BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2023), pp. 000–000.
doi: 10.1111/brv.12992

Biological clocks as age estimation markers in animals: a systematic review and meta-analysis

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ABSTRACT

Various biological attributes associated with individual fitness in animals change predictably over the lifespan of an organism. Therefore, the study of animal ecology and the work of conservationists frequently relies upon the ability to assign animals to functionally relevant age classes to model population fitness. Several approaches have been applied to determining individual age and, while these methods have proved useful, they are not without limitations and often lack standardisation or are only applicable to specific species. For these reasons, scientists have explored the potential use of biological clocks towards creating a universal age-determination method. Two biological clocks, tooth layer annulation and otolith layering have found universal appeal. Both methods are highly invasive and most appropriate for *post-mortem* age-at-death estimation. More recently, attributes of cellular ageing previously explored in humans have been adapted to studying ageing in animals for the use of less-invasive molecular methods for determining age. Here, we review two such methods, assessment of methylation and telomere length, describing (*i*) what they are, (*ii*) how they change with age, and providing (*iii*) a summary and meta-analysis of studies that have explored their utility in animal age determination. We found that both attributes have been studied across multiple vertebrate classes, however, telomere studies were used before methylation studies and telomere length has been modelled in nearly twice as many studies. Telomere length studies included in the review often related changes to stress responses and illustrated that telomere length is sensitive to environmental and social stressors and, in the absence of repair mechanisms such as telomerase or alternative lengthening modes, lacks the ability to recover. Methylation studies, however, while also detecting sensitivity to stressors and toxins, illustrated the ability to recover from such stresses after a period of accelerated ageing, likely due to constitutive expression or reactivation of repair enzymes such as DNA methyl transferases. We also found that both studied attributes have parentally heritable features, but the mode of inheritance differs among taxa and may relate to heterogamy. Our meta-analysis included more than 40 species in common for methylation and telomere length, although both analyses included at least 60 age-estimation models. We found that methylation outperforms telomere length in terms of predictive power evidenced from effect sizes (more than double that observed for telomeres) and smaller prediction intervals. Both methods produced age correlation models using similar sample sizes and were able to classify individuals into young, middle, or old age classes with high accuracy. Our review and meta-analysis illustrate that both methods are well suited to studying age in animals and do not suffer significantly from variation due to differences in the lifespan of the species, genome size, karyotype, or tissue type but rather that quantitative method, patterns of inheritance, and environmental factors should be the main considerations. Thus, provided that complex factors affecting the measured trait can be accounted for, both methylation and telomere length are promising targets to develop as biomarkers for age determination in animals.

Key words: animals, biological clocks, tooth layers, otoliths, epigenetics, methylation, telomeres, biomarker, age determination, meta-analysis.

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I. INTRODUCTION

Age is a key factor in the study of animal populations, as many critical biological attributes change over time. Age classes are often linked to biological functionality in terms of developmental stage (Noren & Edwards, 2007), reproductive maturity (Charpentier *et al.*, 2008), future reproductive potential (Jones *et al.*, 2014), reproductive success

(Bradley & Safran, 2014), disease burden (Starr & Saito, 2014), and risk of mortality (Pérez-Barberìa *et al.*, 2014). These attributes typically differ between discreet age classes and the fraction of a population contained within the different age classes therefore assists conservation biologists to estimate their future fitness (Pérez-Pereira, López-Cortegano & García-Dorado, 2022). Multiple approaches have been used with varying degrees of success to determine the age of animals and often rely upon either external features that change predictably over time, a mark–recapture approach (Clutton-Brock & Sheldon, 2010), or biochemical attributes.

The first, and most common method, uses morphometrics or physical measurements of the skull (Smuts, Anderson & Austin, 1978), body (Marker & Dickman, 2003), scales (Isermann, Wolter & Breeggemann, 2010), wingspan (Erdem *et al.*, 2021), or mass to determine age by comparing measures to an established chart. While this method is particularly useful for juveniles and young adults it generally loses resolution when applied to adults of varying age classes in species where growth arrests at adulthood. To overcome this, morphometrics have also been complemented with other physical attributes such as degree of gumline recession (Hiller & Tyre, 2014) or the condition of the pelage (Marker & Dickman, 2003). Drawbacks of this approach are (*i*) that it requires the capture and prolonged immobilisation of the animal to record the measures, (*ii*) few and sparsely detailed validation studies exist (which may be difficult to conduct), and (*iii*) there is high inter-observer error, particularly in cases where a reasonable amount of expertise is needed to discern age based on observed traits.

The mark–recapture approach either relies upon distinct natural markings and external features that are documented, or artificial markings applied to young individuals upon first encounter that are used to verify the identity and age of the individual upon subsequent encounters. Natural markings that can be used to identify individuals include, for example, pigmentation patterns on the flukes of humpback whales [*Megaptera novaeangliae* (Borowski)] (Polanowski *et al.*, 2014). Artificial markings, on the other hand, include manmade tools of identification such as uniquely numbered bands (Sherley *et al.*, 2014) and geolocators (Saino *et al.*, 2015) attached to birds, tracking collars (Marker *et al.*, 2003) attached to cheetahs [*Acinonyx jubatus* (Schreber)], or teracycline marking (Cailliet, 1990) in fishes. A major pitfall in the use of these methods is that they rely upon the eventual re-encounter of the same individual and require the attachment or injection of a marker to a live animal. In addition, the lifespan of many animals far outreaches the average lifespan and career of conservationists, making long-term tracking unsuitable in some cases.

For these reasons, conservationists have frequently sought a biomarker or method of age determination that is universally applicable to most species, provides a direct measure of age, and does not require extensive experience or detailed individual validation studies. While several biochemical, physiological, and haematological attributes have been suggested (Short, Williams & Bowden, 1987; Cailliet, 1990; Chaney *et al.*, 2003), the most promising method for achieving this is to identify measurable features of biological clocks that change on an annual basis. A biological clock can be defined as: an innate mechanism that controls the physiological activities of an organism which change on a daily, seasonal, yearly, or other regular cycle. Two biological clocks have found widespread applications in the estimation of animal age. In fish, histological features of internal structures such as growth rings in otoliths, small stones found within the auditory system, have been used to determine age by counting stria that form from each annual deposition of a new layer (Campana, 2001). Similarly, the annual cycle of depositing a new layer of dentin (Lockyer, 1993; Lockyer *et al.*, 2007) or cementum (Wittwer-Backofen, Gampe & Vaupel, 2004) on teeth can be used to determine the age of mammals. Tooth cementum annulation (TCA; reviewed in Viciano, López-Lázaro & Tanga, 2022) was first documented across diverse species of mammals and reptiles in the 19th century (Owen, 1840) and has been a reliable method of age estimation in animals for nearly 75 years. TCA has been used to determine the age of more than a hundred species in the class Mammalia alone: across 10 orders and 38 families (see online Supporting Information, Table S1). There are, however, still several shortcomings to these methods, e.g. otoliths are only effective in fish, tooth histology is only accurate in mammals, and both methods are extremely invasive, either requiring the extraction of a tooth or only being appropriate in *post-mortem* age-at-death determination. Birds, on the other hand, represent a unique conundrum as they lack both teeth and otoliths, requiring the exploration of alternatives such as the measurement of pentosidine accumulation in the skin of lilac-breasted rollers, *Coracias caudatus* (Linnaeus), among others (Chaney *et al.*, 2003).

With the dawn of the molecular revolution many methods have been developed that use DNA to ascertain and study critical individual characteristics as well as population attributes within most species. Such attributes include sex (Morin *et al.*, 2005), parentage (Double *et al.*, 1997), population assignment (Kim & Sappington, 2013), hybridization (Brelsford, Milá & Irwin, 2011), speciation (Linck, Freeman & Dumbacher, 2020), and migration (Merlin & Liedvogel, 2019). As natural ageing is in many respects a programmed process under genetic control (Horvath, 2013), or results from unrepaired environmental damage to DNA (Kujoth *et al.*, 2005), there is great potential to exploit these changes to determine age. Some age-related changes, summarised in Fig. 1, include fluctuating levels of transcripts (Cook *et al.*, 2006), changes to the DNA sequence itself, e.g. mitochondrial mutations (Zapico & Ubelaker, 2016), and epigenetic modifications (Jones, Goodman & Kobor, 2015). Epigenetics is a collective term for mechanisms that modify DNA and DNA packaging, independent of changes to the genetic sequence (Bird, 2007).

Studies seeking to establish a genetic method for age estimation have identified several potential targets among the epigenetic regulatory elements including methylation

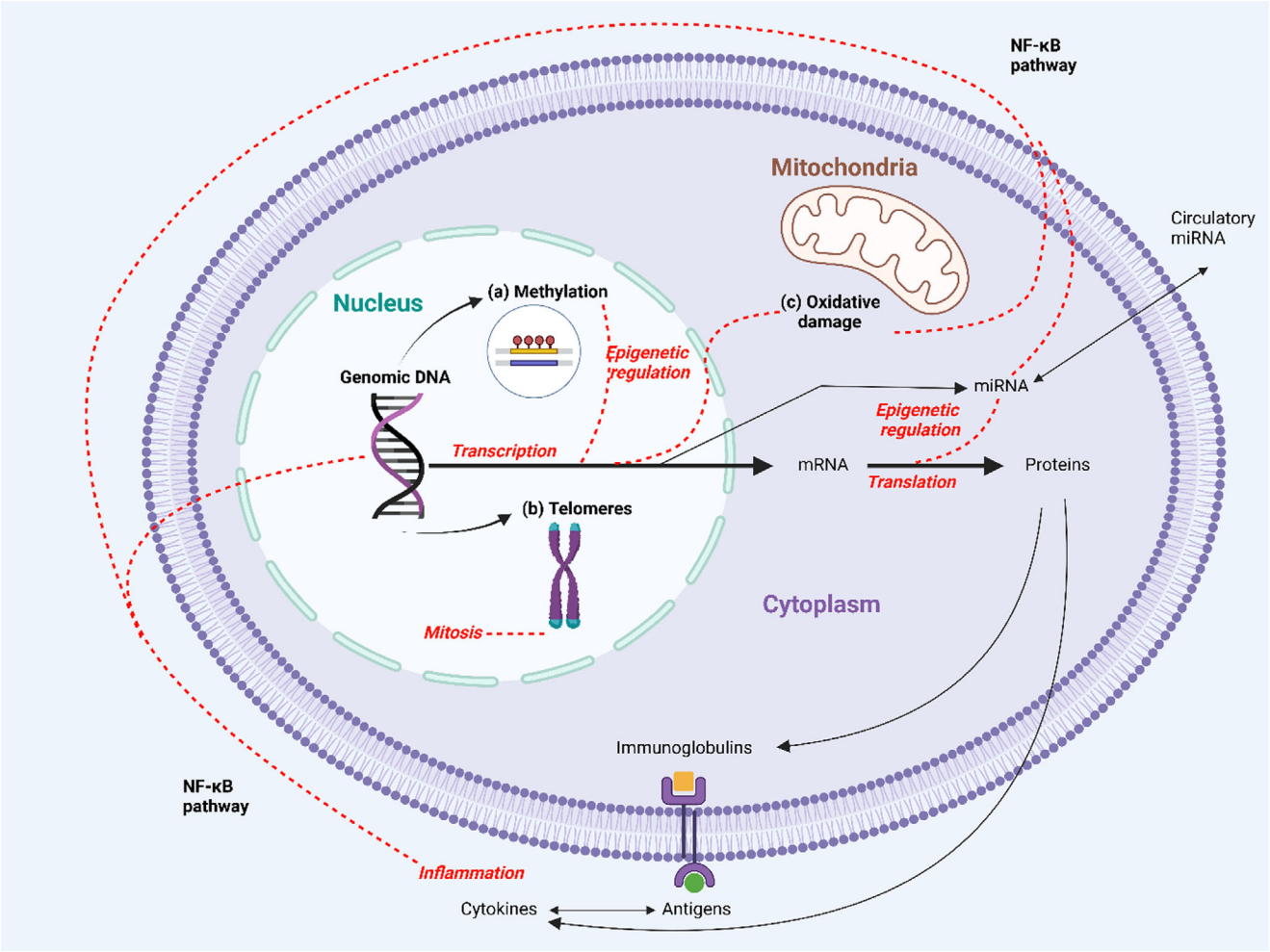

Fig. 1. An animal cell indicating the mechanisms of cellular ageing at the molecular level that are currently used as molecular biomarkers for age. Molecules are labelled in plain text and processes in red italics. Movement of molecules is indicated by lines with arrows; regulatory effects on molecular pathways are indicated by broken red lines. The main pathway of gene expression is shown by thicker arrows in the centre, and comprises the two phases transcription and translation, that turn information stored as DNA into functional molecules including proteins and various types of RNA. (a) DNA methylation levels at several sites change with age. (b) Telomeres have roles in many age-related cellular processes and shorten over time and with age. (c) Oxidative damage due to chronic inflammation through the constitutive activation of the nuclear factor-kappa B (NF-κB) pathway leads to mitochondrial mutations that accumulate over time. miRNA, microRNA; mRNA, messenger RNA. Image created in BioRender.com.

(Johansson, Enroth & Gyllensten, 2013) and telomere shortening (Thomas & Zapico, 2017). Both methylation and telomere shortening were initially tested in humans (Aubert & Lansdorp, 2008; Gopalan *et al.*, 2017) but have since been evaluated as valid methods of less-invasive age determination (Carroll *et al.*, 2018) that is transferable across most species (Jarman *et al.*, 2015; De Paoli-Iseppi *et al.*, 2017*a*). This review provides a synopsis and meta-analysis of two genetic biological clocks for age estimation, methylation and telomeres, in terms of (*i*) what they are, (*ii*) what changes they undergo throughout a lifespan, and (*iii*) the potential use of these features as applied to biological age determination in wildlife, evidenced by a meta-analysis of the literature.

II. METHODS

(1) Literature search and review

A systematic approach was used to search for and synthesise the available literature, as depicted in the PRISMA flow diagrams in Figs S1 and S2 (Haddaway *et al.*, 2022). Literature for methylation-based studies was searched for in the *Scopus* (www.scopus.com) and *Dimensions* (www.dimensions.ai) databases using the following Boolean search string: ('Epigenetics' OR 'Methylation') AND ('age' OR 'aging') AND ('determination' OR 'model') AND ('Animals' OR 'wild'). This process was repeated for telomere-based studies by substituting 'Telomere' for 'Methylation'. Search results

were exported in the comma separated value format and the literature was subsequently summarised, guided by citation networks visualised using CitNetExplorer 1.0.0 and VOSviewer 1.6.16 (van Eck & Waltman, 2017). Results retrieved from *Scopus* were converted to the appropriate format with the R package *Scopus2CitNet 0.1.0.0* in RStudio 1.4.1106 (RStudio Team, 2021), running R version 4.0.5 (R Core Team, 2020). Further studies were identified from the reference lists of articles and free term 'citation searches' on *Google Scholar*. Other background information beyond the studies for systematic review was retrieved with free term searches using 'DNA methylation', 'telomere length', or 'biological clocks for age estimation' and are not included in the PRISMA statements. Studies were summarised based on species covered, publication year, method used, number of sites assayed (methylation), and sample size (N). Individual models reported in studies were summarised by tissue type, species, and method used, with their associated correlation coefficient (R^2), F-test statistic, t-test statistic, or chi-squared (χ^2) test statistic and P-value. Methylation studies that reported more than one model usually presented models using the same method, but different tissue types or species tested, while a few telomere studies reported multiple models generated to compare different measurement methods on the same samples. Where appropriate statistical measures were not reported, values were calculated from data in the online supplement or data extracted from plots using *WebPlotDigitizer* version 4.6 (Rohatgi, 2022). The data set used in this review is available online (see Section 0. Data availability statement). Deviation between predicted and known age are reported as mean error of prediction (MEP) due to variation in reported measure in studies e.g. median absolute error (MAE) or root mean-square error (MSE). Genome information for species, or their closest relative, was retrieved from the *Animal Genome Size Database* (Gregory, 2023), *Animal Chromosome Count Database* (Román-Palacios *et al.*, 2021), the *Bird 10,000 Genomes (B10K) Project* (Zhang, 2015), and/or the *Bird Chromosome Database* (Degrandi *et al.*, 2020).

(2) Meta-analysis

To conduct a meta-analysis, models reported in studies that were adequately similar in terms of tissue sample and method of detection/assay were identified from the systematic review, including 78 studies for methylation and 109 studies for telomeres. Models reported in studies with a sufficient sample size ($N \geq 15$) were included for consideration in the meta-analysis. For methylation, pan-species models with human samples were excluded from further analyses to focus only on non-human models. An additional two methylation models were also excluded (Ito, Yoshimura & Momoi, 2017; Horvath *et al.*, 2022*d*). The first was excluded because neither the correlation coefficient nor the data plots for the combined model were reported, the latter was excluded due to a discrepancy in the reported sample size for their *Macropus* model (59 in the table but 65 in the figure). The final data set included 60 methylation-based models across the five vertebrate classes: fishes ($N = 3$), amphibians ($N = 2$), reptiles ($N = 3$), birds ($N = 3$), mammals ($N = 49$). For mammals this included aquatic mammals ($N = 6$), bats ($N = 2$), carnivores ($N = 9$), elephants ($N = 2$), marsupials ($N = 3$), primates ($N = 10$), rodents ($N = 4$), shrews ($N = 1$), and ungulates ($N = 12$). For methylation we classified models into three groups. Group 1 ($N = 48$) models were those that collected data for a single species and used those data to create a molecular model for predicting age in the same species. For Group 2 ($N = 8$), the models used a large sample size in a related species to create a model, and subsequently tested the model in another species using fewer samples. Group 3 models ($N = 4$) developed and tested a single model using samples from multiple related species such as cetaceans, bats, cod, or pinnipeds. For the included methylation studies the correlation coefficient and sample size were used to calculate the Fisher's-Z_r transform and the variance (v) as a means of approximating the correlation coefficient effect size (r) for cross-study meta-analysis.

Two telomere studies were excluded from the main analyses, the first was completely excluded due to small sample sizes (Whittemore *et al.*, 2019) while the second was analysed separately, as for methylation, due to the pooling of samples for two species (group 2) of marten (Pauli *et al.*, 2011). Due to the large number of studies on birds, 12 avian models were selected at random for inclusion in the cross-taxa analyses ($N = 60$) while the full set of bird models ($N = 51$) were analysed separately to avoid phylogenetic bias. The first telomere data set, for cross-taxa analyses, included 60 models across the five vertebrate classes: fishes ($N = 6$), amphibians ($N = 3$), reptiles ($N = 6$), birds ($N = 12$), mammals ($N = 33$). For mammals this included aquatic mammals ($N = 2$), bats ($N = 6$), carnivores ($N = 13$), elephants ($N = 1$), primates ($N = 2$), rodents ($N = 4$), and ungulates ($N = 5$). Telomere models for cross-taxa analyses were also grouped, as for methylation, with group 1 studies ($N = 59$) representing studies on single species while group 2 studies ($N = 1$) included models based on pooled samples for multiple species.

The second telomere data set, on studies conducted exclusively in birds, included 51 models and were grouped based on key avian taxonomic groupings. This included specific taxonomic groupings such as: chats and flycatchers ($N = 6$); corvids ($N = 4$); cuckoos ($N = 1$); finches ($N = 3$); geese and waterfowl ($N = 1$); gulls, shorebirds, and waders ($N = 4$); juncos, buntings, and sparrows ($N = 2$), magpies ($N = 1$); manakins ($N = 1$); parrots ($N = 1$); penguins ($N = 3$); seabirds ($N = 11$); swallows ($N = 5$); swifts ($N = 1$); tits ($N = 3$); warblers ($N = 3$); and wrens ($N = 1$). For the included telomere studies statistical methods varied, thus effect size was calculated as before or adapted to the tests used in the study e.g. F- or χ^2-test statistics.

The individual meta-analyses were conducted in RStudio 1.4.1106 (RStudio Team, 2021), running R version 4.0.5 (R Core Team, 2020) with the package *meta 5.5-0* (Schwarzer, Carpenter & Rücker, 2015) and effect sizes calculated by *compute.es 0.2-2* (Del Re, 2013). The relative anticipated levels of author bias resulting from multiple

publications by the same authors was assessed using a custom PYTHON script, *Author Bias Computation* (*ABCal*) version 1.0.1 (see Section 0). Meta-analyses were run with grouping based on class, study group, tissue type, and method. A separate meta-analysis was run between biological clocks, rather than studies, to compare the efficacy of either methylation or telomere length for determining age in animals.

Heterogeneity was measured between studies within groups as well as globally using standard heterogeneity percentages (I^2), tau-squared (τ^2), and the *P*-value for the χ^2-test statistic. Potential causes of heterogeneity were explored with a meta-regression of individual factors such as author, publication year, taxonomic class, tissue type, experimental method and either genome size (methylation) or karyotype (telomere). Type I error was avoided by having adequate sample sizes ($N > 50$), carrying out one-tailed analyses when possible, and reporting significance at standard alpha values along with correlation coefficients (R^2). To avoid potential type I error due to multiple testing, introduced by collinearity, each factor was measured in a separate and independent regression. Publication or small study bias, resulting in asymmetry, was assessed in R. The relationships between statistical power, effect size and sample size (N) for study methods were explored using G*Power version 3.1 (Faul *et al.*, 2009; Kang, 2021) and are graphically depicted in Fig. S3. A separate meta-regression analysis was run between study methods, rather than models, to compare the efficacy of either methylation or telomere length for determining age in animals.

III. BIOLOGICAL CLOCKS AND AGE

(1) DNA methylation

(a) *What is DNA methylation?*

DNA methylation is an epigenetic process that modifies DNA through the addition of a methyl group (CH_3) to the 5′ cytosine of cytosine–guanine pairs (CpGs) (Fig. 2), as part of DNA packaging as well as the regulation of gene expression. Active methylation of unmethylated CpGs is catalysed by two DNA methyltransferase enzymes, DNMT3A and DNMT3B (Chédin, 2011), that add a methyl group to the fifth carbon of cytosines to form 5-methylcytosine. Methylation status of methylated CpGs is maintained between replicative cycles through the enzymatic activity of DNA methyltransferase 1 (DNMT1), an enzyme that is able to recognise hemi-methylated DNA after cell division and add a methyl group to the fifth carbon of cytosines on unmethylated daughter strands (Sen *et al.*, 2010).

DNA is demethylated *via* one of two mechanisms, either active or passive demethylation (Guo *et al.*, 2014). Passive demethylation occurs at cell division, where the newly synthesised daughter strands lack a methyl group at the newly incorporated cytosines and methylation cannot be re-established by DNMT1 prior to subsequent division (Kagiwada *et al.*, 2013). As passive demethylation only occurs during highly active sequential cycles of cell division it is most common during embryogenesis and early development (Andergassen *et al.*, 2021), as well as certain types of malignant growths (Gomez *et al.*, 2007; Zhang *et al.*, 2020).

By contrast, active demethylation is an enzymatic process (Ooi & Bestor, 2008) involving TET methylcytosine dioxygenase enzymes, such as TET2, and thymine DNA glycosylase (TDG). TET enzymes demethylate DNA by repeatedly adding a hydrogen to the methyl group on the fifth carbon of cytosines, first forming 5-hydroxymethylcytosine, followed by 5-formylcytosine, and finally 5-carboxylcytosine (Fig. 2C). This then serves as the substrate for TDG, which decarboxylates 5-carboxycytosine back to an unmethylated cytosine (Dalton & Bellacosa, 2012). This process is typically associated with the uncoiling of DNA to provide access to promotor and enhancer elements for transcription factors such as nuclear factor kappa B (Tilstra *et al.*, 2011; Balistreri *et al.*, 2013), as well as polymerase complexes responsible for transcription and replication (Attwood, Yung & Richardson, 2002).

The methylation of CpGs is variable across the genome and is a heritable trait through parental imprinting (Andergassen *et al.*, 2021) (Fig. 3), as well as trans-generational epigenetic inheritance (Fitz-James & Cavalli, 2022), while still being subject to environmental influence (McEwen *et al.*, 2017), and complements other genetic evolutionary processes (Lind & Spagopoulou, 2018; Parrott & Bertucci, 2019; Cavalli & Heard, 2019). Those located within CpG islands, dense clusters of consecutive CpGs, tend to be hypermethylated and occur in proximity to regulatory elements of genes or transposable elements to maintain genomic stability. Those located outside such islands, within genes or in non-regulatory elements, are most often hypomethylated (Johansson *et al.*, 2013).

(b) *Changes with age*

The changes that occur to methylation during the natural ageing process have formed the subject of multiple extensive reviews of both human and rodent models (Horvath & Raj, 2018) and will therefore only be dealt with briefly. Epigenetic remodelling or reprogramming in the form of altered methylation status is most active during embryogenesis (Andergassen *et al.*, 2021) and gametogenesis (Guo *et al.*, 2014) (Fig. 4), while methylation status is generally more stable in differentiated tissue. Gradual changes in methylation status in differentiated tissue have, however, been observed (Bell *et al.*, 2012) and are sub-classified based on causation into two fundamental processes: epigenetic drift (Jones *et al.*, 2015) and an epigenetic clock (Horvath & Raj, 2018). The tendency for an inconsistent increase in variability in the epigenomes of individuals denotes epigenetic drift, whereas the epigenetic clock comprises site-specific changes that consistently relate to age across individuals (Hannum *et al.*, 2013). Studies have revealed that during ageing a genome-wide trend of hypomethylation occurs while CpG islands tend to become hypermethylated (Hannum *et al.*, 2013; Jones *et al.*, 2015) and that within genes, nearly

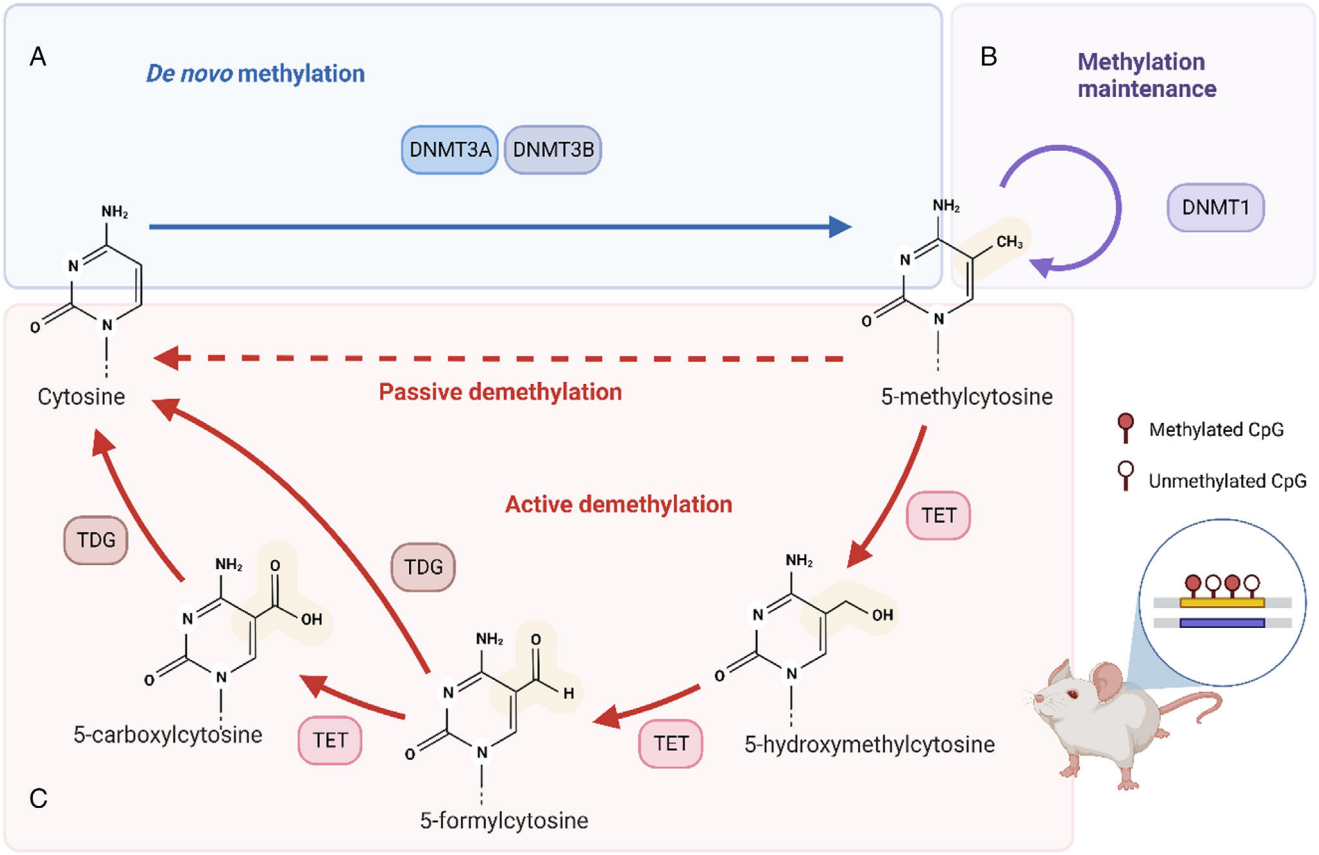

Fig. 2. Diagram of the regulation of methylation. (A) Active *de novo* methylation occurs at unmethylated cytosines through the activity of two DNA methyltransferases 3 (DNMT3A and DNMT3B) that are able to add a methyl group to unmethylated DNA at carbon 5 of cytosines to form 5-methylcytosine. (B) Maintenance of methylation is achieved through the enzymatic activity of DNA methyltransferase 1 (DNMT1) that is able to recognise hemi-methylated DNA after cell division and adds a methyl group to carbon 5 of cytosines on newly synthesised daughter strands. (C) DNA is demethylated through either active or passive demethylation. Passive demethylation happens when cells divide, and the newly synthesised daughter strands do not contain a methyl group at the newly incorporated cytosines. Active demethylation is an enzymatic process involving Tet methylcytosine dioxygenase (TET) enzymes and thymine DNA glycosylase (TDG). TET enzymes sequentially hydroxylate the methyl group on carbon 5 of cytosines to form 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine while TDG decarboxylates 5-carboxycytosine back to an unmethylated cytosine. CpG, cytosine–guanine pair. Image created in BioRender.com.

a third of all CpG sites are influenced by age (Johansson *et al.*, 2013).

(c) *DNA methylation as a biomarker of animal age*

Given its consistency, the epigenetic clock is a promising avenue for chronological age prediction (Lowe *et al.*, 2018; Hong *et al.*, 2019), particularly within the field of conservation (Rey *et al.*, 2020). Methylation levels are usually quantified by bisulfite sequencing (Ashapkin, Kutueva & Vanyushin, 2020), although several methods exist, and the selection of the most appropriate method depends on the relevant application (Kurdyukov & Bullock, 2016). Bisulfite sequencing methods typically provide data in the form of β-methylation values ranging from 0 to 1, and has been subject to extensive statistical validation to optimise sample sizes (Mayne, Berry & Jarman, 2021*b*) and model methods (Snir, vonHoldt & Pellegrini, 2016), including the development of useful tools to design new assays and analyse CpGs (Statham & Csárdi, 2008; Thompson *et al.*, 2009; Wei *et al.*, 2021).

Thus far, methylation studies have successfully been used to establish age models in several animal species and taxa, including: whales (Polanowski *et al.*, 2014; Tanabe *et al.*, 2020; Bors *et al.*, 2021), fishes (Mayne *et al.*, 2020*a*, 2021*a*), dolphins (Beal *et al.*, 2019), birds (De Paoli-Iseppi *et al.*, 2019), non-human primates (Ito *et al.*, 2018), rodents (Stubbs *et al.*, 2017; Lowe *et al.*, 2020), canids (Thompson *et al.*, 2017), bats (Wilkinson *et al.*, 2021) and equids (Larison *et al.*, 2021*a*,*b*). The results for these studies are summarised in Table 1.

