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The Evolution of Aging and Lifespan

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Abstract

Aging is a nearly inescapable trait among organisms yet lifespan varies tremendously among different species spanning several orders of magnitude in vertebrates alone. This vast phenotypic diversity is driven by distinct evolutionary trajectories and tradeoffs that are reflected in patterns of diversification and constraint in organismal genomes. Age-specific impacts of selection also shape allele frequencies in populations impacting disease susceptibility and environment-specific mortality risk. Further, the mutational processes that spawn this genetic diversity both in the germline and somatic cells are strongly influenced by age and life history. Here, we discuss recent advances in our understanding of the evolution of aging and lifespan at organismal, population, and cellular scales and highlight the outstanding questions that remain unanswered.

Keywords

aging; lifespan; genetics; evolution; mutation; diversity

Evolutionary theory of aging

Evolutionary theory predicts that aging is an inevitable result of the increased selective impact of genes that influence early-life survival and fecundity compared with genes that act late in life [1–3]. This observation was first explicitly made by Peter Medawar, building off of R.A. Fisher’s introduction of the concept of age-specific *reproductive value*, which models the age-dependent future genetic contributions of individuals [4]. Indeed, many of the seminal contributions to the evolutionary theory of aging can be traced to the founders of the modern synthesis (reviewed in [5]). Medawar’s *mutation-accumulation* theory posits that late-acting deleterious alleles are likely to accumulate due to the reduced force of natural selection in older individuals (sometimes referred to as a “selection shadow”). A similar but distinct theory of aging, *antagonistic pleiotropy*, proposes that alleles that are beneficial early in life, but deleterious late in life, will accumulate. The key difference between these two theories is that in antagonistic pleiotropy aging evolves due to an evolutionary trade-off between the fitness of old and young individuals while in contrast, in mutation accumulation aging-associated alleles are neutral in young individuals. While the relative contributions

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Declaration of interests

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of *antagonistic pleiotropy* and *mutation accumulation* remain unclear, the extensive and extraordinary variation in lifespan among organisms highlights that different evolutionary scenarios and tradeoffs can favor vastly different outcomes of this phenotype. Medawar also noted the relative ambiguity of the term “*aging*,” which is used to refer to almost any time-dependent change on a biological entity [6]. This is distinct from *lifespan* which refers to the age of death of an individual, or *senescence* which refers to biological changes yielding an increased probability of mortality as a function of age. Recently, several technological advances alongside large-scale genetic datasets have propelled renewed excitement about the evolution of aging and the genetics of age structured populations. Here, we highlight recent progress in our understanding of this topic, ranging from new insights into the life histories of diverse organisms to the new approaches enabling us to identify aging-genes and age-associated biological phenomena (Figure 1).

Insights from extreme agers of the animal kingdom

There exists an extraordinary variation in lifespan among organisms on this planet spanning several orders of magnitude in vertebrates alone [7,8]. Such variation provides an exquisite *natural* dataset in which to probe the evolution of this trait providing insights into the evolutionary trade-offs that constrain and mold this phenotype, as well as the scenarios under which the extremes evolve.

The life history of longevity:

Evolutionary theory predicts that increased lifespan will evolve in the context of low extrinsic mortality, i.e. few environmental threats posing a risk of death. In contrast, short lifespans will evolve in organisms with high extrinsic mortality (for example due to predation). Lifespan, along with many other life history traits, tends to exhibit strong allometric scaling, with larger animals being longer lived. However, even after controlling for body size and phylogenetic relatedness, lifespan exhibits covariation with other life history traits, with the majority of this variance explainable across two independent axes [9,10]. Generation time and age-at-first reproduction co-vary with lifespan along one axis, while the distribution of age-specific mortality and reproduction co-vary along the other. Thus, short lived species tend to be smaller and have shorter generation and maturation times. They also have high rates of iteroparity and many offspring, with highly concentrated mortality risk (e.g. low juvenile survival). Importantly, these life-history correlations mean that extreme lifespan can emerge from indirect selection on a covarying trait.

The relationship between age and mortality is commonly modeled using the Gompertz hazard function (Box 1). This simple empirical model describes the exponential increase in the mortality rate with age. While the increased probability of mortality with age has been considered a universal trait among organisms, recent comparative studies of tortoises [11], and nonavian reptiles and amphibians [9,12] have identified several cases in which the rates of aging are negligibly small, or even negative. Other species that have been suggested to exhibit so-called “negligible senescence” include rockfish [13] and naked mole rats [14]. Many of these species, in particular fish and some reptiles and amphibians, exhibit “*indeterminate*” growth and fecundity, providing a key clue into the basis of their remarkable

longevity: the continual growth and production of offspring throughout lifespan places a strong selective cost on late-acting deleterious mutations. Additionally, in rockfish and many other fishes, fecundity scales disproportionately with body size [15] meaning that larger, older, females will contribute more offspring to the next generation than younger, smaller individuals. Several other correlates of lifespan have been identified including metabolic rate [16,17], temperature and thermoregulatory mode [12], protective phenotypes (e.g. toxins, shells, and flight) [18], and sex. Thus, extreme lifespan evolves across many different phenotypic axes.

