CellPress
OPEN ACCESS

Trends in
Genetics

Review

The evolution of aging and lifespan

Stacy Li,[1,2] Juan Manuel Vazquez,[1] and Peter H. Sudmant[1,2,*]

Aging is a nearly inescapable trait among organisms yet lifespan varies tremendously across different species and spans 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, thus impacting disease susceptibility and environment-specific mortality risk. Further, the mutational processes that spawn this genetic diversity in both germline and somatic cells are strongly influenced by age and life history. We discuss recent advances in our understanding of the evolution of aging and lifespan at organismal, population, and cellular scales, and highlight outstanding questions that remain unanswered.

Highlights

The extensive variation in lifespan among organisms provides a natural dataset to probe the evolutionary tradeoffs that constrain and mold this phenotype across taxa.

Several key pathways repeatedly emerge as the targets of selection from 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.

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 upon 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 tradeoff between the fitness of old and young individuals; by contrast, in the mutation accumulation theory aging-associated alleles are neutral in young individuals. While the relative contributions of antagonistic pleiotropy and mutation accumulation remain unclear, the extensive and extraordinary variation in lifespan across 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 in a biological entity [6]. This is distinct from 'lifespan', which refers to the age of death of an individual, and 'senescence', which refers to biological changes yielding an increased probability of mortality as a function of age. Several technological advances and large-scale genetic datasets have recently propelled renewed excitement about the evolution of aging and the genetics of age-structured populations. We highlight recent progress in our understanding of this topic, ranging from new insights into the life histories of diverse organisms to new approaches that enable us to identify aging genes and age-associated biological phenomena (Figure 1, Key figure).

Insights from extreme agers of the animal kingdom

There is an extraordinary variation in lifespan across organisms on this planet which spans several orders of magnitude in vertebrates alone [7,8]. Such variation provides an exquisite natural

[1]Department of Integrative Biology, University of California, Berkeley, CA, USA
[2]Center for Computational Biology, University of California, Berkeley, CA. USA

*Correspondence:
psudmant@berkeley.edu (P.H. Sudmant).

