural populations might have more subtle effects than laboratory induced mutations [9, 26], mutant analyses have also uncovered alleles of the kind Williams' envisaged. Several null or hypomorphic mutants in *D. melanogaster* and *C. elegans* exhibit life span extension at the expense of reduced fecundity or fertility, a pattern consistent with AP [19, 21-22, 67-68]. Similarly, many mutations that extend life span decrease fitness components other than reproduction, for example causing slow development or adult dwarfism [14, 22, 68]. However, there has generally been a surprising lack of effort to investigate AP, and for many laboratory induced longevity mutants we do not know how they affect fitness components [9, 24].

There is also a growing number of examples suggesting that trade-offs between life span and early fitness traits are either not ubiquitous or can be uncoupled [9, 16, 24, 29-31, 60]. However, sometimes trade-offs might be present but not apparent [9]. Certain mutations in the *C. elegans* genes *age-1* and *daf-2* cause life span extension but with little or no apparent fitness costs in terms of developmental rate, activity, or fertility [69-70]. Yet when the mutants are nutritionally stressed or competed against wild-type individuals, they have lower fitness than wild-type [69-70]. Thus, the AP/trade-off model is at least partly correct, but it remains unclear how many alleles actually exhibit AP [7, 9, 13]. Moreover, neither the AP nor the MA model is fully sufficient to explain the age-dependent patterns of mortality observed in quantitative genetic experiments [61, 71-72]. A better understanding of these issues will require the isolation and characterization of naturally occurring alleles and detailed investigations into the mechanisms underlying trade-offs [29-31].

What about the overall genetic architecture of life span? Evolutionary biologists have proposed that aging is affected by many genes with small, additive effects and that it would thus be difficult to find major mutations with a large impact upon aging [2, 6, 73-74]. However, this notion is at odds with the success of molecular geneticists at identifying major longevity mutants [14-22]. Mutations in homologous genes can clearly have major effects on life span across species, including changes in insulin/insulin-like growth factor (IGF) signaling (IIS), target of rapamycin (TOR) signaling, the dietary restriction (DR) pathway, and other pathways [14-18, 73], although it remains unclear to what extent the identified aging genes contribute to genetic variance in natural populations [26].

How can changes in single genes affect a complex trait such as life span, and how can these observations be reconciled with the notion that aging is a polygenic trait, determined by the effects of many genes? [6] A likely answer is that mutation analyses are biased towards detecting large effects and that many major mutations are found in genes that occupy central regulatory, or upstream, positions in signaling pathways or networks. Even if a polygenic trait is determined by many loci, it is unrealistic to assume that all genes have small additive effects of identical size upon the trait. Some genes will be more pleiotropic than others, or exert stronger regulatory effects than other components of the pathway. Advances in molecular genetics, biochemistry, and evolutionary biology have clearly shown that the genetic architecture of quantitative phenotypes is more complex than simple additive genetic models suggest [73-74]. For example, life span is known to be affected by epistatic gene interactions [74-77], as is expected if aging is determined by complex pathways and networks [47]. One possibility then is that major longevity mutations regulate the activity and effects of many other, more downstream, genes affecting life span [6, 27, 78-80]. Indeed, mutations in the IIS pathway influence life span by converging onto the forkhead transcription factor FOXO/DAF-16, which regulates hundreds of downstream genes [27,

79]. The effects of reduced IIS on life span thus seem to be caused by small changes in many genes, confirming that aging is a highly polygenic trait [6, 27, 79].

2.3. Evolutionary Genetics of Aging in Natural Populations

Patterns of age-specific survivorship and reproduction are essential components of Darwinian fitness and life histories. As such, intense selection on these traits is predicted to result in reduced genetic variance, relative to other quantitative traits, in natural populations [81]. Despite this, the rate of age-related decline in a suite of phenotypes (e.g., survivorship, fecundity, and physiological performance) is variable among individuals, populations, and species. The prerequisite for the evolution of aging is simply the presence of age structure in a population (i.e., fewer individuals survive to each subsequent age class) and the resulting decline in the strength of selection as a function of age [2, 10-11]. In the aging literature, there has been a pronounced emphasis on mortality rates as a function of age. Both the AP and MA hypotheses predict an association between the level of extrinsic mortality and the rate of intrinsic, physiological decline with increasing age: as extrinsic mortality increases, late-onset deleterious mutations accumulate at a faster rate over generational time, leading to accelerated senescence. The extrinsic mortality that drives the evolution of senescence represents the cumulative effects of interactions between organisms and their biotic and abiotic environment. Thus, the analysis of aging, life span, and correlated life history traits in wild populations is essential to generating a comprehensive picture of the aging process [6-7, 82-83].