Death and sex (chromosomes):

Extensive sex differences in lifespan are apparent across many taxa (Reviewed in [19]); intriguingly, the direction of this effect consistently favors the homogametic sex (i.e. females in XY systems like mammals, and males in ZW systems like birds). Indeed, a recent comparison of 299 species found that the homogametic sex lived, on average, 17.6% longer than the heterogametic sex [20] and in-depth analyses of mammals [21] and amphibians [22] have shown similar results. One explanation of this phenomenon, the “unguarded X hypothesis,” posits that the recessive mutations on the X (or Z) chromosomes will be exposed only in the heterogametic context. However, population genetics models have suggested that the effect size of the difference in lifespan between the hetero- and homo-gametic sexes is too large to be explained by the unmasking of recessive variation alone [23]. An alternative explanation is that the Y (or Z) chromosome itself becomes toxic with age, due to the derepression and misexpression of repetitive DNA. This hypothesis is supported by recent work in *Drosophila* demonstrating that increased Y chromosome copy number is negatively correlated with lifespan [24]. Further bioinformatic analyses across several taxa provide further support for this hypothesis and find that the relative sizes of the X and Y chromosomes in mammals (though not Z or W in birds) are associated with lifespan [25]. Heterochromatin becomes de-repressed with age in several species, however, the Y chromosome is particularly rich in heterochromatin-repressed repetitive sequences. Such repetitive, heterochromatic sequences have classically been challenging to interrogate due to limitations in sequencing technologies. The advent of several new long reads sequencing approaches will hopefully open these regions to study across species with different lifespans as well as throughout the lifespan of individual organisms.

Comparative genomics of extreme aging

While classical model organisms including yeast, worms, flies, and mice have provided myriad insights into the molecular underpinnings of lifespan variation, novel long read sequencing and genome assembly approaches are empowering comparative genomics-based interrogation of long-lived wild species across the planet that would be otherwise be intractable to study in the lab (Box 2).

Insights from genome assemblies:

The complete genomes of several extremely long lived non-model species have been generated recently including the bowhead whale (*Balaena mysticetus*, >200 years) [26]; the giant tortoises *Chelonoidis abingdonii* and *Aldabrachelys gigantea* (>100 years) [27,28];

the Asian and African elephant (*Loxodonta africana* and *Elephas maximus*, >80 and >65 years respectively) [28]; naked mole rats (*Heterocephalus glaber*, >35 years) [29]; the Canadian beaver (*Castor canadensis*, >23 years) [29]; several long-lived rockfish of the Sebastes clade (100–200 years) [13]; *immortal* cnidarian jellyfish (*Turritopsis dohrnii*) [30]; and various bats [31]. Substantially less attention has been paid to the genomes of wild, short-lived species with the critical exception of the short-lived African turquoise killifish (*Nothobranchius furzeri*, 4–6mo) [32,33], which has emerged as a key model organism of aging and suspended animation [34]. Individual analyses of the genomes of these diverse species have repeatedly highlighted signatures of selection in key aging pathways including insulin signaling, fatty acid metabolism, DNA repair, inflammation, cell cycle, and tumor suppression pathways among others (pathways reviewed in [35–37]). In long lived species these pathways often exhibit signatures of positive selection [13,29,38] highlighting the evolutionary innovations necessary for extreme lifespan. Intriguingly, in short lived killifish evidence points to these critical age-associated pathways being under relaxed constraint [32,33] due to the reduced selective pressure on later-acting genes with roles in maintenance and longevity.

Such signatures are also observed in structural and copy number changes in the genome. Bowhead whales exhibit duplications in genes associated with DNA damage repair and cancer [26]; rockfish exhibit age-associated copy number expansion of butyrophilin genes, which serve an immuno-suppressive function [13]; *immortal* cnidarian jellyfish exhibit expansions in genes associated with DNA repair and replication, telomere maintenance, oxidative stress, stem cell maintenance and intercellular communication [30]; and bats exhibit an expanded repertoire of *APOBEC3* genes, which have antiviral functions and have known mutational signatures [31,39,40]. In contrast, short-lived annual killifish exhibit increased genome sizes attributed to increased mobile element content, potentially facilitated by relaxed selection on genome maintenance in these species [33].