CellPress
OPEN ACCESS

Key figure

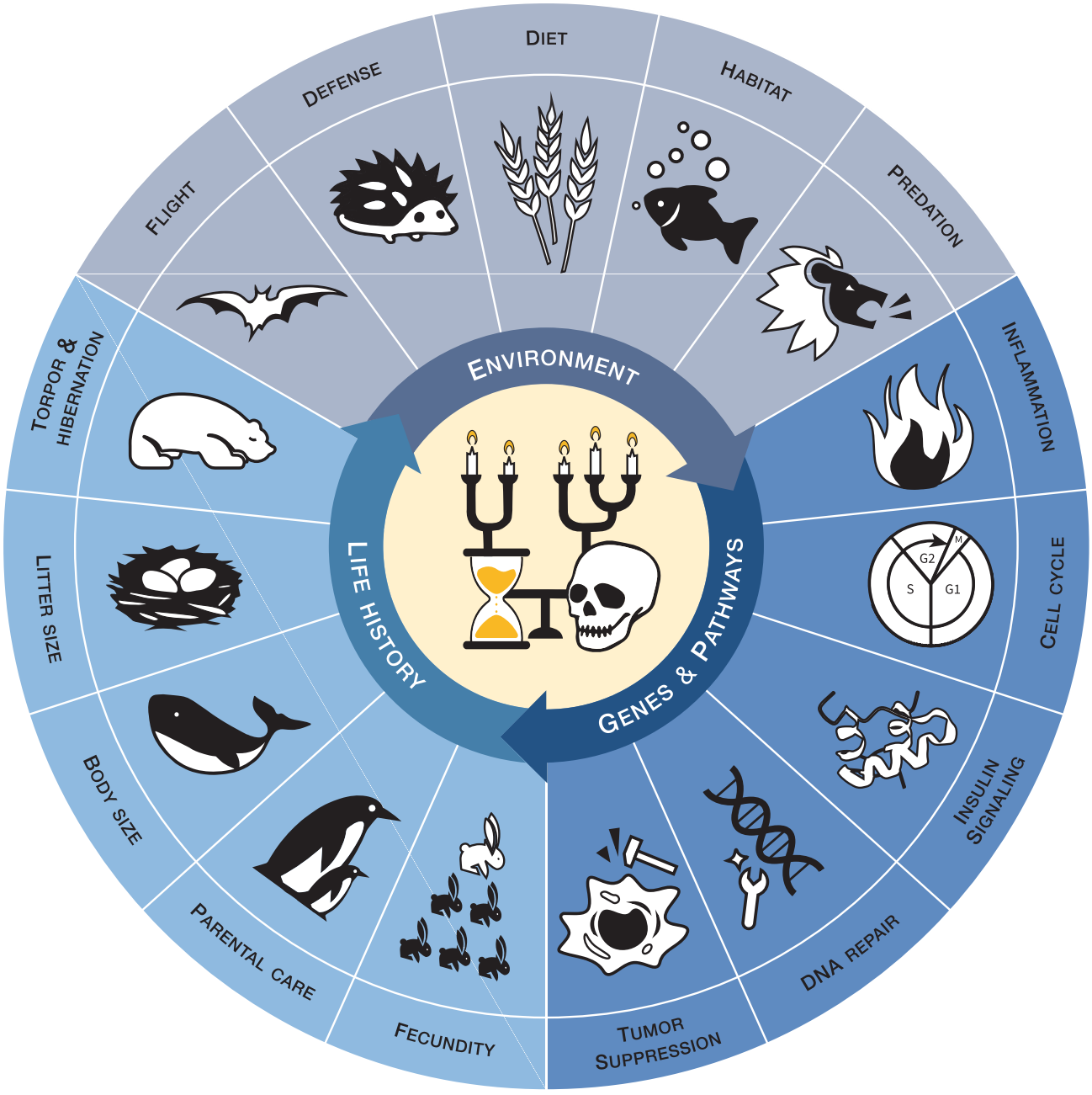

Figure 1. Evolutionary dynamics underlying aging and longevity. The interplay between genes, life-history traits, and environmental factors drives the evolution of aging and lifespan. Highlighted are several of the key elements in each domain that have known associations with aging and longevity. The iconography in the center of the figure depicts differences in lifespan (candle height) and their evolutionary relationships in *Vanitas style*.

dataset in which to probe the evolution of this trait, and can provide insights into the evolutionary tradeoffs 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, in other words when few environmental threats pose a risk of death. By contrast, short lifespans will evolve in organisms with high extrinsic mortality (e.g., due to predation). Lifespan, as well as many other life-history traits, tends to exhibit strong allometric scaling, where larger animals are 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 covary with lifespan along one axis, while the distribution of age-specific mortality and reproduction covary 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, many offspring, and a 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 mortality rate with age. Although the increased probability of mortality with age has been considered to be a universal trait among organisms, recent comparative studies of tortoises [11], and non-avian 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, thus 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. In addition, 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, 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 such as in mammals, and males in ZW systems such as in 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 recessive mutations on the X (or Z) chromosomes will be exposed only in the heterogametic context. However, population genetic models have suggested that the effect size of the difference in lifespan between the hetero- and homogametic 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 correlates negatively 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

Box 1. Modeling mortality

The lifespan of organisms is commonly modeled using the Gompertz–Makeham equation which takes the form of the hazard function (Equation I).

$$m(t) = A_0 e^{Gt} + M_0 \quad [I]$$

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), whereas M_0 is the age-independent mortality rate or extrinsic mortality rate (EMR). Due to the challenge in distinguishing the IMR from the EMR, a simplified Gompertz equation is often used to model mortality in which the M_0 term is dropped. The survival function $S(t)$ (Equation II), which represents the probability of an individual surviving until time t, can be derived from the hazard function and takes the form:

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

More complex mortality functions, such as the Siler model [105], allow mortality to vary more flexibly throughout lifespan, and explicitly quantify the increased mortality in infants and post-reproductive adults. Figure I 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'.

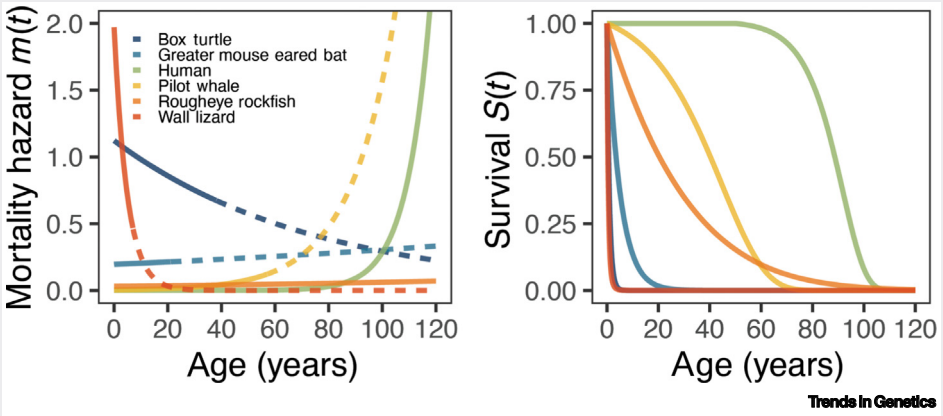

Figure I. Mortality hazard (instantaneous probability of death) and survival curves for six diverse species. Mortality hazard lines become broken after the maximum lifespan of the species.

Y chromosomes in mammals (although not Z or W in birds) are associated with lifespan [25]. Heterochromatin becomes derepressed 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 the limitations of sequencing technologies. The advent of several new long-read sequencing approaches will hopefully open these regions to study across species with different lifespans as well as throughout the lifespans 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 laboratory (Box 2).

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, by contrast, are remarkably fragmented, frustrating analyses. Long-read approaches obviate these difficulties by directly sequencing single molecules of high molecular weight DNA up to many megabases in length, thus 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. By contrast, long-read sequencing alongside recent improvements in genome assembly allows individual laboratories to contribute near-complete genomes. Complete genomes from diverse species enable analysis of previously uncharacterized coding and noncoding 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 I).