(i) Fishes (classes Agnatha, Chondrichthyes, and Osteichthyes). Methylation studies for age estimation have also been expanded to other aquatic species. The first study explored the utility of this biomarker in zebrafish [*Danio rerio* (Hamilton)], as a model organism (Mayne *et al.*, 2020*a*). This

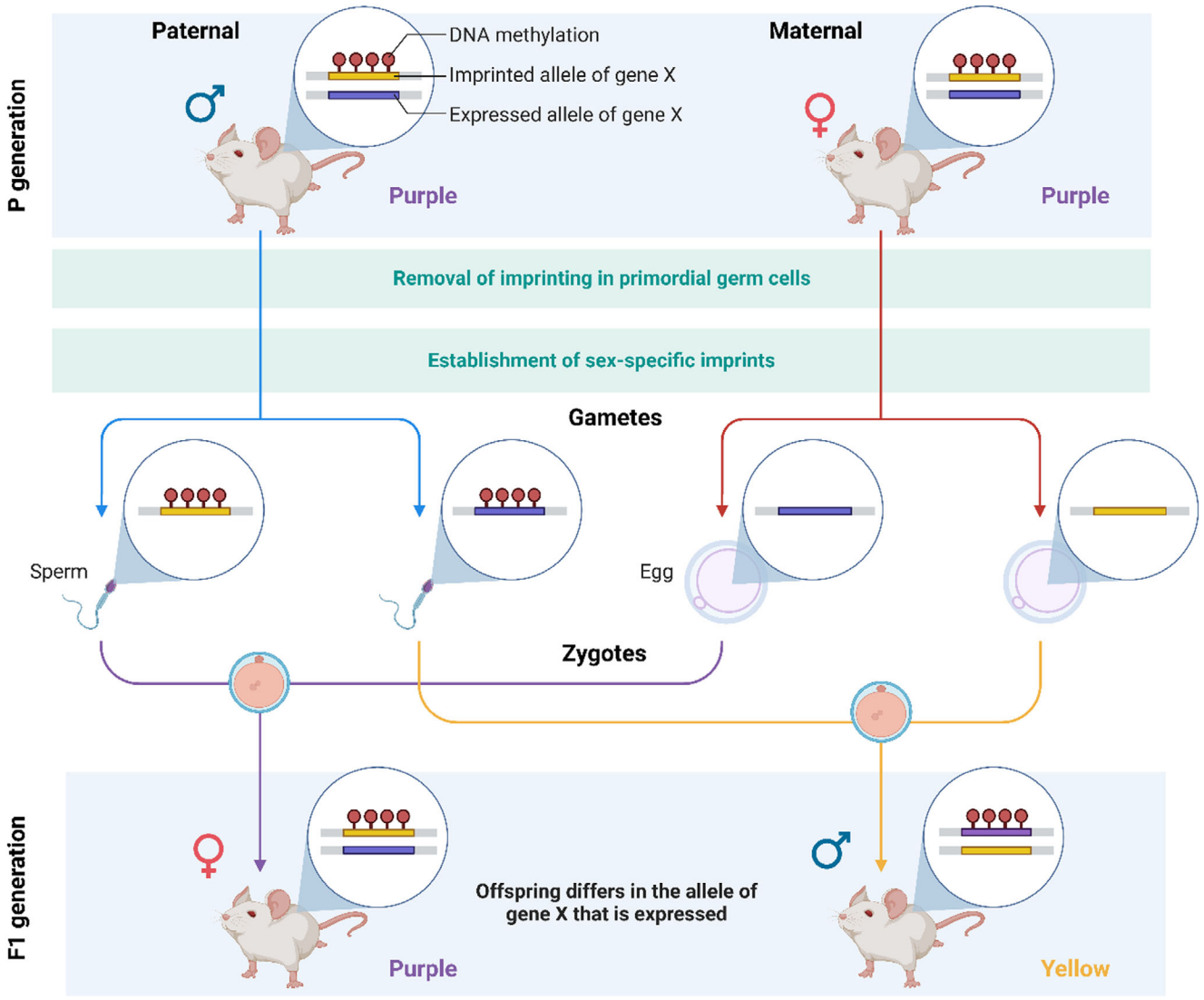

Fig. 3. Diagram of parental imprinting showing the heritability of methylation for a paternally imprinted gene. The top panel shows the parental (P) generation of mice that have identically imprinted (yellow) and expressed (purple) alleles for a hypothetical gene X. Through the removal of inherited imprints in primordial germ cells and the subsequent establishment of sex-specific imprints the alleles inherited paternally are methylated and not expressed, while the maternally inherited alleles are unmethylated and will be expressed. When a zygote is formed through the union of two gametes, the resulting first filial (F1) generation will differ in which allele is expressed, with some expressing the purple allele whilst others express the yellow allele. Image created in BioRender.com.

study used multiplexed methylation-sensitive PCR (MS-PCR) to assay 26 of 29 CpGs shown to follow an age-related decline and was able to establish a predictive model with a standard error of weeks. There was, however, a higher error rate in prediction with a separate test cohort and the authors concluded that MS-PCR may not be sensitive enough to establish age estimation models. The first study on a wild population (Anastasiadi & Piferrer, 2020) assayed methylation in European seabass [*Dicentrarchus labrax* (Linnaeus)]. This study constructed an age clock from 48 CpGs with an error of prediction of 2–3 years, however, the methodology required deep tissue samples and euthanasia similar to otolith age determination. The methods developed in zebrafish were later replicated by the same authors (Mayne *et al.*, 2021*a*) to produce two non-lethal age determination models from skin/fin samples for three threatened fish species: Australian lungfish [*Neoceratodus forsteri* (Krefft)], Murray cod [*Maccullochella peelii* (Mitchell)], and Mary River cod [*Maccullochella mariensis* (Rowland)]. The lungfish clock used 31 CpGs while the cod clock used 26 CpGs, and both had a standard error of prediction of less than a year. The results from a validation test sample showed significantly less error than in zebrafish, which may be related to the different lifespans of these species. Age-related

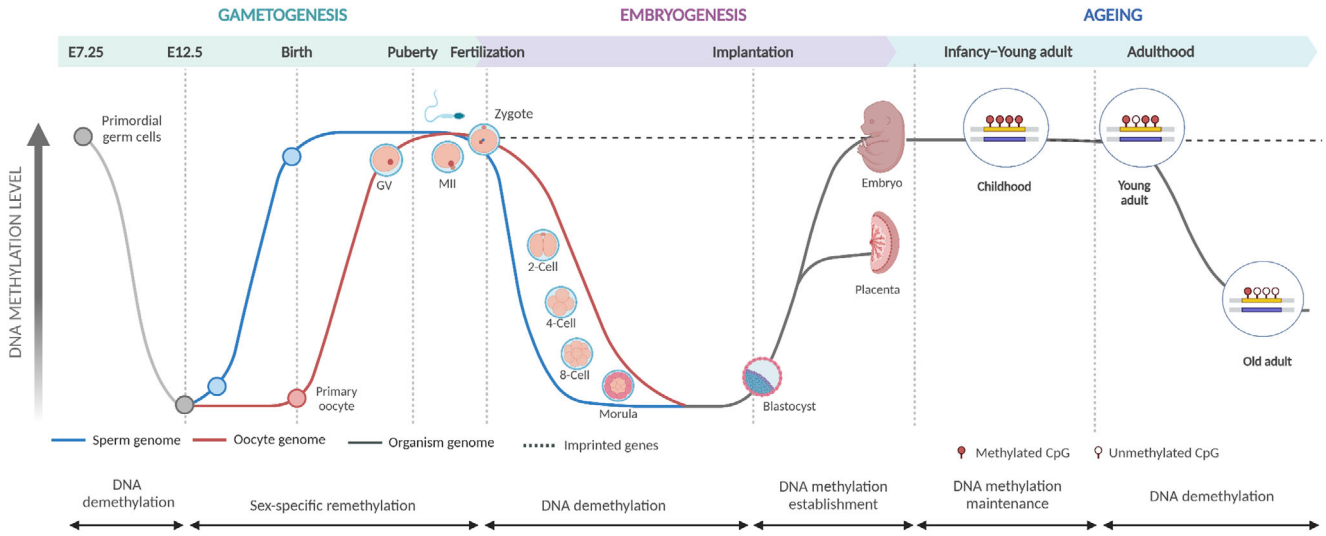

Fig. 4. Diagram of the reprogramming of methylation during key life stages from gametogenesis to embryogenesis and the resulting changes that occur during natural ageing. During gametogenesis the inherited genomic DNA is actively demethylated in primordial germ cells and sex-specific methylation re-established by *de novo* methylation when gametes, either sperm or egg cells, are created. After a zygote is formed from the union of two gametes, passive genomic demethylation takes place due to the rapid replication and division of cells while the methylation of imprinted genes is actively maintained. Once the blastocyst stage/implantation is reached, global methylation is re-established in the embryo. After birth, methylation is maintained throughout childhood from infancy to early adulthood in most tissues with the exception of primordial germ cells forming gametes. During the ageing process methylation is maintained at imprinted genes while global DNA demethylation occurs throughout the remainder of the genome. CpG, cytosine–guanine pair. Image created in BioRender.com.

methylation changes have also been observed in long snout seahorses [*Hippocampus reidi* (Ginsburg)], across different early developmental stages up to adulthood (Suarez-Bregua *et al.*, 2021).

(ii) Amphibians (class Amphibia). The first age clock using methylation for amphibians was recently established in Western clawed frogs [*Xenopus tropicalis* (Gray)] using bisulphite sequencing of the whole genome from web/skin tissue (Morselli *et al.*, 2023). A total of 331 age-correlated CpGs were identified, from which an age clock was established that was able to classify individuals into young, middle, or old age classes with high accuracy; the largest errors occurred in very young or very old individuals. A study published in parallel (Zoller *et al.*, 2022), on both Western clawed frog and African clawed frogs [*Xenopus laevis* (Daudin)], used the same methylation array chip previously developed in mammals, found thousands of conserved CpGs and constructed both individual as well as combined clocks for age. In the Western clawed frog, the mean error of prediction for age was less than a year, while the error for African clawed frog was ∼3 years. The observed difference in accuracy for predictions was likely due to the differences in lifespan as African clawed frogs have a higher average lifespan. A major difference between this and the former study is that the latter models were based on pan-tissue data, including organ and muscle tissue, as the frogs were killed.

(iii) Reptiles (class Reptilia). Early methylation studies in reptiles, done within the context of eco-toxicology (Parrott *et al.*, 2014; Nilsen *et al.*, 2016; Cocci *et al.*, 2018) and sexual differentiation (Venegas *et al.*, 2016), found evidence of an age-related decline in methylation. In the American alligator [*Alligator mississippiensis* (Daudin)], liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used to quantify global methylation patterns in sub-adult and adult groups. The results from both studies revealed a global decline in genomic methylation of ∼8% (Parrott *et al.*, 2014) and 7–9% (Nilsen *et al.*, 2016) respectively. Interestingly both studies also identified a site-directed relationship with the decline in global methylation. This indicates that, while global hypomethylation occurs with ageing, the rate of epigenetic ageing may vary across the genome within a species where specific genes or genomic sites change more slowly or more rapidly than global changes. Subsequently, age clocks based on methylation were investigated in three species of sea turtle, species that have a high infant mortality, are often migratory, and long lived: making long-term tracking for scientific study particularly difficult. The first study exclusively evaluated lifespan prediction in two sea turtles, Australian flatback sea turtle [*Natator depressus* (Garman)] and leatherback sea turtle [*Dermochelys coriacea* (Vandelli)], using CpG promotor density estimates by polymerase chain reaction (PCR) and sequencing (Mayne *et al.*, 2020*b*). The authors were able to predict the lifespan of both turtles as ∼50 and 90 years respectively, in line with previous estimates, and provided the first evidence that methylation studies may be suitable for ageing reptilian species. A subsequent study, using known-age samples from green sea turtle [*Chelonia mydas* (Linnaeus)], developed a methylation-based

Table 1. Summary of studies that assayed global or regional methylation in relation to development or age in animals. Studies that used the data to create a molecular model for predicting animal age, which were included in the meta-analysis, are indicated with an asterisk (*). Species are grouped by class to match the text and listed alphabetically. For each species the genome size in picograms or billions of base-pairs (bbp) is given along with estimates for the average lifespan. For each study, the method of measuring methylation is indicated: a, bisulfite sequencing; b, enzyme-linked immunosorbent assay (ELISA); c, liquid chromatography–tandem mass spectrometry (LC-MS/MS); d, methylation array chip; e, methylation-sensitive polymerase chain reaction (MS-PCR); f, cytosine–guanine (CpG) promotor density (lifespan). Where relevant, citations are also provided for studies on age determination using other biological clocks (tooth annulation/otolith) in the same/similar species. n.a., not available.

Species	Genome (bbp)	Lifespan (years)	Study	Method	Tooth annulation/otolith
(i) Fishes:					
Australian lungfish (*Neoceratodus forsteri*)*	60.73	80–85	Mayne *et al.* (2021*a*)	e	Høie *et al.* (2004)
European seabass (*Dicentrarchus labrax*)*	0.78	15–30	Anastasiadi & Piferrer (2020)	d	Secor *et al.* (1995)
Long snout seahorse (*Hippocampus reidi*)	0.45	1–4	Suarez-Bregua *et al.* (2021)	e	n.a.
Mary River cod (*Maccullochella mariensis*)*	0.83	80–92	Mayne *et al.* (2021*a*)	e	Høie *et al.* (2004)
Murray cod (*Maccullochella peelii*)*	0.83	75–114	Mayne *et al.* (2021*a*)	e	Høie *et al.* (2004)
Zebrafish (*Danio rerio*) – model organism	1.80	1–4	Mayne *et al.* (2020*a*)	e	Higgs *et al.* (2002)
(ii) Amphibians:					
African clawed frog (*Xenopus laevis*)*	3.10	5–15	Zoller *et al.* (2022)	d	n.a.
Western clawed frog (*Xenopus tropicalis*)	3.11	3–12	Morselli *et al.* (2023)	a	n.a.
Western clawed frog (*Xenopus tropicalis*)*	3.11	3–12	Zoller *et al.* (2022)	d	n.a.
(iii) Reptiles:					
American alligator (*Alligator mississippiensis*)*	2.49	30–50	Parrott *et al.* (2014)	c	n.a.
American alligator (*Alligator mississippiensis*)*	2.49	30–50	Nilsen *et al.* (2016)	c	n.a.
Australian flatback sea turtle (*Natator depressus*)	2.64	60–100	Mayne *et al.* (2020*b*)	f	n.a.
Green sea turtle (*Chelonia mydras*)*	2.64	70–80	Mayne *et al.* (2022)	e	n.a.
Leatherback sea turtle (*Dermochelys coriacea*)	2.70	45–50	Mayne *et al.* (2020*b*)	f	n.a.
Loggerhead sea turtle (*Caretta caretta*)	2.70	47–67	Cocci *et al.* (2018)	b	n.a.
(iv) Birds:					
Barn swallow (*Hirundo rustica*)	1.31	1–4	Saino *et al.* (2019)	a	n.a.
Black grouse (*Lyrurus tetrix*)*	1.32	4–5	Soulsbury *et al.* (2018)	a	n.a.
Common tern (*Sterna hirundo*)*	1.44	9–10	Meyer *et al.* (2023)	a	n.a.
Short-tailed shearwater (*Ardenna tenuirostris*)	1.18	19–38	De Paoli-Iseppi *et al.* (2017*b*)	a	n.a.
Short-tailed shearwater (*Ardenna tenuirostris*)*	1.18	19–38	De Paoli-Iseppi *et al.* (2019)	a	n.a.
(v) Mammals:					
Aquatic:					
Antarctic minke whale (*Balaenoptera bonaerensis*)	3.29	100–114	Tanabe *et al.* (2020)	a	Hohn (1980)
Beluga whale (*Delphinapterus leucas*)*	3.29	35–50	Bors *et al.* (2021)	a	Goren *et al.* (1987)
Beluga whale (*Delphinapterus leucas*)	3.29	35–50	Robeck *et al.* (2021*b*)	d	Goren *et al.* (1987)
Bottlenose dolphin (*Tursiops truncatus*)*	3.03	15–16	Beal *et al.* (2019)	a	Kimura (1980)
Bottlenose dolphin (*Tursiops truncatus*)*	3.03	15–16	Robeck *et al.* (2021*a*)	d	Kimura (1980)
Bottlenose dolphin (*Tursiops truncatus*)*	3.03	15–16	Robeck *et al.* (2021*b*)	d	Kimura (1980)
Commerson's dolphin (*Cephalorhynchus commersonii*)*	3.27	10–18	Robeck *et al.* (2021*b*)	d	Myrick *et al.* (1983)
Common dolphin (*Delphinus delphis*)*	3.03	35–40	Robeck *et al.* (2021*b*)	d	Myrick *et al.* (1983)
Harbour porpoise (*Phocoena phocoena*)*	3.46	8–13	Robeck *et al.* (2021*b*)	d	Myrick *et al.* (1983)
Humpback whale (*Megaptera novaeangliae*)*	3.29	45–50	Horvath *et al.* (2022*c*)	d	Ohsumi *et al.* (1965)
Humpback whale (*Megaptera novaeangliae*)*	3.29	45–50	Polanowski *et al.* (2014)	a	Ohsumi *et al.* (1965)
Killer whale (*Orcinus orca*)*	3.18	50–80	Robeck *et al.* (2021*b*)	d	Clark *et al.* (2000)
Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)*	3.46	40–45	Robeck *et al.* (2021*b*)	d	Kasuya & Matsui (1984)
Rough-tooth dolphin (*Steno bredanensis*)*	3.03	32–48	Robeck *et al.* (2021*b*)	d	Myrick *et al.* (1983)
Short-finned pilot whale (*Globicephala macrorhynchus*)*	3.29	45–60	Robeck *et al.* (2021*b*)	d	Kasuya & Matsui (1984)
Sperm whale (*Physeter catodon*)	3.29	50–70	Polanowski *et al.* (2014)	a	Ohsumi *et al.* (1965)
Bats:					
Bechstein's bat (*Myotis bechsteinii*)*	2.72	15–21	Wright *et al.* (2018)	a	Linhart (1973)
Big brown bat (*Eptesicus fuscus*)*	2.25	13–20	Wilkinson *et al.* (2021)	d	Linhart (1973)
Common noctule (*Nyctalus noctule*)*	2.26	5–12	Wilkinson *et al.* (2021)	d	Linhart (1973)
Common vampire bat (*Desmodus rotundus*)*	2.66	12–30	Wilkinson *et al.* (2021)	d	Linhart (1973)

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Table 1. *(Cont.)*

Species	Genome (bbp)	Lifespan (years)	Study	Method	Tooth annulation/otolith
Egyptian fruit bat (*Rousettus aegyptiacus*)*	2.11	10–22	Wilkinson *et al.* (2021)	d	Linhart (1973)
Fish-eating bat (*Myotis vivesi*)*	2.72	8–10	Wilkinson *et al.* (2021)	d	Linhart (1973)
Greater horseshoe bat (*Rhinolophus ferrumequinum*)*	2.68	20–30	Wilkinson *et al.* (2021)	d	Linhart (1973)
Greater mouse-eared bat (*Myotis myotis*)*	2.72	6–13	Wilkinson *et al.* (2021)	d	Linhart (1973)
Greater sac-winged bat (*Saccopteryx bilineata*)*	3.16	1–6	Wilkinson *et al.* (2021)	d	Linhart (1973)
Greater spear-nosed bat (*Phyllostomus hastatus*)*	2.62	10–18	Wilkinson *et al.* (2021)	d	Linhart (1973)
Grey-headed flying fox (*Pteropus poliocephalus*)*	2.32	2–5	Wilkinson *et al.* (2021)	d	Linhart (1973)
Indian flying fox (*Pteropus giganteus*)*	2.18	31–44	Wilkinson *et al.* (2021)	d	Linhart (1973)
Jamaican fruit bat (*Artibeus jamaicensis*)*	2.74	9–19	Wilkinson *et al.* (2021)	d	Linhart (1973)
Large flying fox (*Pteropus vampyrus*)*	2.37	15–30	Wilkinson *et al.* (2021)	d	Linhart (1973)
Lesser short-nosed fruit bat (*Cynopterus brachyotis*)*	2.27	20–30	Wilkinson *et al.* (2021)	d	Linhart (1973)
Little brown bat (*Myotis lucifugus*)*	2.72	7–31	Wilkinson *et al.* (2021)	d	Linhart (1973)
Little golden-mantled flying fox (*Pteropus pumilus*)*	2.32	10–17	Wilkinson *et al.* (2021)	d	Linhart (1973)
Mexican free-tailed bat (*Tadarida brasiliensis*)*	2.94	8–12	Wilkinson *et al.* (2021)	d	Linhart (1973)
Pale spear-nosed bat (*Phyllostomus discolor*)*	2.52	6–9	Wilkinson *et al.* (2021)	d	Linhart (1973)
Pallid bat (*Antrozous pallidus*)*	2.67	9–14	Wilkinson *et al.* (2021)	d	Brook *et al.* (2019)
Proboscis bat (*Rhynchonycteris naso*)*	2.72	1–6	Wilkinson *et al.* (2021)	d	Linhart (1973)
Rodrigues flying fox (*Pteropus rodricensis*)*	2.23	28–30	Wilkinson *et al.* (2021)	d	Linhart (1973)
Seba's short-tailed bat (*Carollia perspicillata*)*	2.92	10–17	Wilkinson *et al.* (2021)	d	Linhart (1973)
Small flying fox (*Pteropus hypomelanus*)*	2.33	9–20	Wilkinson *et al.* (2021)	d	Linhart (1973)
Southern long-nosed bat (*Leptonycteris curasoae*)*	2.36	10–30	Wilkinson *et al.* (2021)	d	Linhart (1973)
Straw-coloured fruit bat (*Eidolon helvum*)*	2.03	15–22	Wilkinson *et al.* (2021)	d	Linhart (1973)
Velvety free-tailed bat (*Molossus molossus*)*	2.41	2–5	Wilkinson *et al.* (2021)	d	Linhart (1973)
Carnivores:					
Australian sea lion (*Neophoca cinerea*)*	3.15	17–25	Robeck *et al.* (2023)	d	Rust *et al.* (2019)
Bobcat (*Lynx rufus*)	2.92	7–13	Cantrell *et al.* (2020)	b	Crowe (1972)
California sea lion (*Zalophus californianus*)*	3.15	15–25	Robeck *et al.* (2023)	d	Rust *et al.* (2019)
Cheetah (*Acinonyx jubatus*)	2.56	10–15	Le Clercq *et al.* (2018)	e	Marker & Dickman (2003)
Cheetah (*Acinonyx jubatus*)*	2.56	10–15	Raj *et al.* (2021)	d	Marker & Dickman (2003)
Domestic cat (*Felis catus*)*	2.91	12–18	Qi *et al.* (2021)	e	Grue & Jensen (1979)
Domestic cat (*Felis catus*)*	2.91	12–18	Raj *et al.* (2021)	d	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)	2.80	10–13	Ito *et al.* (2017)	a	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)*	2.80	10–13	Thompson *et al.* (2017)	a	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)*	2.80	10–13	Horvath *et al.* (2022*e*)	d	Grue & Jensen (1979)
Fisher (*Pekania* [*Martes*] *pennanti*)	n.a.	10–14	Lachance *et al.* (2015)	b	Arthur *et al.* (1992)
Gray wolf (*Canis lupus*)*	2.81	14–16	Thompson *et al.* (2017)	a	Walleser *et al.* (2016)
Harbour seal (*Phoca vitulina*)*	2.94	25–35	Robeck *et al.* (2023)	d	Bowen *et al.* (1983)
Harp/Greenland seal (*Pagophilus groenlandicus*)*	2.94	20–30	Robeck *et al.* (2023)	d	Bowen *et al.* (1983)
Lion (*Panthera leo*)*	2.95	10–16	Raj *et al.* (2021)	d	White & Belant (2016)
Pacific walrus (*Odobenus rosmarus divergens*)*	2.40	20–40	Robeck *et al.* (2023)	d	Stewart *et al.* (1996)
Ringed seal (*Phoca hispida*)	2.94	15–28	Sareisian (2014)	b	Stewart *et al.* (1996)
Snow leopard (*Panthera unica*)	3.54	10–13	Qi *et al.* (2021)	e	McCarthy *et al.* (2005)
Steller sea lion (*Eumetopias jubatus*)*	3.15	20–30	Robeck *et al.* (2023)	d	Rust *et al.* (2019)

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Table 1. *(Cont.)*

Species	Genome (bbp)	Lifespan (years)	Study	Method	Tooth annulation/otolith
Tiger (*Panthera tigris*)*	2.71	8–10	Raj *et al.* (2021)	d	Sharma *et al.* (2021)
Elephants:					
African elephant (*Loxodonta africana*)*	4.44	60–70	Prado *et al.* (2021)	d	Spinage (1976)
Asian elephant (*Elephas maximus*)*	4.03	48–60	Prado *et al.* (2021)	d	Spinage (1976)
Marsupials:					
Eastern grey kangaroo (*Macropus giganteus*)	4.21	6–8	Horvath *et al.* (2022*d*)	d	Grue & Jensen (1979)
Gray short-tailed opossum (*Monodelphis domestica*)*	4.17	1–3	Horvath *et al.* (2022*d*)	d	Pekelharing (1970)
Red kangaroo (*Macropus rufus*)*	3.13	6–20	Horvath *et al.* (2022*d*)	d	Grue & Jensen (1979)
Red-necked wallaby (*Macropus rufogriseus*)	5.92	7–15	Horvath *et al.* (2022*d*)	d	Grue & Jensen (1979)
Tasmanian devil (*Sarcophilus harrisii*)*	3.63	5–8	Horvath *et al.* (2022*d*)	d	Pekelharing (1970)
Western grey kangaroo (*Macropus fuliginosus*)	3.91	10–29	Horvath *et al.* (2022*d*)	d	Grue & Jensen (1979)
Primates:					
Baboon (*Papio cynocephalus x P. anubis*)*	3.53	20–30	Anderson *et al.* (2021)	a	Wada *et al.* (1978)
Chimpanzee (*Pan troglodytes*)*	3.76	32–39	Guevara *et al.* (2020)	d	Wittwer-Backofen *et al.* (2004)
Chimpanzee (*Pan troglodytes*)*	3.76	32–39	Ito *et al.* (2018)	a	Wittwer-Backofen *et al.* (2004)
Common marmoset (*Callithrix jacchus*)*	3.43	7–16	Horvath *et al.* (2021*b*)	d	Scott *et al.* (1980)
Rhesus macaque (*Macaca mulatta*)*	3.59	25–40	Horvath *et al.* (2021*a*)	d	Newham *et al.* (2021)
Vervet monkey (*Chlorocebus pygerythrus*)*	4.19	15–30	Jasinska *et al.* (2022)	d	Scott *et al.* (1980)
Rodents:					
Mouse (*Mus musculus*)–Model organism	3.25	1–1.5	Spiers *et al.* (2016)	a	Grue & Jensen (1979)
Mouse (*Mus musculus*)–Model organism	3.25	1–1.5	Stubbs *et al.* (2017)	a	Grue & Jensen (1979)
Naked mole-rat (*Heterocephalus glaber*)*	2.90	22–31	Lowe *et al.* (2020)	a	Grue & Jensen (1979)
Naked mole-rat (*Heterocephalus glaber*)*	2.90	22–31	Horvath *et al.* (2022*a*)	d	Grue & Jensen (1979)
Naked mole-rat (*Heterocephalus glaber*) *	2.90	22–31	Kerepesi *et al.* (2022)	a	Grue & Jensen (1979)
Wood mouse (*Apodemus sylvaticus*) *	3.29	1–4	Little *et al.* (2020)	a	Grue & Jensen (1979)
Shrews:					
Masked shrew (*Sorex cinereus*)*	2.91	1–2	Cossette *et al.* (2022)	d	Grue & Jensen (1979)
Ungulates:					
Cattle (*Bos taurus*)*	3.70	10–20	Kordowitzki *et al.* (2021)	d	Grue & Jensen (1979)
Common zebra (*Equus quagga*)*	3.15	20–25	Larison *et al.* (2021*a*)	d	Penzhorn (1982)
Domestic horse (*Equus caballus*)*	3.15	25–30	Larison *et al.* (2021*a*)	d	Penzhorn (1982)
Domestic horse (*Equus caballus*)*	3.15	25–30	Horvath *et al.* (2022*b*)	d	Penzhorn (1982)
Domestic pig (*Sus scrofa domesticus*)*	3.00	15–20	Schachtschneider *et al.* (2021)	d	Grue & Jensen (1979)
Domestic sheep (*Ovis aries*)*	3.30	10–12	Sugrue *et al.* (2021)	d	Saxon & Higham (1968)
Grévy's zebra (*Equus grevyi*)*	3.15	20–25	Larison *et al.* (2021*a*)	d	Penzhorn (1982)
Roe deer (*Capreolus capreolus*)*	3.41	7–10	Lemaître *et al.* (2022)	d	Grue & Jensen (1979)
Somali wild ass (*Equus africanus somaliensis*)*	4.12	27–40	Larison *et al.* (2021*a*)	d	Penzhorn (1982)

clock for age determination that would be transferable across several species of marine turtles (Mayne *et al.*, 2022). This model, based on 17 CpGs, was able to predict age with an error rate of 3–4 years among adult individuals. The method was developed also to include sites that could be assayed in hawksbill sea turtle [*Eretmochelys imbricata* (Linnaeus)], loggerhead sea turtle [*Caretta caretta* (Linnaeus)], and olive Ridley sea turtle [*Lepidochelys olivacea* (Eschscholtz)]; in addition to previously studied flatback and leatherback turtles. Ecotoxicology studies on methylation in loggerhead sea turtles found similar patterns in global methylation as compared to studies conducted in alligators (Cocci *et al.*, 2018).

(iv) Birds (class Aves). Similar to reptiles, many of the early methylation studies in birds had a focus on ecotoxicology (Brandenburg, 2016; Romano *et al.*, 2017) or other aspects of animal ecology. For example, several studies have focussed on the association between methylation levels and migration or breeding phenology (Saino *et al.*, 2017; Liebl *et al.*, 2021), temporal variation on an annual basis in both methylation levels (Viitaniemi *et al.*, 2019; Mäkinen *et al.*, 2019) and the expression of key regulatory elements within the methylation circuitry (Sharma *et al.*, 2018; Trivedi *et al.*, 2019). While these studies were not focussed on age estimation, they did establish the heritability of methylation (Romano *et al.*, 2017) and inter-generational resemblance of methylation patterns (Saino *et al.*, 2019). Studies aimed at exploiting methylation to model age found that 67 CpGs in 13 genes, previously used in mammals, were not well conserved in a long-lived seabird species, the short-tailed shearwater [*Ardenna tenuirostris* (Temminck)], with only 12 CpGs showing a weakly correlated relationship with age (De Paoli-Iseppi *et al.*, 2017*a*). The same authors were, however, able to construct an age clock for this species with seven newly developed CpGs that predicted age with a mean error of 2–3 years (De Paoli-Iseppi *et al.*, 2019). A similar trend of age–dependent changes was later observed in the black grouse [*Lyrurus tetrix* (Linnaeus)] that found four CpGs within one gene to be highly correlated with age (Soulsbury *et al.*, 2018). A recent study in the common tern [*Sterna hirundo* (Linnaeus)], identified several genomic regions that showed shifts in methylation equivalent to epigenetic clocks that changed predictably over time within individuals in a sex-specific manner mostly affecting females (Meyer *et al.*, 2023).