The biology of aging and tests of the evolutionary theory of aging have historically been focused on model genetic systems in a laboratory environment (see section 2.2.). The use of model systems offers a wide range of advantages: ease of culture, controlled matings and quantitative genetics, selection analyses, and molecular genetics. However, generalizing such investigations to aging in the wild may not be straightforward [44, 84]. Life span and rates of aging, as with other quantitative traits, are greatly influenced by environmental parameters and variance. The laboratory is an obviously artificial and often optimal environment; adaptation to the culture environment proceeds very quickly and has widespread effects on life histories [e.g., 85]. Life span and patterns of aging are also distinct between wild and laboratory populations, with natural strains exhibiting longer life span and delayed onset of senescence [85-86]. Conversely, the study of aging in wild populations presents a different set of challenges, including the tracking of individuals in longitudinal studies, the availability of detailed records and phenotypic data to permit quantitative genetic analysis, and the identification and functional analysis of candidate genes for aging. New methodologies may permit estimation of demographic parameters and studies of senescence in a variety of taxa that are difficult to monitor in the field [87-88].

Another consideration in the translation from laboratory to wild populations is whether or not aging actually occurs in a natural setting. A decline in various parameters as a function of age may be documented in an optimal laboratory environment in which such processes as inter- and intra-specific competition, predation, and parasitism may be removed; in wild populations, the level of extrinsic mortality may be so high that aging is never realized. However, a number of studies have documented senescence in natural populations of a variety of taxa [89-91]. Furthermore, aging has also been demonstrated in short-lived insect species with an appreciably high rate of daily mortality [92-93]. There is strong selection on age-specific fitness parameters in natural populations [92, 94], and this may also influence the strength of sexual selection [93] and different rates of senescence between the sexes in nature [95]. Nevertheless, comparisons between wild and laboratory populations suggest that aging profiles may be quite distinct in these two environments [96]. For example, Kawasaki et al. analyzed life span and rates of aging in natural and laboratory populations of the dipteran *Telostylinus angusticollis*; while aging phenotypes were similar between

environments for females, males demonstrated marked differences in life span and rates of senescence [97]. The genes that underlie observed variance in longevity may also be dependent on environment [98-99] (see section 3.3.). A general challenge for the biology of aging is to synthesize studies of life span and aging in wild and laboratory populations, utilizing the relative strengths of each empirical system [3, 26, 44, 82-83, 100].

The evolutionary theory of aging makes predictions that can be readily tested in natural populations, although case studies are relatively few in number. A basic prediction of the classical theory is that there is an association between the level of extrinsic mortality and the subsequent expression of intrinsic mortality (aging or senescence): as the level of extrinsic mortality increases senescence is predicted to accelerate [43, 100], although this may depend on the reproductive potential of older individuals [101]. The prediction that high mortality leads to a high rate of aging has been supported in some studies in which aging profiles were compared between natural populations that differ in mortality risk [102]. However, recent work in the guppy *Poecilia reticulata* suggests that the association between extrinsic mortality and senescence may be quite complex in natural populations [44].

Reznick et al. examined swimming performance, mortality rates, and patterns of reproduction in guppies derived from high and low predation risk populations [44]. Individuals from the high-risk environment did demonstrate accelerated senescence in physiological performance, but not in mortality or reproductive rates; no difference between populations was observed for post-reproductive life span and aging [103]. As discussed by Reznick et al. [44], such results may be counter to prediction from classical theory but are in complete accord with more derived models of senescence [43, 100, 104]. Bronikowski and Promislow [105] use this case study to highlight the importance of how aging is specifically defined, the actual source of mortality events, and the variation in mortality risk among individuals in a population (e.g., condition-dependence) [104]. Essentially, the factors that determine mortality risk, and thus the evolution of senescence profiles, may be quite distinct in the natural world vs. the laboratory environment.

While the MA hypothesis predicts an increase in genetic variance for fitness as a function of age [10, 39, 106-107], AP predicts genetically based trade-offs between early and late life fitness parameters [2, 9, 66]. Trade-offs are evidenced by negative genetic correlations between early and late mortality rates and/or reproductive investment. Such negative genetic correlations are a general prediction for fitness traits subjected to selection in different directions [108-109]. In AP, covariance across age classes is predicted to result specifically from the presence of alleles that confer a fitness advantage at one time point but a fitness cost at another [2, 9]. Thus, mortality and reproductive rates early in life are predicted to covary with patterns late in life. Such covariance is commonly observed in laboratory populations [12, 42, 71-72, 110], providing support for the AP hypothesis [2, 9, 24] (see section 2.2.).

The evolutionary theory of aging also assumes the widespread occurrence of genetic variation for aging in wild populations. A phenotypic association exists between early life investment in reproduction and reproductive/survival rate later in life [111], but the genetic basis of this association has rarely been tested in a natural setting. By analyzing longitudinal data in the mute swan, Charmantier et al. demonstrated that phenotypic and additive genetic variance for the timing of reproduction vary significantly across age classes [94]. Similarly, genetic variance for aging is evident in both natural populations of soay sheep and red deer [112]. The availability of long term monitoring of individuals has also allowed tests of the AP hypothesis. The age of first and last reproduction exhibit genetic covariance in mute swans [113], and maternal investment in early life reproduction is negatively genetically correlated with senescence rates in red deer populations [114]. This research supports the

hypothesis that natural populations are segregating for alleles that have antagonistic pleiotropic effects on fitness. However, the identities of the genes that contribute to the genetic variance for aging in the wild are unknown (see section 3.1.).