Table I. 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 later)	Vertebrate Genome Project [48]; Human Pangenome Reference Consortium [108]
T2T genomes	Complete analyses of autosomal and sex chromosome gene content; full characterization of complex regions including segmental duplications, centromeres, telomeres, and rDNA repeats	T2T Consortium [82]

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], Asian and African elephants (*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], the '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*, 4–6 months) [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 the evidence indicates that these critical age-associated pathways are 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 immunosuppresive 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]. By 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 ensembles

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 noncoding 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]. By contrast, short-lived killifish populations exhibit small effective population sizes and the accumulation of deleterious mutations in similar pathways [45]. Taken together with insights from individual genomes, it emerges that, in general, key aging pathways are under strong purifying selection in long-lived taxa, and independent distinct innovations are 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 act 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 have some drawbacks, such as a reliance on large multiple sequence alignments (MSAs). While advances have been made in this area [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 (SV), 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 taxonomic 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 dissect 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 that can induce apoptosis upon expression [49,52]. By contrast, similar work in the long-lived bowhead whale has demonstrated a preference for repair mechanisms over the

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 healthspan 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, but it is crucial that appropriate models are utilized to avoid wasted effort.

Many processes operating in aging are tissue-specific and non-cell-autonomous. Although 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 to resolve 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 (iPSCs). Compared with *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 and possibly lethal sampling of target species, an alternative approach is to construct organoid models *de novo* using stem cell-based approaches. Specifically, iPSCs promise a robust system for generating any tissue of interest with minimal nonlethal 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 challenging outside key model species such as humans and mice. Although there are many methods to transform cells into iPSCs, many rely upon 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.

Table I 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 I. Select studies leveraging cell culture or organoid models in long-lived clades of interest[a]

Species	Cell types	iPSC protocols	Organoids
Human	Various	Various	Various (iPSCs, *ex vivo*); (e.g., [112])
Non-human primate	Various	Various [113–116]	Various (iPSCs, *ex vivo*); (e.g., [117])
Mouse	Various	Various	Various (iPSCs, *ex vivo*); (e.g., [117])
Naked mole rat	Fibroblasts (various tissues) [55,118–121]	Integrative plasmid; viral transduction [122–125]	Aorta (*ex vivo)* [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 (iPSCs) [133,134]
Turtles	Fibroblasts (various tissues) [135–137]	N/A	Liver [138]
Bats	Fibroblasts (skin, kidney) [139–143]	Integrative plasmid [144,145]; viral transformation [146]	Trachea (*ex vivo*) [147], intestines (*ex vivo*) [148,149]

[a]Abbreviation: N/A, not applicable.

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 of which are 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 the 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 genome-wide association studies (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 only 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, and 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. By contrast, polygenically predicted increases in body mass index (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 as a result of assortative mating, and the true heritability is likely <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 sociocultural and environmental factors in influencing human lifespan.

Interactions between genes and the environment (G×E) also almost certainly play an important role, although these are exceptionally hard to dissect in humans. A recent study in *Drosophila* identified alleles associated with lifespan under dietary stress (high sugar versus control) [71]. Remarkably, one third of alleles mediated an environment-specific (G×E) 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', namely that differences between ancient and modern environments contribute to disease [72]. Humans notably have undergone massive dietary changes over the past several thousand years that accompanied the Neolithic transition from hunter-gatherer to agricultural sustenance.

Although many late-acting disease-causing genes appear to be under strong purifying selection, genes expressed in old age generally tend to exhibit signatures of relaxed selection compared with genes expressed early in life. Analyses of gene expression across several mammals and insects have found that the rate of nonsynonymous substitution (d_n/d_s) is significantly higher in genes expressed later in life [73–75]. Late-expressed genes also exhibit more segregating nonsynonymous substitutions and have a reduced effective strength of selection (N_es). Similar signatures have been identified in killifish, where genes expressed early in life recapitulate 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 the force of purifying selection was indeed 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, and play critical roles both in early development and 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 and thus link 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 1548 pedigrees showed that the paternal mutation rate increases with age at a rate approximately fourfold 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, and several egg-specific mutation hotspots were 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 SV formation [79], although 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]. However, these observations are at odds with analyses highlighting significant age-associated increases in instability and fragmentation in sperm [81]. Future studies and new sequencing technologies will be necessary 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 between 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, and that higher mutation rates were driven primarily by greater parental age at reproduction [83]. However, per-generation mutation rates varied by up to 40-fold between

different species. A paternal bias in the mutation rate was observed for mammals and birds, but not for 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 correlated negatively with mutation rate. The mutational spectrum also differed substantially between vertebrate classes. The largest differences were A>C and C>A mutations in fish. Changes in life history can have strong effects on germline 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 deamination of methylated cytosines, 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 not yet been performed. However, 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 to be a 'hallmark' of aging [84], although 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 1–2 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 tradeoff in their investment in germline versus 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 has provided 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, although 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 interspecies 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 threefold 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, supports life history-dependent evolution of cancer mitigation. However, the genetic drivers underpinning increased cancer resistance in larger organisms are still largely unresolved, and recent evidence suggests a plurality of mechanisms (Box 3).