(v) Mammals (class Mammalia)

(*i*.1) *Aquatic mammals (infraorder Cetacea).* The first study to develop an age-estimation model in wildlife based on methylation was conducted on humpback whales and assayed 37 CpGs in eight genes, of which seven CpGs had a significant linear correlation with age (Polanowski *et al.*, 2014). This allowed the creation of a model able to estimate the age of several whale species with a standard error of prediction of ∼3 years. A similar study on humpbacks was reported more recently (Horvath *et al.*, 2022*c*) and used a conserved site mammalian methylation array which was able to reduce the MAE to 2 years. Several similar models have been developed in other whale species as well. Two of the genes assayed by Polanowski *et al.* (2014) were assessed in the Antarctic minke whale [*Balaenoptera bonaerensis* (Burmeister)] in 17 CpGs (Tanabe *et al.*, 2020). The authors, however, only found a significant relationship between methylation and age in one gene and concluded that the methylation–age relationship identified in one species may not be conserved between species. An analysis of 37,491 CpGs using a conserved site mammalian methylation array (Arneson *et al.*, 2022) applied to Beluga whale [*Delphinapterus leucas* (Pallas)] found 23 highly age-correlated CpGs, of which 15 were in genes that enabled the development of an age-estimation model able to determine age with comparable accuracy to the model developed in humpback whales (Bors *et al.*, 2021). Similar methods were later applied to bottlenose dolphins [*Tursiops truncatus* (Montagu)] in a study that replicated previous findings in humpback whales by assaying 17 CpGs in three genes on skin biopsies (Beal *et al.*, 2019). The authors found two of the genes to have comparable methylation levels between species while the third was significantly hypomethylated. Two of the assayed CpGs had significant age-correlated methylation which enabled the construction of a linear ageing model that could accurately predict the age of bottlenose dolphins with a standard error of ∼4 years (Beal *et al.*, 2019). Another study on the same species was able to construct a combined age-estimation model from skin and blood samples with an error in prediction of ∼2–3 years (Robeck *et al.*, 2021*a*).

These studies culminated in the recent validation of a methylation array chip which assayed between 79 and 142 CpGs, depending on tissue type, in nine species of cetaceans to construct a single age-estimation model that is transferable across species (Robeck *et al.*, 2021*b*). This study included four additional species of dolphins, two additional species of whales, and porpoises. The dolphins included the common dolphin [*Delphinus delphis* (Linnaeus)], Commerson's dolphin [*Cephalorhynchus commersonii* (Lacépède)], Pacific white-sided dolphin [*Lagenorhynchus obliquidens* (Gill)], and rough-tooth dolphin [*Steno bredanensis* (Cuvier)], as well as the harbour porpoise [*Phocoena phocoena* (Linnaeus)]. The whales included the killer whale [*Orcinus orca* (Linnaeus)] and short-finned pilot whale [*Globicephala macrorhynchus* (Gray)]. The final model generated from this study was able to predict age accurately in all tested species with a standard error of between 3 and 4 years, depending on tissue type, similar to findings in individual, species-specific studies.

(*i*.2) *Bats (order Chiroptera).* Among the only true flying mammals in the order Chiroptera, which includes bats, megabats, and microbats, two studies have aimed at developing methylation-based clocks for age (Wright *et al.*, 2018; Wilkinson *et al.*, 2021). The first used methylation levels at seven CpGs in three genes to construct an age clock from wing skin samples from Bechstein's bat [*Myotis bechsteinii* (Kuhl)] able to determine age with a MEP of 2–3 years (Wright *et al.*, 2018). A pan-species model was later developed using 162 CpGs from a conserved mammalian methylation array able to predict the age of 26 species within the order Chiroptera with a MEP of less than a year (Wilkinson *et al.*, 2021).

(*i*.3) *Carnivores (order Carnivora).* In carnivores, age clocks were assessed for canids (Ito *et al.*, 2017; Thompson

et al., 2017, Horvath *et al.*, 2022*e*), felines (Cantrell *et al.*, 2020; Raj *et al.*, 2021; Qi *et al.*, 2021), mustelids (Lachance *et al.*, 2015) and pinnipeds (Sareisian, 2014; Robeck *et al.*, 2023). The first model in the domestic dog, *Canis familiaris* (Linnaeus) assayed CpGs in six genes and constructed a clock from the four highly age-correlated CpGs, able to predict age with a MEP of 34–37 months (Ito *et al.*, 2017). The number of breeds included was not stated but likely less than 50 (based on sample size) and the authors postulated that the error in clocks could be improved by screening for age-correlated CpGs to assay prior to modelling age using methylation. The second study (Thompson *et al.*, 2017), which also included both domestic dog and gray wolf [*Canis lupus* (Linnaeus)], was able to construct age clocks for each species from 41 and 67 CpGs respectively with a MEP of ∼10 months. This study also used fewer than 50 breeds but represented a significant improvement in the accuracy of the prediction. Furthermore, a relationship between size (mass) and accelerated epigenetic age was detected in line with the knowledge that smaller breeds live longer while larger breeds have shorter lifespans. A follow-up study in domestic dog by some of the same co-authors (Horvath *et al.*, 2022*e*) was able further to improve the epigenetic clock (MEP of 5–6 months) and predict age-to-death by assaying CpGs in 93 dog breeds.

The first study in felines (Qi *et al.*, 2021) studied methylation in multiple CpGs in two genes, across several breeds of domestic cat [*Felis catus* (Linnaeus)]. Significant correlates with age were detected using high-resolution melting analyses combined with MS-PCR for both genes and a combined ageing model with a MEP of 3.5–4 years was constructed for both healthy and chronically ill individuals. The authors also tested this method in a smaller sample of snow leopard [*Panthera uncia* (Schreber)] and found the trends observed in domestic cat to be sufficiently conserved to determine age in this species with an improved MEP of ∼2 years. The second feline study (Raj *et al.*, 2021), also in domestic cat, used a methylation array to identify 34 CpGs that have a conserved correlation with age and created a model with a MEP of ∼10 months, similar to that observed in canids. The authors further tested the model on smaller sample subsets ($N < 15$) of wild felines including cheetah, lion [*Panthera leo* (Linnaeus)], and tiger [*Panthera tigris* (Linnaeus)]. The cat clock was able to determine age in the related wild species with a MEP of 1–3 years.

Global methylation has also been studied in pinnipeds in relation to age in the ringed seal [*Phoca hispida* (Schreber)], using liver and kidney tissue, however, no clear difference was observed between the sub-adult and adult groups – possibly due to small sample sizes or tissue type (Sareisian, 2014). Similar studies were performed measuring global methylation using enzyme-linked immunosorbent assay (ELISA) in bobcats [*Lynx rufus* (Schreber)], and fishers [*Pekania pennanti* (Erxleben)], using liver samples (Lachance *et al.*, 2015; Cantrell *et al.*, 2020). While both identified the same trend of a decrease in methylation with age, supporting the possibility that these species may be well suited to the development of epigenetic clocks for age determination, neither was able to detect a statistically significant difference between young and mature individuals. This may indicate that site-directed methylation detection methods, such as bisulfite sequencing, are more useful for this purpose than ELISA or MS-PCR (Le Clercq, Dalton & Kotzé, 2018). This was illustrated by a later study in pinnipeds (Robeck *et al.*, 2023) that included six species: three species of sea lion, the Australian sea lion [*Neophoca cinerea* (Péron)], California sea lion [*Zalophus californianus* (Lesson)], and Steller sea lion [*Eumetopias jubatus* (Schreber)]; two seal species, harbour seal [*Phoca vitulina* (Linnaeus)], and harp/Greenland seal [*Pagophilus groenlandicus* (Erxleben)]; as well as one species of walrus, the Pacific walrus [*Odobenus rosmarus divergens* (Linnaeus)]. This study did find several age-correlated changes in methylation in pinnipeds that enabled the construction of an epigenetic clock, from combined skin and blood samples, able to predict age with a MEP of 1–1.5 years.

(*i*.4) *Elephants (order Proboscidea, suborder Elephantiformes)*. Methylation has been used to model age in both extant genera of elephants in the family Elephantidae: the African elephant [*Loxodonta africana* (Blumenbach)] and the Asian elephant [*Elephas maximus* (Linnaeus)]. Prado *et al.* (2021) created three epigenetic models of age, one for each of the genera as well as one general model for all elephants. For the African elephant, 2341 CpGs from a methylation array were found to be significantly correlated with age while the Asian elephant only had approximately a quarter as many. Nevertheless ∼366 of these age-correlated CpGs were shared by both species. The most accurate clock was reconstructed for the African elephant with a MEP of 2–3 years while the clock for Asian elephants had a MEP of 3–4 years. The combined clock for both species performed similarly to the latter, with a MEP of 3–4 years. Overall, this study illustrated that the accuracy of clocks may be improved by a higher number of targets rather than just increased sampling, while also highlighting that substantial differences may exist in age-related CpGs between closely related species.

(*i*.5) *Marsupials (infraorder Marsupialia)*. The first study to assay methylation in relation to age in marsupials (Horvath *et al.*, 2022*d*) was conducted in gray short-tailed opossum [*Monodelphis domestica* (Wagner)], a South American species with a pseudo-pouch, similar to those of male thylacines [*Thylacinus cynocephalus* (Harris)]. This study used a universal mammal array to develop a clock able to predict age with a MEP of 3–4 months. This model was subsequently validated in several other marsupials including the Tasmanian devil [*Sarcophilus harrisii* (Boitard)], eastern grey kangaroo [*Macropus giganteus* (Shaw)], western grey kangaroo [*Macropus fuliginosus* (Desmarest)], red kangaroo [*Macropus rufus* (Desmarest)], and red-necked wallaby [*Macropus rufogriseus* (Desmarest)] from the Australasian region. Despite the taxonomic and phylogenetic distance in the relatedness of these species, the model developed for opossums was well conserved in other marsupials and was able to predict the age of these species with a MEP of 12–13 and 11–13 months for Tasmanian devils and kangaroos/wallabies respectively.

(*i*.6) *Primates (order Primates)*. Epigenetic clocks for age have been studied extensively in humans, *Homo sapiens sapiens* (Linnaeus) (Jarman *et al*., 2015; De Paoli-Iseppi *et al*., 2017*a*). Such studies were expanded into non-human primates including hybrid baboons (*Papio cynocephalus* × *P. anubis*) (Anderson *et al*., 2021), chimpanzees [*Pan troglodytes* (Blumenbach)] (Ito *et al*., 2018; Guevara *et al*., 2020), rhesus macaques [*Macaca mulatta* (Zimmermann)] (Horvath *et al*., 2021*a*), and monkeys (Horvath *et al*., 2021*b*; Jasinska *et al*., 2022). Ito *et al*. (2018) developed the first primate age clock in chimpanzees based on methylation differences in several CpGs analysed in three genes. The most accurate model used two CpGs and was able to predict age with a mean error of prediction of 5–6 years. Improved results were later achieved using a human methylation array (Guevara *et al*., 2020) owing to the high amounts of largely conserved DNA between humans and chimps. This study was able to construct a highly accurate age clock from as little as 10 CpGs with an error rate comparable to that observed in humans (2–3 years). A study conducted on a hybridised baboon population, between congeneric yellow baboon [*Papio cynocephalus* (Linnaeus)], and Anubis baboon [*Papio anubis* (Lesson)], used genome-wide methylation analysis refined to ∼573 CpG sites to create a model with predictive precision of 1–2 years.

In the rhesus macaque, three clocks were constructed using blood, skin, and multi-tissue methylation data (Horvath *et al*., 2021*a*). The most accurate models were derived from blood and multi-tissue data with a MEP of between 12 and 18 months, while the model from skin samples had a MEP of ∼2 years. The pan-tissue models in macaques were based on 71 CpGs from a custom mammalian methylation array. These clocks were also tested on vervet monkeys [*Chlorocebus pygerythrus* (Cuvier)] and were able to predict age with a MEP of 2–3 years using blood or multi-tissue models, and ∼8 years using skin. More detailed analyses of this species were published in a subsequent companion article (Jasinska *et al*., 2022) where clocks were constructed using blood, liver, and brain tissue. Overall performance was improved using species-specific data, with liver and cortex models found to be the most accurate with a MEP of 3–6 months while the blood-derived clock had a MEP of approximately 1 year. A similar study in common marmosets [*Callithrix jacchus* (Linnaeus)] constructed an age clock from blood samples from over 1000 age-related CpGs able to estimate age with a MEP of 8–9 months (Horvath *et al*., 2021*b*). The studies on macaques as well as vervet and marmoset monkeys also compared the accuracy of these models on human data and between species. They found most models to retain a relatively high predictive power across multiple primates and tissue types, despite the ∼12 million years divergence distance between macaques and vervets and 29 million years between humans and vervets (Horvath *et al*., 2021*a*; Jasinska *et al*., 2022).

(*i*.7) *Rodents (order Rodentia)*. Laboratory murine mouse models such as *Mus musculus* (Linnaeus) were used to construct age clocks in multiple tissue types (Stubbs *et al*., 2017). This was later replicated in two wild rodent species, the wood mouse [*Apodemus sylvaticus* (Linnaeus)], and naked mole-rat [*Heterocephalus glaber* (Rüppell)]. In wood mouse (Little *et al*., 2020), ear punches from a laboratory-reared and formerly wild population (Clerc *et al*., 2019) were used to develop an age clock based on 9 CpGs in four genes. The resulting clock provided the first evidence with a high accuracy for the prediction of age in wild mice with a MEP of 59 days. For the naked mole-rat, 23 CpGs that are age correlated were used to construct an initial age clock using liver tissue (Lowe *et al*., 2020). To enable non-lethal age determination of wild individuals this clock was validated using skin samples, with a resulting model able to predict age with a MEP of 3–4 years. The model was found to have slightly lower accuracy between skin and liver samples, indicating that skin may age slower. More recent studies in the same species used reduced-representation bisulfite sequencing (Kerepesi *et al*., 2022) and a universal mammalian methylation array (Horvath *et al*., 2022*a*) to construct more accurate models. The first was able to construct an age-prediction model from blood samples that could predict age with a MEP of 1.1 years (Kerepesi *et al*., 2022) while the second constructed a pan-tissue model able to predict age with a MEP of less than 1 year (Horvath *et al*., 2022*a*).

(*i*.8) *Shrews (order Eulipotyphla)*. To date, one study has explored epigenetic ageing in shrews (Cossette *et al*., 2022), an order closely related to Carnivora, Chiroptera, and Pholidota (pangolins), in the superorder Laurasiatheria. Using a conserved mammalian methylation array, this study explored both the utility of methylation in estimating the age of masked shrew, *Sorex cinereus* (Kerr), as well as the possible divergence in epigenetic ageing between two highly differentiated mainland and insular island populations. The epigenetic clock, constructed from 26 age-correlated CpGs, was able to predict age with a MEP of 0.1 years, however, some evidence was found for the accelerated ageing of island populations. Further epigenome-wide association studies also identified several genes without significant age associations that have differential methylation and possibly expression patterns that may contribute to the morphological differences between mainland and island populations.

(*i*.9) *Ungulates (orders Artiodactyla and Perissodactyla)*. In hooved mammals, distant relatives of whales and other aquatic mammals, age clocks were established in several species of both even-toed (Artiodactyla) and odd-toed (Perissodactyla) ungulates. In odd-toed ungulates, studies included wild and domesticated species of the family Equidae. Epigenetic clocks were modelled in common zebra [*Equus quagga* (Boddaert)] using 70 CpG sites for blood and 99 CpGs for blood and skin pooled samples (Larison *et al*., 2021*a*). The clock developed using blood samples outperformed the skin-based model and was able to predict age with a MEP of 6–7 months, as compared to a MEP of 1–2 years. The blood model was tested further in other species in the genus *Equus*, including domestic horses [*E. caballus* (Linnaeus)], Grévy's zebras [*E. grevyi* (Oustalet)], and Somali wild asses [*E. africanus somaliensis* (Noack)], and achieved results comparable to the skin-based model. A second study on domestic horses created three epigenetic clocks based

on data derived from blood, liver, and pan-tissue sampling (Horvath *et al.*, 2022*b*). The most accurate model was derived from liver tissue with a MEP of 7–8 months while both the blood and pan-tissue models had a MEP of ∼1 year.

Studies conducted on even-toed ungulates included species from the families Bovidae (Kordowitzki *et al.*, 2021; Sugrue *et al.*, 2021), Cervidae (Lemaître *et al.*, 2022), and Suidae (Schachtschneider *et al.*, 2021). A study in roe deer [*Capreolus capreolus* (Linnaeus)], from wild populations in Eastern France, used the same methods as for equines to create sex-specific clocks for age determination with a MEP of 6–8 months (Lemaître *et al.*, 2022). Interestingly, this study also identified acceleration methylation ageing in males and several CpGs that showed differential methylation patterns based on sex. Due to the basal genetic similarities between this species and white-tailed deer [*Odocoileus virginianus* (Zimmermann)], this method may also be transferable to other deer species. Among domesticated species, epigenetic clocks were constructed for cattle [*Bos taurus* (Linnaeus)], domestic sheep [*Ovis aries* (Linnaeus)], and domestic pig [*Sus scrofa domesticus* (Linnaeus)]. Epigenetic clocks constructed for cattle and domestic sheep were able to estimate age with a MEP of 8–9 and 3–5 months, respectively (Kordowitzki *et al.*, 2021; Sugrue *et al.*, 2021), based on between 80 and 140 CpGs with conserved age-related methylation patterns across tissue types. For domestic pig an age-estimation clock was constructed from more than 900 CpGs with a MEP of 1–2 months (Schachtschneider *et al.*, 2021).

(2) Telomeres

(*a*) *What are telomeres?*

Early work in cytogenetics identified knobs of heterochromatin, visualised at the end of linear chromosomes (Fig. 5), which could be considered the natural ends of chromosomes (McClintock, 1941). These ends would later be called telomeres, from the Greek words for 'end' (telos) and 'part' (meros). These studies would pave the way towards the discovery of basic telomere structure, several decades later, and the advancement of science and technology towards the further characterization of their molecular composition and biology (Shay & Wright, 2019).

The telomeres are formed by a tandem repeat of a short nucleotide sequence. In humans, this sequence was identified to be a repeat of 5′-TTAGGG-3′ (Moyzis *et al.*, 1988) which is conserved in most eukaryotes, including all mammals (Gomes *et al.*, 2011). The full length of the telomere sequence varies; from 10 to 15 kilobases (kb) in humans (de Lange *et al.*, 1990) to 20–50 kb in laboratory mice (Kipling & Cooke, 1990). The tandem repeat sequence is partially double stranded (Fig. 6), ending with a free 3′ overhang which protrudes past the 5′ strand by ∼50–500 nucleotides (Wu, Takai & De Lange, 2012). It was initially unclear how the 3′ strand is formed (Palm & de Lange, 2008), as telomerase had already been excluded (Yuan *et al.*, 1999), but further studies elucidated the role of several nucleases (Wu *et al.*, 2012) including Exonuclease I. The 3′ strand, containing the triple G of the repeat is rich in guanosine and thus termed the G-strand, whilst the 5′ strand is termed the C-strand. The 5′-end is well defined and mostly ends with the ATC-5′ codon, in contrast to the variable 3′-end (Sfeir *et al.*, 2005). The telomere sequence folds in upon itself to form a laureate structure (Fig. 6) as a higher order chromatin configuration consisting of two distinct loops, the double-stranded DNA telomere loop (T-loop) and the single-stranded displacement loop (D-loop; Cleal, Norris & Baird, 2018). The D-loop is formed when the 3′ overhang of the free end invades and displaces a 50–300 nucleotide region of the telomeric sequence to close the loop, thereby protecting the complex from recognition by DNA repair

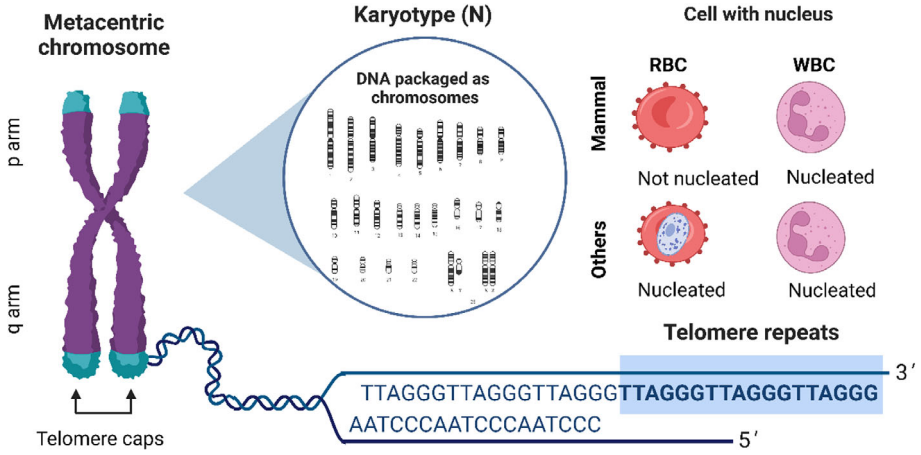

Fig. 5. Picture of metacentric chromosomes showing the telomere caps at either end. On the right the typical mammalian cell is shown with the nucleus highlighted. The nucleus contains the genomic DNA which is packaged in densely coiled structures called chromosomes for which the karyotype (chromosomal organisation) is illustrated in the central circle. The ends of the chromosomes (blue) are capped with telomeres – non-coding repeats of the same 6–8 base pair sequence – to protect the coding genomic DNA (purple) from damage or loss during replications. Cells from the tissue most commonly used in telomere length studies are also indicated showing the nucleated red blood cells (RBCs) found in non-mammalian species as well as the nucleated white blood cells (WBCs).

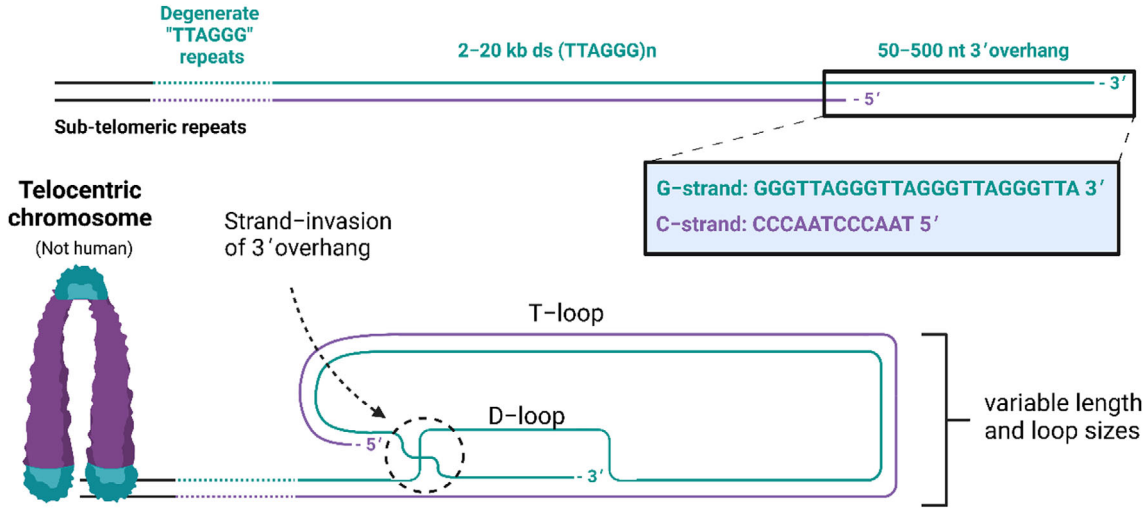

Fig. 6. Segments and higher chromatin structure of telomeric DNA. The DNA strand at the distal end of a chromosome ends is formed by sub-telomeric repeats, followed by degenerate tandem repeats, adjacent to the double stranded (ds) telomeric tandem repeats of 5′-TTAGGG-3′ that span ∼2–20 kilobase pairs (kb). The terminal ends of the telomere DNA comprise the longer, guanine rich, G-strand that forms the 3′ overhang of ∼50–500 nucleotides (nt), as well as the complementary C-strand of the 5′ terminal DNA. The terminal ends of the telomere strand bend back to form a hairpin-like loop, known as the telomere loop (T-loop), where the 3′ overhang inserts itself between a double-stranded segment forming the displacement or D-loop. The structure of telocentric chromosomes found in non-human and non-mammalian species is also illustrated. Image created in BioRender.com.

machinery as single strand breaks (Oganesian & Karlseder, 2011; Kazda *et al.*, 2012; Wu *et al.*, 2012).

Telomere length is maintained through the activity of telomerase (Fig. 7), a reverse transcriptase that elongates telomeres (Greider & Blackburn, 1985), as well as by the alternative lengthening of telomeres (ALT) pathway (Cesare & Reddel, 2010), which relies upon homologous recombination between telomeres. Telomerase is a ribonucleoprotein complex with RNA subunits that elongate chromosomes by adding telomeric repeat sequences to the terminal ends of chromosomes. This complex comprises two catalytic subunits, telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), which serve as the template to synthesise telomeric repeat sequences, as well as a stabilising protein complex of Dyskerin protein and nucleolar protein family A member proteins (Greider & Blackburn, 1985).

(*b*) *Changes with age*

Similar to methylation, telomeres undergo several phases of alteration throughout gametogenesis, following zygote formation, during embryogenesis, and throughout the lifespan of an organism in different tissues (Fig. 8). For example, telomeres in oocytes are typically shorter than in somatic tissue and undergo activation following zygote formation to elongate throughout the two- and four-cell phases until blastocyst formation (Zhao *et al.*, 2014). This initially happens independent of telomerase, which has been found to be absent from spermatozoa, oocytes and morula stage cells (Wright *et al.*, 1996), followed by an upregulation of telomerase during the morula–blastocyst phase (Schaetzlein *et al.*, 2004). Elongation in the absence of telomerase is likely due to extensive genome-wide demethylation providing a favourable milieu for telomere lengthening via an ALT mechanism (Liu *et al.*, 2007). After birth, telomerase activity is restricted to germline cells and stem cells.

As DNA polymerase would not be able to replicate the segment of DNA to which it binds, telomeres serve as barriers protecting coding sequences located towards the end of chromosomes. In the absence of telomerase, however, telomeres are also subject to the gradual loss of sequence due to the terminal replication problem, and telomere length has since become a well-studied attribute of cellular ageing (Olsson, Wapstra & Friesen, 2018). The terminal replication problem is attributed to DNA polymerase requiring an RNA primer to initiate replication. During replication the RNA primer, which binds to the terminal nucleotides of a replicating strand, dissociates from the newly synthesised strand without being incorporated resulting in a daughter strand that is shorter than the template strand. Consequently, telomere length is typically lost at a rate of 30–50 bp per cell division and ultimately leads to cellular senescence. Furthermore, telomeres have been shown to be highly susceptible to oxidative damage due to reactive oxygen species (ROS), for example because of high stress levels, persistent inflammation, or in response to environmental toxins (Zhao *et al.*, 2014).

(*c*) *Telomeres as a biomarker of animal age*

Following the observations of telomere length reflecting age and cellular senescence in humans there has been increased

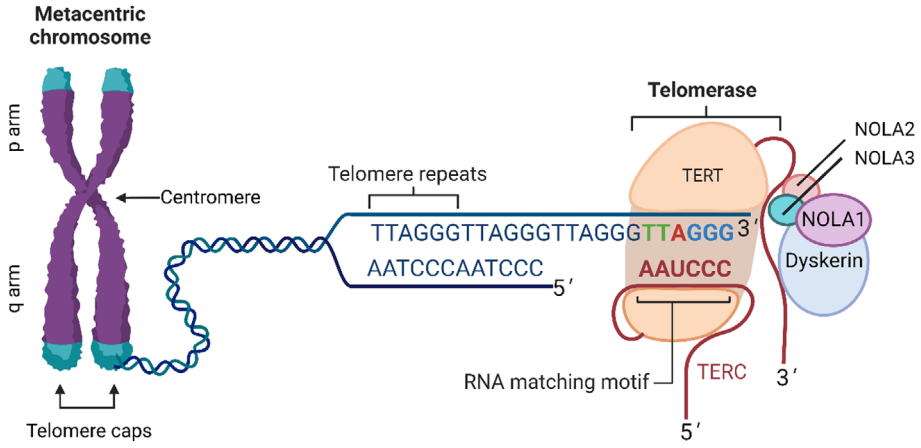

Fig. 7. Diagrammatic representation of the telomerase complex binding to the single–stranded loop of telomeres for elongation. The telomerase complex has a telomerase RNA component (TERC) that contains an RNA matching motif to incorporate nucleotides of the telomeric repeat sequence to the strand being elongated in the 5′ to 3′ direction. The telomerase reverse transcriptase (TERT) element enzymatically catalyses the addition of the newly synthesised telomeric repeat to the existing strand. Other elements of the telomerase complex are also indicated, including small nucleolar ribonucleoproteins Dyskerin, and the three nucleolar protein family A member proteins (NOLA1, NOLA2, and NOLA3). Image created in BioRender.com.

interest in its ability to determine age in animals (Haussmann & Vleck, 2002; Horn, Robertson & Gemmell, 2010) and such correlations have been illustrated in several non-human species including birds (Tricola *et al.*, 2018; Vedder *et al.*, 2022), aquatic mammals (Garde *et al.*, 2010; Izzo *et al.*, 2011), and reptiles (Hatase *et al.*, 2008). Telomere length has been assayed using several methods (Mensà *et al.*, 2019) ranging from terminal restriction fragment (TRF) analyses followed by southern blotting to fluorescence-based methods that include *in situ* hybridization (FISH), flow cytometry coupled FISH (Flow-FISH), and quantitative real time polymerase chain reaction (qPCR). Animal studies that assayed telomere length, included in this review and meta-analysis, are summarised in Table 2. As telomeres and age have been the subject of several reviews (Dunshea *et al.*, 2011; Wilbourn *et al.*, 2018; Remot *et al.*, 2021) this section will highlight a few key studies across

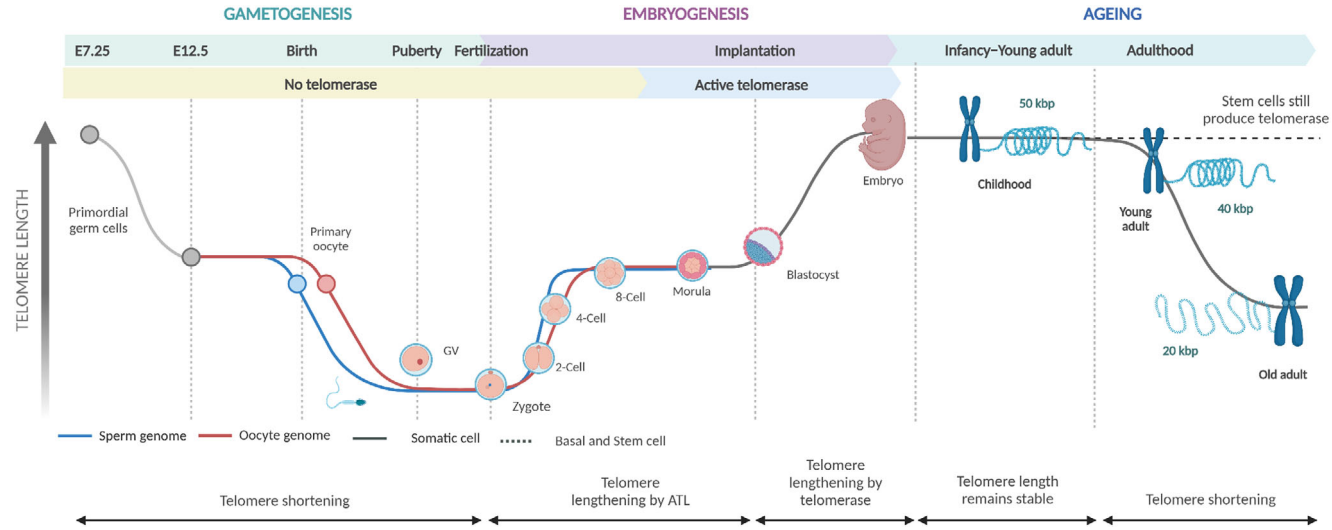

Fig. 8. Diagrammatic illustration of the alterations to telomere length during key life stages from gametogenesis to embryogenesis and the resulting changes that occur during natural ageing in most mammals. During gametogenesis the telomeres shorten in primordial germ cells and gametes, either sperm or egg cells, due to the absence of telomerase. After a zygote is formed from the union of two gametes, passive genomic demethylation happens which aids in telomere lengthening by enabling alternative telomere lengthening (ALT). Between the morula and blastocyst stage, telomerase expression is reactivated, and active telomere lengthening occurs prior to implantation. After birth, telomere length remains stable in most tissues throughout childhood from infancy to early adulthood. During the ageing process telomere length is maintained in stem cells while telomere shortening happens throughout the remainder of somatic cells. Image created in BioRender.com.