Using the information generated by molecular genetic studies of aging in model systems, it is possible to test for the functional contribution of candidate genes to aging in wild populations (also see section 3.1.). Obvious candidates would be genes involved in cellular stress response [115-119] and IIS [120], among others. An alternative approach is to examine aging in wild populations of the genetic models [121]. Although data on aging in the wild are completely lacking for such organisms as *D. melanogaster*, studies of longevity in wild-derived populations have demonstrated significant and predictable variation in aging phenotypes [122-123].

The examples reviewed here demonstrate a pronounced need for synthesis in the biology of aging: synthesis of laboratory models and natural systems, increased focus on aspects of senescence other than age at death (e.g., reproductive senescence, physiological performance), longitudinal studies and estimation of multiple demographic parameters, and the separation of distinct causes of mortality. In particular, answering some of the most important open questions about aging requires an improved integration of evolutionary and molecular approaches.

Below we focus on four key areas where such an interdisciplinary synthesis is likely to be fruitful: (1) the genetics of longevity genes in natural populations; (2) the evolution and mechanisms of longevity trade-offs; (3) the genetic basis of plasticity in life span; and (4) phylo-“genetic” and -“genomic” aspects of the evolution of aging among species. Ultimately, the analysis of aging genes in both the field and the laboratory will generate a more comprehensive understanding of why and how organisms age.

3. Integrating Evolutionary and Molecular Genetics of Aging

3.1. Candidate Aging Genes in Natural Populations

Two general approaches, distinct yet highly complementary, have been used to identify the genes and molecular pathways that regulate aging and life span. The first is mutational analysis, where mutants are screened for life span extension or a correlated trait and then the gene is identified by forward genetics [124]. Such analyses have generated an extensive list of “aging genes” that extend life and/or reduce age-specific mortality rates when gene function is impaired (hypomorphic expression or gene knockout) or accentuated (overexpression) [18-22]. This list has been reviewed elsewhere [13-17, 123]. While the role of a subset of these genes in the aging process may be unique to a particular taxonomic group (e.g., *mth*, [19]; *EcR* [125]), others appear to be conserved across metazoans, although only very few species have been investigated up to date [28] (see section 3.4.). The most intensively studied pathway that influences aging is IIS pathway. A reduction in IIS results in life span extension and/or reduced age-specific mortality rates in worms [18, 67], flies [22, 68, 126], and mice [127-128], and variation at the downstream transcription factor FOXO3A explains a significant amount of variance for longevity in humans [120].

Forward genetic screens and subsequent molecular analysis identify genes involved in the aging process, but they do not address whether candidate genes are of functional significance in natural populations: i.e., whether or not these genes harbor allelic variation that contributes to the standing genetic variance for longevity that is commonly observed. In addition to the identification and genetic manipulation of aging pathways, it is of fundamental importance to determine the genetic basis for variation in longevity among individual genotypes [26, 129]. This can be addressed in two ways, by quantitative trait

locus (QTL) mapping of longevity phenotypes [130-131] and direct functional analysis of candidate genes for aging [123, 132-133].

While QTL mapping of longevity has been done in a variety of systems [134-137], it has been particularly effective in identifying candidate genes in *D. melanogaster*. A variety of mapping methodologies has been used to identify chromosomal regions containing QTL for life span (reviewed in [131]), including recombination mapping in [74-75, 99-99, 138-139], selection mapping [140], deficiency mapping [132, 141-143], composite interval mapping [144], and speed mapping [145]. In classical QTL mapping the major obstacle in the identification of a gene is the size of the chromosomal region that is associated with the phenotype of interest; this depends on the extent of genetic differentiation between the strains used for mapping, marker density, and the recombinational landscape. Even in high-resolution deficiency mapping, a given QTL for life span may contain more than 50 genes [131].

While most studies have not resolved QTLs for life span at the level of the gene or nucleotide, a robust methodology has emerged that allows for tests of the phenotypic contribution of individual genes within identified QTLs. This methodology proceeds in four steps: (1) classical QTL mapping to identify the chromosomal region(s); (2) high resolution deficiency mapping to narrow the interval; (3) the use of genetic complementation analysis to test for the contribution of single genes to the phenotype; and (4) functional analysis of allelic variation at the candidate gene(s). The quantitative complementation tests (QCT) require a mutant for the gene that is being tested [26, 141, 146-148]. Essentially, the phenotype of a given parental allele is examined over a mutant and wild-type chromosome (mutational revertant or balancer); a significant interaction term indicates failure to complement. Provided this interaction cannot be explained by epistasis, failure to complement indicates that the tested gene has a functional impact on the trait of interest. In genetic models such as *D. melanogaster*, mutations exist for many genes and available *piggyBac* deletions cover 56% of the euchromatic genome (~ 7,000 genes). If a deletion is unavailable, one can be readily created via FRT-FLP facilitated deletion [149] and used in the complementation scheme [150].