Insights from genome pensembls:

While studies of individual genomes have been highly informative, methods leveraging comparative analyses of phylogenetically diverse taxa with broad variation in a phenotype of interest can also yield unique insights. Broadly speaking, such methods seek to identify correlations between the rate of evolution of a gene or region with the phenotype of interest across taxa. This approach has been successfully used to quantify genetic constraint associated with extended lifespan across the entire mammalian clade [38], longevity among different rockfish species [13,41], and differences in the constraint of both genes and non-coding regions associated with several different phenotypes in other mammals [42–44]. These approaches have highlighted that overall, long-lived species tend to exhibit signatures of increased *constraint* in many key aging pathways including DNA repair, cell cycle, cell death, insulin signaling and immunity [38]. In contrast, short-lived killifish populations exhibit small effective population sizes and the accumulation of deleterious mutations in similar pathways [45]. Taken together alongside insights from individual genomes it emerges that *in general*, key aging pathways are under strong purifying selection in long lived taxa with independent distinct innovations observed in select genes in individual species. This is well illustrated in rockfish where several long lived species exhibit

signatures of positive selection in DNA repair genes, however different positively selected genes are found in different species [13]. In rockfish these approaches have also been used to dissect apart the *direct* genetic drivers of extreme lifespan (largely associated with nutrient signaling) from the *indirect* genetic drivers which acted to increase lifespan by increasing body size (associated with DNA repair and mTOR signaling associated genes).

Despite the power of these comparative approaches they still exhibit some drawbacks, such as a reliance on large multiple sequence alignments (MSAs). While advances have been made in this space [46], whole genome MSAs are still extremely computationally expensive to generate. Furthermore, genetic variation that is not well described by MSAs, such as structural variation, will be ignored. Another important caveat is that poor quality input genomes will likely lead to unreliable inferences and exclude complex and rapidly evolving loci (Box 2). While recent efforts have prioritized broad taxonomical sampling over genome quality [47], future projects such as the Vertebrate Genome Project [48] are focused on constructing high quality resources which will be of substantial value for comparative genomics of extreme phenotypes.

Insights from functional genomics:

Functionally characterizing the genes and pathways identified from comparative genomics studies is essential to fully dissecting the mechanistic underpinnings of differences in lifespan. However, a primary challenge to such work is the intractability of exploring molecular mechanisms in non-model, long-lived organisms (Box 3). Nonetheless, several studies have leveraged cell culture models to explore hypotheses from comparative genomics studies. Work in elephants and their large-bodied relatives (Proboscideans) has identified gene duplications in several tumor suppressor genes [49–52]; two of these genes, *LIF6* and *TP53-Retrogene9*, represent functional retrogenes with the ability to induce apoptosis upon expression [49,52]. In contrast, similar work in the long-lived bowhead whale has demonstrated a preference for repair mechanisms over the elimination of damaged cells. This includes work demonstrating the effectiveness of a cloned retrogene of the cell cycle regulator *CDKN2C* that serves to enhance cell cycle arrest and cell viability in response to DNA damage [53]; and additional work in bowhead whale cells demonstrating an enhanced DNA damage repair response [54]. Work in long-lived rodents has shown a spectrum of responses to various aging-related stresses, many private to each species [55–58]. These studies highlight the exciting possibilities of using cellular models of diverse and intractable species to understand even highly complex phenotypes such as body size and aging (Box 3).

Population genetics of aging

The age-specific impacts of selection:

Increasingly large-scale cohort studies such as the UK Biobank [59] and All of Us Research Program [60] present new opportunities to study human aging from a population genetics perspective. Trends in allele frequencies across age strata have the potential to reveal longitudinal dynamics of variant effects. In humans, both *mutation accumulation* and *antagonistic pleiotropy* have been postulated as explanations for the presence of segregating

disease-causing alleles. However, studies of the GWAS catalog [60] purporting to have identified evidence of this [61] have suffered from statistical and logical flaws [62]. Taking advantage of GWAS data from more than 175,000 participants, Mostafavi et al identified just a handful of common variants with strong impacts on age-specific mortality [63]. These variants were found at the *APOE ε4* and *CHRNA3* loci, which predispose to Alzheimer's disease and smoking behavior respectively. These results highlight the strong impact of purifying selection to purge deleterious alleles, even if they act later in life.

Epidemiological studies have documented an association between reproductive traits, such as age at onset of puberty or first childbirth, on female lifespan [64,65]. By calculating polygenic prediction scores for these traits, Mostafavi et al were able to show that genetic variants that delay puberty and increase age at first birth in mothers are associated with increased lifespan. In contrast, polygenically predicted increases in BMI, cholesterol, and coronary artery disease risk are associated with decreased lifespan.

The observation in humans that purifying selection strongly influences late-acting disease-causing alleles is intriguing given that these alleles should exert their effect well after menopause and peak reproductive ages in women. Indeed, for many late-onset diseases, causal variants tend to be evolutionarily recent and segregate at low frequencies. In an attempt to reconcile these observations with theory Pavard and Coste [66] developed an evolutionary demographic model that accounted for male fertility extending into old age, and the role of familial care by parents or grandparents. This model predicted that variants that cause disease later in life are indeed expected to be under strong purifying selection in many cases due to the impact of post-menopausal parental and grandparental care.

The many complexities inherent to dissecting the evolution and genetics of lifespan have also been highlighted in attempts to estimate the heritability of this trait. While estimates as high as 30% have been reported [67], recent analyses have suggested that these estimates are substantially inflated due to assortative mating, with the true estimated heritability <10% [68]. Indeed, some of the strongest predictors of lifespan are geographic and environmental in nature [69,70]. Together, these data highlight the clear importance of socio-cultural and environmental factors in influencing human lifespan.