Table 2. Summary of studies that assayed animal telomere length in relation to age, the environment, or transgenerational heredity. Studies that used the data to create a molecular model for animal age, which were included in the meta-analysis, are indicated with an asterisk (*) for across-class analyses and a double asterisk (**) for the birds-only analyses. Species are grouped by class and listed alphabetically. For each species the karyotype of diploid chromosomes is given along with estimates for the average lifespan. For each study, the method of measuring telomere length is indicated: a, fluorescent *in situ* hybridization–flow cytometry (Flow-FISH); b, terminal restriction fragment (TRF) length & Southern blot; c, quantitative real-time polymerase chain reaction (qPCR); d, quantitative-fluorescent *in situ* hybridization (Q-FISH). Where relevant, citations are also provided for studies on age determination using other biological clocks (tooth annulation/otolith) in the same/similar species. n.a., not available.

Species	Karyotype	Lifespan (years)	Study	Method	Tooth annulation/otolith
(i) Fishes:					
Common carp (*Cyprinus carpio*)*	100	11–13	Izzo *et al.* (2014)	c	Brown *et al.* (2011)
Common carp (*Cyprinus carpio*)*	100	11–13	Izzo (2010)	c	Brown *et al.* (2011)
European chub (*Squalius cephalus*)*	50	25–30	Molbert *et al.* (2021)	c	Brown *et al.* (2011)
European seabass (*Dicentrarchus labrax*)*	48	15–30	Horn *et al.* (2008)	b	Secor *et al.* (1995)
Rainbow trout (*Oncorhynchus mykiss*)*	60	4–6	Panasiak *et al.* (2020)	d	Payan *et al.* (1998)
Siberian sturgeon (*Acipenser baerii*)*	248	40–60	Simide *et al.* (2016)	c	Campana (2001)
Turquoise killifish (*Nothobranchius furzeri*)	36	4–8 months	Hartmann *et al.* (2009)	b, c	Reichenbacher *et al.* (2007)
Zebrafish (*Danio rerio*) – model organism	48	1–4	Lund *et al.* (2009)	b	Higgs *et al.* (2002)
Zebrafish (*Danio rerio*) – model organism	48	1–4	Anchelin *et al.* (2011)	a, b	Higgs *et al.* (2002)
(ii) Amphibians:					
Australian green tree frog (*Litoria caerulea*)*	26	16–21	Lundsgaard *et al.* (2022)	c	n.a.
Natterjack toad (*Epidalea calamita*)*	22	10–15	Sánchez-Montes *et al.* (2020)	c	n.a.
(iii) Reptiles:					
Algerian psammodromus (*Psammodromus algirus*)*	38	3–5	Burraco *et al.* (2020)	c	n.a.
American alligator (*Alligator mississippiensis*)	32	30–50	Bae *et al.* (2021)	c	n.a.
American alligator (*Alligator mississippiensis*)*	32	30–50	Scott *et al.* (2006)	b	n.a.
Frilled lizard (*Chlamydosaurus kingii*)*	32	10–20	Ujvari *et al.* (2017)	c	n.a.
Loggerhead sea turtle (*Caretta caretta*)*	56	47–67	Hatase *et al.* (2008)	c	n.a.
Leatherback sea turtle (*Dermochelys coriacea*)*	56	45–50	Plot *et al.* (2012)	c	n.a.
Przewalski's toadhead agama (*Phrynocephalus przewalskii*)	46	3–10	Zhang *et al.* (2018)	c	n.a.
Water python (*Liasis fuscus*)*	36	20–30	Ujvari & Madsen (2009)	b	n.a.
(iv) Birds:					
Chats & flycatchers:					
African stonechat (*Saxicola torquatus*)*,**	80	4–5	Apfelbeck *et al.* (2019)	b	n.a.
Bluethroat (*Luscinia svecica*)	82	1–5	Sørheim (2020)	c	n.a.
Collared flycatcher (*Ficedula albicollis*)*,**	80	5–9	Stier *et al.* (2020)	c	n.a.
Collared flycatcher (*Ficedula albicollis*)*,**	80	5–9	Voillemot *et al.* (2012)	c	n.a.
European pied flycatcher (*Ficedula hypoleuca*)**	80	2–15	Kärkkäinen *et al.* (2019)	c	n.a.
European pied flycatcher (*Ficedula hypoleuca*)**	80	2–15	Kärkkäinen *et al.* (2021)	c	n.a.
European stonechat (*Saxicola rubicola*)*,**	80	4–5	Apfelbeck *et al.* (2019)	b	n.a.
Corvids:					
Florida scrub-jay (*Aphelocoma coerulescens*)*,**	80	4–9	Tricola *et al.* (2018)	b	n.a.

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Table 2. *(Cont.)*

Species	Karyotype	Lifespan (years)	Study	Method	Tooth annulation/otolith
Jackdaw (*Corvus monedula*)**	80	3–5	Bauch *et al.* (2021)	b	n.a.
Jackdaw (*Corvus monedula*)**	80	3–5	Salomons *et al.* (2009)	b	n.a.
Mexican jay (*Aphelocoma ultramarina*)*,***	80	9–15	Tricola *et al.* (2018)	b	n.a.
Cuckoos:					
Great spotted cuckoo (*Clamator glandarius*)**	78	3–6	Soler *et al.* (2015)	c	n.a.
Eagles:					
White-tailed eagle (*Haliaeetus albicilla*)	66	21–25	Hansen *et al.* (2022)	c	n.a.
Finches:					
Bengalese finch (*Lonchura striata*)*,***	78	4–7	Tricola *et al.* (2018)	b	n.a.
Pine siskin (*Spinus pinus*)	78	5–10	Vernasco & Watts (2022)	c	n.a.
Zebra finch (*Taeniopygia guttata*)*,***	78	2–3	Heidinger *et al.* (2012)	c	n.a.
Zebra finch (*Taeniopygia guttata*)*,***	78	2–3	Tricola *et al.* (2018)	b	n.a.
Geese & waterfowl:					
Barnacle goose (*Branta leucopsis*)*,***	80	14–24	Pauliny *et al.* (2012)	b	n.a.
Gulls, shorebirds & waders:					
Common ruff (*Calidris pugnax*)*,***	86	4–5	Tricola *et al.* (2018)	b	n.a.
Dunlin (*Calidris alpina*)*,***	88	5–9	Pauliny *et al.* (2006)	b	n.a.
Oystercatcher (*Haematopus longirostris*)*,***	72	12–17	Tricola *et al.* (2018)	b	n.a.
Yellow-legged gull (*Larus michahellis*)**	66	18–20	Noguera & Velando (2021)	c	n.a.
Juncos, buntings & sparrows:					
Dark-eyed junco (*Junco hyemalis*)*,***	82	3–11	Bauer *et al.* (2018)	c	n.a.
Dark-eyed junco (*Junco hyemalis*)	82	3–11	Graham (2018)	c	n.a.
House sparrow (*Passer domesticus*)	76	3–5	Meillère *et al.* (2015)	c	n.a.
Savannah sparrow (*Passerculus sandwichensis*)*,***	74	2–6	Tricola *et al.* (2018)	b	n.a.
Magpies:					
Eurasian magpie (*Pica pica*)**	82	3–4	Soler *et al.* (2015)	c	n.a.
Manakins:					
Wire-tailed manakin (*Pipra filicauda*)**	78	10–15	Vernasco *et al.* (2021)	c	n.a.
Ovenbirds:					
Thorn-tailed Rayadito (*Aphrastura spinicauda*)	82	4–9	Quirici *et al.* (2016)	c	n.a.
Parrots:					
African Grey parrot (*Psittacus erithacus erithacus*)*,***	66	60–80	Aydinonat *et al.* (2014)	c	n.a.
Penguins:					
Adelie penguin (*Pygoscelis adeliae*)*,***	96	10–20	Tricola *et al.* (2018)	b	n.a.
King penguin (*Aptenodytes patagonicus*)**	72	26–41	Reichert *et al.* (2015)	c	n.a.
Magellanic penguin (*Spheniscus magellanicus*)*,***	68	20–30	Cerchiara *et al.* (2017)	c	n.a.
Seabirds:					
Black guillemot (*Cepphus grille*)*,***	50	10–11	Tricola *et al.* (2018)	b	n.a.
Common tern (*Sterna hirundo*)*,***	68	9–10	Bauch *et al.* (2014)	b	n.a.
Common tern (*Sterna hirundo*)*,***	68	9–10	Haussmann *et al.* (2003)	b	n.a.
Common tern (*Sterna hirundo*)*,***	68	9–10	Tricola *et al.* (2018)	b	n.a.
Common tern (*Sterna hirundo*)	68	9–10	Vedder *et al.* (2017)	b	n.a.
Common tern (*Sterna hirundo*)	68	9–10	Vedder *et al.* (2022)	b	n.a.
Cory's shearwater (*Calonectris borealis*)*,***	80	20–25+	Bauch *et al.* (2020)	b	n.a.
	80	20–25+	Bauch *et al.* (2022)	b	n.a.

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Table 2. *(Cont.)*

Species	Karyotype	Lifespan (years)	Study	Method	Tooth annulation/otolith
Cory's shearwater (*Calonectris borealis*)					
Great frigatebird (*Fregata minor*)*,***	78	30–34	Juola *et al.* (2006)	b	n.a.
Great frigatebird (*Fregata minor*)*,***	78	30–34	Tricola *et al.* (2018)	b	n.a.
Leach's storm petrel (*Oceanodroma leucorhoa*)*,***	74	20–36	Tricola *et al.* (2018)	b	n.a.
Northern fulmar (*Fulmarus glacialis*)*,***	78	30–42	Tricola *et al.* (2018)	b	n.a.
Red-footed booby (*Sula sula*)*,***	78	24–40	Tricola *et al.* (2018)	b	n.a.
Thick-billed murre (*Uria lomvia*)*,***	80	20–29	Tricola *et al.* (2018)	b	n.a.
Starlings:					
Spotless starling (*Sturnus unicolor*)	80	8–9	Soler *et al.* (2017)	c	n.a.
Swallows:					
Barn swallow (*Hirundo rustica*)**	80	1–4	Parolini *et al.* (2015)	c	n.a.
Barn swallow (*Hirundo rustica*)**	80	1–4	Tricola *et al.* (2018)	b	n.a.
Mangrove swallow (*Tachycineta albilinea*)*,***	78	4–8	Tricola *et al.* (2018)	b	n.a.
Sand martin (*Riparia riparia*)*,***	80	2–9	Pauliny *et al.* (2006)	b	n.a.
Tree swallow (*Tachycineta bicolor*)*,***	78	4–8	Tricola *et al.* (2018)	b	n.a.
Swifts:					
Alpine swift (*Tachymarptis melba*)*,***	78	10–15	Bize *et al.* (2009)	c	n.a.
Thrushes:					
Eastern bluebird (*Sialia sialis*)	80	6–10	de la Iglesia (2018)	c	n.a.
Eurasian blackbird (*Turdus merula*)	80	3–21	Ibáñez-Álamo *et al.* (2018)	c	n.a.
Tits:					
Blue tit (*Cyanistes caeruleus*)**	80	3–10	Sudyka *et al.* (2014)	c	n.a.
Great tit (*Parus major*)	80	2–3	Grunst *et al.* (2020)	c	n.a.
Great tit (*Parus major*)*,***	80	2–3	Salmón *et al.* (2016)	c	n.a.
Great tit (*Parus major*)*,***	80	2–3	Tricola *et al.* (2018)	b	n.a.
Warblers:					
American redstart (*Setophaga ruticilla*)**	78	5–10	Angelier *et al.* (2013)	c	n.a.
Great reed warbler (*Acrocephalus arundinaceus*)**	80	2–6	Asghar *et al.* (2015)	c	n.a.
Seychelles warbler (*Acrocephalus sechellensis*)*,***	78	5–17	Barrett *et al.* (2013)	c	n.a.
Seychelles warbler (*Acrocephalus sechellensis*)	78	5–17	Spurgin *et al.* (2018)	c	n.a.
Wrens:					
Purple-crowned fairywren (*Malurus coronatus*)	72	3–5	Eastwood *et al.* (2019)	c	n.a.
Purple-crowned fairywren (*Malurus coronatus*)*,***	72	3–5	Roast *et al.* (2022)	c	n.a.
(v) Mammals:					
Aquatic:					
Dugong (*Dugong dugon*)*	48	50–70	Cherdsukjai *et al.* (2020)	c	Marsh, Heinsohn & Marsh (1984)
Humpback whale (*Megaptera novaeangliae*)*	44	45–50	Olsen *et al.* (2014)	c	Ohsumi *et al.* (1965)
Bats:					
Bechstein's bat (*Myotis bechsteinii*)*	44	15–21	Foley *et al.* (2018)	c	Linhart (1973)
Big brown bat (*Eptesicus fuscus*)*	50	6–19	Ineson *et al.* (2020)	c	Linhart (1973)
Common bent-wing bat (*Miniopterus schreibersii*)*	46	13–20	Foley *et al.* (2018)	c	Linhart (1973)
Greater horseshoe bat (*Rhinolophus ferrumequinum*)*	58	20–30	Foley *et al.* (2018)	c	Linhart (1973)
	44	6–13	Foley *et al.* (2018)	c	Linhart (1973)

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Table 2. *(Cont.)*

Species	Karyotype	Lifespan (years)	Study	Method	Tooth annulation/otolith
Greater mouse-eared bat (*Myotis myotis*)*					
Greater mouse-eared bat (*Myotis myotis*)	44	6–13	Foley *et al.* (2020)	c	Linhart (1973)
Little brown bat (*Myotis lucifugus*)*	44	7–31	Ineson *et al.* (2020)	c	Linhart (1973)
Carnivores:					
American black bear (*Ursus americanus*)*	74	18–23+	Kirby *et al.* (2017)	c	Marks & Erickson (1966)
American marten (*Martes americana*)*	38	6–15	Pauli *et al.* (2011)	c	Belant *et al.* (2011)
Australian sea lion (*Neophoca cinerea*)*	36	17–25	Izzo *et al.* (2011)	c	Rust *et al.* (2019)
Domestic cat (*Felis catus*)*	38	12–18	Brümmendorf *et al.* (2002)	a	Grue & Jensen (1979)
Domestic cat (*Felis catus*)*	38	12–18	McKevitt *et al.* (2003)	b	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)	78	10–13	Dutra *et al.* (2020)	c	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)*	78	10–13	Fick *et al.* (2012)	c	Grue & Jensen (1979)
Domestic dog (*Canis familiaris*)*	78	10–13	McKevitt *et al.* (2002)	b	Grue & Jensen (1979)
European badger (*Meles meles*)*	44	6–16	Beirne *et al.* (2014)	c	Ahnlund (1976)
European badger (*Meles meles*)	44	6–16	van Lieshout *et al.* (2019)	c	Ahnlund (1976)
European badger (*Meles meles*)	44	6–16	van Lieshout *et al.* (2021)	c	Ahnlund (1976)
Grizzly bear (*Ursus arctos horribilis*)*	74	20–25	Wong (2017)	b	Craighead *et al.* (1970)
Harp/Greenland seal (*Pagophilus groenlandicus*)*	32	20–30	Garde *et al.* (2010)	b, c	Bowen *et al.* (1983)
Meerkat (Suricata suricatta)	36	12–14	Cram *et al.* (2018)	c	Rausch & Pearson (1972)
Pacific marten (*Martes caurina*)*	38	6–15	Pauli *et al.* (2011)	c	Belant *et al.* (2011)
Polar bear (*Ursus maritimus*)*	74	25–30	Wong (2017)	c	Christensen-Dalsgaard *et al.* (2010)
Spotted hyena (*Crocuta crocuta*)*	40	12–25	Lewin *et al.* (2015)	b	Grue & Jensen (1979)
Elephants:					
Asian elephant (*Elephas maximus*)*	56	48–60	Buddhachat *et al.* (2017)	c	Spinage (1976)
Marsupials:					
Tasmanian devil (*Sarcophilus harrisii*)	14	5–8	Smith *et al.* (2020)	c	Pekelharing (1970)
Primates:					
Baboon (*Papio hamadryas*)*	42	27–37	Baerlocher *et al.* (2003)	a	Wada *et al.* (1978)
Baboon (*Papio hamadryas*)	42	27–37	Karere *et al.* (2019)	c	Wada *et al.* (1978)
Chimpanzee (*Pan troglodytes*)	48	32–39	Tackney *et al.* (2014)	c	Wittwer-Backofen *et al.* (2004)
Chimpanzee (*Pan troglodytes*)	48	32–39	Eisenberg *et al.* (2017)	c	Wittwer-Backofen *et al.* (2004)
Crab-eating macaque (*Macaca fascicularis*)*	42	15–30	Lee *et al.* (2002)	a, b	Wada *et al.* (1978)
Rodents:					
American red squirrel (*Tamiasciurus hudsonicus*)	46	3–8	Dantzer *et al.* (2020)	b	Adams & Watkins (1967)
Columbian ground squirrel (*Urocitellus columbianus*)*	32	5–10	Viblanc *et al.* (2022)	c	Adams & Watkins (1967)
Damaraland mole-rat (*Fukomys damarensis*)*	74	15–16	Leonida *et al.* (2020)	d	Grue & Jensen (1979)
Eastern chipmunk (*Tamias striatus*)*	38	3–8	Tissier *et al.* (2022)	c	Adams & Watkins (1967)
Hottentot mole-rat (*Cryptomys hottentotus*)*	54	11–15	Leonida *et al.* (2020)	d	Grue & Jensen (1979)
Shrews:					
Iberian shrew (*Sorex granarius*)	42	10–14 months	Zhdanova *et al.* (2010)	d	Grue & Jensen (1979)
Ungulates:					
Domestic horse (*Equus caballus*)*	64	25–30	Denham *et al.* (2019)	b	Penzhorn (1982)
Moose (*Alces alces*)	68	15–25	Fohringer *et al.* (2022)	c	Rolandsen *et al.* (2008)

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Table 2. *(Cont.)*

Species	Karyotype	Lifespan (years)	Study	Method	Tooth annulation/otolith
Roe deer (*Capreolus capreolus*)*	70	7–10	Wilbourn *et al.* (2017)	c	Grue & Jensen (1979)
Soay sheep (*Ovis aries*)	54	10–12	Fairlie *et al.* (2016)	c	Saxon & Higham (1968)
Soay sheep (*Ovis aries*)*	54	10–12	Froy *et al.* (2021)	c	Saxon & Higham (1968)
Water buffalo (*Bubalus bubalis*)*	50	25–35+	Seibt *et al.* (2019)	c	Grimsdell (1973)

the same taxonomic range as those done comparing methylation to age.

(i) Fishes (classes Agnatha, Chondrichthyes, and Osteichthyes). In fishes, telomere length has been studied in relation to ecotoxicology and environmental stressors in addition to ageing, and the validity of telomeres as proxies of life history in ectotherms has been the subject of considerable research (Friesen, Wapstra & Olsson, 2021). With regards to age, a relationship has been illustrated in several species including model organisms such as zebrafish (Anchelin *et al.*, 2011) and several wild populations such as rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (Panasiak, Dobosz & Ocalewicz, 2020), European seabass (Horn *et al.*, 2008), common carp [*Cyprinus carpio* (Linnaeus)] (Izzo *et al.*, 2014), turquoise killifish [*Nothobranchius furzeri* (Jubb)] (Hartmann *et al.*, 2009), and Siberian sturgeon [*Acipenser baerii* (Brandt)] (Simide *et al.*, 2016). Although most of these studies used non-lethal sampling techniques to explore telomere length as an alternative to otolith ageing techniques, a few did include lethal sampling and even explored telomere length in the head kidney, an organ unique to teleost fishes (Panasiak *et al.*, 2020, 2022).

(ii) Amphibians (class Amphibia). Two amphibian species have been studied in the context of telomere length changes throughout their lifespan. One was principally focussed on differential changes in the Australian green tree frog [*Litoria caerulea* (White)], as a result of early life exposure to ultraviolet (UV) and different periods of light (Lundsgaard, Cramp & Franklin, 2022). The authors found that differences in early life exposure led to differences in telomere length later in life, as well as a relatedness to several key aspects that influence long-term survival in frogs. The other specifically studied telomere shortening in a wild population of naturally ageing natterjack toads [*Epidalea calamita* (Laurenti)], over a period of 3 years (Sánchez-Montes *et al.*, 2020). Their study found significant telomere shortening in the first 2–3 years of life in most of the studied individuals and a general trend of telomere shortening throughout the lifespan of toads.

(iii) Reptiles (class Reptilia). The observation that telomere length is affected by temperature or light exposure in ectotherms was replicated in both experimental and wild populations of reptiles. Under experimental conditions, telomeres were found to shorten faster and result in shortened lifespans in Przewalski's toadhead agama [*Phrynocephalus przewalskii* (Strauch)] (Zhang *et al.*, 2018). In the wild, Algerian psammodromus [*Psammodromus algirus* (Linnaeus)] lizards showed a cline of variable telomere length across a latitudinal gradient of their habitat (Burraco *et al.*, 2020). In terms of age-related telomere studies, telomere length has been shown to decline with age in American alligators (Scott *et al.*, 2006; Bae *et al.*, 2021), as well as loggerhead sea turtles (Hatase *et al.*, 2008; Plot *et al.*, 2012). Conversely, telomeres were found to increase in length in tropical water python [*Liasis fuscus* (Peters)] (Ujvari & Madsen, 2009) and follow a curvilinear relationship in frilled lizards [*Chlamydosaurus kingii* (Gray)] with both increases and decreases across their full lifespan (Ujvari *et al.*, 2017).

(iv) Birds (class Aves). Birds represent the best studied class of wild vertebrates in terms of telomeres (Criscuolo, Dobson & Schull, 2021) with most studies falling into one of four broad categories: ecotoxicological studies, stress-associated studies, migration-related studies, and age-model studies. As in other organisms, telomere length has been shown to decrease at an increased rate in response to toxins or contaminants in birds, including Arctic-breeding black-legged kittiwakes [*Rissa tridactyla* (Linnaeus)] (Blévin *et al.*, 2016), and Cory's shearwaters [*Calonectris borealis* (Cory)] (Bauch *et al.*, 2022). In stress-associated studies, telomere length dynamics were compared to environmental stressors including noise (Dorado-Correa *et al.*, 2018; Grunst *et al.*, 2020), an urban environment (Salmón *et al.*, 2016; Ibáñez-Álamo *et al.*, 2018), or anthropogenic activity (Caccavo *et al.*, 2021) as well as social factors such as brood size (Voillemot *et al.*, 2012; Quirici *et al.*, 2016; Criscuolo, Zahn & Bize, 2017, Noguera & Velando, 2021), brood parasitism (Soler *et al.*, 2015), proximity to predators (Kärkkäinen *et al.*, 2019), and social isolation (Aydinonat *et al.*, 2014). In most cases, telomere length was negatively impacted by stress and cohort-wide increases in telomere loss were observed in response to measured stressors. It should, however, be noted that many of these studies solely included birds in their early life stages and did not access telomere length changes across the full lifespan of all species.

In terms of the relationship between telomere length and attributes of migration differences have been observed for the differential timing of breeding (Graham, 2018) and migration (Vernasco & Watts, 2022) as well as return rate (Angelier *et al.*, 2013) and reproductive success (Eastwood *et al.*, 2019). Studies have also illustrated that habitat quality

differentially affects telomere shortening within species with different breeding habitats (Angelier *et al.*, 2013) and that generally advanced shortening is a consequence of the metabolic stain that migration creates when comparing migratory and resident populations of the same species (Bauer *et al.*, 2018).

Studies with a focus on cross-sectional changes in telomere length with increased age found varied results depending on the study species (Tricola *et al.*, 2018). For example, small effects were observed for an age correlation in Magellanic penguin [*Spheniscus magellanicus* (Forster)] (Cerchiara *et al.*, 2017), and other penguins (Reichert *et al.*, 2015) while an intermediate effect was observed in several other bird species (Bize *et al.*, 2009; Vedder *et al.*, 2017; Bauer *et al.*, 2018; Roast *et al.*, 2022). The largest effect was observed in three species: common tern (Haussmann, Vleck & Nisbet, 2003), great frigatebird [*Fregata minor* (Gmelin)] (Juola *et al.*, 2006), and sand martin *Riparia riparia* (Linnaeus) (Pauliny *et al.*, 2006). Although telomere length declines were evident in birds from most lineages the rate of decline varies and appears to be correlated with maximum lifespan (Tricola *et al.*, 2018).

(v) Mammals (class Mammalia)

(*i*.1) *Aquatic mammals (infraorder Cetacea and order Sirenia)*. Telomere length was found to decline with age in humpback whales but showed a high level of variation among individuals of the same age, resulting in a model with high error (∼28 years) in the predicted ages. The authors attributed this to the low sample size for calibration as well as the sensitivity of the qPCR assay as even small experimental deviations significantly altered the assay values (Olsen *et al.*, 2014). A recent study that included dolphins found a significant decline in telomere length with age, however, the study was limited by small sample sizes ($N < 10$) with no or few replicates across the same age. One key finding was that telomere length tended to decrease at an increased rate in species with shorter lifespans (Whittemore *et al.*, 2019). In wild dugong [*Dugong dugon* (Müller)], relatives of manatees in the herbivorous aquatic mammal order Sirenia, telomere length was highly correlated with age and showed less variability at the individual level (Cherdsukjai *et al.*, 2020).

(*i*.2) *Bats (order Chiroptera)*. Telomeres have been studied in at least six species of bats including several members of the long lived *Myotis* genus (Foley *et al.*, 2018, 2020; Ineson *et al.*, 2020). In both greater horseshoe bat [*Rhinolophus ferrumequinum* (Schreber)] and common bent-wing bat [*Miniopterus schreibersii* (Kuhl)] telomere length was found to decrease rapidly with age while telomeres in *Myotis* species, including Bechstein's bat, were well maintained throughout their lifespan (Foley *et al.*, 2018). Due to the absence of telomerase in mature blood cells this was likely due to the upregulation of genes involved in the ALT pathway (Foley *et al.*, 2020). A study on two American bat species (Ineson *et al.*, 2020) found telomere length to be correlated with age in both big brown bats [*Eptesicus fuscus* (Beauvois)] and little brown bats [*Myotis lucifugus* (Le Conte)]. The authors did, however, note that the relative predictive power for unknown-age individuals was low and the relationship between telomere attrition and age in *M. lucifugus* was correlated with advanced ageing in response to white nose syndrome, a common fungal infection in some bats.

(*i*.3) *Carnivores (order Carnivora)*. Some of the first studies in non-model animals with regards to telomeres and ageing used domesticated companion animals including the domestic dog and cat. In canids, telomere length was found to shorten at an advanced rate (McKevitt *et al.*, 2002) and to be correlated with the maximum lifespan of breeds (Fick *et al.*, 2012). Similar results were obtained from longitudinal studies in felids (Brümmendorf *et al.*, 2002; McKevitt *et al.*, 2003) including the observation that telomere length varies in sub-populations of leucocytes. Other studies on other members of the suborder Feliformia included the spotted hyena [*Crocuta crocuta* (Erxleben)], and meerkat [*Suricata suricatta* (Schreber)]. For both species, studies focussed on socio-ecological factors affecting variable telomere attrition primarily in relation to social rank and found higher ranking members to have a slower rate of telomere attrition and a corresponding longer life expectancy than low-ranking members (Lewin *et al.*, 2015; Cram *et al.*, 2018).

A study on two species of martens, the American marten [*Martes americana* (Turton)], and Pacific marten [*M. caurina* (Merriam)], specifically used tooth cementum annulation to determine age for cross-validation of a telomere-based PCR method of assigning age (Pauli *et al.*, 2011). The authors were able to establish a model that can assign the age of individuals to one of five functionally relevant classes with ∼80% accuracy, which improved to over 90% when including morphometric data. Several studies in the related European badger [*Meles meles* (Linnaeus)] found diverse factors to contribute to telomere biology in these species including age-related declines (Beirne *et al.*, 2014), disease-related declines (Beirne *et al.*, 2014), transgenerational effects (van Lieshout *et al.*, 2021), and differential fitness (van Lieshout *et al.*, 2019).

Other studied carnivores include several species of bears (Wong, 2017; Kirby, Alldredge & Pauli, 2017) and pinnipeds (Garde *et al.*, 2010; Izzo *et al.*, 2011) for which a small to intermediate effect for age was observed.

(*i*.4) *Elephants (order Proboscidea, suborder Elephantiformes)*. Telomere length has been studied in relation to age in the Asian elephant using 122 samples across the full lifespan of the species (Buddhachat *et al.*, 2017). A significant decline in telomere length was found ($P < 0.05$) independent of any observed effect for sex-linked differences in ageing rate. Furthermore, this study found telomere length to be markedly increased in younger individuals in comparison to shorter lived species, while the general trend and rate of telomere shortening was consistent in both Asian elephants and shorter-lived species. Another study (Whittemore *et al.*, 2019) evaluated differential telomere shortening in species with different lifespans, including Sumatran subspecies of Asian elephants (*Elephas maximus sumatranus*), but was limited in resolution by small sample sizes ($N = 4$). Nevertheless,

this study did detect a decline in telomere length and differential rates of attrition among species of varied lifespans.

(*i*.5) *Marsupials (infraorder Marsupialia)*. Few studies have been conducted on telomere dynamics in marsupials. One study, in Tasmanian devils, focussed on telomere length as a predictive risk factor for facial tumours and found longer telomeres to have a protective role that delays the age of onset for facial tumours in different populations (Smith *et al*., 2020). Studies on sexual dimorphism in telomere length in Tasmanian devils and other marsupials have illustrated that while telomeres shorten during oogenesis they tend to elongate during spermatogenesis and the inherited length therefore shows strong parental effects. In facial tumours, however, all cells showed characteristically short telomeres even though telomerase activity is typically upregulated in cancer cells (Bender *et al*., 2012).

(*i*.6) *Primates (order Primates)*. Beyond the cornucopia of telomere studies conducted in humans, telomeres have been assayed in relation to age in at least three other primates. The first analysed telomere length in an ageing population of laboratory-kept crab-eating macaque [*Macaca fascicularis* (Raffles)] using flow-FISH (Lee *et al*., 2002). This study highlighted two important aspects of ageing in primates. Firstly, telomere length was shown to decline with age in a predictable manner and, secondly, the ratios of different leucocyte sub-types changes with age. A similar study in baboons (Baerlocher *et al*., 2003, 2007) further supported these findings. In chimpanzees, studies have been done on telomere dynamics in relation to sex and age. A study on female chimpanzees found telomere length is increased as compared to humans, however, the rate of telomere attrition is conserved in both species and declines predictably with age (Tackney *et al*., 2014). In male chimpanzees, the heritability of telomere length based on paternal age at conception was explored and an increase in the telomeres of spermatozoa with increased paternal age was observed with consequently high levels of paternal inheritance of telomere length (Eisenberg *et al*., 2017).