Concluding remarks and future perspectives

The scope of the field of aging and evolution is extraordinarily broad, and encompasses a myriad of topics beyond those we have covered, including epigenetic modifications [99–101], telomere attrition [102], and other hallmarks of aging [84]. We also choose here to focus primarily on vertebrates, although 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. Longer and shorter lifespans covary 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 with those expressed late in life, except in the case of a handful of highly proliferative 'non-Medawarian' tissues. However, the cell type-specific patterning of expression timing and constraint remains to be explored. 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 allow individual cellular phenomena to be linked 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 mutations, our understanding of both germline and somatic SV 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 by comparative genomics studies and clarify 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 to be applied at scale to explore fundamental questions of evolutionary biology. The future (see Outstanding questions) 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.

Outstanding questions

Gene-by-environment (G×E) interactions play an extremely important role in influencing lifespan and other life-history traits. However, identifying G×E interactions is challenging even in model organisms, and remains underexplored in humans. A major outstanding challenge is the development of both study designs and statistical approaches that are empowered to identify G×E interactions 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 by comparative genomics studies, 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 ribosomal (r)DNA repeats, as well as large SVs 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 concerns how these structural rearrangements influence phenotypes. Answering this question will require novel computational and statistical comparative approaches in addition to high-quality genomic and phenotypic data.

Heterochromatic repeat sequences on sex chromosomes have been implicated in aging, but the longitudinal dynamics of their derepression in different species and their mechanism of toxicity are 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 SV remains incomplete.

Acknowledgments

This work was supported by National Institute of General Medical Sciences grant R35GM142916 to P.H.S, a Vallee Scholars Award to P.H.S, National Science Foundation (NSF) grant PRFB 2109915 to J.M.V. and National Institutes of Health (NIH) National Institute on Aging (NIA) grant T32 AG000266 to J.M.V.

Declaration of interests

The authors declare no competing interests.