The efficacy of this method is demonstrated by the analysis of the *dopa decarboxylase* (*Ddc*) gene in *D. melanogaster*, which is involved in the synthesis of the neurotransmitters dopamine and serotonin. De Luca et al. [132] used deficiency mapping to generate higher resolution of a previously identified QTL for life span [74]. Multiple QTLs were identified in this region of the second chromosome, including the *Ddc* locus. Complementation tests subsequently demonstrated that *Ddc* contributes to the observed difference in life span between the two parental strains used in the mapping studies. If *Ddc* contributes to the variance for life span in natural populations, it would be predicted that the gene is segregating for specific molecular polymorphisms that have functional effects on life span. Sequence analysis of a sample of 173 *Ddc* alleles collected from a single wild population (North Carolina, USA) demonstrated extensive nucleotide polymorphism and patterns of variation that were consistent with the action of balancing selection. Of the polymorphisms segregating within the North Carolina population, three were shown by linkage disequilibrium mapping to contribute to the standing genetic variance for life span associated with the second chromosome [132]. This elegant study provided the first demonstration of the functional significance of naturally occurring polymorphism at a candidate gene for aging, and provides a standard for the assessment of the genetic basis of variation for longevity in natural populations [26].

While the analysis of De Luca et al. [132] remains the most comprehensive, other studies have used gene-specific QCT schemes to dissect the genetics of longevity in *D.*

melanogaster populations and identify specific candidate genes for aging. Such examples include *catecholamines up* (*catsup*, [133]), *shuttle craft* (*stc*, [151]), and *tailup* (*tup*, [131]). In these case studies, complementation and subsequent sequence analysis were preceded by QTL studies that identified a genomic region of interest. However, one of the advantages of QCT and subsequent functional analysis is that they can be applied to any phenotype and gene, provided a mutant is available. In this respect, any of the candidate genes for aging identified in mutational screens can be examined for their contribution to longevity variance in natural populations.

The *Drosophila* gene *methuselah* (*mth*) was originally identified in a mutational screen for life span extension [19]; it encodes a G-protein coupled receptor thought to be involved in modulation of synaptic strength [152]. Disruption of the gene encoding *mth* peptide ligands extends life span [153-154]. Paaby and Schmidt [123] investigated the effects of allelic variation at *mth* on life span, fecundity, and oxidative stress resistance. Previous work had identified *mth* alleles whose frequencies varied predictably with latitude [25] and among natural populations characterized by differences in life span [155]. QCT using a constructed random sample of *mth* alleles demonstrated that these variants have a functional effect on all three assayed traits [123].

These studies illustrate that it is now possible to examine the evolutionary genetics and dynamics of longevity genes in natural populations in great detail. While mapping variation in life span down to single loci or single nucleotide polymorphisms (SNPs) can be technically challenging, future population genetic research on aging genes will undoubtedly improve our understanding of the evolution of aging.

3.2. Evolution and Mechanisms of Longevity Trade-Offs

Another research area which requires a better integration of evolutionary and molecular genetics is the study of trade-offs between reproduction and life span (so-called “survival costs of reproduction”), as well as trade-offs between life span and fitness traits other than reproduction [2-3, 7, 9, 11, 13, 29, 156-158]. Here we focus our discussion predominantly on the commonly observed trade-off between life span and reproduction.

Williams hypothesized that aging evolves because selection acts on pleiotropic alleles that have positive effects on reproduction (or other fitness traits) early but negative effects late in life, with the deleterious effects unopposed by weak selection at advanced age [2]. Kirkwood put Williams’ model on a physiological basis by proposing that aging evolves because selection favors alleles that increase the competitive allocation of energetic resources into reproduction at the expense of investment into maintenance, repair, and survival [37]. Selection experiments, phenotypic manipulations, and mutant analyses broadly confirm these ideas [3, 5, 9, 11, 13, 15-16], however, little is known about the actual mechanisms whereby reproduction or other fitness traits affect life span [16, 24, 29, 31, 157-158].

In *Drosophila*, direct selection for extended life span increases adult survival but decreases early reproduction [58], and selection for reproduction at old ages increases life span but reduces early fecundity [53, 57]. Sterilizing short-lived control and long-lived selection lines abolishes the evolved difference in longevity, suggesting that prolonged life span evolved through a trade-off with reproduction [159]. Consistent with Kirkwood’s DS hypothesis and the concept of resource allocation trade-offs [37, 160], dietary restriction (DR) extends life span but reduces fecundity in fruit flies [161-164]. Phenotypic manipulations that reduce reproduction can also increase longevity: flies are long-lived when mating opportunities are restricted or when oviposition substrate is removed [165-167]. Moreover, some longevity mutants have reduced fecundity or fertility [15-16, 22, 67]. However, these data are also

compatible with alternative explanations, and recent progress in molecular biogerontology has begun to challenge the evolutionary framework, in particular the notion of a resource allocation trade-off between reproduction and life span.