Interactions between genes and the environment (GxE) also almost certainly play an important role, although are exceptionally hard to dissect in humans. A recent study in *Drosophila* identified alleles associated with lifespan under dietary stress (high sugar versus a control) [71]. Remarkably, one third of alleles mediated an environment-specific (GxE) effect on shortening lifespan. These alleles were evolutionary younger and exhibited signatures of selection in the wild. These results provide support for the “evolutionary mismatch hypothesis,” i.e. differences between ancient and modern environments contribute to disease [72]. Humans notably have undergone massive dietary changes over the last several thousand years with the neolithic transition from hunter-gatherer to agricultural sustenance.

Though many late-acting disease-causing genes appear to be under strong purifying selection, in general genes expressed in old age do tend to exhibit signatures of relaxed

selection compared to genes expressed early in life. Analyses of gene expression across several mammals and insects have found that the rate of non-synonymous substitution (d_n/d_s) is significantly higher in genes expressed later in life [73–75]. Late-expressed genes also exhibit more segregating non-synonymous substitutions and have a reduced effective strength of selection ($N_e s$). Similar signatures have been identified in killifish, with genes expressed early in life recapitulating stringencies in selection [33]. To explore these patterns more broadly in human populations, Yamamoto et al developed a statistical model to quantify the proportion of variance in gene expression that is attributable to age or genetics in 948 humans across 27 different tissues [76]. Intriguingly, while indeed the force of purifying selection was stronger on genes expressed earlier in life for the majority of tissues, recapitulating work in other species, several highly proliferative tissues exhibited the opposite trend. These ‘*non-Medawarian*’ tissues displayed high rates of cancer and age-of-expression associated somatic mutation. The genes responsible for driving this signature were highly enriched for pathways associated with DNA repair, cellular proliferation, differentiation, and cancer. One explanation for these signatures is that these genes are highly pleiotropic with critical roles both in early development as well as later in life in these specific “*non-Medawarian*” tissues.

Mutation: cause and/or consequence of aging and death

The aging germline:

Mutation is the fundamental source of genetic variation. Observations of an association of advanced paternal age with achondroplasia provided some of the first clues that the germline mutation rate increases with age [77]. Recent advances in sequencing methodologies have allowed us to directly quantify this mutation rate across a multitude of organisms linking aging and several other life history traits to this key biological trait.

Some of the most in depth analyses of the impact of age on mutation rate have been performed in humans. A landmark study of 1,548 pedigrees showed the paternal mutation rate to increase with age at a rate approximately four-times higher than the maternal rate (1.51 versus 0.37 mutations per year) [78]. The types of de-novo mutations from sperm and eggs also differed significantly with several egg-specific mutation hotspots observed. Intriguingly, analyses of almost 10,000 individuals from multiple single-generation families both with and without autism spectrum disorder (ASD) did not find any age-association with the rate of structural variant (SV) formation [79], though SVs were observed at a significantly higher rate in ASD probands and were more likely to be paternal in origin. These results are consistent with recent analyses of macaque parent-offspring trios, which also failed to identify any association of SV formation with age [80]. These observations are however at odds with analyses highlighting significant age-associated increases in instability and fragmentation in sperm [81]. Future studies and new sequencing technologies are required to reconcile the differences in mutations identified between family studies and direct observation of gametes. In particular, de-novo SVs are extremely challenging to identify using short read sequencing. Long-read based approaches, including those leveraging new telomere-to-telomere assemblies [82], may provide more sensitivity in detecting de-novo events (Box 2).

Germline mutation rates also vary substantially by species, reflecting differences in life history and reproductive strategies. A recent hallmark study of 151 trios from different mammals, fish, birds, and reptiles identified an average germline mutation rate of 1.17×10^{-8} across vertebrates, with higher mutation rates driven primarily by greater parental age at reproduction [83]. However, per-generation mutation rates varied by up to 40-fold among different species. A paternal bias in the mutation rate was observed for mammals and birds, but not reptiles and fishes. Age at maturity and generation time were both positively associated with increased mutation rates, and in mammals the number of offspring per generation was negatively correlated with mutation rate. The mutational spectrum also differed substantially between vertebrate classes with the largest differences being observed in fish in A>C and C>A mutations. Changes in life history can have strong effects on germ-line mutation rates and profiles. One extreme example is in domesticated animals which have been selected for short generation times resulting in exceptionally high per-year mutation rates [83]. In rockfish, species with increased lifespans exhibit more segregating CpG->TpG mutations [13]. This mutational signature is characteristic of spontaneous methylated cytosine deamination, highlighting that shifts in the average generation time of a population can influence the spectrum of segregating genetic variation. Extensive comparisons of the rate and spectrum of de-novo SVs between species have yet to be performed. Together these results highlight the close relationship between the evolution of mutation rates and the evolution of aging.