References

1. Medawar, P.B. (1952) *An Unsolved Problem of Biology*.
2. Williams, G.C. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11, 398–411
3. Hamilton, W.D. (1966) The moulding of senescence by natural selection. *J. Theor. Biol.* 12, 12–45
4. Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon Press
5. Charlesworth, B. (2000) Fisher, Medawar, Hamilton and the evolution of aging. *Genetics* 156, 927–931
6. Finch, C.E. (1994) *Longevity, Senescence, and the Genome*, University of Chicago Press
7. Nielsen, J. *et al.* (2016) Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). *Science* 353, 702–704
8. Depczynski, M. and Bellwood, D.R. (2005) Shortest recorded vertebrate lifespan found in a coral reef fish. *Curr. Biol.* 15, R288–R289
9. Healy, K. *et al.* (2019) Animal life history is shaped by the pace of life and the distribution of age-specific mortality and reproduction. *Nat. Ecol. Evol.* 3, 1217–1224
10. Healy, K. *et al.* (2014) Ecology and mode-of-life explain lifespan variation in birds and mammals. *Proc. Biol. Sci.* 281, 20140298
11. da Silva, R. *et al.* (2022) Slow and negligible senescence among testudines challenges evolutionary theories of senescence. *Science* 376, 1466–1470
12. Reinke, B.A. *et al.* (2022) Diverse aging rates in ectothermic tetrapods provide insights for the evolution of aging and longevity. *Science* 376, 1459–1466
13. Kolora, S.R.R. *et al.* (2021) Origins and evolution of extreme life span in Pacific Ocean rockfishes. *Science* 374, 842–847
14. Ruby, J.G. *et al.* (2018) Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age. *Elife* 7, e31157
15. Barneche, D.R. *et al.* (2018) Fish reproductive-energy output increases disproportionately with body size. *Science* 360, 642–645
16. Auer, S.K. *et al.* (2018) Metabolic rate evolves rapidly and in parallel with the pace of life history. *Nat. Commun.* 9, 14
17. Pinho, G.M. *et al.* (2022) Hibernation slows epigenetic ageing in yellow-bellied marmots. *Nat. Ecol. Evol.* 6, 418–426
18. Wilkinson, G.S. and Adams, D.M. (2019) Recurrent evolution of extreme longevity in bats. *Biol. Lett.* 15, 20180860
19. Austad, S.N. and Fischer, K.E. (2016) Sex differences in lifespan. *Cell Metab.* 23, 1022–1033
20. Xirocostas, Z.A. *et al.* (2020) The sex with the reduced sex chromosome dies earlier: a comparison across the tree of life. *Biol. Lett.* 16, 20190867
21. Lemaître, J.-F. *et al.* (2020) Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proc. Natl. Acad. Sci. U. S. A.* 117, 8546–8553
22. Cayuela, H. *et al.* (2022) Sex-related differences in aging rate are associated with sex chromosome system in amphibians. *Evolution* 76, 346–356
23. Connallon, T. *et al.* (2022) How much does the unguarded X contribute to sex differences in life span? *Evol. Lett.* 6, 319–329
24. Brown, E.J. *et al.* (2020) The Y chromosome may contribute to sex-specific ageing in *Drosophila*. *Nat. Ecol. Evol.* 4, 853–862
25. Sultanova, Z. *et al.* (2023) Genetic sex determination, sex chromosome size and sex-specific lifespans across tetrapods. *J. Evol. Biol.* 36, 480–494
26. Keane, M. *et al.* (2015) Insights into the evolution of longevity from the bowhead whale genome. *Cell Rep.* 10, 112–122
27. Quesada, V. *et al.* (2019) Giant tortoise genomes provide insights into longevity and age-related disease. *Nat. Ecol. Evol.* 3, 87–95
28. Çilingir, F.G. *et al.* (2022) Chromosome-level genome assembly for the Aldabra giant tortoise enables insights into the genetic health of a threatened population. *Gigascience* 11, giac090
29. Zhou, X. *et al.* (2020) Beaver and naked mole rat genomes reveal common paths to longevity. *Cell Rep.* 32, 107949
30. Pascual-Torner, M. *et al.* (2022) Comparative genomics of mortal and immortal cnidarians unveils novel keys behind rejuvenation. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2118763119
31. Jebb, D. *et al.* (2020) Six reference-quality genomes reveal evolution of bat adaptations. *Nature* 583, 578–584
32. Valenzano, D.R. *et al.* (2015) The African turquoise killifish genome provides insights into evolution and genetic architecture of lifespan. *Cell* 163, 1539–1554
33. Cui, R. *et al.* (2019) Relaxed selection limits lifespan by increasing mutation load. *Cell* 178, 385–399
34. Hu, C.-K. *et al.* (2020) Vertebrate diapause preserves organisms long term through Polycomb complex members. *Science* 367, 870–874
35. Ma, S. and Gladyshev, V.N. (2017) Molecular signatures of longevity: Insights from cross-species comparative studies. *Semin. Cell Dev. Biol.* 70, 190–203
36. Singh, P.P. *et al.* (2019) The genetics of aging: a vertebrate perspective. *Cell* 177, 200–220
37. Tian, X. *et al.* (2017) Molecular mechanisms determining lifespan in short- and long-lived species. *Trends Endocrinol. Metab.* 28, 722–734
38. Kowalczyk, A. *et al.* (2020) Pan-mammalian analysis of molecular constraints underlying extended lifespan. *Elife* 9, e51089
39. Alexandrov, L.B. *et al.* (2020) The repertoire of mutational signatures in human cancer. *Nature* 578, 94–101
40. Stavrou, S. and Ross, S.R. (2015) APOBEC3 proteins in viral immunity. *J. Immunol.* 195, 4565–4570
41. Treaster, S. *et al.* (2023) Convergent genomics of longevity in rockfishes highlights the genetics of human life span variation. *Sci. Adv.* 9, eadd2743
42. Kowalczyk, A. *et al.* (2022) Complementary evolution of coding and noncoding sequence underlies mammalian hairlessness. *Elife* 11, e76911
43. Partha, R. *et al.* (2017) Subterranean mammals show convergent regression in ocular genes and enhancers, along with adaptation to tunneling. *Elife* 6, e25884
44. Kowalczyk, A. *et al.* (2019) RERconverge: an R package for associating evolutionary rates with convergent traits. *Bioinformatics* 35, 4815–4817
45. Willemsen, D. *et al.* (2020) Intra-species differences in population size shape life history and genome evolution. *Elife* 9, e55794
46. Armstrong, J. *et al.* (2020) Progressive Cactus is a multiple-genome aligner for the thousand-genome era. *Nature* 587, 246–251
47. Christmas, M.J. *et al.* (2023) Evolutionary constraint and innovation across hundreds of placental mammals. *Science* 380, eabn3943
48. Rhie, A. *et al.* (2021) Towards complete and error-free genome assemblies of all vertebrate species. *Nature* 592, 737–746
49. Vazquez, J.M. *et al.* (2018) A zombie LIF gene in elephants is upregulated by TP53 to induce apoptosis in response to DNA damage. *Cell Rep.* 24, 1765–1776
50. Vazquez, J.M. and Lynch, V.J. (2021) Pervasive duplication of tumor suppressors in Afrotherians during the evolution of large bodies and reduced cancer risk. *Elife* 10, e65041