Studies in *C. elegans*, *D. melanogaster*, and the mouse suggest that life span can sometimes be increased without obvious fitness costs [9, 16, 24, 29-31, 60, 69-70]. For example, laboratory mutants can be long-lived without apparent impairment of fertility or other fitness components [16, 69], although fitness trade-offs are often subsequently found under different environmental conditions [16, 69-70]. In *C. elegans*, worms mutant for the insulin-like receptor gene *daf-2* are long-lived even when the gonad of the weakly fertility-impaired mutant and of the fertile control wild-type strain is removed by laser ablation; thus, life span is extended in the mutant relative to the control even when there is no difference in energy allocation into egg production among the two genotypes since they are both sterile [18]. Silencing *daf-2* with RNAi in pre-adult stages increases nematode life span but decreases fertility, whereas silencing the gene in the adult causes longevity extension with little or no effect on reproduction, although sample sizes in this study were somewhat small [168]. These results in *C. elegans* might have two important implications. First, *daf-2* might affect reproduction and life span pleiotropically, but the connection between these traits can potentially be broken. Second, since life span is extended even when mutant and control worms are gonadectomized, there can be no difference in energy allocation into egg production. Interestingly, long-lived flies subject to DR have a greater ratio of investment to somatic tissue relative to allocation to eggs, but contrary to expectation from the classic resource allocation trade-off model short-lived flies on full diet have greater net somatic investment [169]. Thus, DR might extend life span independent of reduced reproduction, a notion that is supported by the fact that DR increases the life span of a *Drosophila* mutant with oogenic arrest [170].

The concept of a resource-based allocation trade-off between life span and reproduction has also been challenged by the finding that laser ablation of the entire gonad in wild-type *C. elegans* does not extend life span [18], whereas ablation of germline stem cells in the presence of the somatic gonad increases longevity [30]. This observation is consistent with a model whereby putative signals from the germline accelerate aging whereas the somatic gonad produces counteracting signals that favor survival [29-31]. Germline ablation also extends life span in other nematodes [171], and in *D. melanogaster* [31], suggesting that the germline regulation of aging is evolutionarily conserved. However, these results do not necessarily exclude metabolism-based trade-offs as an explanation for the antagonistic relationship between reproduction and longevity [157-158].

Abolishing reproduction by gonad removal might not eliminate all costs of reproduction since damage or nutrient consumption induced by reproductive processes might continue outside the gonad even after the gonad has been removed [157]. Moreover, signals from the gonad might communicate levels of metabolic activity or demand to peripheral tissues, and the metabolic consequences might affect life span, either because metabolic processes that enable reproduction are energy consuming and withdraw resources from investment into somatic maintenance or because they cause direct damage [157-158]. Such signals would ensure that the somatically costly or deleterious processes that promote reproduction are enabled only when a proliferating germline is present [157]. Thus, the results on gonad and germline removal in worms and flies are easily compatible either with a resource-based trade-off or with a trade-off between low somatic damage and reproduction [157-158].

In the classical resource allocation trade-off model, resources are allocated to reproduction at the cost of somatic maintenance when they are abundant, but allocated to somatic maintenance at the cost of reproduction when they are limiting. Alternatively, in the “direct

constraints” model, the gonad does not act as an energetic resource sink that limits investment into somatic maintenance, but reproductive processes cause damage to the soma or directly inhibit processes of somatic maintenance [29, 157, 169, 172]. This might occur if upregulation of reproductive functions impairs or represses repair and maintenance systems, or if reproductive metabolism causes the accumulation of reactive oxygen species and thus oxidative stress [29, 157, 169, 172]. Consistent with this notion, many long-lived mutants in flies and worms are resistant to heat and oxidative stress [15-17], and this is also the case in germline ablated long-lived worms [173]. Interestingly, increased reproduction in flies causes elevated susceptibility to oxidative stress [174], and flies that overexpress the heat shock protein Hsp70, a chaperone, live longer but have reduced egg hatchability [175]. Reproduction is also known to impair immune function [157], and this could potentially also occur independent of resource allocation: for example, many sterile *C. elegans* mutants are pathogen-resistant, whereas pathogen-resistant mutants tend to be sterile [176].

Although we do not presently know whether reproduction shortens life span because it limits investment into somatic maintenance or because it generates damage, five lines of evidence suggest that reproduction, metabolism, diet, and aging are intimately coupled. First, the effects of germline ablation on *C. elegans* life span depend on the forkhead transcription factor DAF-16/FOXO downstream of IIS [173], a pathway known to be important for nutrient metabolism and growth [14]. Second, germline-less long-lived *Drosophila* exhibit hyperinsulinemia and hypoglycemia (low levels of glucose and trehalose), suggesting that signals from the gonad can modulate IIS and metabolism in peripheral tissues [31]. Third, *C. elegans* that lack germ cells (rather than the gonad) are long-lived, and DR cannot further extend longevity in these individuals, suggesting that germline removal and food scarcity might converge onto the same mechanisms that affect aging [177]. Fourth, germ cell loss in *C. elegans* causes systemic fat depletion from fat stores throughout the body via induction of fat lipase; the constitutive expression of this lipase in the intestine is sufficient to extend lifespan [178]. Fifth, the expression of trade-offs between reproduction and life span can critically depend on nutrient levels: long-lived fly mutants of the gene *Indy* (*I am not dead yet*) have normal metabolism and reproduction on normal diet, but reduced fecundity on DR diet [179]. While these findings indicate that metabolism, reproduction, and aging represent interconnected regulatory axes, the mechanisms by which they are coupled remain poorly understood. Thus, the relationship between life span and reproduction is more complicated than the models by Williams and Kirkwood suggest [2, 29, 31, 37, 157-158].