(*i*.7) *Rodents (order Rodentia)*. Studies on telomeres in rodents revealed that food availability differentially affects telomere attrition rates in dormouse species (Hoelzl *et al*., 2016*a*) while hibernation tends to increase telomere length (Hoelzl *et al*., 2016*b*). A similar trend was also observed for post-hibernation differences in telomere length among female Columbian ground squirrels [*Urocitellus columbianus* (Ord)] (Viblanc *et al*., 2022). Experimental studies in glucocorticoid-treated mothers with offspring in the American red squirrel [*Tamiasciurus hudsonicus* (Erxleben)] (Dantzer *et al*., 2020) found no evidence of maternal stress as measured by endocrine responses adversely affecting telomere length in offspring either during parturition, lactation, or rearing. This indicates that stress-associated telomere attrition in the young may be independent of a biological maternal effect. Further evidence of telomere elongation in hibernating rodent species was recently established in a longitudinal study of Eastern chipmunks [*Tamias striatus* (Linnaeus)] (Tissier *et al*., 2022). In the non-hibernating mole-rat, two species were, however, found to have shortening telomeres with advanced age in mature bone marrow cells (Leonida *et al*., 2020).

(*i*.8) *Shrews (order Eulipotyphla)*. Studies on telomeres in shrews included species such as the Iberian shrew [*Sorex granaries* (Miller)], however the complex biology of telomeres in these species, including telocentric chromosomes and the inclusion of ribosomal DNA between telomere repeats, makes them unsuitable as a biomarker of age (Zhdanova *et al*., 2010).

(*i*.9) *Ungulates (orders Artiodactyla and Perissodactyla)*. Several ungulate species for which methylation clocks of age have been established were also studied in telomere–age studies. Among deer species, telomeres of both roe deer and moose [*Alces alces* (Linnaeus)] from cross-sectional studies of populations from different ecoregions found telomere length changes with age to be highly correlated with geographic location, likely due to differential exposure to environmental stressors and food availability (Wilbourn *et al*., 2017; Fohringer *et al*., 2022). In Soay sheep [*Ovis aries* (Linnaeus)], a recently domesticated breed of sheep, longitudinal studies of a largely unmanaged population found evident differences in telomere shortening based on biological sex (Watson *et al*., 2017); while no evidence was found for an effect of parental age at conception (Froy *et al*., 2017) despite the high heredity (Froy *et al*., 2021) previously linked to individual lifespans (Fairlie *et al*., 2016) in this species. Telomere length was also found to decrease with age in domesticated horses (Denham, Stevenson & Denham, 2019) and domesticated water buffalo [*Bubalus bubalis* (Linnaeus)] (Seibt *et al*., 2019).

IV. META-ANALYSIS RESULTS

(1) Methylation-based clocks

Results from the meta-analysis of studies using methylation as a biological clock for age determination are summarised in Fig. 9. The global pooled cross-study effect was significant in favour of methylation being an accurate method of age prediction ($R^2 = 0.92$, $k = 60$, $P < 0.01$). No significant differences were observed in effect size between classes ($Q = 5.53$, df = 4, $P = 0.24$), however, a separate meta-analysis with clustering based on groups found a significant difference in effect sizes between the groups (Fig. S4; $Q = 10.13$, df = 2, $P < 0.01$). Further subgroup differences were also evident for effect size between models based on tissue type (Fig. S5; $Q = 17.52$, df = 6, $P < 0.01$) and study method (Fig. S6; $Q = 232.24$, df = 4, $P < 0.01$).

Between-study heterogeneity was high ($I^2 = 97\%$, $\tau^2 = 0.29$, $P < 0.01$). This heterogeneity was less for within-group analyses for groups 2 and 3 (Fig. S4) indicating that much of the heterogeneity was likely due to the smaller sample size used to validate models in related species not used to train the model. There was, however, still a high level of heterogeneity in group 1. Results from meta-regressions assessing individual factors as possible sources of heterogeneity are summarised in Table 3. As expected, heterogeneity and author were correlated with more than half of the

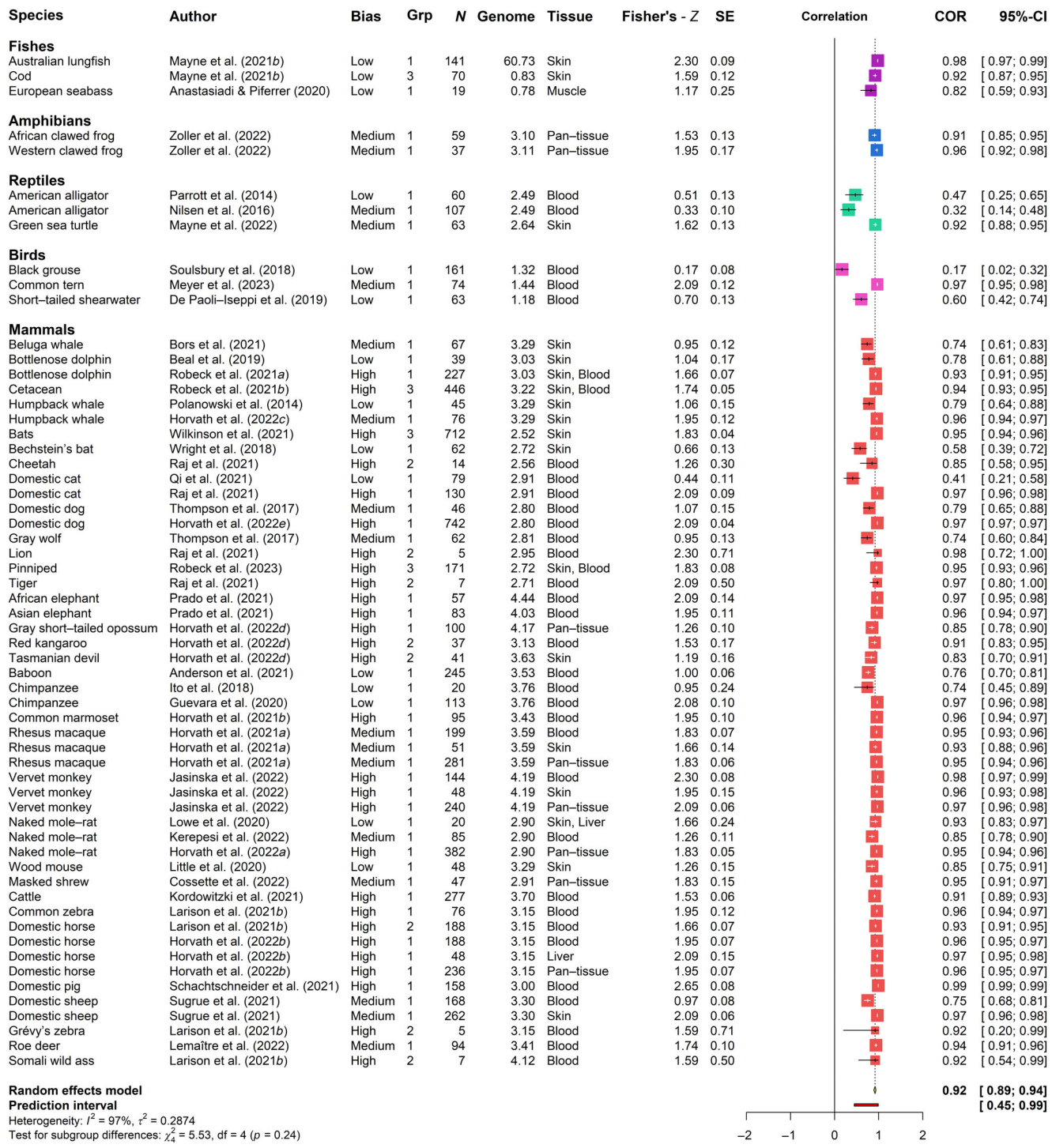

Fig. 9. Forest plot and results for the meta-analysis of studies using methylation to estimate animal age grouped by class. The pooled correlation from the random effects model is indicated (yellow diamond) along with measures of between-study heterogeneity and subgroup differences. The prediction interval is indicated with a maroon bar. Key study attributes such as sample size (*N*), genome size (in bbp), and tissue type are indicated along with the computed level of possible bias. CI, confidence interval; COR, correlation; *N*, sample size; SE, standard error.

heterogeneity being accounted for by within-study variances. A relationship with heterogeneity was also illustrated for both year and study method ($P < 0.01$) as well as class ($P < 0.05$) and sample size ($P < 0.10$), however, no relationship was found with maximum lifespan estimates, tissue type, or genome size. There were some significant indicators for

Table 3. Summary of results for multiple meta-regressions for heterogeneity. Lifespan estimates are derived from the lower, average/middle, and upper estimates of average lifespans reported in Tables 1 and 2. Significance: $*P < 0.10$, $**P < 0.05$, and $***P < 0.01$

Factor	Z-value	P-value	Degrees of freedom (df)	Correlation (R^2)
Methylation:				
Author	3.10	<0.01***	40	0.724
Year	2.65	<0.01***	8	0.467
Bias	20.32	<0.01***	2	0.297
Sample size (N)	1.90	0.057*	1	0.049
Tissue	14.61	0.762	6	<0.01
Method	9.58	<0.01***	4	0.492
Class	5.65	0.022**	4	0.126
Genome size (bbp)	1.63	0.103	1	0.030
Lifespan:				
Lower	11.70	0.290	1	0.054
Middle	10.80	0.390	1	<0.01
Upper	10.32	0.497	1	<0.01
Telomeres (All):				
Author	4.66	<0.01***	46	0.513
Year	3.07	0.060*	17	0.147
Bias	5.63	0.251	2	0.013
Sample size (N)	8.94	0.057*	1	0.043
Tissue	6.83	0.368	7	0.057
Method	2.91	0.225	3	0.020
Class	3.54	0.499	4	<0.01
Karyotype (diploid)	2.26	0.024**	1	0.065
Lifespan:				
Lower	4.48	0.172	1	0.014
Middle	4.08	0.149	1	0.019
Upper	3.87	0.139	1	0.020
Telomeres (Birds):				
Author	3.123	<0.01***	24	0.378
Year	0.008	0.991	1	<0.01
Sample size (N)	6.553	0.507	1	<0.01

asymmetry and publication bias or small study effects (t-statistic = −2.30, df = 58, $P = 0.03$). The quantification of potential author bias using *ABCal* identified several studies (28 models) with a higher risk of bias considering they share a significant number of authors and co-authors, compared to medium- (17 models) and low-risk (15 models) studies. The computed risk of bias also accounted for a significant ($P < 0.01$) amount of the heterogeneity. Notably, eight authors contributed to more than five of the included studies while four contributed to more than 10.

(2) Telomere-based clocks

Results for the meta-analysis of studies using telomeres as a biological clock for age determination across taxa are summarised in Fig. 10 and the separate analysis for birds in Fig. 11. The global pooled cross-study effect was significant in favour of telomere length being an accurate method of age prediction ($R^2 = 0.31$, $k = 60$, $P < 0.01$). A significant difference was observed in effect size between classes ($Q = 46.43$, df = 4, $P < 0.01$), between study groups (Fig. S7; $Q = 28.96$, df = 1, $P < 0.01$), between models based on tissue used (Fig. S8; $Q = 522.36$, df = 7, $P < 0.01$), and study method (Fig. S9; $Q = 44.10$, df = 3, $P < 0.01$). When analysing only studies in birds, the pooled effect was slightly larger ($R^2 = 0.43$, $k = 51$, $P < 0.01$) with a similar prediction interval to cross-taxa studies.

Between-study heterogeneity was high for both cross-taxa studies ($I^2 = 100\%$, $\tau^2 = 0.076$, $P < 0.01$) as well as birds alone. Results from the meta-regression assessing possible contributors to heterogeneity are summarised in Table 3. As for methylation, a correlation was detected for heterogeneity and author (as well as year) accounting for more than half of the heterogeneity, however, no relationship was evident for study method, tissue type, or lifespan. A small effect was observed for sample size and karyotype, however, both only accounted for <10% of the observed heterogeneity. There were some significant indicators for asymmetry and publication bias or small study effects for the cross taxa analyses (t-statistic = 3.33, df = 58, $P < 0.01$), as for methylation, but not for the birds alone (t-statistic = −0.18, df = 49, $P = 0.86$). The quantification of potential author bias using *ABCal* identified a near-equal number of studies (26 models) with a higher risk of bias for the between-study meta-analyses considering they share a significant number of authors and co-authors. This was, however, not found to be a significant contributor to the heterogeneity of studies ($P = 0.25$). This risk of bias was generally lower than for methylation studies as most of the highly represented authors only contributed to 2–4 of the included models.

(3) Methylation *versus* telomeres

Results for the comparison between telomere length and methylation as biomarkers for age determination are indicated in Table 4 and Fig. S10. The overall effect size for methylation-based studies was higher than that observed for telomeres, 0.92 *versus* 0.31, with a narrower prediction interval. The observed difference was statistically significant (Z-value = 21.58, $P < 0.01$) while no significant difference was found between the sample size distributions of the two methods (t-statistic = 0.422, df = 104.5, $P = 0.67$).

V. DISCUSSION

Our systematic review on the use of biological clocks as markers for age determination in wildlife clearly illustrates that several biological clocks are well suited to this purpose. Two methods, tooth cementum annulation and otoliths, have been in use for nearly a century, however, both methods have limitations on their use in studying age in living animals as part of continued conservation efforts. We therefore identified and synthesised the molecular mechanisms and existing

Species	Author	Bias	Grp	*N*	Karyo	Tissue	Fisher's - *Z*	SE	COR	95%-CI
Fishes										
Common carp	Izzo (2010)	Medium	1	84	100	Muscle	0.10	0.01	0.10	[0.07; 0.12]
European chub	Molbert et al. (2021)	Medium	1	98	50	Skin	0.41	0.01	0.39	[0.37; 0.40]
European seabass	Horn et al. (2008)	Low	1	24	48	Blood	0.02	0.05	0.02	[−0.07; 0.12]
Rainbow trout	Panasiak et al. (2020)	Low	1	64	60	Organ	0.34	0.02	0.33	[0.29; 0.36]
Siberian sturgeon	Simide et al. (2016)	Low	1	22	248	Blood	1.01	0.05	0.77	[0.72; 0.80]
Siberian sturgeon	Simide et al. (2016)	Low	1	121	248	Skin	0.55	0.01	0.50	[0.49; 0.52]
Amphibians										
Australian green tree frog	Lundsgaard et al. (2022)	Low	1	36	26	Muscle	0.06	0.03	0.06	[0.00; 0.12]
Natterjack toad	Sánchez–Montes et al. (2020)	Medium	1	175	22	Skin (male)	0.04	0.01	0.04	[0.02; 0.06]
Natterjack toad	Sánchez–Montes et al. (2020)	Medium	1	69	22	Skin (female)	0.09	0.01	0.09	[0.06; 0.12]
Reptiles										
Algerian psammodromus	Burraco et al. (2020)	Low	1	147	38	Skin	0.26	0.01	0.25	[0.24; 0.26]
American alligator	Scott et al. (2006)	High	1	34	32	Blood	0.18	0.03	0.18	[0.12; 0.24]
Frilled lizard	Ujvari et al. (2017)	High	1	93	32	Blood	0.42	0.01	0.40	[0.38; 0.41]
Loggerhead sea turtle	Hatase et al. (2008)	High	1	20	56	Blood	0.12	0.06	0.12	[0.01; 0.23]
Loggerhead sea turtle	Hatase et al. (2008)	High	1	20	56	Skin	0.44	0.06	0.41	[0.31; 0.50]
Water python	Ujvari & Madsen (2009)	Low	1	70	36	Blood	0.65	0.01	0.57	[0.55; 0.59]
Birds										
Alpine swift	Bize et al. (2009)	High	1	96	78	Blood	0.24	0.01	0.24	[0.22; 0.25]
Barnacle goose	Pauliny et al. (2012)	Low	1	30	80	Blood	0.09	0.04	0.09	[0.02; 0.16]
Common tern	Bauch et al. (2014)	Medium	1	184	68	Blood	0.21	0.01	0.21	[0.19; 0.23]
Common tern	Haussmann et al. (2003)	High	1	44	68	Blood	0.71	0.02	0.61	[0.58; 0.64]
Cory's shearwater	Bauch et al. (2020)	High	1	258	80	Blood	0.13	0.00	0.13	[0.13; 0.13]
Dark–eyed junco	Bauer et al. (2018)	Medium	1	24	82	Blood	0.25	0.05	0.24	[0.15; 0.33]
Dunlin	Pauliny et al. (2006)	Medium	1	28	88	Blood	0.42	0.04	0.40	[0.33; 0.46]
Great frigatebird	Juola et al. (2006)	High	1	36	78	Blood	0.95	0.03	0.74	[0.71; 0.77]
Magellanic penguin	Cerchiara et al. (2017)	Medium	1	73	68	Blood	0.01	0.01	0.01	[−0.01; 0.03]
Purple–crowned fairywren	Roast et al. (2022)	High	1	331	72	Blood	0.30	0.00	0.29	[0.29; 0.29]
Sand martin	Pauliny et al. (2006)	Medium	1	20	80	Blood	0.65	0.06	0.57	[0.49; 0.64]
Seychelles warbler	Barrett et al. (2013)	Low	1	373	78	Blood	0.34	0.00	0.33	[0.32; 0.34]
Mammals										
Dugong	Cherdsukjai et al. (2020)	High	1	24	48	Skin	1.29	0.05	0.86	[0.83; 0.88]
Humpback whale	Olsen et al. (2014)	Low	1	28	44	Skin	0.23	0.04	0.22	[0.15; 0.30]
Bechstein's bat	Foley et al. (2018)	High	1	49	44	Skin	0.16	0.02	0.16	[0.12; 0.20]
Big brown bat	Ineson et al. (2020)	Low	1	73	50	Skin	0.24	0.01	0.24	[0.21; 0.27]
Common bent–wing bat	Foley et al. (2018)	High	1	45	46	Skin	0.71	0.02	0.61	[0.59; 0.63]
Greater horseshoe bat	Foley et al. (2018)	High	1	160	58	Skin	0.26	0.01	0.25	[0.24; 0.27]
Greater mouse–eared bat	Foley et al. (2018)	High	1	239	44	Skin	0.09	0.00	0.09	[0.09; 0.09]
Little brown bat	Ineson et al. (2020)	Low	1	32	44	Skin	0.12	0.04	0.12	[0.05; 0.19]
American black bear	Kirby et al. (2017)	Low	1	245	74	Hair	0.00	0.00	0.00	[−0.01; 0.01]
Australian sea lion	Izzo et al. (2011)	Low	1	86	36	Skin	0.24	0.01	0.24	[0.22; 0.25]
Domestic cat	McKevitt et al. (2003)	High	1	32	38	Pan–tissue	0.34	0.04	0.33	[0.27; 0.39]
Domestic cat	Brümmendorf et al. (2002)	High	1	79	38	Blood	0.55	0.01	0.50	[0.48; 0.52]
Domestic dog	McKevitt et al. (2002)	High	1	47	78	Blood	0.23	0.02	0.23	[0.19; 0.27]
Domestic dog	Fick et al. (2012)	High	1	175	78	Blood	0.03	0.01	0.03	[0.01; 0.05]
European badger	Beirne et al. (2014)	Low	1	173	44	Blood	0.14	0.04	0.14	[0.06; 0.21]
Grizzly bear	Wong (2017)	Low	1	21	74	Blood	0.19	0.07	0.19	[0.05; 0.32]
Harp seal	Garde et al. (2010)	Medium	1	52	32	Pan–tissue (PCR)	0.05	0.02	0.05	[0.01; 0.09]
Harp seal	Garde et al. (2010)	Medium	1	22	32	Pan–tissue (TRL)	0.14	0.05	0.14	[0.04; 0.23]
Martens	Pauli et al. (2011)	Medium	2	399	38	Pan–tissue	0.13	0.00	0.13	[0.13; 0.13]
Polar bear	Wong (2017)	Low	1	29	74	Muscle	0.24	0.04	0.24	[0.16; 0.31]
Spotted hyena	Lewin et al. (2015)	High	1	64	40	Blood	0.11	0.02	0.11	[0.07; 0.15]
Asian elephant	Buddhachat et al. (2017)	High	1	122	56	Blood	0.02	0.01	0.02	[0.00; 0.04]
Baboon	Baerlocher et al. (2003)	High	1	32	42	Blood	0.91	0.03	0.72	[0.69; 0.75]
Crab–eating macaque	Lee et al. (2002)	Low	1	55	42	Blood	0.58	0.02	0.52	[0.49; 0.55]
Columbian ground squirrel	Viblanc et al. (2022)	High	1	43	32	Buccal	0.38	0.03	0.36	[0.31; 0.41]
Damaraland mole–rat	Leonida et al. (2020)	Low	1	12	74	Bone marrow	0.82	0.11	0.68	[0.54; 0.78]
Eastern chipmunk	Tissier et al. (2022)	High	1	204	38	Skin	0.42	0.00	0.40	[0.40; 0.40]
Hottentot mole–rat	Leonida et al. (2020)	Low	1	27	54	Bone marrow	0.58	0.04	0.52	[0.46; 0.58]
Domestic horse	Denham et al. (2019)	Low	1	43	64	Blood	0.51	0.02	0.47	[0.43; 0.51]
Roe deer	Wilbourn et al. (2017)	High	1	73	70	Blood (TF)	0.23	0.01	0.23	[0.21; 0.24]
Roe deer	Wilbourn et al. (2017)	High	1	66	70	Blood (CH)	0.20	0.02	0.20	[0.16; 0.23]
Soay sheep	Froy et al. (2021)	High	1	1586	54	Blood	0.04	0.00	0.04	[0.04; 0.04]
Water buffalo	Seibt et al. (2019)	Medium	1	73	50	Blood	0.27	0.01	0.27	[0.24; 0.29]
Random effects model									**0.31**	**[0.25; 0.37]**
Prediction interval										**[−0.23; 0.71]**

Heterogeneity: I^2 = 100%, τ^2 = 0.0763

Test for subgroup differences: χ^2_4 = 46.43, df = 4 (p < 0.01)

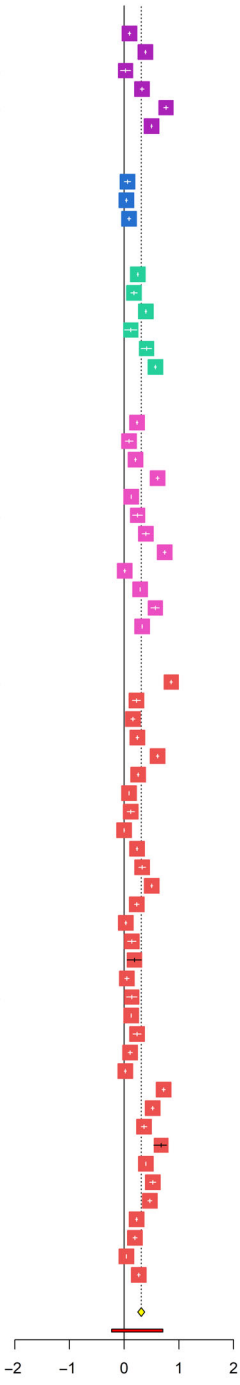

Fig. 10. Forest plot and results for the meta-analysis of studies using telomere length to estimate animal age grouped by class. The pooled correlation from the random effects model is indicated (yellow diamond) along with measures of between-study heterogeneity and the prediction interval is indicated with a red bar. Key study attributes such as sample size (*N*), karyotype (Karyo), and tissue type are indicated along with the computed level of possible bias. CH, Chizé region, France; CI, confidence interval; COR, correlation; *N*, sample size; PCR, polymerase chain reaction; SE, standard error; TF, Trois-Fontaines region, France; TRL, terminal restriction length.

primary evidence for the use of two genetic biological clocks, methylation and telomere length, that are suitable alternatives for studying age in living animals.

Methylation has previously been studied in several organisms in the context of eco-toxicology (Sareisian, 2014; Nilsen *et al.*, 2016; Romano *et al.*, 2017), relating changes in methylation to exposure to environmental pollutants, as well as

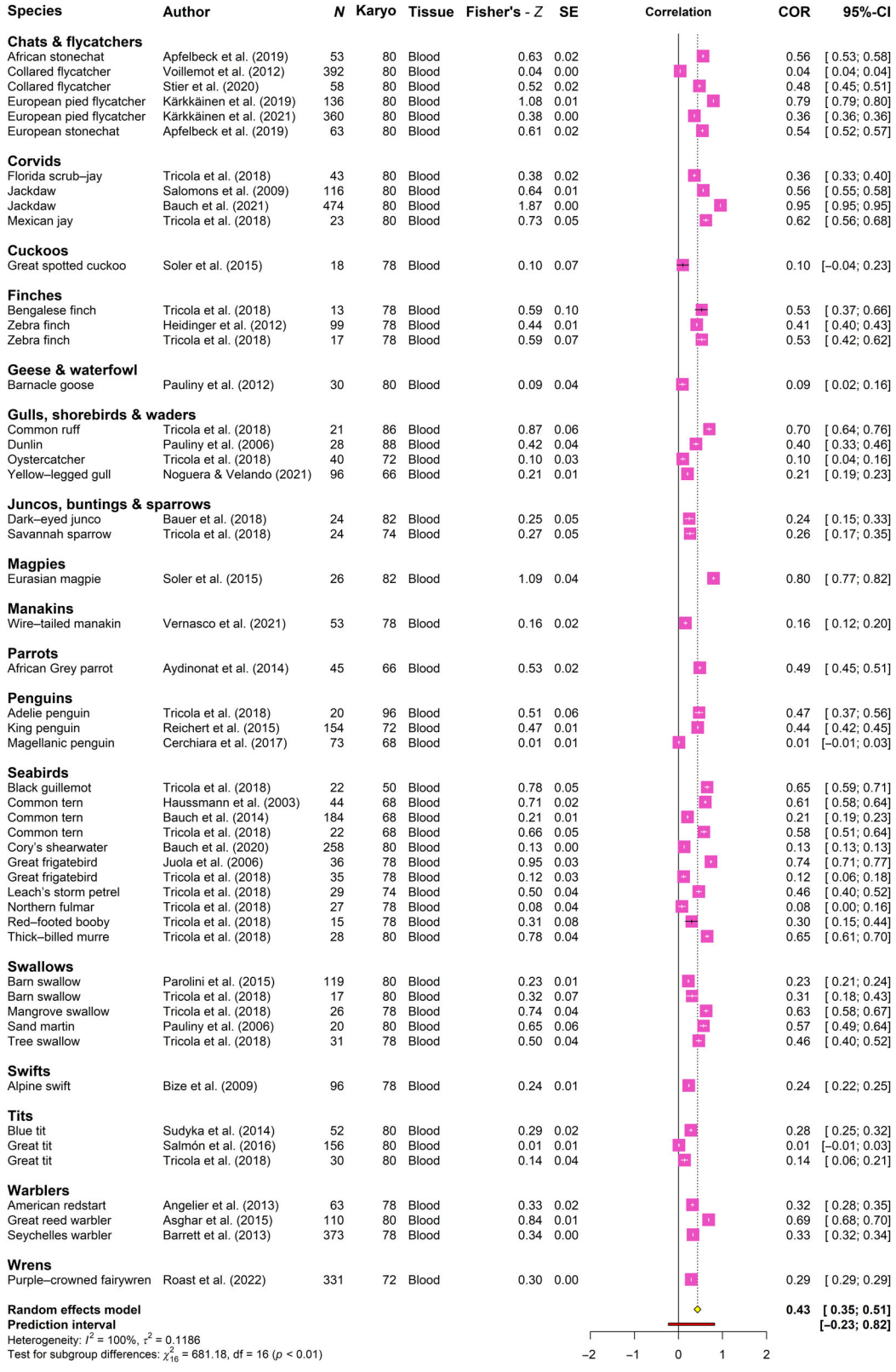

Fig. 11. Forest plot and results for the meta-analysis of studies using telomere length to estimate age in birds. Key study attributes such as sample size (*N*), karyotype (Karyo), and tissue type are indicated. The pooled correlation from the random effects model is indicated (yellow diamond) along with measures of between-study heterogeneity and the prediction interval is indicated with a red bar. CI, confidence interval; COR, correlation; *N*, sample size; SE, standard error.

Table 4. Results from the comparison of meta-analysis results from studies on methylation and telomeres for age determination in animals. A fixed-effect method was used with model ($k = 2$) as a moderator. The overall z-value and P-value indicate that the observed effect sizes and variances between the methylation studies ($\mathcal{N} = 60$) and telomere studies ($\mathcal{N} = 60$) is statistically significant (***$P < 0.01$) with the methylation studies having a substantially higher estimation efficiency than telomeres

Meta-analysis	$\mathcal{N}$	Estimate	Standard error (σ_M)
Methylation	60	0.92	0.29
Telomeres	60	0.31	0.07
***Z*-value**		**21.58*****	
***P*-value**		**<0.01*****	

***$P < 0.01$.

several developmental or embryological studies (Suarez-Bregua *et al.*, 2021). More recently, however, many studies have shifted in focus towards the potential use of methylation to determine age in wildlife. This followed the great successes in human and murine mouse models and the accumulating evidence that methylation is conserved across most vertebrate (Jarman *et al.*, 2015), and even invertebrate (Macchi, Edsinger & Sadler, 2022), species. Thus far, methylation clocks for age determination have been developed or tested in more than 50 species across six classes in less than 10 years, including reptiles and bird species for which other biological clocks were not suitable.

Variable methods have been applied to several different tissue types to establish these models ranging from studies that used only a small, targeted selection of genes or subsets of CpGs to studies that used methylation arrays to assay thousands of CpGs at once to identify suitable targets empirically. Our review and meta-analysis illustrate that methylation is generally a highly accurate method able to predict age within weeks, months or years in many species and does not suffer significantly from variation due to changes in the lifespan of the organism or the tissue type. By contrast, one study did find a lack of age correlates in liver tissue (Sareisian, 2014) which may be related to the higher regenerative capacity of liver cells causing differential ageing in the liver compared to other tissue. Although our meta-analysis identified substantial study heterogeneity, this was mostly confined either to differences in sample size or to differences in the sensitivity of the specific study method used to assay methylation. Sample size requirements have been explored (Mayne *et al.*, 2021*b*) but remain fundamentally tied to effect size and the number of predictors used. In the case of methylation studies, calibrated with highly age-correlated CpGs, the effect sizes are generally large and sample sizes of 40 of above should be well suited for adequate statistical power, as measured by β-values (Fig. S3).

It should be noted that several authors did suggest that the accuracy of prediction is not always linear across the lifespan of organisms and that age estimates of older individuals are typically under- or overestimated. This does not, however, reduce the utility of this method to classify animals into functionally relevant age classes for conservation purposes (Wright *et al.*, 2018). Several other potential confounding factors have also been identified that may need to be taken into consideration in studies analysing methylation for age determination. For example, some evidence suggests that ageing of the epigenome in terms of methylation may be dependent on biological sex (Beal *et al.*, 2019; Meyer *et al.*, 2023), with females ageing slower, and transgenerational epigenetic inheritance in offspring may be linked to maternal age or brood size in some species (De Paoli-Iseppi *et al.*, 2017*a*). Methylation levels were also found to decrease during moulting and periods of fasting and return to normal later, highlighting the importance of taking life histories into account at the time of sampling (Poganik *et al.*, 2023). Other methods of correction such as accounting for leucocyte composition when blood cells are used have also been suggested (Adalsteinsson *et al.*, 2012), however, while variation in leucocyte ratios with age has been illustrated in some primates it is absent in others (Sato *et al.*, 2005), and the heredity or repeatability of such ratio changes is low across different generations for cross-sectional studies (Webb *et al.*, 2020).