51. Sulak, M. *et al.* (2016) copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. *Elife* 5, e11994
52. Preston, A.J. *et al.* (2023) Elephant TP53-RETROGENE 9 induces transcription-independent apoptosis at the mitochondria. *Cell Death Dis.* 9, 66
53. Vazquez, J.M. *et al.* (2022) A CDKN2C retroduplication in Bowhead whales is associated with the evolution of extremely long lifespans and alerted cell cycle dynamics. *BioRxiv* Published online September 7, 2022. https://doi.org/10.1101/2022.09.07.506958
54. Firsanov, D. *et al.* (2023) DNA repair and anti-cancer mechanisms in the longest-living mammal: the bowhead whale. *BioRxiv* Published online May 8, 2023. https://doi.org/10.1101/2023.05.07.539748
55. Salmon, A.B. *et al.* (2008) Fibroblasts from naked mole-rats are resistant to multiple forms of cell injury, but sensitive to peroxide, ultraviolet light, and endoplasmic reticulum stress. *J. Gerontol. A Biol. Sci. Med. Sci.* 63, 232–241
56. Gorbunova, V. *et al.* (2012) Cancer resistance in the blind mole rat is mediated by concerted necrotic cell death mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 109, 19392–19396
57. Gorbunova, V. *et al.* (2008) Rodents for comparative aging studies: from mice to beavers. *Age* 30, 111–119
58. Zhang, Q. *et al.* (2021) Genomic expansion of Aldh1a1 protects beavers against high metabolic aldehydes from lipid oxidation. *Cell Rep.* 37, 109965
59. Sudlow, C. *et al.* (2015) UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* 12, e1001779
60. All of Us Research Program Investigators *et al.* (2019) The 'All of Us' Research Program. *N. Engl. J. Med.* 381, 668–676
61. Rodríguez, J.A. *et al.* (2017) Antagonistic pleiotropy and mutation accumulation influence human senescence and disease. *Nat. Ecol. Evol.* 1, 55
62. Long, E. and Zhang, J. (2019) Retesting the influences of mutation accumulation and antagonistic pleiotropy on human senescence and disease. *Nat. Ecol. Evol.* 3, 992–993
63. Mostafavi, H. *et al.* (2017) Identifying genetic variants that affect viability in large cohorts. *PLoS Biol.* 15, e2002458
64. Smith, K.R. *et al.* (2009) Familial aggregation of survival and late female reproduction. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 740–744
65. Shadyab, A.H. *et al.* (2017) Maternal age at childbirth and parity as predictors of longevity among women in the United States: the Women's Health Initiative. *Am. J. Public Health* 107, 113–119
66. Pavard, S. and Coste, C.F.D. (2021) Evolutionary demographic models reveal the strength of purifying selection on susceptibility alleles to late-onset diseases. *Nat. Ecol. Evol.* 5, 392–400
67. Ljungquist, B. *et al.* (1998) The effect of genetic factors for longevity: a comparison of identical and fraternal twins in the Swedish Twin Registry. *J. Gerontol. A Biol. Sci. Med. Sci.* 53, M441–M446
68. Ruby, J.G. *et al.* (2018) Estimates of the heritability of human longevity are substantially inflated due to assortative mating. *Genetics* 210, 1109–1124
69. Dwyer-Lindgren, L. *et al.* (2017) Inequalities in life expectancy among US counties, 1980 to 2014: temporal trends and key drivers. *JAMA Intern. Med.* 177, 1003–1011
70. Vierboom, Y.C. and Preston, S.H. (2020) Life beyond 65: changing spatial patterns of survival at older ages in the united states, 2000–2016. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 75, 1093–1103
71. Pallares, L.F. *et al.* (2023) Dietary stress remodels the genetic architecture of lifespan variation in outbred *Drosophila*. *Nat. Genet.* 55, 123–129
72. Corbett, S. *et al.* (2018) The transition to modernity and chronic disease: mismatch and natural selection. *Nat. Rev. Genet.* 19, 419–430
73. Cheng, C. and Kirkpatrick, M. (2021) Molecular evolution and the decline of purifying selection with age. *Nat. Commun.* 12, 2657