Somatic mutation:

Somatic mutations accumulate with age in cells throughout the body. Indeed, this accumulation of somatic mutation is considered a “hallmark” of aging [84], though it is not clear to what degree such mutations are a “cause” of aging. Somatic mutations are difficult to measure due to their low frequency and the challenge of distinguishing them from artifactual sequencing errors. Newly developed single-molecule sequencing techniques, such as Nano-seq (duplex sequencing), enable highly accurate somatic mutation calls and to establish somatic mutation rates [85]. Somatic mutation rates tend to exceed their matched germline rates by 1- to 2-orders of magnitude [86–88], however they differ between tissues and cell types [89,90]. This increased somatic mutation rate is consistent with the *disposable soma* theory, which posits that organisms face a resource trade-off in their investment in germline vs somatic repair [91].

While the vast majority of work on somatic mutations has focused on humans, a recent analysis of intestinal crypts from 16 mammalian species provides several key insights into the evolution of somatic mutation [87]. Endogenous mutational processes were found to dominate the observed mutations, as opposed to environmentally associated mutations, though this trend may differ between cell types and tissues. Across mammals, the mutational signatures observed were largely the same, mirroring results in de-novo germline mutations which only found differences in the mutational spectrum between vertebrate classes. Most strikingly, the somatic mutation rate showed an inverse relationship with lifespan, with 82% of inter-species variation explained by this trait. Variation in body size was surprisingly not associated with somatic mutation rate. Moreover, the end-of-lifespan burden across species varied by only 3-fold regardless of lifespan, supporting the theory that somatic mutation

accumulation may be a key contributing factor to lifespan. Together these findings suggest that, at least in some cell types, somatic mutation burden represents a highly accurate estimator of absolute age across species.

Cancer is caused by somatic mutations that activate uncontrolled cell proliferation [92], with cancer risk varying between cell types. Thus, if every cell possesses some intrinsic risk of tumorigenesis, it follows that larger organisms with a greater number of cells should be at a greater risk of developing cancer [93]. This is indeed the case within species: in humans for example, the risk of many different cancer types increases with height [94]. Likewise in dogs, larger breeds have a higher cancer incidence [95]. However, there is no apparent correlation between lifespan, body size, and cancer risk between species [96], a phenomenon referred to as Peto's paradox [97]. Organisms with longer lifespans should have a similarly increased risk of cancer, as a consequence of increased mutation burden.

A recent study of 110,148 captive individuals across 191 mammalian species did identify a dramatic range in cancer mortality risk (CMR, i.e. the likelihood of dying from cancer) among species [98]. Over 20% of species examined exhibited substantial risk (exceeding 10% CMR) of cancer-related death [98]. However, body mass and lifespan accounted for only 0.78% and 2.94% of cross-species variance in CMR respectively, robustly supporting Peto's paradox in mammals. This line of evidence, taken alongside findings of an inverse relationship between lifespan and somatic mutation rate, support life history-dependent evolution of cancer mitigation. However, the genetic drivers underpinning increased cancer resistance in larger organisms are still largely unresolved, with recent evidence suggesting a plurality of mechanisms (Box 3).

Concluding Remarks & Future Perspectives

The scope of the field of aging and evolution is extraordinarily broad encompassing a myriad of topics beyond what we have covered here including epigenetic modifications [99–101], telomere attrition [102], other hallmarks of aging [84]. We also choose here to focus primarily on vertebrates, however many of the molecular mechanisms underlying aging are conserved in invertebrate and even in plant models, as described in other reviews [103,104].

Differences in lifespan have evolved countless times throughout the tree of life with longer and shorter lifespans covarying strongly with several distinct phenotypes and environmental conditions. This means that extreme lifespans can emerge indirectly from selection on covarying traits or adaptations to new environments, signatures of which will be left in their genomes. Dissecting the functional impact of selected genes and pathways remains challenging, however cellular and organoid models have emerged as potentially transformative tools to explore these lifespan-extending adaptations.

The age-specific forces of selection are now for the first time being revealed in humans demonstrating that strongly deleterious alleles, even when late-acting, exhibit signatures of purifying selection. However, in general, genes expressed earlier in life exhibit increased constraint compared to those expressed late in life, except in the case of a handful of highly proliferative '*non-medawarian*' tissues. The cell type-specific patterning of expression

timing and constraint have yet to be explored however. Such studies have the potential to reveal “*medawarian*” and “*non-medawarian*” patterns in individual cell types. Future profiling of cellular phenotypes throughout lifespan will also enable linking individual cellular phenomena to organism level phenotypes and evolutionary patterns.

The evolution of aging and lifespan is tightly linked to the evolution of mutation rates and processes. While rapid advances have been observed in dissecting single nucleotide mutation, our understanding of both germline and somatic structural variation remains incomplete. As somatic mutation profiling approaches become more cost effective, future studies also have the potential to increase our understanding of cellular aging across taxa. These studies will hopefully provide further insights into the lifespan-associated pathways identified from comparative genomics studies, clarifying their cell-type specific actions.