Another important but much neglected concept for future work is the distinction between evolutionary and physiological trade-offs [180-181]. Trade-offs at the physiological level are manifest within individuals in the same generation, for instance when increased reproductive effort temporarily decreases the probability of survival in a plastic way. Such physiological, plastic changes are likely to be mediated by endocrine signaling and metabolism [14, 60, 158, 180, 182]. By contrast, evolutionary or genetic trade-offs are manifest at the population level. Their existence requires genetic covariance among individuals in the populations, with antagonistic effects on reproduction and survival, so that some genotypes have reduced fecundity but increased life span, whereas others have reduced life span but increased fecundity [60, 180-181]. Trade-offs at the physiological level do not necessarily imply trade-offs at the evolutionary, genetic level. For example, a physiological trade-off can be genetically fixed in the population, and all individuals will physiologically respond in the same way to increased reproductive effort, even if individuals differ genetically in their levels of reproduction or survival. Alternatively, physiological trade-offs might be genetically variable and contribute to, or modulate, evolutionary trade-offs among individuals in the population [60, 180-181].

Experimental evidence supports the significance of this distinction [60]. Juvenile hormone (JH), a hormone thought to promote reproduction at the expense of longevity, physiologically increases egg production but shortens life span when fruit flies are exposed to exogenous JH [60], as had been suggested previously [183]. Conversely, when flies were selected to become insensitive to exogenously supplied JH, the effects of JH were much smaller for life span and not detectable for fecundity. Selected, JH-resistant flies also lived longer than unselected control flies, even when not exposed to JH, suggesting that flies had evolved insensitivity to their endogenously produced hormone. However, these long-lived flies had normal fecundity. Thus, although these results confirmed that JH is a physiological regulator of the trade-off between reproduction and life span, JH signaling did apparently not mediate the evolutionary trade-off between these traits [60]. An important question for future work will be to address how often trade-offs observed at the physiological and evolutionary level involve the same mechanisms.

To better understand how reproduction physiologically affects aging, and to see how it impacts the evolution of life span, we need studies combining evolutionary and molecular approaches [157-158]. While independent selection experiments have shown that the negative relationship between reproduction and survival is a general evolutionary outcome in genetically variable laboratory populations, molecular work has demonstrated that this trade-off can be context-dependent and uncoupled. To understand this paradox, we must isolate naturally occurring longevity genotypes and characterize their effects on reproduction and metabolism [26, 158]. On the mechanistic level, we need to measure how specific nutrients are acquired and allocated into reproduction versus the soma [169], examine how reproduction modulates physiology and metabolism [31, 178, 182], and assess whether the mechanisms underlying the trade-off are conserved [31, 158, 171, 184].

3.3. The Genetics of Life Span Plasticity

While classical theory postulates that aging should be affected by different mechanisms in different species [2, 6, 73], molecular biogerontology has revealed that life span is influenced by environmentally sensitive, conserved signaling pathways [7, 14-17]. While these findings do not mean that aging is “programmed”, they imply that survival might be regulated by homologous signaling processes in different organisms [7, 182]. It is possible then that such pathways allow organisms to plastically adjust their survival rate, or life span, in response to environmental cues [7, 182, 185-186]. Such phenotypic plasticity is defined as the ability of a genotype to change phenotypically when exposed to environmental change [181]. Indeed, life span is a remarkably plastic trait that readily responds to variation in the environment [6-7, 15, 33, 182].

The potential adaptive benefit of such plasticity is obvious. Since environmental change is often challenging and unpredictable, regulatory plasticity might allow organisms to maintain homeostasis and optimize their fitness despite environmental change [7, 181-182, 187]. In optimal environments organisms might invest into reproductive success at the expense of future survival, whereas in stressful environments organisms might switch to increased investment into survival until conditions for reproduction have improved [7, 181-183, 187]. This is of course only one out of many possible life history strategies: the detailed response will depend on ecological details and the biology of the organisms involved. Nevertheless, fluctuating environments are expected to select for organisms that can sense the state of the environment and adjust their life history accordingly [7, 181-183, 187]. Since the same genotype has the ability to express different life history phenotypes in different environments, plastic differences in life span and related traits must ultimately be due to environmentally induced changes in gene expression [188-190].

An extreme case of plasticity in aging is the difference in life span between queen and worker castes in social insects. In ants and bees, phenotypically distinct castes develop from the same genotype and can differ in life span by several orders of magnitude [191-194]. For example, ant queens can live 500 times longer than males and 10 times longer than non-reproductive workers [191]. Life span plasticity is also found within castes, for example between long-lived worker honey bees performing nest tasks and short-lived workers performing foraging tasks [192-194]. Recent studies have begun to uncover the underlying transcriptional and physiological changes involved in the environmental regulation of aging in social insects [189-194]. The plastic difference in longevity among honey bee workers, for instance, seems to be caused by changes in the social environment and has been linked to changes in endocrine signaling [192, 194].

Dauer diapause in larval nematodes [182] and reproductive diapause in adult insects [183] are other examples of plasticity in survival ability. In adult insects such as grasshoppers, butterflies, and fruit flies, individuals respond to low temperatures and short day length by downregulating metabolism, arresting reproduction, and increasing stress resistance and survival, coordinated life history adjustments mediated by neuroendocrine signaling [150, 183, 195-197]. Similar to the environmental cues inducing diapause, low temperatures are known to dramatically extend life span in fruit flies and nematode worms [198-202].