Here we found that telomeres have been studied in relation to age in many of the same species as methylation, however, the total number of publications are nearly double the number of methylation studies with approximately half of the published studies focused on bird species. The abundance of studies on birds is likely attributable to the fact that, as jawless terrestrial vertebrates, neither tooth cementum annulation nor otoliths are suitable for age estimation in these species (Chaney *et al.*, 2003). Telomere length showed a moderate effect size in relation to age and the efficacy in relating telomere changes to age was affected by the average lifespan of some species, consistent with previous findings (Dantzer & Fletcher, 2015; Tricola *et al.*, 2018). Some studies have however found that telomere length can be heritable (Reichert *et al.*, 2015) and that both social (Lewin *et al.*, 2015; Cram *et al.*, 2018) and environmental factors can differentially affect telomere shortening rates within populations (Salmón *et al.*, 2016; Dorado-Correa *et al.*, 2018). While similar effects have been observed on methylation, telomere length tends to be permanently altered, likely due to a lack of telomerase activity in most tissue, while methylation levels recover after periods of stress (Poganik *et al.*, 2023) due to DNMT enzymes being persistently expressed. As a result, methylation outperformed telomere length in its ability to change predictably with age.

Both methylation and telomere length show distinct patterns of inheritance that vary across taxa. For example, methylation is known to follow parental imprinting in subsets of genes where different patterns of methylation are inherited from different parents (Andergassen *et al.*, 2021) while global changes can result in offspring that resemble the maternal methylome (Romano *et al.*, 2017; Saino *et al.*, 2019). Telomere length, however, showed strong paternal inheritance in mammals (Eisenberg *et al.*, 2017) but maternal inheritance in birds (Horn *et al.*, 2011; Reichert *et al.*, 2015), possibly

indicating that the parental inheritance of telomere length is driven by the heterogamous sex as most male mammals are XY while most female birds are ZW. Interestingly, paternal inheritance has also been illustrated in sand lizard species that follow a similar ZZ/ZW pattern of sex chromosome heterogamy (Olsson *et al.*, 2011). More studies are needed to elucidate if similar patterns are absent from vertebrate species that either lack sex chromosomes or are completely homogamous, including most amphibians and mammals with unusual sex-determination systems (Saunders & Veyrunes, 2021).

Using attributes of cellular and molecular ageing as biomarkers for age across many taxa is fairly complicated. It requires not only a high level of consistency but also substantial heritability in the trait to ensure comparability in the measurements from a cross-sectional study accessing levels in age groups for different generations: as opposed to longitudinal studies that track changes in specific individuals across their lifetime. Furthermore, it needs to be correlated with all relevant biological and environmental factors that contribute to variability. Biological factors include biological sex, diet, stress, differences in physiology between ectotherms and endotherms, and differences in growth patterns such as the arrested growth in mammals and birds compared to continuous growth in most fish, reptiles, and amphibians. Environmental factors may include the effects of temperature, latitude, environmental stressors, and possible exposure to toxins or contaminants. When controlling for such complex variables, both methylation and telomere length are promising targets for the development of methods to determine age in animals.

VI. CONCLUSIONS

(1) The first biological clocks used to determine age in animals were tooth cementum/dentin annulation and otoliths but are mostly applicable to toothed vertebrates and fish and remain superior tools for age estimation in the *post-mortem* interval.
(2) Methylation is a conserved molecular ageing attribute able to predict age across most classes, orders and infraorders using as little as two CpGs in calibrated clocks.
(3) Telomere shortening is another biological clock with sufficient age prediction capabilities to measure age in animals but is less conserved.
(4) Methylation may be superior to telomere length in terms of accuracy and cross-taxa portability; however, costs may be higher depending on the method.

VII. ACKNOWLEDGEMENTS

Images were created in BioRender.com. Figure 1 was reproduced with permission from the authors (Jarman *et al.*, 2015).

This work is based on research supported wholly/in part by the National Research Foundation of South Africa (Grant Number: 112062).

VIII. DATA AVAILABILITY STATEMENT

Data and code used in this study are available for download from the Zenodo repository at the following link: https://doi.org/10.5281/zenodo.7893443. The custom PYTHON script, ABCal *version* 1.0.1., used to quantitate possible bias by virtue of authorship contributions is freely available on GitHub (https://github.com/LSLeClercq/ABCal).

IX. REFERENCES

References identified with an asterisk (*) are cited only within the supporting information.

Adalsteinsson, B. T., Gudnason, H., Aspelund, T., Harris, T. B., Launer, L. J., Eiriksdottir, G., Smith, A. V. & Gudnason, V. (2012). Heterogeneity in white blood cells has potential to confound DNA methylation measurements. *PLoS One* **7**, e46705.

*Adams, D. M. & Blanchong, J. A. (2020). Precision of cementum annuli method for aging male white-tailed deer. *PLoS One* **15**, e0233421.

Adams, L. & Watkins, S. G. (1967). Annuli in tooth cementum indicate age in California ground squirrels. *The Journal of Wildlife Management* **31**, 836.

Ahnlund, H. (1976). Age determination in the European badger, *Meles meles* L. *Zeitschrift fur Saugetierkunder* **41**, 119–125.

Anastasiadi, D. & Piferrer, F. (2020). A clockwork fish. Age-prediction using DNA methylation-based biomarkers in the European seabass. *Molecular Ecology Resources* **20**, 387–397.

Anchelin, M., Murcia, L., Alcaraz-Pérez, F., García-Navarro, E. M. & Cayuela, M. L. (2011). Behaviour of telomere and telomerase during aging and regeneration in zebrafish. *PLoS One* **6**, e16955.

Andergassen, D., Smith, Z. D., Kretzmer, H., Rinn, J. L. & Meissner, A. (2021). Diverse epigenetic mechanisms maintain parental imprints within the embryonic and extraembryonic lineages. *Developmental Cell* **56**, 2995–3005.e4.

Anderson, J. A., Johnston, R. A., Lea, A. J., Campos, F. A., Voyles, T. N., Akinyi, M. Y., Alberts, S. C., Archie, E. A. & Tung, J. (2021). High social status males experience accelerated epigenetic aging in wild baboons. *eLife* **10**, e66128.

Angelier, F., Vleck, C. M., Holberton, R. L. & Marra, P. P. (2013). Telomere length, non-breeding habitat and return rate in male American redstarts. *Functional Ecology* **27**, 342–350.

*Ansorge, H. & Suchentrunk, F. (2001). Aging steppe polecats (*Mustela eversmanni*) and polecats (*Mustela putorius*) by canine cementum layers and skull characters. *Wissenschaftliche Mitteilungen des Niederösterreichischen Landesmuseums* **14**, 79–106.

Apfelbeck, B., Haussmann, M. F., Boner, W., Flinks, H., Griffiths, K., Illera, J. C., Mortega, K. G., Sisson, Z., Smiddy, P. & Helm, B. (2019). Divergent patterns of telomere shortening in tropical compared to temperate stonechats. *Ecology and Evolution* **9**, 511–521.

Arneson, A., Haghani, A., Thompson, M. J., Pellegrini, M., Kwon, S. B., Vu, H., Maciejewski, E., Yao, M., Li, C. Z., Lu, A. T., Morselli, M., Rubbi, L., Barnes, B., Hansen, K. D., Zhou, W., *et al.* (2022). A mammalian methylation array for profiling methylation levels at conserved sequences. *Nature Communications* **13**, 1–13.

Arthur, S. M., Cross, R., Paragi, T. F. & Krohn, W. B. (1992). Precision and utility of cementum annuli for estimating ages of fishers. *Wildlife Society Bulletin* **20**, 402–405.

Asghar, M., Bensch, S., Tarka, M., Hansson, B. & Hasselquist, D. (2015). Maternal and genetic factors determine early life telomere length. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20142263.

Ashapkin, V. V., Kutueva, L. I. & Vanyushin, B. F. (2020). Quantitative analysis of DNA methylation by bisulfite sequencing. *Methods in Molecular Biology* **2138**, 297–312.

Attwood, J. T., Yung, R. L. & Richardson, B. C. (2002). DNA methylation and the regulation of gene transcription. *Cellular and Molecular Life Sciences* **59**, 241–257.

Aubert, G. & Lansdorp, P. M. (2008). Telomeres and aging. *Physiological Reviews* **88**, 557–579.

Aydinonat, D., Penn, D. J., Smith, S., Moodley, Y., Hoelzl, F., Knauer, F. & Schwarzenberger, F. (2014). Social isolation shortens telomeres in African Grey parrots (*Psittacus erithacus erithacus*). *PLoS One* **9**, e93839.

*Azorit, C., Analla, M., Hervas, J., Carrasco, R. & Muñoz-Cobo, J. (2002). Growth marks observation: preferential techniques and teeth for ageing of Spanish red deer (*Cervus elaphus hispanicus*). *Anatomia, Histologia, Embryologia* **31**, 303–307.

Bae, J., Bertucci, E. M., Bock, S. L., Hale, M. D., Moore, J., Wilkinson, P. M., Rainwater, T. R., Bowden, J. A., Koal, T., PhamTuan, H. & Parrott, B. B. (2021). Intrinsic and extrinsic factors interact during development to influence telomere length in a long-lived reptile. *Molecular Ecology* **31**, 6114–6127.

Baerlocher, G. M., Mak, J., Röth, A., Rice, K. S. & Lansdorp, P. M. (2003). Telomere shortening in leukocyte subpopulations from baboons. *Journal of Leukocyte Biology* **73**, 289–296.

Baerlocher, G. M., Rice, K., Vulto, I. & Lansdorp, P. M. (2007). Longitudinal data on telomere length in leukocytes from newborn baboons support a marked drop in stem cell turnover around 1 year of age. *Aging Cell* **6**, 121–123.

*Baitchman, E. J. & Kollias, G. V. (2000). Clinical anatomy of the North American river otter (*Lontra canadensis*). *Journal of Zoo and Wildlife Medicine* **31**, 473–483.

Balistreri, C. R., Candore, G., Accardi, G., Colonna-Romano, G. & Lio, D. (2013). NF-κB pathway activators as potential ageing biomarkers: targets for new therapeutic strategies. *Immunity and Ageing* **10**, 1–16.

Barrett, E. L. B., Burke, T. A., Hammers, M., Komdeur, J. & Richardson, D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology* **22**, 249–259.

Bauch, C., Becker, P. H. & Verhulst, S. (2014). Within the genome, long telomeres are more informative than short telomeres with respect to fitness components in a long-lived seabird. *Molecular Ecology* **23**, 300–310.

Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E. & Verhulst, S. (2021). High heritability of telomere length and low heritability of telomere shortening in wild birds. *Molecular Ecology* **31**, 6308–6323.

Bauch, C., Gatt, M. C., Granadeiro, J. P., Verhulst, S. & Catry, P. (2020). Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory's shearwaters. *Molecular Ecology* **29**, 1344–1357.

Bauch, C., Gatt, M. C., Verhulst, S., Granadeiro, J. P. & Catry, P. (2022). Higher mercury contamination is associated with shorter telomeres in a long-lived seabird – a direct effect or a consequence of among-individual variation in phenotypic quality? *Science of the Total Environment* **839**, 156359.

Bauer, C. M. C. M., Graham, J. L. J. L., Abolins-Abols, M., Heidinger, B. J. B. J., Ketterson, E. D. E. D. & Greives, T. J. T. J. (2018). Chronological and biological age predict seasonal reproductive timing: an investigation of clutch initiation and telomeres in birds of known age. *The American Naturalist* **191**, 777–782.

Beal, A. P., Kiszka, J. J., Wells, R. S. & Eirin-Lopez, J. M. (2019). The bottlenose dolphin epigenetic aging tool (BEAT): a molecular age estimation tool for small cetaceans. *Frontiers in Marine Science* **6**, 561.

Beirne, C., Delahay, R., Hares, M. & Young, A. (2014). Age-related declines and disease-associated variation in immune cell telomere length in a wild mammal. *PLoS One* **9**, e108964.

Belant, J. L., Etter, D. R., Friedrich, P. D., Cosgrove, M. K., Williams, B. W. & Scribner, K. T. (2011). Comparison of techniques for sex determination of American martens. *Journal of Wildlife Management* **75**, 256–260.

Bell, J. T., Tsai, P. C., Yang, T. P., Pidsley, R., Nisbet, J., Glass, D., Mangino, M., Zhai, G., Zhang, F., Valdes, A., Shin, S. Y., Dempster, E. L., Murray, R. M., Grundberg, E., Hedman, A. K., *et al.* (2012). Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genetics* **8**, e1002629.

Bender, H. S., Murchison, E. P., Pickett, H. A., Deakin, J. E., Strong, M. A., Conlan, C., McMillan, D. A., Neumann, A. A., Greider, C. W., Hannon, G. J., Reddel, R. R. & Graves, J. A. M. (2012). Extreme telomere length dimorphism in the Tasmanian devil and related marsupials suggests parental control of telomere length. *PLoS One* **7**, e46195.

Bird, A. (2007). Perceptions of epigenetics. *Nature* **447**, 396–398.

Bize, P., Criscuolo, F., Metcalfe, N. B., Nasir, L. & Monaghan, P. (2009). Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B: Biological Sciences* **276**, 1679–1683.

Blévin, P., Angelier, F., Tartu, S., Ruault, S., Bustamante, P., Herzke, D., Moe, B., Bech, C., Gabrielsen, G. W., Bustnes, J. O. & Chastel, O. (2016). Exposure to oxychlordane is associated with shorter telomeres in Arctic breeding kittiwakes. *Science of the Total Environment* **563**, 125–130.

Bors, E. K., Baker, C. S., Wade, P. R., O'Neill, K. B., Shelden, K. E. W., Thompson, M. J., Fei, Z., Jarman, S. & Horvath, S. (2021). An epigenetic clock to estimate the age of living beluga whales. *Evolutionary Applications* **14**, 1263–1273.

Bowen, W. D., Sergeant, D. E. & Oritsland, T. (1983). Validation of age estimation in the harp seal, *Phoca groenlandica*, using dentinal annuli. *Canadian Journal of Fisheries and Aquatic Sciences* **40**, 1430–1441.

Bradley, R. J. & Safran, R. J. (2014). Conceptual revision and synthesis of proximate factors associated with age-related improvement in reproduction. *Ethology* **120**, 411–426.

Brandenburg, J. (2016). Persistent effects of early life exposure to polycyclic aromatic hydrocarbons on DNA methylation in birds. Masters dissertation: McGill University, Montreal. https://escholarship.mcgill.ca/concern/theses/xd07gw307.

Brelsford, A., Milá, B. & Irwin, D. E. (2011). Hybrid origin of Audubon's warbler. *Molecular Ecology* **20**, 2380–2389.

Brook, C. E., Ranaivoson, H. C., Andriafidison, D., Ralisata, M., Razafimanahaka, J., Héraud, J. M., Dobson, A. P. & Metcalf, C. J. (2019). Population trends for two Malagasy fruit bats. *Biological Conservation* **234**, 165–171.

Brown, P., Green, C., Sivakumaran, K. P., Stoessel, D. & Giles, A. (2011). Validating otolith annuli for annual age determination of common carp. *Transactions of the American Fisheries Society* **133**, 190–196.

Brümmendorf, T. H., Mak, J., Sabo, K. M., Baerlocher, G. M., Dietz, K., Abkowitz, J. L. & Lansdorp, P. M. (2002). Longitudinal studies of telomere length in feline blood cells: implications for hematopoietic stem cell turnover in vivo. *Experimental Hematology* **30**, 1147–1152.

Buddhachat, K., Kriangwanich, W., Kumoun, S., Brown, J. L., Chailangkarn, S., Somgird, C., Thitaram, C., Prasitwattanaseree, S. & Nganvongpanit, K. (2017). Telomeric attrition with increasing age in short- (Chihuahua dog) and long- (Asian elephant) life span animals. *Kafkas Universitesi Veteriner Fakultesi Dergisi* **23**, 643–649.

Burraco, P., Comas, M., Reguera, S., Zamora-Camacho, F. J. & Moreno-Rueda, G. (2020). Telomere length mirrors age structure along a 2200-m altitudinal gradient in a Mediterranean lizard. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **247**, 110741.

Caccavo, J. A., Raclot, T., Poupart, T., Ropert-Coudert, Y. & Angelier, F. (2021). Anthropogenic activities are associated with shorter telomeres in chicks of Adélie penguin (*Pygoscelis adeliae*). *Polar Biology* **44**, 1391–1399.

Cailliet, G. M. (1990). Elasmobranch age determination and verification: an updated review Elasmobranch age determination and verification: an updated review. In *Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries. National Oceanic and Atmospheric Administration Technical Report* **90**, pp. 157–165. NOAA, Seattle, USA.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**, 197–242.

Cantrell, B., Friedman, S., Lachance, H., Bernier, C., Murdoch, B., Frattini, S., Talenti, A., Crepaldi, P. & McKay, S. (2020). A novel understanding of global DNA methylation in bobcat (*Lynx rufus*). *Genome* **63**, 125–130.

Carroll, E. L., Bruford, M. W., DeWoody, J. A., Leroy, G., Strand, A., Waits, L. & Wang, J. (2018). Genetic and genomic monitoring with minimally invasive sampling methods. *Evolutionary Applications* **11**, 1094–1119.

*Casey, G. A. & Webster, W. A. (1975). Age and sex determination of striped skunks (*Mephitis mephitis*) from Ontario, Manitoba, and Quebec. *Canadian Journal of Zoology* **53**, 223–226.

Cavalli, G. & Heard, E. (2019). Advances in epigenetics link genetics to the environment and disease. *Nature* **571**, 489–499.

Cerchiara, J. A., Risques, R. A., Prunkard, D., Smith, J. R., Kane, O. J. & Boersma, P. D. (2017). Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. *Ecology and Evolution* **7**, 5682–5691.

Cesare, A. J. & Reddel, R. R. (2010). Alternative lengthening of telomeres: models, mechanisms and implications. *Nature Reviews Genetics* **11**, 319–330.

Chaney, R. C., Blemings, K. P., Bonner, J. & Klandorf, H. (2003). Pentosidine as a measure of chronological age in wild birds. *Auk* **120**, 394–399.

Charpentier, M. J. E., Tung, J., Altmann, J. & Alberts, S. C. (2008). Age at maturity in wild baboons: genetic, environmental and demographic influences. *Molecular Ecology* **17**, 2026–2040.

Chédin, F. (2011). The DNMT3 family of mammalian *de novo* DNA methyltransferases. *Progress in Molecular Biology and Translational Science* **101**, 255–285.

Cherdsukjai, P., Buddhachat, K., Brown, J., Kaewkool, M., Poommouang, A., Kaewmong, P., Kittiwattanawong, K. & Nganvongpanit, K. (2020). Age relationships with telomere length, body weight and body length in wild dugong (*Dugong dugon*). *PeerJ* **8**, e10319.

Christensen-Dalsgaard, S. N., Aars, J., Andersen, M., Lockyer, C. & Yoccoz, N. G. (2010). Accuracy and precision in estimation of age of Norwegian Arctic polar bears (*Ursus maritimus*) using dental cementum layers from known-age individuals. *Polar Biology* **33**, 589–597.

Clark, S. T., Odell, D. K. & Lacinak, C. T. (2000). Aspects of growth in captive killer whales (*Orcinus orca*). *Marine Mammal Science* **16**, 110–123.

Cleal, K., Norris, K. & Baird, D. (2018). Telomere length dynamics and the evolution of cancer genome architecture. *International Journal of Molecular Sciences* **19**, 1–17.

Clerc, M., Fenton, A., Babayan, S. A. & Pedersen, A. B. (2019). Parasitic nematodes simultaneously suppress and benefit from coccidian coinfection in their natural mouse host. *Parasitology* **146**, 1096–1106.

Clutton-Brock, T. & Sheldon, B. C. (2010). Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology and Evolution* **25**, 562–573.

Cocci, P., Mosconi, G., Bracchetti, L., Nalocca, J. M., Frapiccini, E., Marini, M., Caprioli, G., Sagratini, G. & Palermo, F. A. (2018). Investigating the potential impact of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) on gene biomarker expression and global DNA methylation in loggerhead sea turtles (*Caretta caretta*) from the Adriatic Sea. *Science of the Total Environment* **619**, 49–57.

Cook, P. E., Hugo, L. E., Iturbe-Ormaetxe, I., Williams, C. R., Chenoweth, S. F., Ritchie, S. A., Ryan, P. A., Kay, B. H., Blows, M. W. & O'Neill, S. L. (2006). The use of transcriptional profiles to predict adult mosquito age under field conditions. *Proceedings of the National Academy of Sciences* **103**, 18060–18065.

Cossette, M.-L., Stewart, D. T., Haghani, A., Zoller, J. A., Shafer, A. B. A. & Horvath, S. (2022). Epigenetics and Island-mainland divergence in an insectivorous small mammal. *Molecular Ecology* **32**, 152–166.

Craighead, J. J., Craighead, F. C. & McCutchen, H. E. (1970). Age determination of grizzly bears from fourth premolar tooth sections. *The Journal of Wildlife Management* **34**, 353.

Cram, D. L., Monaghan, P., Gillespie, R., Dantzer, B., Duncan, C., Spence-Jones, H. & Clutton-Brock, T. (2018). Rank-related contrasts in longevity arise from extra-group excursions not delayed senescence in a cooperative mammal. *Current Biology* **28**, 2934–2939.e4.

Criscuolo, F., Dobson, F. S. & Schull, Q. (2021). The influence of phylogeny and life history on telomere lengths and telomere rate of change among bird species: a meta-analysis. *Ecology and Evolution* **11**, 12908–12922.

Criscuolo, F., Zahn, S. & Bize, P. (2017). Offspring telomere length in the long lived Alpine swift is negatively related to the age of their biological father and foster mother. *Biology Letters* **13**, 20170188.

Crowe, D. M. (1972). The presence of annuli in bobcat tooth cementum layers. *The Journal of Wildlife Management* **36**, 1330.

*Crowe, D. M. & Strickland, M. D. (1975). Dental annulation in the American badger. *Journal of Mammalogy* **56**, 269–272.

Dalton, S. R. & Bellacosa, A. (2012). DNA demethylation by TDG. *Epigenomics* **4**, 459–467.

Dantzer, B. & Fletcher, Q. E. (2015). Telomeres shorten more slowly in slow-aging wild animals than in fast-aging ones. *Experimental Gerontology* **71**, 38–47.

Dantzer, B., van Kesteren, F., Westrick, S. E., Boutin, S., McAdam, A. G., Lane, J. E., Gillespie, R., Majer, A., Haussmann, M. F. & Monaghan, P. (2020). Maternal glucocorticoids promote offspring growth without inducing oxidative stress or shortening telomeres in wild red squirrels. *Journal of Experimental Biology* **223**, jeb212373.

de la Iglesia, G. (2018). Telomere length as a biomarker for stress in eastern bluebird nestlings (*Sialis sialis*) reared in urban habitats. Masters dissertation: Western Carolina University: Cullowhee. http://libres.uncg.edu/ir/wcu/listing.aspx?id=23338.

de Lange, T., Shiue, L., Myers, R. M., Cox, D. R., Naylor, S. L., Killery, A. M. & Varmus, H. E. (1990). Structure and variability of human chromosome ends. *Molecular and Cellular Biology* **10**, 518–527.

De Paoli-Iseppi, R., Deagle, B. E., McMahon, C. R., Hindell, M. A., Dickinson, J. L. & Jarman, S. N. (2017*b*). Measuring animal age with DNA methylation: from humans to wild animals. *Frontiers in Genetics* **8**, 2010–2017.

De Paoli-Iseppi, R., Deagle, B. E., Polanowski, A. M., McMahon, C. R., Dickinson, J. L., Hindell, M. A. & Jarman, S. N. (2019). Age estimation in a long-lived seabird (*Ardenna tenuirostris*) using DNA methylation-based biomarkers. *Molecular Ecology Resources* **19**, 411–425.

De Paoli-Iseppi, R., Polanowski, A. M., McMahon, C., Deagle, B. E., Dickinson, J. L., Hindell, M. A. & Jarman, S. N. (2017*a*). DNA methylation levels in candidate genes associated with chronological age in mammals are not conserved in a long-lived seabird. *PLoS One* **12**, 1–20.

Degrandi, T. M., Barcellos, S. A., Costa, A. L., Garnero, A. D. V., Hass, I. & Gunski, R. J. (2020). Introducing the bird chromosome database: an overview of cytogenetic studies in birds. *Cytogenetic and Genome Research* **160**, 199–205.

Del Re, A. C. (2013). compute.es: Compute Effect Sizes. *R Package*. Electronic file available at https://cran.r-project.org/package=compute.es Accessed 31.10.2022.

Denham, J., Stevenson, K. & Denham, M. M. (2019). Age-associated telomere shortening in thoroughbred horses. *Experimental Gerontology* **127**, 110718.

*Dietz, R., Heide-Jørgensen, M. P., Härkönen, T., Teilmann, J. & Valentin, N. (1991). Age determination of European harbour seal, *Phoca vitulina* L. *Sarsia* **76**, 17–21.

*Divljan, A., Parray-Jones, K. & Wardle, G. M. (2006). Age determination in the grey-headed flying fox. *Journal of Wildlife Management* **70**, 607–611.

Dorado-Correa, A. M., Zollinger, S. A., Heidinger, B. & Brumm, H. (2018). Timing matters: traffic noise accelerates telomere loss rate differently across developmental stages. *Frontiers in Zoology* **15**, 1–8.

Double, M. C., Dawson, D., Burke, T. & Cockburn, A. (1997). Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Molecular Ecology* **6**, 691–693.

Dunshea, G., Duffield, D., Gales, N., Hindell, M., Wells, R. S. & Jarman, S. N. (2011). Telomeres as age markers in vertebrate molecular ecology. *Molecular Ecology Resources* **11**, 225–235.

Dutra, L., Souza, F., Friberg, I., Araújo, M., Vasconcellos, A. & Young, R. (2020). Validating the use of oral swabs for telomere length assessment in dogs. *Journal of Veterinary Behavior* **40**, 16–20.

*Earle, R. D. & Kramm, K. R. (1980). Techniques for age determination in the Canadian porcupine. *The Journal of Wildlife Management* **44**, 413.

Eastwood, J. R., Hall, M. L., Teunissen, N., Kingma, S. A., Hidalgo Aranzamendi, N., Fan, M., Roast, M., Verhulst, S. & Peters, A. (2019). Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology* **28**, 1127–1137.

Eisenberg, D. T. A., Tackney, J., Cawthon, R. M., Cloutier, C. T. & Hawkes, K. (2017). Paternal and grandpaternal ages at conception and descendant telomere lengths in chimpanzees and humans. *American Journal of Physical Anthropology* **162**, 201–207.

Erdem, E., Özbaşer, F. T., Gürcan, E. K. & Soysal, M. I. (2021). The morphological and morphometric characteristics of Alabadem pigeons. *Turkish Journal of Veterinary and Animal Sciences* **45**, 372–379.

Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L. & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell* **15**, 140–148.

Faul, F., Erdfelder, E., Buchner, A. & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* **41**, 1149–1160.

Fick, L. J., Fick, G. H., Li, Z., Cao, E., Bao, B., Heffelfinger, D., Parker, H. G., Ostrander, E. A. & Riabowol, K. (2012). Telomere length correlates with life span of dog breeds. *Cell Reports* **2**, 1530–1536.

Fitz-James, M. H. & Cavalli, G. (2022). Molecular mechanisms of transgenerational epigenetic inheritance. *Nature Reviews Genetics* **23**, 325–341.

Fohringer, C., Hoelzl, F., Allen, A. M., Cayol, C., Ericsson, G., Spong, G., Smith, S. & Singh, N. J. (2022). Large mammal telomere length variation across ecoregions. *BMC Ecology and Evolution* **22**, 1–10.

Foley, N. M., Hughes, G. M., Huang, Z., Clarke, M., Jebb, D., Whelan, C. V., Petit, E. J., Touzalin, F., Farcy, O., Jones, G., Ransome, R. D., Kacprzyk, J., O'Connell, M. J., Kerth, G., Rebelo, H., *et al.* (2018). Growing old, yet staying young: the role of telomeres in bats' exceptional longevity. *Science Advances* **4**, eaao0926.

Foley, N. M., Petit, E. J., Brazier, T., Finarelli, J. A., Hughes, G. M., Touzalin, F., Puechmaille, S. J. & Teeling, E. C. (2020). Drivers of longitudinal telomere dynamics in a long-lived bat species, *Myotis myotis*. *Molecular Ecology* **29**, 2963–2977.

Friesen, C. R., Wapstra, E. & Olsson, M. (2021). Of telomeres and temperature: measuring thermal effects on telomeres in ectothermic animals. *Molecular Ecology* **31**, 6069–6086.

Froy, H., Bird, E. J., Wilbourn, R. V., Fairlie, J., Underwood, S. L., Salvo-Chirnside, E., Pilkington, J. G., Bérénos, C., Pemberton, J. M. & Nussey, D. H. (2017). No evidence for parental age effects on offspring leukocyte telomere length in free-living Soay sheep. *Scientific Reports* **7**, 9991.

Froy, H., Underwood, S. L., Dorrens, J., Seeker, L. A., Watt, K., Wilbourn, R. V., Pilkington, J. G., Harrington, L., Pemberton, J. M. & Nussey, D. H. (2021). Heritable variation in telomere length predicts mortality in Soay sheep. *Proceedings of the National Academy of Sciences of the United States of America* **118**, e2020563118.

Garde, E., Frie, A. K., Dunshea, G., Hansen, S. H., Kovacs, K. M. & Lydersen, C. (2010). Harp seal ageing techniques—teeth, aspartic acid racemization, and telomere sequence analysis. *Journal of Mammalogy* **91**, 1365–1374.

Gomes, N. M. V., Ryder, O. A., Houck, M. L., Charter, S. J., Walker, W., Forsyth, N. R., Austad, S. N., Venditti, C., Pagel, M., Shay, J. W. & Wright, W. E. (2011). Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination. *Aging Cell* **10**, 761–768.

Gomez, A., Karlgren, M., Edler, D., Bernal, M. L., Mkrtchian, S. & Ingelman-Sundberg, M. (2007). Expression of CYP2W1 in colon tumors: regulation by gene methylation. *Pharmacogenomics* **8**, 1315–1325.

Gopalan, S., Carja, O., Fagny, M., Patin, E., Myrick, J. W., McEwen, L. M., Mah, S. M., Kobor, M. S., Froment, A., Feldman, M. W., Quintana-Murci, L. & Henn, B. M. (2017). Trends in DNA methylation with age replicate across diverse human populations. *Genetics* **206**, 1659–1674.

Goren, A. D., Brodie, P. F., Spotte, S., Ray, G. C., Kaufman, H. W., Gwinnett, A. J., Sciubba, J. J. & Buck, J. D. (1987). Growth layer groups (GLGs) in the teeth of an adult beluga whale (*Delphinaptherus leucas*) of known age: evidence for two annual layers. *Marine Mammal Science* **3**, 14–21.