74. Turan, Z.G. *et al.* (2019) Molecular footprint of Medawar's mutation accumulation process in mammalian aging. *Aging Cell* 18, e12965
75. Jia, K. *et al.* (2018) An analysis of aging-related genes derived from the Genotype-Tissue Expression project (GTEx). *Cell Death Dis.* 4, 26
76. Yamamoto, R. *et al.* (2022) Tissue-specific impacts of aging and genetics on gene expression patterns in humans. *Nat. Commun.* 13, 5803
77. Penrose, L.S. (1955) Parental age and mutation. *Lancet* 269, 312–313
78. Jónsson, H. *et al.* (2017) Parental influence on human germline de novo mutations in 1,548 trios from Iceland. *Nature* 549, 519–522
79. Belyeu, J.R. *et al.* (2021) De novo structural mutation rates and gamete-of-origin biases revealed through genome sequencing of 2,396 families. *Am. J. Hum. Genet.* 108, 597–607
80. Thomas, G.W.C. *et al.* (2021) Origins and long-term patterns of copy-number variation in Rhesus macaques. *Mol. Biol. Evol.* 38, 1460–1471
81. Laurentino, S. *et al.* (2020) A germ cell-specific ageing pattern in otherwise healthy men. *Aging Cell* 19, e13242
82. Nurk, S. *et al.* (2022) The complete sequence of a human genome. *Science* 376, 44–53
83. Bergeron, L.A. *et al.* (2023) Evolution of the germline mutation rate across vertebrates. *Nature* 615, 285–291
84. López-Otín, C. *et al.* (2023) Hallmarks of aging: an expanding universe. *Cell* 186, 243–278
85. Abascal, F. *et al.* (2021) Somatic mutation landscapes at single-molecule resolution. *Nature* 593, 405–410
86. Milholland, B. *et al.* (2017) Differences between germline and somatic mutation rates in humans and mice. *Nat. Commun.* 8, 15183
87. Cagan, A. *et al.* (2022) Somatic mutation rates scale with lifespan across mammals. *Nature* 604, 517–524
88. Moore, L. *et al.* (2021) The mutational landscape of human somatic and germline cells. *Nature* 597, 381–386
89. Rockweiler, N.B. *et al.* (2023) The origins and functional effects of postzygotic mutations throughout the human life span. *Science* 380, eabn7113
90. Yizhak, K. *et al.* (2019) RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science* 364
91. Kirkwood, T.B. (1977) Evolution of ageing. *Nature* 270, 301–304
92. Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell* 100, 57–70
93. Leroi, A.M. *et al.* (2003) Cancer selection. *Nat. Rev. Cancer* 3, 226–231
94. Green, J. *et al.* (2011) Height and cancer incidence in the Million Women Study: prospective cohort, and meta-analysis of prospective studies of height and total cancer risk. *Lancet Oncol.* 12, 785–794
95. Dobson, J.M. (2013) Breed-predispositions to cancer in pedigree dogs. *ISRN Vet. Sci.* 2013, 941275
96. Abegglen, L.M. *et al.* (2015) Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. *JAMA* 314, 1850–1860
97. Peto, R. (1977) *Origins of Human Cancer*, Cold Spring Harbor Laboratory
98. Vincze, O. *et al.* (2022) Cancer risk across mammals. *Nature* 601, 263–267
99. Lu, A.T. *et al.* (2023) Universal DNA methylation age across mammalian tissues. *Nat. Aging* Published online August 10, 2023. https://doi.org/10.1038/s43587-023-00462-6
100. Moqri, M. *et al.* (2022) PRC2 clock: a universal epigenetic biomarker of aging and rejuvenation. *BioRxiv* Published online June 5, 2022. https://doi.org/10.1101/2022.06.03.494609
101. Haghani, A. *et al.* (2023) DNA methylation networks underlying mammalian traits. *Science* 381, eabq5693
102. Whittemore, K. *et al.* (2019) Telomere shortening rate predicts species life span. *Proc. Natl. Acad. Sci. U. S. A.* 116, 15122–15127
103. Nooden, L.D. (2012) *Senescence and Aging in Plants*, Elsevier
104. Zhang, S. *et al.* (2020) *Caenorhabditis elegans* as a useful model for studying aging mutations. *Front. Endocrinol.* 11, 554994
105. Siler, W. (1979) A competing-risk model for animal mortality. *Ecology* 60, 750–757