Perhaps the best known case of life span plasticity is the dietary restriction (DR, or caloric restriction, CR) response [164, 203]. In many organisms, from invertebrates to mammals, nutrient limitation without malnutrition extends life span but typically reduces reproduction [161-163]. In fruit flies, reduced yeast levels extend life span at the expense of fecundity [161-163]. Remarkably, mortality rates in *Drosophila* change rapidly and reversibly within 2-3 days when flies are switched between high and low food concentrations [201]. Over recent years, molecular geneticists have identified several key genes affecting the DR response, including the histone deacetylases Sir2 and Rpd3 and the forkhead transcription factor Pha-4 [15-17, 164, 203].

However, despite progress in our understanding of the mechanisms underlying regulatory plasticity in aging [150, 164, 182-183, 185-194, 196-197, 202], evolutionary aspects remain little understood. For example, theory predicts that the response to DR represents a case of adaptive plasticity, allowing individuals to survive conditions of poor nutrition by reallocating resources normally invested in reproduction to survival [7, 160, 164, 204]. Since DR extends life span at the expense of reproduction in many species, this plastic change might represent an evolutionarily conserved adaptive strategy [7, 160, 164, 204]. The benefit of such a strategy would be that individuals could survive a famine while preserving their reproductive potential for a time when conditions have improved [204]. However, although a model by Shanley and Kirkwood supports this idea [160], this model has been criticized on theoretical grounds by Mitteldorf [205], and experimental evidence for the adaptive value of DR and other cases of life span plasticity is largely lacking. Interestingly, a new model by Ratcliff et al. suggests that under stressful conditions such as DR selection might favor facultative (plastic) delays in reproduction (which might cause life span extension as a side-effect) when environmental cues predict a decline in population size [206]. However, these models [160, 205] await empirical validation, especially since it remains unclear how life span and reproduction evolve when populations adapt to novel diets (or other environments) and whether there are long-term benefits and costs associated with responding to such environmental change. Moreover, despite the identification of genes required for the DR response [164], or other forms of life span plasticity [150, 182-183, 197, 202], the genetic factors underlying the evolution of such plasticity remain largely unknown.

To address the extent of standing genetic variation for life span plasticity in natural populations future work must quantify genotype by environment interactions (GxE) in samples from natural populations using quantitative genetics [181] or, at higher resolution, by sequencing genes thought or known to be involved in the plastic response (also see section 3.1.). To investigate how life span evolves when organisms adapt to novel environments, and to examine the costs and benefits of life span plasticity in changing environments, experimental evolution approaches in short-lived laboratory organisms might be used [60, 207]. To determine the genetic basis of adaptation to constant versus changing environments in such experiments, genetic targets of selection might be identified by using, for example, microarray-based QTL speed mapping [145], sequencing, or other SNP genotyping technologies [208]. If possible, the significance of allelic variation at the identified candidate loci should then be tested using functional assays. In *Drosophila*, for example, candidate polymorphisms within a chromosome can be isolated using introgression [209], QCT [26, 123, 148], or by using multiple replicates with randomly recombined backgrounds, and then subjected to phenotypic tests (see section 3.1.). In addition, plastic changes in response to environmental change can be assessed at the transcriptional level using microarrays [188]. Such experiments will be particularly informative if they examine the effects of a range of environments (e.g., multiple nutrient levels) [162-163].

3.4. Phylo-“Genetics” and -“Genomics” of Aging

The perhaps greatest unresolved problem in the biology of aging is to determine the genetic basis of variation in life span among species. We currently know little about why some species live for hundreds or thousands of years (tortoises, bristlecone pines), or only for days or weeks (fruit flies, nematodes), and about whether the mechanisms of aging are evolutionarily conserved (“public”) or lineage-specific (“private”) [3, 6, 9, 27-28, 210].

On the one hand, aging is a polygenic trait which evolves as a maladaptive byproduct of MA or AP; it does not serve a biological function, and the underlying mechanisms have not evolved, or been “programmed”, by natural selection to cause age-progressive deterioration and death [1-3, 6-7]. If so, one might surmise that the genetics of aging is different among species. On the other hand, aging (or rather its flip-side, survival or life span) might be “regulated” by different signaling pathways that play important roles in ensuring proper development or adult function and thus critically contribute to fitness [7, 14-17, 73, 182, 187]. If so, we might expect that the central mechanisms of aging might have been shaped by selection, as a byproduct of selection on pleiotropic functions that enhance early fitness [2, 6-7, 182, 187].