The proliferation of several genomic technologies has propelled our understanding of the evolution of aging enormously. However, many of these technologies have only recently started being applied at scale to explore fundamental questions of evolutionary biology. The future thus holds unprecedented promise as we begin to explore the full extent of organismal diversity to uncover the many different origins of life history differences on earth.

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Box 1 -**Modeling mortality**

The lifespan of organisms is commonly modeled using the Gompertz-Makeham equation which takes the form of the hazard function:

$$m(t) = A_0 e^{Gt} + M_0$$

where $m(t)$, the mortality hazard, represents the instantaneous probability of death at time t and $m(t)dt$ is the fraction of the population that dies from t to $t + dt$. This equation states that the probability of mortality of an organism can be modeled by an age-dependent component that increases exponentially with age (the Gompertzian component), and an age-independent component (the Makeham component). The parameter G reflects the exponential mortality rate coefficient, or “rate of aging”. A_0 is a constant representing the intrinsic vulnerability or initial mortality rate (IMR) while M_0 is the age independent mortality rate or extrinsic mortality rate (EMR). Due to the challenge in distinguishing IMR from EMR often a simplified Gompertz equation is used to model mortality in which the M_0 term is dropped. The survival function $S(t)$, which represents the probability of an individual surviving until time t , can be derived from the hazard function and takes the form:

$$S(t) = \exp\left(\frac{A_0}{G}(1 - e^{-Gt})\right)$$

More complex mortality functions, such as the Siler model [105], allow mortality to vary more flexibly throughout lifespan quantifying the increased mortality in infants and post-reproductive adults explicitly. The figure below shows mortality and survival curves for several different species, including long-lived humans and ultra long-lived rockfish, as well as a turtle and lizard that exhibit “negative senescence.”

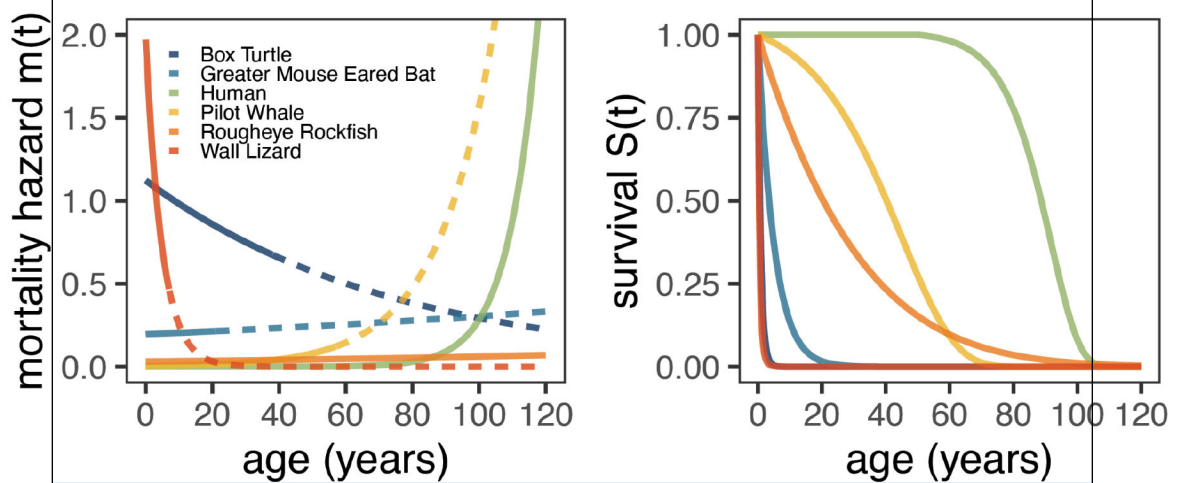


Figure Box 1:

Mortality hazard (instantaneous probability of death) and survival curves for six diverse species. Mortality hazard lines become dotted after the maximum lifespan of the species.

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Box 2 -**The evolution of comparative genomics**

Long-read sequencing technologies have dramatically reshaped modern approaches to evolutionary genomics. Over the past decade, the rapid improvement of nanopore and PacBio sequencing technologies has enabled remarkable advances in genome assembly. These technologies also allow us to access notoriously challenging regions of the genome, including segmental duplications, telomeres, centromeres, and other repeat-rich regions which can span many megabases. Short read assemblies in contrast are remarkably fragmented, frustrating analyses. Long-read approaches obviate these difficulties by directly sequencing single molecules of HMW (high molecular weight) DNA up to many megabases in length, permitting coverage across the aforementioned regions. Complete, reference quality genomes have historically only been available for a handful of species (e.g. human and mouse) and were the result of massive-scale consortia efforts. In contrast, long read sequencing alongside recent improvements in genome assembly allow individual labs to contribute near-complete genomes. Complete genomes from diverse species enables analysis of previously uncharacterized coding and non-coding sequences. The many structural rearrangements present in genomes can also be assessed for the first time: linking genome structural variants to phenotypes has remained relatively unexplored due to technological shortcomings in short-read assembly. Ultimately, comprehensive comparative genomics across the tree of life requires so-called telomere-to-telomere assemblies which are gapless covering even the most structurally challenging loci. Nevertheless, currently comparative genomics relies on genomes assembled to varying levels of completion which are suited for different comparative genomics analyses (Table Box II).