106. Zoonomia Consortium (2020) A comparative genomics multitool for scientific discovery and conservation. *Nature* 587, 240–245
107. Kuderna, L.F.K. *et al.* (2023) A global catalog of whole-genome diversity from 233 primate species. *Science* 380, 906–913
108. Liao, W.-W. *et al.* (2023) A draft human pangenome reference. *Nature* 617, 312–324
109. Ferreira, J.V. *et al.* (2022) Cell non-autonomous proteostasis regulation in aging and disease. *Front. Neurosci.* 16, 878296
110. Mertens, J. *et al.* (2018) Aging in a dish: iPSC-derived and directly induced neurons for studying brain aging and age-related neurodegenerative diseases. *Annu. Rev. Genet.* 52, 271–293
111. Browder, K.C. *et al.* (2022) In vivo partial reprogramming alters age-associated molecular changes during physiological aging in mice. *Nat. Aging* 2, 243–253
112. Torrens-Mas, M. *et al.* (2021) Organoids: an emerging tool to study aging signature across human tissues. Modeling aging with patient-derived organoids. *Int. J. Mol. Sci.* 22, 10547
113. Liu, H. *et al.* (2008) Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* 3, 587–590
114. Hong, S.G. *et al.* (2014) Path to the clinic: assessment of iPSC-based cell therapies in vivo in a nonhuman primate model. *Cell Rep.* 7, 1298–1309
115. Pollen, A.A. *et al.* (2019) Establishing cerebral organoids as models of human-specific brain evolution. *Cell* 176, 743–756
116. Pavlovic, B.J. *et al.* (2018) A comparative assessment of human and chimpanzee iPSC-derived cardiomyocytes with primary heart tissues. *Sci. Rep.* 8, 15312
117. Mitchell, S.J. *et al.* (2015) Animal models of aging research: implications for human aging and age-related diseases. *Annu. Rev. Anim. Biosci.* 3, 283–303
118. Tian, X. *et al.* (2013) High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* 499, 346–349
119. Hadi, F. *et al.* (2020) Transformation of naked mole-rat cells. *Nature* 583, E1–E7
120. Zhao, J. *et al.* (2020) Reply to: Transformation of naked mole-rat cells. *Nature* 583, E8–E13
121. Zhao, S. *et al.* (2014) High autophagy in the naked mole rat may play a significant role in maintaining good health. *Cell. Physiol. Biochem.* 33, 321–332
122. Miura, K. *et al.* (2021) Induced pluripotent stem cells from cancer-resistant naked mole-rats. *Adv. Exp. Med. Biol.* 1319, 329–339
123. Tan, L. *et al.* (2017) Naked mole rat cells have a stable epigenome that resists iPSC reprogramming. *Stem Cell Rep.* 9, 1721–1734
124. Miyawaki, S. *et al.* (2016) Tumour resistance in induced pluripotent stem cells derived from naked mole-rats. *Nat. Commun.* 7, 11471
125. Lee, S.-G. *et al.* (2017) Naked mole rat induced pluripotent stem cells and their contribution to interspecific chimera. *Stem Cell Rep.* 9, 1706–1720
126. Labinskyy, N. *et al.* (2006) Comparison of endothelial function, $O2^{-\bullet}$ and H_2O_2 production, and vascular oxidative stress resistance between the longest-living rodent, the naked mole rat, and mice. *Am. J. Physiol. Heart Circ. Physiol.* 291, H2698–H2704
127. Burkard, M. *et al.* (2015) Establishment of the first humpback whale fibroblast cell lines and their application in chemical risk assessment. *Aquat. Toxicol.* 167, 240–247
128. Pereiro, X. *et al.* (2022) Characteristics of whale Müller glia in primary and immortalized cultures. *Front. Neurosci.* 16, 854278
129. Yajing, S. *et al.* (2018) Establishment and characterization of pygmy killer whale (*Feresa attenuata*) dermal fibroblast cell line. *PLoS One* 13, e0195128
130. Hightower, L.E. and Renfro, J.L. (1988) Recent applications of fish cell culture to biomedical research. *J. Exp. Zool.* 248, 290–302
131. Goswami, M. *et al.* (2022) Role and relevance of fish cell lines in advanced in vitro research. *Mol. Biol. Rep.* 49, 2393–2411
132. Peng, L. *et al.* (2019) Generation of stable induced pluripotent stem-like cells from adult zebra fish fibroblasts. *Int. J. Biol. Sci.* 15, 2340–2349
133. Schwarz, J.S. *et al.* (2015) Value of organoids from comparative epithelia models. *Yale J. Biol. Med.* 88, 367–374
134. Zilova, L. *et al.* (2021) Fish primary embryonic pluripotent cells assemble into retinal tissue mirroring in vivo early eye development. *Elife* 10, e66998
135. Tan, F. *et al.* (2010) Validation of an in vitro cytotoxicity test for four heavy metals using cell lines derived from a green sea turtle (*Chelonia mydas*). *Cell Biol. Toxicol.* 26, 255–263
136. Glaberman, S. *et al.* (2021) Concurrent evolution of antiaging gene duplications and cellular phenotypes in long-lived turtles. *Genome Biol. Evol.* 13, evab244
137. Martins, G.S. *et al.* (2016) Cytochemical characteristics of blood cells from Brazilian tortoises (Testudines: Testudinidae). *Genet. Mol. Res.* 15, gmr7549
138. Zdyrski, C. *et al.* (2023) Characterization of the first turtle organoids: a model for investigating unique adaptations with biomedical potential. *BioRxiv* Published online February 21, 2023. https://doi.org/10.1101/2023.02.20.527070
139. Brook, C.E. *et al.* (2020) Accelerated viral dynamics in bat cell lines, with implications for zoonotic emergence. *Elife* 9, e48401
140. Jacquet, S. *et al.* (2022) Adaptive duplication and genetic diversification of protein kinase R contribute to the specificity of bat–virus interactions. *Sci. Adv.* 8, eadd7540
141. Koh, J. *et al.* (2019) ABCB1 protects bat cells from DNA damage induced by genotoxic compounds. *Nat. Commun.* 10, 2820
142. Tarigan, R. *et al.* (2021) Distinct interferon response in bat and other mammalian cell lines infected with Pteropine orthoreovirus. *Virus Genes* 57, 510–520
143. Cosby, R.L. *et al.* (2021) Recurrent evolution of vertebrate transcription factors by transposase capture. *Science* 371, eabc6405
144. Aurine, N. *et al.* (2021) Reprogrammed *Pteropus* bat stem cells as a model to study host–pathogen interaction during Henipavirus infection. *Microorganisms* 9, 2567
145. Mo, X. *et al.* (2014) Generation and characterization of bat-induced pluripotent stem cells. *Theriogenology* 82, 283–293
146. Déjosez, M. *et al.* (2023) Bat pluripotent stem cells reveal unusual entanglement between host and viruses. *Cell* 186, 957–974
147. Chan, L.L.Y. *et al.* (2023) Generation of self-replicating airway organoids from the cave nectar bat as a model system for studying host–pathogen interactions in the bat airway epithelium. *Emerg. Microbes Infect.* 12, e2148561
148. Elbadawy, M. *et al.* (2021) Establishment of intestinal organoid from *Rousettus leschenaultii* and the susceptibility to bat-associated viruses, SARS-CoV-2 and pteropine orthoreovirus. *Int. J. Mol. Sci.* 22, 10763
149. Zhou, J. *et al.* (2020) Infection of bat and human intestinal organoids by SARS-CoV-2. *Nat. Med.* 26, 1077–1083