Recent studies have begun to suggest that at least some mechanisms might be public determinants of aging among species [14-17, 27-28, 210-214]. The best example of a probably conserved longevity mechanism might be the DR response. While DR does not extend life span in all species investigated up to date, this dietary manipulation promotes longevity in a wide range of species, from yeast, flies, and worms to fish and mammals (e.g., including hamsters, rats, mice, dogs, and rhesus monkeys) [17, 164, 203], and in several of these organisms DR has been linked to the histone deacetylase gene *Sir2* [17, 20, 213]. Another important example is the regulation of aging by IIS in worms, flies, and mice [14-18]. Mutations in *C. elegans daf-2* and its *Drosophila* homolog *dInR* extend worm and fly life span, and impairing the function of both the insulin receptor and the IGF-1 receptor promotes longevity in the mouse [14, 18, 22, 127-128, 212]. Remarkably, heterozygous mutations in the IGF-1 receptor confer reduced receptor activity in transformed lymphocytes and are significantly enriched in female centenarians of Ashkenazi Jews, suggesting that IIS might also regulate longevity in humans [214]. The potential importance of genetic variation in IIS in affecting human longevity is also underscored by the finding that polymorphisms in

FOXO3A, a human ortholog of dFOXO/DAF-16, are associated with exceptional longevity in two independent studies [120, 215].

Two recent comparative genomic studies have also provide novel insights into the question of public versus private mechanisms of aging. Using gene expression profiling, J. McElwee et al. asked whether the downstream mechanisms whereby IIS regulates life span are conserved among species [27]. By comparing transcript profiles in flies, worms, and mice with mutations in IIS, they found that there is little evidence for conservation at the level of orthologous or paralogous downstream genes but that two IIS regulated processes (reduced protein biosynthesis, cellular detoxification) are conserved across species [27]. This suggests that some of the downstream targets of IIS might be lineage-specific, whereas the pathway might have conserved effects on aging at the process level [27]. In another study, E.D. Smith et al. systematically examined life span phenotypes of single-gene deletions of yeast orthologs of 276 known *C. elegans* aging genes and found that many of these loci are conserved, both in sequence and function [211]. In particular, among the conserved ortholog pairs, genes involved in nutrient sensing and protein translation downstream of TOR signaling were significantly enriched [211].

Even less is known about the evolution of life span among closely related species. Similar to studies in evolutionary developmental biology (“evo-devo”) [e.g., 216], which examine the genetics of interspecific variation in development and morphology, it will be of major interest to determine the genetics of differences in life span among closely related species, an approach which Partridge and Gems have called evolutionary gerontology (“evo-gero”) [6]. We suggest that such an approach might be particularly fruitful in *Drosophila*. This genus contains 12 relatively closely related species for which genome sequence information is available as well as a powerful model system (*D. melanogaster*) with extensive genetic tools and information on the mechanisms of aging. Such studies will likely provide important insights into the relationship between genetic variation segregating within populations and genetic differences in life span among species. Rapid advances in sequencing technology and the availability of genome information for a growing number of species will make the comparative genomics and genetics of aging a promising area of future research.

4. Conclusions

Together with Partridge and Gems [6] and Ackermann and Pletcher [7] we believe that the time is ripe for a synthesis of evolutionary and molecular genetics of aging. In recent years molecular genetics has shed light upon many aspects of aging of major interest to evolutionary biologists, while molecular geneticists have become increasingly interested in answering questions with an evolutionary angle [6-7, 9]. Although the core ideas of the evolutionary theory of aging are well supported today, the evolution of aging is likely to be much more complex than classical theory suggests. Several aspects of the theory require corroboration and refinement, particularly with regard to the frequency and distribution of age-dependent mutational effects on mortality and other fitness components [7, 217]. At the same time, molecular biogerontology has made remarkable progress at uncovering the mechanisms of aging, yet many fundamental questions about aging remain unresolved. To answer them, researchers in the field should combine the advances of molecular biology with evolutionary thinking.

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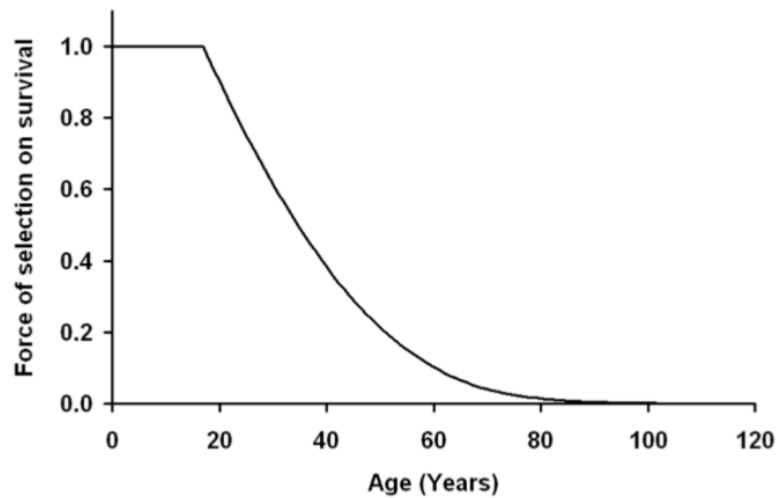


Figure 1.

Intensity of selection on survival. The force of selection on survival rate declines as a function of age, a key insight first developed by Haldane and Medawar and later mathematically formalized by Hamilton [1, 8, 10, 38]; see Baudisch [41] for a qualifier. Haldane [8], Medawar [1], and Williams [2] realized that the declining strength of selection “opens the door” for mutations with either neutral or beneficial effects during youth, when selection is intense, but with deleterious effects at older ages, when selection is negligible (so-called “selection shadow”). Since such alleles have unchecked negative consequences at old age when selection is weak, these alleles can spread in the population and lead to the evolution of aging.