Table Box II:

A brief overview of reference genome resources for comparative and evolutionary genomics.

Assembly type	Strengths	Recent resources
Short-read genomes	Resequencing, population genetics, initial characterizations of genome diversity	Zoonomia [106], Primate Genome Project [107]
Long-read genomes	Near-complete analyses of autosomal gene content and diversity, characterization of structural variation, near-complete haplotype assembly, initial characterizations of complex loci (see below)	Vertebrate Genome Project [48]; Human Pangenome Reference Consortium [108]
T2T genomes	Complete analyses of autosomal and sex chromosomal gene content, full characterization of complex regions including segmental duplications, centromeres, telomeres, and rDNA repeats	T2T Consortium [82]

Box 3 -**Aging in a dish**

While the ultimate tests of aging-related hypotheses and treatment are *in vivo*, longitudinal studies of putative life- or health-span extensions are costly and complex, if not impossible in exceptionally long lived taxa. Cell culture models have long served as a bridge between the trials of research and the tribulations of clinical trials; yet it is critical that appropriate models are utilized to avoid wasted effort.

Many processes in aging are tissue-specific, and non-cell autonomous. While any cell type can be used in principle for initial exploration of candidate genes and pathways, robust characterization of aging-associated genes and interventions requires more faithful experimental systems. Organoid models and organism-on-a-chip studies have been proposed as a means of resolving this in humans [109]. These approaches involve either *ex vivo* culturing of organ tissues under controlled conditions; or the establishment of organoids derived from induced pluripotent stem cells. Compared to *in vivo* studies, *ex vivo* organoid systems provide an unmatched increase in experimental tractability and a level of control of extrinsic, confounding factors.

Because these approaches require invasive, possibly lethal sampling of target species, an alternative approach is to construct organoid models *de novo* using stem-cell-based approaches. Specifically, induced pluripotent stem cells (iPSCs) promise a robust system for generating any tissue of interest with minimal non-lethal sampling of individual animals. Both a key strength and limitation of iPSC-based approaches is that epigenetic changes associated with aging are lost during the reprogramming process [110,111]. The generation of iPSCs, however, remains a challenging process outside of key model species such as humans and mice. While many methods exist to transform cells into iPSCs, many of these methods rely or can result in alterations to the genome or to the fundamental cellular biology of the species, and special care must be taken to ensure that the resulting iPSCs replicate the true biology of their original host.

The following table highlights a selection of some of the ongoing work in different long-lived clades of interest using cell culture models, and the progress that has been made towards improving the state-of-the-art of *ex vivo* aging research.

Table Box III:

A select list of studies leveraging cell culture or organoid models in long-lived clades of interest.

Species	Cell Types	iPSC protocols	Organoids
Human	Various; see references for reviews	Various; see references for reviews	Various (iPSC, <i>ex vivo</i>); see references for reviews, eg [112]
Non-human primate	Various; see references for reviews	Various [113–116]	Various (iPSC, <i>ex vivo</i>); see references for reviews, eg [117]
Mouse	Various; see references for reviews	Various; see references for reviews	Various (iPSC, <i>ex vivo</i>); see references for reviews, eg [117]

Species	Cell Types	iPSC protocols	Organoids
Naked Mole Rat	fibroblasts (various tissues)[55,118–121]	Integrative plasmid; Viral transduction [122–125]	Aorta (<i>ex vivo</i>) [117,126]
Elephant	fibroblasts[49,51]	N/A	N/A
Whale	Fibroblasts [53,54,127–129]	N/A	N/A
Fish and Sharks	Various [130,131]	Viral transformation [132]	Retina (iPSC) [133,134]
Turtles	fibroblasts (various tissues)[135–137]	NA	Liver [138]
Bats	Fibroblasts (skin, kidney) [139–143]	Integrative plasmid [144,145]; Viral transformation [146]	Trachea (<i>ex vivo</i>) [147], Intestines (<i>ex vivo</i>) [148,149]

Highlights

- The extensive variation in lifespan among organisms provides a *natural* dataset to probe the evolutionary trade-offs that constrain and mold this phenotype across taxa
- Several key pathways repeatedly emerge as the targets of selection in comparative genomics of long lived species. Overall, these pathways exhibit increased constraint in long lived species, and reduced constraint in short lived species with distinct signatures of diversifying selection observed in individual taxa.
- Large cohort studies demonstrate that even late-acting deleterious alleles appear to be under strong purifying selection in human populations.
- Somatic mutation rates scale with lifespan and are significantly higher than matched germline mutation rates.
- Cell culture models of extreme agers are beginning to facilitate characterization of key genotype-phenotype interactions in biological aging.