*Gosselink, T. E., van Deelen, T. R., Warner, R. E. & Joselyn, M. G. (2003). Temporal habitat partitioning and spatial use of coyotes and red foxes in east-Central Illinois. *The Journal of Wildlife Management* **67**, 90.
*Gourichon, L. & Parmigiani, V. (2016). Preliminary analysis of dental cementum of *Lama guanicoe* for the estimation of age and season at death: studies of modern specimens and further archaeological applications. *Journal of Archaeological Science: Reports* **6**, 856–861.
Graham, J. L. (2018). Reproductive timing in a changing world: understanding mechanisms and costs associated with reproductive timing decisions. Doctoral Thesis: North Dakota State University of Agriculture and Applied Science Fargo. https://hdl.handle.net/10365/27478.
*Grau, G. A., Sanderson, G. C. & Rogers, J. P. (1970). Age determination of raccoons. *The Journal of Wildlife Management* **34**, 364.
Gregory, T. R. (2023). Animal genome size database. Electronic file available at http://www.genomesize.com Accessed 8.5.2023.
Greider, C. W. & Blackburn, E. H. (1985). Identification of a specific telomere terminal transferase activity in tetrahymena extracts. *Cell* **43**, 405–413.
Grimsdell, J. J. R. (1973). Age determination of the African buffalo, *Syncerus caffer* Sparrman. *African Journal of Ecology* **11**, 31–53.
Grue, H. & Jensen, B. (1979). Review of the formation of incremental lines in tooth cementum of terrestrial mammals. *Danish Review of Game Biology* **11**, 1–48.
Grunst, M. L., Grunst, A. S., Pinxten, R. & Eens, M. (2020). Anthropogenic noise is associated with telomere length and carotenoid-based coloration in free-living nestling songbirds. *Environmental Pollution* **260**, 114032.
Guevara, E. E., Lawler, R. R., Staes, N., White, C. M., Sherwood, C. C., Ely, J. J., Hopkins, W. D. & Bradley, B. J. (2020). Age-associated epigenetic change in chimpanzees and humans: chimpanzee epigenetic age. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**, 20190616.
Guo, F., Li, X., Liang, D., Li, T., Zhu, P., Guo, H., Wu, X., Wen, L., Gu, T. P., Hu, B., Walsh, C. P., Li, J., Tang, F. & Xu, G. L. (2014). Active and passive demethylation of male and female pronuclear DNA in the mammalian zygote. *Cell Stem Cell* **15**, 447–459.
Haddaway, N. R., Page, M. J., Pritchard, C. C. & McGuinness, L. A. (2022). PRISMA2020: an R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and open synthesis. *Campbell Systematic Reviews* **18**, e1230.
Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S. V., Klotzle, B., Bibikova, M., Fan, J. B., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., *et al.* (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Molecular Cell* **49**, 359–367.
Hansen, E., Skotnes, T., Bustnes, J. O., Helander, B., Eulaers, I., Sun, J., Covaci, A., Bårdsen, B. J., Zahn, S., Criscuolo, F. & Bourgeon, S. (2022). Telomere length in relation to persistent organic pollutant exposure in white-tailed eagle (*Haliaeetus albicilla*) nestlings from Sweden sampled in 1995–2013. *Environmental Research* **208**, 112712.
Hartmann, N., Reichwald, K., Lechel, A., Graf, M., Kirschner, J., Dorn, A., Terzibasi, E., Wellner, J., Platzer, M., Rudolph, K. L., Cellerino, A. & Englert, C. (2009). Telomeres shorten while Tert expression increases during ageing of the short-lived fish *Nothobranchius furzeri*. *Mechanisms of Ageing and Development* **130**, 290–296.
Hatase, H., Sudo, R., Watanabe, K. K., Kasugai, T., Saito, T., Okamoto, H., Uchida, I. & Tsukamoto, K. (2008). Shorter telomere length with age in the loggerhead turtle: a new hope for live sea turtle age estimation. *Genes and Genetic Systems* **83**, 423–426.
Haussmann, M. F. & Vleck, C. M. (2002). Telomere length provides a new technique for aging animals. *Oecologia* **130**, 325–328.
Haussmann, M. F., Vleck, C. M. & Nisbet, I. C. T. (2003). Calibrating the telomere clock in common terns, *Sterna hirundo*. *Experimental Gerontology* **38**, 787–789.
Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B. & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 1743–1748.
Higgs, D. M., Souza, M. J., Wilkins, H. R., Presson, J. C. & Popper, A. N. (2002). Age- and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). *Journal of the Association for Research in Otolaryngology* **3**, 174–184.
Hiller, T. L. & Tyre, A. J. (2014). Comparison of two age-estimation techniques for cougars. *Northwestern Naturalist* **95**, 77–82.
Hoelzl, F., Cornils, J. S., Smith, S., Moodley, Y. & Ruf, T. (2016*a*). Telomere dynamics in free-living edible dormice (*Glis glis*): the impact of hibernation and food supply. *Journal of Experimental Biology* **219**, 2469–2474.
Hoelzl, F., Smith, S., Cornils, J. S., Aydinonat, D., Bieber, C. & Ruf, T. (2016*b*). Telomeres are elongated in older individuals in a hibernating rodent, the edible dormouse (*Glis glis*). *Scientific Reports* **6**, 36856.
Hohn, A. A. (1980). Age determination and age related factors in the teeth of Western North Atlantic bottlenose dolphins. *Scientific Reports of the Whales Research Institute* **32**, 39–66.
Høie, H., Andersson, C., Folkvord, A. & Karlsen, Ø. (2004). Precision and accuracy of stable isotope signals in otoliths of pen-reared cod (*Gadus morhua*) when sampled will a high-resolution micromill. *Marine Biology* **144**, 1039–1049.
Hong, S. R., Shin, K. J., Jung, S. E., Lee, E. H. & Lee, H. Y. (2019). Platform-independent models for age prediction using DNA methylation data. *Forensic Science International: Genetics* **38**, 39–47.
Horn, T., Gemmell, N. J., Robertson, B. C. & Bridges, C. R. (2008). Telomere length change in European sea bass (*Dicentrarchus labrax*). *Australian Journal of Zoology* **56**, 207–210.
Horn, T., Robertson, B. C. & Gemmell, N. J. (2010). The use of telomere length in ecology and evolutionary biology. *Heredity* **105**, 497–506.
Horn, T., Robertson, B. C., Will, M., Eason, D. K., Elliott, G. P. & Gemmell, N. J. (2011). Inheritance of telomere length in a bird. *PLoS One* **6**, e17199.
Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biology* **14**, 3156.
Horvath, S., Haghani, A., Macoretta, N., Ablaeva, J., Zoller, J. A., Li, C. Z., Zhang, J., Takasugi, M., Zhao, Y., Rydkina, E., Zhang, Z., Emmrich, S., Raj, K., Seluanov, A., Faulkes, C. G., *et al.* (2022*a*). DNA methylation clocks tick in naked mole rats but queens age more slowly than nonbreeders. *Nature Aging* **2**, 46–59.
Horvath, S., Haghani, A., Peng, S., Hales, E. N., Zoller, J. A., Raj, K., Larison, B., Robeck, T. R., Petersen, J. L., Bellone, R. R. & Finno, C. J. (2022*b*). DNA methylation aging and transcriptomic studies in horses. *Nature Communications* **13**, 1–13.
Horvath, S., Haghani, A., Zoller, J. A., Fei, Z., Bérubé, M. & Robbins, J. (2022*c*). DNA methylation age studies of humpback whales. *bioRxiv* 2022.08.15.503952. https://doi.org/10.1101/2022.08.15.503952.
Horvath, S., Haghani, A., Zoller, J. A., Raj, K., Sinha, I., Robeck, T. R., Black, P., Couzens, A., Lau, C., Manoyan, M., Ruiz, Y. A., Talbott, A., Belov, K., Hogg, C. J. & Sears, K. E. (2022*d*). Epigenetic clock and methylation studies in marsupials: opossums, Tasmanian devils, kangaroos, and wallabies. *GeroScience* **44**, 1825–1845.
Horvath, S., Lu, A. T., Haghani, A., Zoller, J. A., Li, C. Z., Lim, A. R., Brooke, R. T., Raj, K., Serres-Armero, A., Dreger, D. L., Hogan, A. N., Plassais, J. & Ostrander, E. A. (2022*e*). DNA methylation clocks for dogs and humans. *Proceedings of the National Academy of Sciences of the United States of America* **119**, e2120887119.
Horvath, S. & Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews Genetics* **19**, 371–384.
Horvath, S., Zoller, J. A., Haghani, A., Jasinska, A. J., Raj, K., Breeze, C. E., Ernst, J., Vaughan, K. L. & Mattison, J. A. (2021*a*). Epigenetic clock and methylation studies in the rhesus macaque. *GeroScience* **43**, 2441–2453.
Horvath, S., Zoller, J. A., Haghani, A., Lu, A. T., Raj, K., Jasinska, A. J., Mattison, J. A. & Salmon, A. B. (2021*b*). DNA methylation age analysis of rapamycin in common marmosets. *GeroScience* **43**, 2413–2425.
Ibáñez-Álamo, J. D., Pineda-Pampliega, J., Thomson, R. L., Aguirre, J. I., Díez-Fernández, A., Faivre, B., Figuerola, J. & Verhulst, S. (2018). Urban blackbirds have shorter telomeres. *Biology Letters* **14**, 20180083.
Ineson, K. M., O'Shea, T. J., Kilpatrick, C. W., Parise, K. L. & Foster, J. T. (2020). Ambiguities in using telomere length for age determination in two North American bat species. *Journal of Mammalogy* **101**, 958–969.
Isermann, D. A., Wolter, M. H. & Breeggemann, J. J. (2010). Estimating black crappie age: an assessment of dorsal spines and scales as nonlethal alternatives to otoliths. *North American Journal of Fisheries Management* **30**, 1591–1598.
Ito, G., Yoshimura, K. & Momoi, Y. (2017). Analysis of DNA methylation of potential age-related methylation sites in canine peripheral blood leukocytes. *Journal of Veterinary Medical Science* **79**, 745–750.
Ito, H., Udono, T., Hirata, S. & Inoue-Murayama, M. (2018). Estimation of chimpanzee age based on DNA methylation. *Scientific Reports* **8**, 1–5.
Izzo, C. (2010). Patterns of telomere length change with age in aquatic vertebrates and the phylogenetic distribution of the pattern among jawed vertebrates. Doctoral Thesis: University of Adelaide, Adelaide. https://hdl.handle.net/2440/63477.
Izzo, C., Bertozzi, T., Gillanders, B. M. & Donnellan, S. C. (2014). Variation in telomere length of the common carp, *Cyprinus carpio* (Cyprinidae), in relation to body length. *Copeia* **2014**, 87–94.
Izzo, C., Hamer, D. J., Bertozzi, T., Donnellan, S. C. & Gillanders, B. M. (2011). Telomere length and age in pinnipeds: the endangered Australian sea lion as a case study. *Marine Mammal Science* **27**, 841–851.
Jarman, S. N., Polanowski, A. M., Faux, C. E., Robbins, J., De Paoli-Iseppi, R., Bravington, M. & Deagle, B. E. (2015). Molecular biomarkers for chronological age in animal ecology. *Molecular Ecology* **24**, 4826–4847.
Jasinska, A. J., Haghani, A., Zoller, J. A., Li, C. Z., Arneson, A., Ernst, J., Kavanagh, K., Jorgensen, M. J., Mattison, J. A., Wojta, K., Choi, O. W., DeYoung, J., Li, X., Rao, A. W., Coppola, G., *et al.* (2022). Epigenetic clock and methylation studies in vervet monkeys. *GeroScience* **44**, 699–717.

Johansson, Å., Enroth, S. & Gyllensten, U. (2013). Continuous aging of the human DNA methylome throughout the human lifespan. *PLoS One* **8**, 67378.
Jones, M. J., Goodman, S. J. & Kobor, M. S. (2015). DNA methylation and healthy human aging. *Aging Cell* **14**, 924–932.
Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., Dahlgren, J. P., Ehrlén, J., García, M. B., Menges, E. S., Quintana-Ascencio, P. F., Caswell, H., Baudisch, A. & Vaupel, J. W. (2014). Diversity of ageing across the tree of life. *Nature* **505**, 169–173.
Juola, F. A., Haussmann, M. F., Dearborn, D. C. & Vleck, C. M. (2006). Telomere shortening in a long-lived marine bird: cross-sectional analysis and test of an aging tool. *The Auk* **123**, 775–783.
Kagiwada, S., Kurimoto, K., Hirota, T., Yamaji, M. & Saitou, M. (2013). Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. *EMBO Journal* **32**, 340–353.
*Kamler, J. F. & Macdonald, D. W. (2006). Longevity of a wild bat-eared fox. *African Journal of Wildlife Research* **36**, 199–200.
Kang, H. (2021). Sample size determination and power analysis using the G*power software. *Journal of Educational Evaluation for Health Professions* **18**, 1–12.
Karere, G. M., Mahaney, M. C., Newman, D. E., Riojas, A. M., Christensen, C., Birnbaum, S., VandeBerg, J. L. & Cox, L. (2019). Diet-induced leukocyte telomere shortening in a baboon model for early stage atherosclerosis. *Scientific Reports* **9**, 1–9.
Kärkkäinen, T., Laaksonen, T., Burgess, M., Cantarero, A., Martínez-Padilla, J., Potti, J., Moreno, J., Thomson, R. L., Tilgar, V. & Stier, A. (2021). Population differences in the length and early-life dynamics of telomeres among European pied flycatchers. *Molecular Ecology* **31**, 5966–5978.
Kärkkäinen, T., Teerikorpi, P., Panda, B., Helle, S., Stier, A. & Laaksonen, T. (2019). Impact of continuous predator threat on telomere dynamics in parent and nestling pied flycatchers. *Oecologia* **191**, 757–766.
Kasuya, T. & Matsui, S. (1984). Age determination and growth of the short-finned pilot whale off the Pacific coast of Japan. *Scientific Reports of the Whales Research Institute Tokyo* **35**, 57–92.
Kazda, A., Zellinger, B., Rössler, M., Derboven, E., Kusenda, B. & Riha, K. (2012). Chromosome end protection by blunt-ended telomeres. *Genes and Development* **26**, 1703–1713.
*Kenyon, K. W. & Fiscus, C. H. (1963). Age determination in the Hawaiian monk seal. *Journal of Mammalogy* **44**, 280.
Kerepesi, C., Meer, M. V., Ablaeva, J., Amoroso, V. G., Lee, S. G., Zhang, B., Gerashchenko, M. V., Trapp, A., Yim, S. H., Lu, A. T., Levine, M. E., Seluanov, A., Horvath, S., Park, T. J., Gorbunova, V., *et al.* (2022). Epigenetic aging of the demographically non-aging naked mole-rat. *Nature Communications* **13**, 1–10.
Kim, K. S. & Sappington, T. W. (2013). Microsatellite data analysis for population genetics. In *Microsatellites: Methods in Molecular Biology* (Volume **1006**, ed. S. Kantartzi), pp. 271–295. Humana Press, Totowa.
Kimura, M. (1980). Variability in techniques of counting dentinal growth layer groups in a tooth of a known-age dolphin, Tursiops truncatus. *Reports of the International Whaling Commission, Special Issue* **3**, 161–163.
*Kingsmill, E. (1962). An investigation of criteria for estimating age in the marsupials *Trichosurus vulpecula* kerr and *Perameles nasuta* geoffroy. *Australian Journal of Zoology* **10**, 597–616.
Kipling, D. & Cooke, H. J. (1990). Hypervariable ultra-long telomeres in mice. *Nature* **347**, 400–402.
Kirby, R., Alldredge, M. W. & Pauli, J. N. (2017). Environmental, not individual, factors drive markers of biological aging in black bears. *Evolutionary Ecology* **31**, 571–584.
*Klevezal, G. A. & Pucek, Z. (1987). Bisoniana XCIII. Growth layers in tooth cement and dentine of European bison and its hybrids with domestic cattle. *Acta Theriologica* **32**, 115–128.
*Klevezal, G. A. & Stewart, B. S. (1994). Patterns and calibration of layering in tooth cementum of female northern elephant seals, *Mirounga angustirostris*. *Journal of Mammalogy* **75**, 483–487.
Kordowitzki, P., Haghani, A., Zoller, J. A., Li, C. Z., Raj, K., Spangler, M. L. & Horvath, S. (2021). Epigenetic clock and methylation study of oocytes from a bovine model of reproductive aging. *Aging Cell* **20**, e13349.
Kujoth, C. C., Hiona, A., Pugh, T. D., Someya, S., Panzer, K., Wohlgemuth, S. E., Hofer, T., Seo, A. Y., Sullivan, R., Jobling, W. A., Morrow, J. D., Van Remmen, H., Sedivy, J. M., Yamasoba, T., Tanokura, M., *et al.* (2005). Medicine: mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* **309**, 481–484.
Kurdyukov, S. & Bullock, M. (2016). DNA methylation analysis: choosing the right method. *Biology* **5**, 3.
Lachance, H., Cantrell, B., Friedman, S., Mckay, S. & Yee, R. (2015). Genome-wide epigenetic survey of Vermont bobcat (*Lynx rufus*) and fisher (*Martes pennanti*). In *74th Annual Conference of the Northeast Association of Fish and Wildlife Agencies*. Northeast Association of Fish and Wildlife Agencies, Vermont.
Larison, B., Pinho, G. M., Hagani, A., Zoller, J. A., Li, C. Z., Finno, C. J., Farrell, C., Kaelin, C. B., Barsh, G. S., Wooding, B., Robeck, T. R., Maddox, D., Pellegrini, M. & Horvath, S. (2021*b*). Epigenetic models predict age and aging in plains zebras and other equids. *bioRxiv* 2021.03.29.437607. https://doi.org/10.1101/2021.03.29.437607.
Larison, B., Pinho, G. M., Haghani, A., Zoller, J. A., Li, C. Z., Finno, C. J., Farrell, C., Kaelin, C. B., Barsh, G. S., Wooding, B., Robeck, T. R., Maddox, D., Pellegrini, M. & Horvath, S. (2021*a*). Epigenetic models developed for plains zebras predict age in domestic horses and endangered equids. *Communications Biology* **4**, 1–9.
*Larter, N. C. & Allaire, D. G. (2016). Longevity and mortality of boreal woodland caribou (*Rangifer tarandus caribou*) of the dehcho region, northwest territories. *Canadian Field-Naturalist* **130**, 222–223.
*Laws, R. M. (1952). A new method of age determination for mammals. *Nature* **169**, 972–973.
*Laws, R. M. (1958). Growth rates and ages of crabeater seals, *Lobodon carcinophagus*. *Proceedings of the Zoological Society of London* **130**, 275–288.
Le Clercq, L. S., Dalton, D. L. & Kotzé, A. (2018). Molecular age estimation based on promotor CpG methylation using methylation sensitive PCR. In *Poster Presented at: 9th Annual Research Symposium of the National Zoological Gardens of South Africa*. SANBI, Pretoria. https://doi.org/10.13140/RG.2.2.11352.21768.
Lee, W. W., Nam, K. H., Terao, K. & Yoshikawa, Y. (2002). Age-related telomere length dynamics in peripheral blood mononuclear cells of healthy cynomolgus monkeys measured by flow FISH. *Immunology* **105**, 458–465.
Lemaître, J. F., Rey, B., Gaillard, J. M., Régis, C., Gilot-Fromont, E., Débias, F., Duhayer, J., Pardonnet, S., Pellerin, M., Haghani, A., Zoller, J. A., Li, C. Z. & Horvath, S. (2022). DNA methylation as a tool to explore ageing in wild roe deer populations. *Molecular Ecology Resources* **22**, 1002–1015.
Leonida, S. R. L., Bennett, N. C., Leitch, A. R. & Faulkes, C. G. (2020). Patterns of telomere length with age in African mole-rats: new insights from quantitative fluorescence *in situ* hybridisation (qFISH). *PeerJ* **8**, e10498.
Lewin, N., Treidel, L. A., Holekamp, K. E., Place, N. J. & Haussmann, M. F. (2015). Socioecological variables predict telomere length in wild spotted hyenas. *Biology Letters* **11**, 20140991.
Liebl, A. L., Wesner, J. S., Russell, A. F. & Schrey, A. W. (2021). Methylation patterns at fledging predict delayed dispersal in a cooperatively breeding bird. *PLoS One* **16**, e0252227.
Linck, E., Freeman, B. G. & Dumbacher, J. P. (2020). Speciation and gene flow across an elevational gradient in New Guinea kingfishers. *Journal of Evolutionary Biology* **33**, 1643–1652.
Lind, M. I. & Spagopoulou, F. (2018). Evolutionary consequences of epigenetic inheritance. *Heredity* **121**, 205–209.
Linhart, S. B. (1973). Age determination and occurrence of incremental growth lines in the dental cementum of the common vampire bat (*Desmodus rotundus*). *Journal of Mammalogy* **54**, 493–496.
Little, T. J., O'Toole, A. N., Rambaut, A., Chandra, T., Marioni, R. & Pedersen, A. B. (2020). Methylation-based age estimation in a wild mouse. *bioRxiv* 2020.07.16.203687. https://doi.org/10.1101/2020.07.16.203687.
Liu, L., Bailey, S. M., Okuka, M., Muñoz, P., Li, C., Zhou, L., Wu, C., Czerwiec, E., Sandler, L., Seyfang, A., Blasco, M. A. & Keefe, D. L. (2007). Telomere lengthening early in development. *Nature Cell Biology* **9**, 1436–1441.
Lockyer, C. H. (1993). A report on patterns of deposition of dentine and cement in teeth of pilot whales, genus *Globicephala*. In *Reports of the International Whaling Commission, Special Issue* (Volume **14**), pp. 137–161. IWC, Impington, UK.
Lockyer, C. H., Hohn, A. A., Doidge, W. D., Heide-Jørgensen, M. P. & Suydam, R. (2007). Age determination in belugas (*Delphinapterus leucas*): a quest for validation of dentinal layering. *Aquatic Mammals* **33**, 293–304.
*Lombaard, L. J. (1971). Age determination and growth curved in the black-backed jackal *Canis mesomelas* Schreber, 1775 (Carnivora: Canidae). *Annals of the Transvaal Museum* **27**, 135–169.
*Low, W. A. (1970). The influence of aridity on reproduction of the collared peccary (*Picotyles tajacu* (Linn)) in Texas. Doctoral Thesis: University of British Columbia, Vancouver. http://hdl.handle.net/2429/34781.
*Low, W. A. & Cowan, I. M. (1963). Age determination of deer by annular structure of dental cementum. *The Journal of Wildlife Management* **27**, 466.
Lowe, R., Barton, C., Jenkins, C. A. C. A., Ernst, C., Forman, O., Fernandez-Twinn, D. S. D. S., Bock, C., Rossiter, S. J. S. J., Faulkes, C. G. C. G., Ozanne, S. E. S. E., Walter, L., Odom, D. T., Mellersh, C. & Rakyan, V. K. V. K. (2018). Ageing-associated DNA methylation dynamics are a molecular readout of lifespan variation among mammalian species. *Genome Biology* **19**, 1–8.
Lowe, R., Danson, A. F., Rakyan, V. K., Yildizoglu, S., Saldmann, F., Viltard, M., Friedlander, G. & Faulkes, C. G. (2020). DNA methylation clocks as a predictor for ageing and age estimation in naked mole-rats, *Heterocephalus glaber*. *Aging* **12**, 4394–4406.

Lund, T. C., Glass, T. J., Tolar, J. & Blazar, B. R. (2009). Expression of telomerase and telomere length are unaffected by either age or limb regeneration in *Danio rerio*. *PLoS One* **4**, e7688.
Lundsgaard, N. U., Cramp, R. L. & Franklin, C. E. (2022). Early exposure to UV radiation causes telomere shortening and poorer condition later in life. *Journal of Experimental Biology* **225**, jeb243924.
Macchi, F., Edsinger, E. & Sadler, K. C. (2022). Epigenetic machinery is functionally conserved in cephalopods. *BMC Biology* **20**, 1–23.
Mäkinen, H., Vitaniemi, H. M., Visser, M. E., Verhagen, I., van Oers, K. & Husby, A. (2019). Temporally replicated DNA methylation patterns in great tit using reduced representation bisulfite sequencing. *Scientific Data* **6**, 1–7.
Marker, L. L. & Dickman, A. J. (2003). Morphology, physical condition, and growth of the cheetah (*Acinonyx jubatus jubatus*). *Journal of Mammalogy* **84**, 840–850.
Marker, L. L., Dickman, A. J., Jeo, R. M., Mills, M. G. L. & Macdonald, D. W. (2003). Demography of the Namibian cheetah, *Acinonyx jubatus jubatus*. *Biological Conservation* **114**, 413–425.
Marks, S. A. & Erickson, A. W. (1966). Age determination in the black bear. *The Journal of Wildlife Management* **30**, 389–410.
Marsh, H., Heinsohn, G. E. & Marsh, L. M. (1984). Breeding cycle, life history and population dynamics of the dugong, *Dugon dugon*. *Australian Journal of Zoology* **32**, 767–788.
Mayne, B., Berry, O. & Jarman, S. (2021*b*). Optimal sample size for calibrating DNA methylation age estimators. *Molecular Ecology Resources* **21**, 2316–2323.
Mayne, B., Espinoza, T., Roberts, D., Butler, G. L., Brooks, S., Korbie, D. & Jarman, S. (2021*a*). Nonlethal age estimation of three threatened fish species using DNA methylation: Australian lungfish, Murray cod and Mary River cod. *Molecular Ecology Resources* **21**, 2324–2332.
Mayne, B., Korbie, D., Kenchington, L., Ezzy, B., Berry, O. & Jarman, S. (2020*a*). A DNA methylation age predictor for zebrafish. *Aging* **12**, 24817–24835.
Mayne, B., Mustin, W., Baboolal, V., Casella, F., Ballorain, K., Barret, M., Vanderklift, M. A., Tucker, A. D., Korbie, D., Jarman, S. & Berry, O. (2022). Age prediction of green turtles with an epigenetic clock. *Molecular Ecology Resources* **22**, 2275–2284.
Mayne, B., Tucker, A. D., Berry, O. & Jarman, S. (2020*b*). Lifespan estimation in marine turtles using genomic promoter CpG density. *PLoS One* **15**, e0236888.
*Mbizah, M. M., Steenkamp, G. & Groom, R. J. (2016). Evaluation of the applicability of different age determination methods for estimating age of the endangered African wild dog (*Lycaon pictus*). *PLoS One* **11**, e0164676.
McCarthy, T. M., Fuller, T. K. & Munkhtsog, B. (2005). Movements and activities of snow leopards in southwestern Mongolia. *Biological Conservation* **124**, 527–537.
McClintock, B. (1941). The stability of broken ends of chromosomes in *Zea mays*. *Genetics* **26**, 234–282.
*McCutchen, H. E. (1969). Age determination of pronghorns by the incisor cementum. *The Journal of Wildlife Management* **33**, 172.
McEwen, L. M., Goodman, S. J., Kobor, M. S. & Jones, M. J. (2017). The DNA methylome: an interface between the environment, immunity, and ageing. In: *The Ageing Immune System and Health* pp. 35–52. Springer, Cham.
McKevitt, T. P., Nasir, L., Devlin, P. & Argyle, D. J. (2002). Telomere lengths in dogs decrease with increasing donor age. *Journal of Nutrition* **132**, 1604S–1606S.
McKevitt, T. P., Nasir, L., Wallis, C. V. & Argyle, D. J. (2003). A cohort study of telomere and telomerase biology in cats. *American Journal of Veterinary Research* **64**, 1496–1499.
*Mclaren, I. (1958). The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian Arctic. *Bulletin of the Fisheries Research Board of Canada* **118**, 1–97.
Meillère, A., Brischoux, F., Ribout, C. & Angelier, F. (2015). Traffic noise exposure affects telomere length in nestling house sparrows. *Biology Letters* **11**, 20150559.
Mensà, E., Latini, S., Ramini, D., Storci, G., Bonafè, M. & Olivieri, F. (2019). The telomere world and aging: analytical challenges and future perspectives. *Ageing Research Reviews* **50**, 27–42.
Merlin, C. & Liedvogel, M. (2019). The genetics and epigenetics of animal migration and orientation: birds, butterflies and beyond. *The Journal of Experimental Biology* **222**, jeb191890.
Meyer, B. S., Moiron, M., Caswara, C., Chow, W., Fedrigo, O., Formenti, G., Haase, B., Howe, K., Mountcastle, J., Uliano-Silva, M., Wood, J., Jarvis, E. D., Liedvogel, M. & Bouwhuis, S. (2023). Sex-specific changes in autosomal methylation rate in ageing common terns. *Frontiers in Ecology and Evolution* **11**, 20.
*Miller, F. L. (1974). Age determination of caribou by annulations in dental cementum. *The Journal of Wildlife Management* **38**, 47.
*Mitchell, B. (1967). Growth layers in dental cement for determining the age of red deer (*Cervus elaphus* L.). *The Journal of Animal Ecology* **36**, 279.
*Miura, S. (1985). Horn and cementum annulation as age criteria in Japanese serow. *The Journal of Wildlife Management* **49**, 152.
Molbert, N., Angelier, F., Alliot, F., Ribout, C. & Goutte, A. (2021). Fish from urban rivers and with high pollutant levels have shorter telomeres: telomeres and contamination in fish. *Biology Letters* **17**, 20200819.
Morin, P. A., Nestler, A., Rubio-Cisneros, N. T., Robertson, K. M. & Mesnick, S. L. (2005). Interfamilial characterization of a region of the ZFX and ZFY genes facilitates sex determination in cetaceans and other mammals. *Molecular Ecology* **14**, 3275–3286.
Morselli, M., Bennett, R., Shaidani, N.-I., Horb, M., Peshkin, L. & Pellegrini, M. (2023). Age-associated DNA methylation changes in *Xenopus* frogs. *Epigenetics* **18**, 2201517.
Moyzis, R. K., Buckingham, J. M., Crams, L. S., Dani, M., Deavent, L. L., Jones, M. D., Meyne, J., Ratliff, R. L. & Wu, J.-R. (1988). A highly conserved repetitive DNA sequence, $(TTAGGG)_n$, present at the telomeres of human chromosomes. *Proceedings of the National Academy of Sciences* **85**, 6622–6626.
Myrick, A. C. J., Hohn, A. A., Sloan, P. A., Makoto, K. & Stanley, D. D. (1983). Estimating age of spotted and spinner dolphins (*Stenella attenuata* and *Stenella longirostris*) from teeth. NOAA Technical Memorandum NMFS, SWFC-30.
*Nakanishi, N., Ichinose, F., Higa, G. & Izawa, M. (2009). Age determination of the iriomote cat by using cementum annuli. *Journal of Zoology* **279**, 338–348.
Newham, E., Gill, P. G., Robson Brown, K., Gostling, N. J. & Schneider, P. (2021). A robust, semi-automated approach for counting cementum increments imaged 1 with X-ray computed tomography. *PLoS One* **16**, e0249743.
*Nicholson, W. S., Hill, E. P. & Briggs, D. (1985). Denning, pup-rearing, and dispersal in the gray fox in east-Central Alabama. *The Journal of Wildlife Management* **49**, 33.
Nilsen, F. M., Parrott, B. B., Bowden, J. A., Kassim, B. L., Somerville, S. E., Bryan, T. A., Bryan, C. E., Lange, T. R., Delaney, J. P., Brunell, A. M., Long, S. E. & Guillette, L. J. (2016). Global DNA methylation loss associated with mercury contamination and aging in the American alligator (*Alligator mississippiensis*). *Science of the Total Environment* **545**, 389–397.
Noguera, J. C. & Velando, A. (2021). Telomerase activity can mediate the effects of growth on telomeres during post-natal development in a wild bird. *Journal of Experimental Biology* **224**, jeb242465.
Noren, S. R. & Edwards, E. F. (2007). Physiological and behavioral development in delphinid calves: implications for calf separation and mortality due to tuna purse-seine sets. *Marine Mammal Science* **23**, 15–29.
Oganesian, L. & Karlseder, J. (2011). Mammalian 5′ C-rich telomeric overhangs are a mark of recombination-dependent telomere maintenance. *Molecular Cell* **42**, 224–236.
Ohsumi, S., Kasuya, T. & Nishiwaki, M. (1965). Accumulation rate of dentinal growth layers in the maxillary tooth of the sperm whale. *Scientific Reports The Whales Research Institute* **17**, 15–34.
*Olivier, G. C. & Greyling, F. J. (1991). Criteria for age assessment of blesbok. *South African Journal of Wildlife Research* **21**, 1–5.
Olsen, M. T., Robbins, J., Bérubé, M., Rew, M. B. & Palsbøll, P. J. (2014). Utility of telomere length measurements for age determination of humpback whales. *NAMMCO Scientific Publications* **10**, 1–17.
Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T. & Blomqvist, D. (2011). Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *PLoS One* **6**, e17473.
Olsson, M., Wapstra, E. & Friesen, C. R. (2018). Evolutionary ecology of telomeres: a review. *Annals of the New York Academy of Sciences* **1422**, 5–28.
Ooi, S. K. T. & Bestor, T. H. (2008). The colorful history of active DNA demethylation. *Cell* **133**, 1145–1148.
*Oosthuizen, W. H. (1997). Evaluation of an effective method to estimate age of Cape fur seals using ground tooth sections. *Marine Mammal Science* **13**, 683–693.
*Østbye, E., Lauritzen, S.-E. E., Østbye, K. & Wiig, Ø. (2006). Holocene brown bear (*Ursus arctos* L.) from Norwegian caves. *Boreas* **35**, 296–316.
Owen, R. (1840). *Odontography, or, A Treatise on the Comparative Anatomy of the Teeth: Their Physiological Relations, Mode of Development, and Microscopic Structure in the Vertebrate Animals*, Edition (Volume **1**). Balliere, London, UK. https://doi.org/10.5962/bhl.title.16281.
Palm, W. & de Lange, T. (2008). How shelterin protects mammalian telomeres. *Annual Review of Genetics* **42**, 301–334.
Panasiak, L., Dobosz, S. & Ocalewicz, K. (2020). Telomere dynamics in the diploid and triploid rainbow trout (*Oncorhynchus mykiss*) assessed by Q-FISH analysis. *Genes* **11**, 1–12.
Panasiak, L., Szubert, K., Polonis, M. & Ocalewicz, K. (2022). Telomere length variation does not correspond with the growth disturbances in the rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Genetics* **63**, 133–139.
Parolini, M., Romano, A., Khoriauli, L., Nergadze, S. G., Caprioli, M., Rubolini, D., Santagostino, M., Saino, N. & Giulotto, E. (2015). Early-life telomere dynamics differ between the sexes and predict growth in the barn swallow (*Hirundo rustica*). *PLoS One* **10**, e0142530.