Outstanding questions

- Gene-by-environment (GxE) interactions play an extremely important role in influencing lifespan and other life history traits. However, identifying GxE interactions is challenging even in model organisms, and still underexplored in humans. A major outstanding challenge is the development of both study designs and statistical approaches that are empowered to identify GxE in large human cohorts and to elucidate the impacts of these interactions on human health and lifespan.
- The covariance of lifespan with several other life history traits implies that shared genetic pathways shape the biology of aging. Many of these pathways are extremely deeply conserved and essential. While these pathways are discovered repeatedly in comparative genomics, disentangling the many pleiotropic and epistatic effects of perturbations in these pathways remains extremely challenging.
- Long read sequencing is unveiling the extensive structural differences that exist within and between species in previously intractable regions such as centromeres, telomeres, and rDNA repeats as well as large structural variants such as inversions and translocations. However, current comparative genomics approaches based on multiple sequence alignments are not empowered to assess this variation. An outstanding question is how these structural rearrangements influence phenotypes. Answering this question will require novel computational and statistical comparative approaches in addition to high quality genomes and phenotype data.
- While heterochromatic repeat sequences found on sex chromosomes have been implicated in aging, the longitudinal dynamics of their de-repression in different species and the mechanism of their toxicity is not well understood.
- While recent work highlights the tight linkage between the evolution of lifespan and both germline and somatic single nucleotide mutation patterns, our understanding of age-associated somatic and germline structural variation remains incomplete.

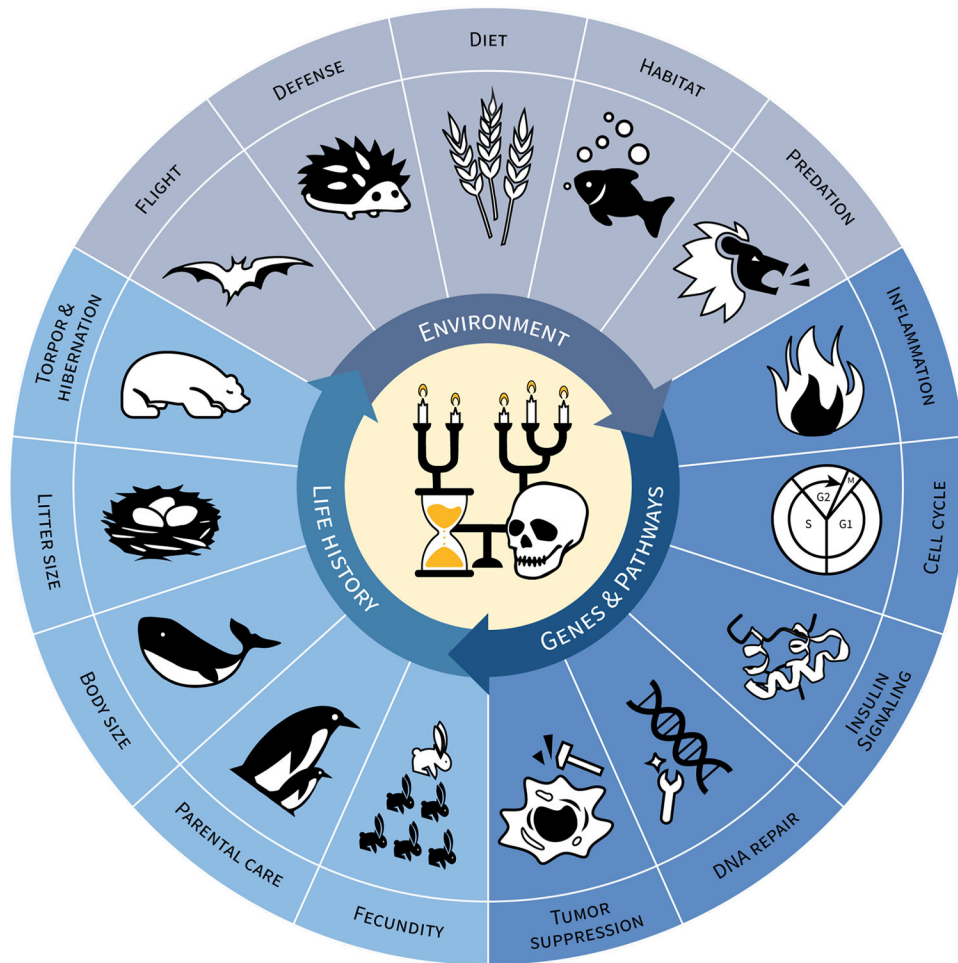


Figure 1:

Evolutionary dynamics underlying aging and longevity.

The interplay between genes, life history traits, and environmental factors drives the evolution of aging and lifespan. Highlighted above are several of the key elements in each domain that have known associations with aging and longevity. The iconography in the center of the figure depicts differences in lifespan (candle height) and their evolutionary relationships, in the Vanitas style.