Parrott, B. B. & Bertucci, E. M. (2019). Epigenetic aging clocks in ecology and evolution. *Trends in Ecology and Evolution* **34**, 767–770.
Parrott, B. B., Bowden, J. A., Kohno, S., Cloy-McCoy, J. A., Hale, M. D., Bangma, J. T., Rainwater, T. R., Wilkinson, P. M., Kucklick, J. R. & Guillette, L. J. (2014). Influence of tissue, age, and environmental quality on DNA methylation in *Alligator mississippiensis*. *Reproduction* **147**, 503–513.
Pauli, J. N., Whiteman, J. P., Marcot, B. G., McClean, T. M. & Ben-David, M. (2011). DNA-based approach to aging martens (*Martes americana* and *M. caurina*). *Journal of Mammalogy* **92**, 500–510.
Pauliny, A., Larsson, K. & Blomqvist, D. (2012). Telomere dynamics in a long-lived bird, the barnacle goose. *BMC Evolutionary Biology* **12**, 1–8.
Pauliny, A., Wagner, R. H., Augustin, J., Szép, T. & Blomqvist, D. (2006). Age-independent telomere length predicts fitness in two bird species. *Molecular Ecology* **15**, 1681–1687.
Payan, P., Borelli, G., Boeuf, G. & Mayer-Gostan, N. (1998). Relationship between otolith and somatic growth: consequence of starvation on acid-base balance in plasma and endolymph in the rainbow trout *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **19**, 35–41.
Pekelharing, C. J. (1970). Cementum deposition as an age indicator in the brush-tailed possum, *Trichosurus vulpecula* Kerr (Marsupiala). *Australian Journal of Zoology* **18**, 71–76.
Penzhorn, B. L. (1982). Age determination in Cape mountain zebras *Equus zebra zebra* in the Mountain Zebra National Park. *Koedoe* **25**, 89–102.
Pérez-Barberìa, F. J., Duff, E. I., Brewer, M. J. & Guinness, F. E. (2014). Evaluation of methods to age Scottish red deer: the balance between accuracy and practicality. *Journal of Zoology* **294**, 180–189.
*Perez-Barberia, F. J. & Fernandez-Lopez, J. M. (1996). Using cementum annuli to estimate Cantabrian chamois age. *The Journal of Wildlife Management* **60**, 62–67.
Pérez-Pereira, N., López-Cortegano, E. & García-Dorado, A. (2022). Prediction of fitness under different breeding designs in conservation programs. *Animal Conservation* **26**, 86–102.
*Pires, A. E., Caldeira, I. S., Petrucci-Fonseca, F., Viegas, I., Viegas, C., Bastos-Silveira, C. & Requicha, J. F. (2020). Dental pathology of the wild Iberian wolf (*Canis lupus signatus*): the study of a 20th century Portuguese museum collection. *Veterinary and Animal Science* **9**, 100100.
Plot, V., Criscuolo, F., Zahn, S. & Georges, J. Y. (2012). Telomeres, age and reproduction in a long-lived reptile. *PLoS One* **7**, e40855.
Poganik, J. R., Zhang, B., Baht, G. S., Kerepesi, C., Yim, S. H., Lu, A. T., Haghani, A., Gong, T., Hedman, A. M., Andolf, E., Pershagen, G., Almqvist, C., White, J. P., Horvath, S. & Gladyshev, V. N. (2023). Biological age is increased by stress and restored upon recovery. *Cell Metabolism* **35**, 807–820.
Polanowski, A. M., Robbins, J., Chandler, D. & Jarman, S. N. (2014). Epigenetic estimation of age in humpback whales. *Molecular Ecology Resources* **14**, 976–987.
Prado, N. A., Brown, J. L., Zoller, J. A., Haghani, A., Yao, M., Bagryanova, L. R., Campana, M. G., Maldonado, J. E., Raj, K., Schmitt, D., Robeck, T. R. & Horvath, S. (2021). Epigenetic clock and methylation studies in elephants. *Aging Cell* **20**, e13414.
Qi, H., Kinoshita, K., Mori, T., Matsumoto, K., Matsui, Y. & Inoue-Murayama, M. (2021). Age estimation using methylation-sensitive high-resolution melting (MS-HRM) in both healthy felines and those with chronic kidney disease. *Scientific Reports* **11**, 1–10.
Quirici, V., Guerrero, C. J., Krause, J. S., Wingfield, J. C. & Vásquez, R. A. (2016). The relationship of telomere length to baseline corticosterone levels in nestlings of an altricial passerine bird in natural populations. *Frontiers in Zoology* **13**, 1–11.
R Core Team (2020). *R: A Language and Environment for Statisitical Computing*. R Foundation for Statistical Computing, Vienna https://www.r-project.org/.
Raj, K., Szladovits, B., Haghani, A., Zoller, J. A., Li, C. Z., Black, P., Maddox, D., Robeck, T. R. & Horvath, S. (2021). Epigenetic clock and methylation studies in cats. *GeroScience* **43**, 2363–2378.
Rausch, R. A. & Pearson, A. M. (1972). Notes on the wolverine in Alaska and the Yukon territory. *The Journal of Wildlife Management* **36**, 249–268.
*Rautenbach, I. L. (1970). Ageing criteria in the springbok, *Antidorcas marsupialis* (Zimmermann, 1780) (Artiodactyla: Bovidae). *Annals of the Transvaal Museum* **27**, 83–133.
Reichenbacher, B., Sienknecht, U., Küchenhoff, H. & Fenske, N. (2007). Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (Aphanius, Prolebias). *Journal of Morphology* **268**, 898–915.
Reichert, S., Rojas, E. R., Zahn, S., Robin, J. P., Criscuolo, F. & Massemin, S. (2015). Maternal telomere length inheritance in the king penguin. *Heredity* **114**, 10–16.
*Reimers, E. & Nordby, O. (1968). Relationship between age and tooth cementum layers in Norwegian reindeer. *The Journal of Wildlife Management* **32**, 957.
Remot, F., Ronget, V., Froy, H., Rey, B., Gaillard, J. M., Nussey, D. H. & Lemaitre, J. F. (2021). Decline in telomere length with increasing age across nonhuman vertebrates: a meta-analysis. *Molecular Ecology* **31**, 5917–5932.
Rey, O., Eizaguirre, C., Angers, B., Baltazar-Soares, M., Sagonas, K., Prunier, J. G. & Blanchet, S. (2020). Linking epigenetics and biological conservation: towards a conservation epigenetics perspective. *Functional Ecology* **34**, 414–427.
Roast, M. J., Eastwood, J. R., Aranzamendi, N. H., Fan, M., Teunissen, N., Verhulst, S. & Peters, A. (2022). Telomere length declines with age, but relates to immune function independent of age in a wild passerine. *Royal Society Open Science* **9**, 212012.
Robeck, T. R., Fei, Z., Haghani, A., Zoller, J. A., Li, C. Z., Steinman, K. J., Dirocco, S., Staggs, L., Schmitt, T., Osborn, S., Montano, G., Rodriguez, M. & Horvath, S. (2021*a*). Multi-tissue methylation clocks for age and sex estimation in the common bottlenose dolphin. *Frontiers in Marine Science* **8**, 1318.
Robeck, T. R., Fei, Z., Lu, A. T., Haghani, A., Jourdain, E., Zoller, J. A., Li, C. Z., Steinman, K. J., DiRocco, S., Schmitt, T., Dold, C. & Horvath, S. (2021*b*). Multi-species and multi-tissue methylation clocks for age estimation in toothed whales and dolphins. *Communications Biology* **4**, 642.
Robeck, T. R., Haghani, A., Fei, Z., Lindemann, D. M., Russell, J., Herrick, K. E. S., Montano, G., Steinman, K. J., Katsumata, E., Zoller, J. A. & Horvath, S. (2023). Multi-tissue DNA methylation aging clocks for sea lions, walruses and seals. *Communications Biology* **6**, 359.
*Robinette, W. L. & Archer, A. L. (1971). Notes on aging criteria and reproduction of Thomson's gazelle. *African Journal of Ecology* **9**, 83–98.
Rohatgi, A. (2022). Webplotdigitizer: Version 4.6. https://automeris.io/WebPlotDigitizer.
Rolandsen, C. M., Solberg, E. J., Heim, M., Holmstrøm, F., Solem, M. I. & Saether, B.-E. (2008). Accuracy and repeatability of moose (*Alces alces*) age as estimated from dental cement layers. *European Journal of Wildlife Research* **54**, 6–14.
Romano, A., De Giorgio, B., Parolini, M., Favero, C., Possenti, C. D., Iodice, S., Caprioli, M., Rubolini, D., Ambrosini, R., Gianfranceschi, L., Saino, N. & Bollati, V. (2017). Methylation of the circadian clock gene in the offspring of a free-living passerine bird increases with maternal and individual exposure to PM10. *Environmental Pollution* **220**, 29–37.
Román-Palacios, C., Medina, C. A., Zhan, S. H. & Barker, M. S. (2021). Animal chromosome counts reveal a similar range of chromosome numbers but with less polyploidy in animals compared to flowering plants. *Journal of Evolutionary Biology* **34**, 1333–1339.
*Rosell, F., Zedrosser, A. & Parker, H. (2010). Correlates of body measurements and age in Eurasian beaver from Norway. *European Journal of Wildlife Research* **56**, 43–48.
RStudio Team (2021). *RStudio: Integrated Development Environment for R*. IDE, Boston http://www.rstudio.com/.
Rust, L. B., Danil, K., Melin, S. R. & Wilkerson, B. (2019). Accuracy and precision of age determination using growth layer groups for California Sea lions (*Zalophus californianus*) with known ages. *Marine Mammal Science* **35**, 1355–1368.
Saino, N., Albetti, B., Ambrosini, R., Caprioli, M., Costanzo, A., Mariani, J., Parolini, M., Romano, A., Rubolini, D., Formenti, G., Gianfranceschi, L. & Bollati, V. (2019). Inter-generational resemblance of methylation levels at circadian genes and associations with phenology in the barn swallow. *Scientific Reports* **9**, 1–16.
Saino, N., Ambrosini, R., Albetti, B., Caprioli, M., De Giorgio, B., Gatti, E., Liechti, F., Parolini, M., Romano, A., Romano, M., Scandolara, C., Gianfranceschi, L., Bollati, V. & Rubolini, D. (2017). Migration phenology and breeding success are predicted by methylation of a photoperiodic gene in the barn swallow. *Scientific Reports* **7**, 45412.
Saino, N., Rubolini, D., Ambrosini, R., Romano, M., Scandolara, C., Fairhurst, G. D., Caprioli, M., Romano, A., Sicurella, B. & Liechti, F. (2015). Light-level geolocators reveal covariation between winter plumage molt and phenology in a trans-Saharan migratory bird. *Oecologia* **178**, 1105–1112.
Salmón, P., Nilsson, J. F., Nord, A., Bensch, S. & Isaksson, C. (2016). Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biology Letters* **12**, 20160155.
Salomons, H. M., Mulder, G. A., Van De Zande, L., Haussmann, M. F., Linskens, M. H. K. & Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3157–3165.
Sánchez-Montes, G., Martínez-Solano, Í., Díaz-Paniagua, C., Vilches, A., Ariño, A. H. & Gomez-Mestre, I. (2020). Telomere attrition with age in a wild amphibian population. *Biology Letters* **16**, 20200168.
Sareisian, L. (2014). Global DNA methylation and persistent organic pollutants in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Masters dissertation: University of Oslo, Oslo. http://urn.nb.no/URN:NBN:no-46760.
Sato, A., Fairbanks, L. A., Lawson, P. T. & Lawson, G. W. (2005). Effects of age and sex on hematologic and serum biochemical values of vervet monkeys (*Chlorocebus aethiops sabaeus*). *Contemporary Topics in Laboratory Animal Science* **44**, 29–34.

Saunders, P. A. & Veyrunes, F. (2021). Unusual mammalian sex determination systems: a cabinet of curiosities. *Genes* **12**, 1170.
Saxon, A. & Higham, C. F. W. (1968). Identification and interpretation of growth rings in the secondary dental cementum of *Ovis aries* L. *Nature* **219**, 634–635.
Schachtschneider, K. M., Schook, L. B., Meudt, J. J., Shanmuganayagam, D., Zoller, J. A., Haghani, A., Li, C. Z., Zhang, J., Yang, A., Raj, K. & Horvath, S. (2021). Epigenetic clock and DNA methylation analysis of porcine models of aging and obesity. *GeroScience* **43**, 2467–2483.
Schaetzlein, S., Lucas-Hahn, A., Lemme, E., Kues, W. A., Dorsch, M., Manns, M. P., Niemann, H. & Rudolph, K. L. (2004). Telomere length is reset during early mammalian embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 8034–8038.
*Scheffer, V. B. (1950). Growth layers on the teeth of pinnipedia as an indication of age. *Science* **112**, 309–311.
*Schuler, K. L., Baker, B. B., Mayer, K. A., Perez-Heydrich, C., Holahan, P. M., Thomas, N. J. & White, C. L. (2018). Refining aging criteria for northern sea otters in Washington State. *Journal of Fish and Wildlife Management* **9**, 208–221.
Schwarzer, G., Carpenter, J. R. & Rücker, G. (2015). *Meta-Analysis with R*. Springer, Cham..
Scott, G. G., Sis, R. F., Levy, B. M., Stott, G. G., Sis, R. F. & Levy, B. M. (1980). Cemental annulation as an age criterion in the common marmoset (*Callithrix jacchus*). *Journal of Medical Primatology* **9**, 274–285.
Scott, N. M., Haussmann, M. F., Elsey, R. M., Trosclair, P. L. & Vleck, C. M. (2006). Telomere length shortens with body length in *Alligator mississippiensis*. *Southeastern Naturalist* **5**, 685–692.
Secor, D. H., Trice, T. M. & Hornick, H. T. (1995). Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured Chesapeake Bay striped bass, *Morone saxatilis*. *Fishery Bulletin* **93**, 186–190.
Seibt, K. D., Häussler, S., Vecchio, D., DeCarlo, E., Ceciliani, F. & Sauerwein, H. (2019). Comparison of telomere lengths in leukocytes and in nasal and vaginal epithelial cells from water buffaloes (*Bubalus bubalis*) of different ages. *Research in Veterinary Science* **124**, 328–333.
Sen, G. L., Reuter, J. A., Webster, D. E., Zhu, L. & Khavari, P. A. (2010). DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* **463**, 563–567.
Sfeir, A. J., Chai, W., Shay, J. W. & Wright, W. E. (2005). Telomere-end processing: the terminal nucleotidesof human chromosomes. *Molecular Cell* **18**, 131–138.
Sharma, A., Singh, D., Malik, S., Gupta, N. J., Rani, S. & Kumar, V. (2018). Difference in control between spring and autumn migration in birds: insight from seasonal changes in hypothalamic gene expression in captive buntings. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20181531.
Sharma, V., Sharma, C. P., Sharma, V., Goyal, S. P. & Gupta, S. K. (2021). Fast and cost-effective age estimation in Bengal tiger and Asiatic lion: applicability of cementum analysis method. *bioRxiv* 2021.09.27.461978. https://doi.org/10.1101/2021.09.27.461978.
Shay, J. W. & Wright, W. E. (2019). Telomeres and telomerase: three decades of progress. *Nature Reviews Genetics* **20**, 299–309.
Sherley, R. B., Abadi, F., Ludynia, K., Barham, B. J., Clark, A. E. & Altwegg, R. (2014). Age-specific survival and movement among major African penguin *Spheniscus demersus* colonies. *Ibis* **156**, 716–728.
Short, R., Williams, D. D. & Bowden, D. M. (1987). Cross-sectional evaluation of potential biological markers of aging in pigtailed macaques: effects of age, sex, and diet. *Journals of Gerontology* **42**, 644–654.
Simide, R., Angelier, F., Gaillard, S. & Stier, A. (2016). Age and heat stress as determinants of telomere length in a long-lived fish, the Siberian sturgeon. *Physiological and Biochemical Zoology* **89**, 441–447.
*Simpson, C. D. (1983). *Tooth Replacement, Growth, and Ageing Criteria for the Zambezi Bushbuck, Tragelaphus Scriptus Ornatus Pocock*. National Museums and Monuments of Rhodesia. Harare, Zimbabwe.
*Simpson, C. D. & Elder, W. H. (1969). Tooth cementum as an index of age in greater kudu (*Trageluphus strepsireros*). *Artioldia* **4**, 1–10.
*Smith, J. R. (1975). Age determination of the woodchuck, *Marmota monax*, by annulations in dental cementum and in the periosteal zone of bones. Doctoral Thesis: Eastern Kentucky University, Richmond, United States of America.
Smith, L. E., Jones, M. E., Hamede, R., Risques, R., Patton, A. H., Carter, P. A. & Storfer, A. (2020). Telomere length is a susceptibility marker for Tasmanian devil facial tumor disease. *EcoHealth* **17**, 280–291.
Smuts, G. L., Anderson, J. L. & Austin, J. C. (1978). Age determination of the African lion (*Panthera leo*). *Journal of Zoology* **185**, 115–146.
Snir, S., vonHoldt, B. M. & Pellegrini, M. (2016). A statistical framework to identify deviation from time linearity in epigenetic aging. *PLoS Computational Biology* **12**, e1005183.
Soler, J. J., Ruiz Castellano, C., Martínez-de la Puente, J., Tomás, G., Ruiz-Rodríguez, M. & Figuerola, J. (2015). Telomere dynamics in parasitic great spotted cuckoos and their magpie hosts. *Journal of Evolutionary Biology* **28**, 1610–1617.
Soler, J. J., Ruiz-Castellano, C., Figuerola, J., Martín-Vivaldi, M., Martínez-de la Puente, J., Ruiz-Rodríguez, M. & Tomás, G. (2017). Telomere length and dynamics of spotless starling nestlings depend on nest-building materials used by parents. *Animal Behaviour* **126**, 89–100.
Sørheim, V. C. (2020). Telomere dynamics during nestling development in bluethroats (*Luscinia s. svecica*). Masters dissertation: University of Oslo, Oslo.
Soulsbury, C. D., Lipponen, A., Wood, K., Mein, C. A., Hoffman, J. I. & Lebigre, C. (2018). Age- and quality-dependent DNA methylation correlate with melanin-based coloration in a wild bird. *Ecology and Evolution* **8**, 6547–6557.
Spiers, H., Hannon, E., Wells, S., Williams, B., Fernandes, C. & Mill, J. (2016). Age-associated changes in DNA methylation across multiple tissues in an inbred mouse model. *Mechanisms of Ageing and Development* **154**, 20–23.
Spinage, C. A. (1976). Incremental cementum lines in the teeth of tropical African mammals. *Journal of Zoology* **178**, 117–131.
Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T., Dugdale, H. L. & Richardson, D. S. (2018). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology* **87**, 187–198.
Starr, M. E. & Saito, H. (2014). Sepsis in old age: review of human and animal studies. *Aging and Disease* **5**, 126–136.
Statham, A. & Csárdi, G. (2008). *RSeqMeth: Package for Analysis of Sequenom EpiTYPER Data*. Sydney. https://github.com/cran/RSeqMeth.
Stewart, R. E. A., Stewart, B. E., Stirling, I. & Street, E. (1996). Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). *Marine Mammal Science* **12**, 383–401.
Stier, A., Hsu, B. Y., Marciau, C., Doligez, B., Gustafsson, L., Bize, P. & Ruuskanen, S. (2020). Born to be young? Prenatal thyroid hormones increase early-life telomere length in wild collared flycatchers. *Biology Letters* **16**, 20200364.
*Stirling, I., Archibald, W. R. & DeMaster, D. (1977). Distribution and abundance of seals in the eastern Beaufort Sea. *Journal of the Fisheries Research Board of Canada* **34**, 976–988.
Stubbs, T. M., Bonder, M. J., Stark, A. K., Krueger, F., von Meyenn, F., Stegle, O., Reik, W., Bolland, D., Butcher, G., Chandra, T., Clark, S. J., Corcoran, A., Eckersley-Maslinc, M., Field, L., Frising, U. C., *et al.* (2017). Multi-tissue DNA methylation age predictor in mouse. *Genome Biology* **18**, 1–14.
Suarez-Bregua, P., Rosendo, S., Comesaña, P., Sánchez-Ruiloba, L., Morán, P., Planas, M. & Rotllant, J. (2021). Dynamic changes in DNA methylation during seahorse (*Hippocampus reidi*) postnatal development and settlement. *Frontiers in Zoology* **18**, 1–10.
Sudyka, J., Arct, A., Drobniak, S., Dubiec, A., Gustafsson, L. & Cichoń, M. (2014). Experimentally increased reproductive effort alters telomere length in the blue tit (*Cyanistes caeruleus*). *Journal of Evolutionary Biology* **27**, 2258–2264.
Sugrue, V. J., Zoller, J. A., Narayan, P., Lu, A. T., Ortega-Recalde, O. J., Grant, M. J., Bawden, C. S., Rudiger, S. R., Haghani, A., Bond, D. M., Hore, R. R., Garratt, M., Sears, K. E., Wang, N., Yang, X. W., *et al.* (2021). Castration delays epigenetic aging and feminizes DNA methylation at androgen-regulated loci. *eLife* **10**, e64932.
Tackney, J., Cawthon, R. M., Coxworth, J. E. & Hawkes, K. (2014). Blood cell telomere lengths and shortening rates of chimpanzee and human females. *American Journal of Human Biology* **26**, 452–460.
Tanabe, A., Shimizu, R., Osawa, Y., Suzuki, M., Ito, S., Goto, M., Pastene, L. A. L. A., Fujise, Y. & Sahara, H. (2020). Age estimation by DNA methylation in the Antarctic minke whale. *Fisheries Science* **86**, 35–41.
Thomas, C. & Zapico, S. C. (2017). Role of telomeres in aging. In *Mechanisms Linking Aging, Diseases and Biological Age Estimation*, First Edition (ed. S. C. Zapico), pp. 141–149. CRC Press. Boca Raton, USA.
*Thomas, D. C. (1977). Metachromatic staining of dental cementum for mammalian age determination. *The Journal of Wildlife Management* **41**, 207.
Thompson, M. J., von Holdt, B., Horvath, S. & Pellegrini, M. (2017). An epigenetic aging clock for dogs and wolves. *Aging* **9**, 1055–1068.
Thompson, R. F., Suzuki, M., Lau, K. W. & Greally, J. M. (2009). A pipeline for the quantitative analysis of CG dinucleotide methylation using mass spectrometry. *Bioinformatics* **25**, 2164–2170.
Tilstra, J. S., Clauson, C. L., Niedernhofer, L. J. & Robbins, P. D. (2011). NF-κB in aging and disease. *Aging and Disease* **2**, 449–465.
Tissier, M. L., Bergeron, P., Garant, D., Zahn, S., Criscuolo, F. & Réale, D. (2022). Telomere length positively correlates with pace-of-life in a sex- and cohort-specific way and elongates with age in a wild mammal. *Molecular Ecology* **31**, 3812–3826.
Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., Divoky, G., Eimes, J. A., Huntington, C. E., Kitaysky, A. S., Juola, F. A., Lank, D. B., Litwa, H. P., Mulder, E. G. A., Nisbet, I. C. T., *et al.* (2018). The rate of telomere loss is related to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20160445.

Trivedi, A. K., Sur, S., Sharma, A., Taufique, S. T., Gupta, N. J. & Kumar, V. (2019). Temperature alters the hypothalamic transcription of photoperiod responsive genes in induction of seasonal response in migratory redheaded buntings. *Molecular and Cellular Endocrinology* **493**, 110454.
Ujvari, B., Biro, P. A., Charters, J. E., Brown, G., Heasman, K., Beckmann, C. & Madsen, T. (2017). Curvilinear telomere length dynamics in a squamate reptile. *Functional Ecology* **31**, 753–759.
Ujvari, B. & Madsen, T. (2009). Short telomeres in hatchling snakes: erythrocyte telomere dynamics and longevity in tropical pythons. *PLoS One* **4**, e7493.
*Van Aarde, R. J. (1985). Age determination of Cape porcupines, *Hystrix africaeaustralis*. *African Zoology* **20**, 232–236.
van Eck, N. J. & Waltman, L. (2017). Citation-based clustering of publications using CitNetExplorer and VOSviewer. *Scientometrics* **111**, 1053–1070.
van Lieshout, S. H. J., Bretman, A., Newman, C., Buesching, C. D., Macdonald, D. W. & Dugdale, H. L. (2019). Individual variation in early-life telomere length and survival in a wild mammal. *Molecular Ecology* **28**, 4152–4165.
Van Lieshout, S. H. J., Sparks, A. M., Bretman, A., Newman, C., Buesching, C. D., Burke, T., Macdonald, D. W. & Dugdale, H. L. (2021). Estimation of environmental, genetic and parental age at conception effects on telomere length in a wild mammal. *Journal of Evolutionary Biology* **34**, 296–308.
*Van Nostrand, F. C. & Stephenson, A. B. (1964). Age determination for beavers by tooth development. *The Journal of Wildlife Management* **28**, 430.
Vedder, O., Moiron, M., Bichet, C., Bauch, C., Verhulst, S., Becker, P. H. & Bouwhuis, S. (2022). Telomere length is heritable and genetically correlated with lifespan in a wild bird. *Molecular Ecology* **31**, 6297–6307.
Vedder, O., Verhulst, S., Bauch, C. & Bouwhuis, S. (2017). Telomere attrition and growth: a life-history framework and case study in common terns. *Journal of Evolutionary Biology* **30**, 1409–1419.
Venegas, D., Marmolejo-Valencia, A., Valdes-Quezada, C., Govenzensky, T., Recillas-Targa, F. & Merchant-Larios, H. (2016). Dimorphic DNA methylation during temperature-dependent sex determination in the sea turtle *Lepidochelys olivacea*. *General and Comparative Endocrinology* **236**, 35–41.
Vernasco, B. J., Dakin, R., Majer, A. D., Haussmann, M. F., Brandt Ryder, T. & Moore, I. T. (2021). Longitudinal dynamics and behavioural correlates of telomeres in male wire-tailed manakins. *Functional Ecology* **35**, 450–462.
Vernasco, B. J. & Watts, H. E. (2022). Telomere length predicts timing and intensity of migratory behaviour in a nomadic songbird. *Biology Letters* **18**, 20220176.
Viblanc, V. A., Criscuolo, F., Sosa, S., Schull, Q., Boonstra, R., Saraux, C., Lejeune, M., Roth, J. D., Uhlrich, P., Zahn, S. & Dobson, F. S. (2022). Telomere dynamics in female Columbian ground squirrels: recovery after emergence and loss after reproduction. *Oecologia* **199**, 301–312.
Viciano, J., López-Lázaro, S. & Tanga, C. (2022). *Post-mortem* dental profile as a powerful tool in animal forensic investigations—a review. *Animals* **12**, 2038.
Viitaniemi, H. M., Verhagen, I., Visser, M. E., Honkela, A., Van Oers, K., Husby, A. & Meyer, M. (2019). Seasonal variation in genome-wide DNA methylation patterns and the onset of seasonal timing of reproduction in great tits. *Genome Biology and Evolution* **11**, 970–983.
Voillemot, M., Hine, K., Zahn, S., Criscuolo, F., Gustafsson, L., Doligez, B. & Bize, P. (2012). Effects of brood size manipulation and common origin on phenotype and telomere length in nestling collared flycatchers. *BMC Ecology* **12**, 1–8.
Wada, K., Ohtaishi, N. & Hachiya, N. (1978). Determination of age in the Japanese monkey from growth layers in the dental cementum. *Primates* **19**, 775–784.
Walleser, L. R., Crimmins, S. M. & Roberts, N. M. (2016). Estimating the age of male gray wolves (*Canis lupus*) using baculum measurements. *Canadian Field-Naturalist* **130**, 212–215.
Watson, R. L., Bird, E. J., Underwood, S., Wilbourn, R. V., Fairlie, J., Watt, K., Salvo-Chirnside, E., Pilkington, J. G., Pemberton, J. M., McNeilly, T. N., Froy, H. & Nussey, D. H. (2017). Sex differences in leucocyte telomere length in a free-living mammal. *Molecular Ecology* **26**, 3230–3240.
Webb, S. J. N., Schapiro, S. J., Sherwood, C. C., Raghanti, M. A. & Hopkins, W. D. (2020). Neutrophil to lymphocyte ratio (NLR) in captive chimpanzees (*Pan troglodytes*): the effects of sex, age, and rearing. *PLoS One* **15**, e0244092.
Wei, T., Nie, J., Larson, N. B., Ye, Z., Eckel-Passow, J. E., Robertson, K. D., Kocher, J. P. A. & Wang, L. (2021). CpGtools: a python package for DNA methylation analysis. *Bioinformatics* **37**, 1598–1599.
White, P. A. & Belant, J. L. (2016). Individual variation in dental characteristics for estimating age of African lions. *Wildlife Biology* **22**, 71–77.
Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C. & Blasco, M. A. (2019). Telomere shortening rate predicts species life span. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 15122–15127.
Wilbourn, R. V., Froy, H., McManus, M. C., Cheynel, L., Gaillard, J. M., Gilot-Fromont, E., Regis, C., Rey, B., Pellerin, M., Lemaître, J. F. & Nussey, D. H. (2017). Age-dependent associations between telomere length and environmental conditions in roe deer. *Biology Letters* **13**, 20170434.
Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H. & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20160447.
Wilkinson, G. S., Adams, D. M., Haghani, A., Lu, A. T., Zoller, J., Breeze, C. E., Arnold, B. D., Ball, H. C., Carter, G. G., Cooper, L. N., Dechmann, D. K. N., Devanna, P., Fasel, N. J., Galazyuk, A. V., Günther, L., *et al.* (2021). DNA methylation predicts age and provides insight into exceptional longevity of bats. *Nature Communications* **12**, 1615.
*Wilson, V. J., Schmidt, J. L. & Hanks, J. (1984). Age determination and body growth of the common duiker *Sylvicapra grimmia* (Mammalia). *Journal of Zoology* **202**, 283–297.
Wittwer-Backofen, U., Gampe, J. & Vaupel, J. W. (2004). Tooth cementum annulation for age estimation: results from a large known-age validation study. *American Journal of Physical Anthropology* **123**, 119–129.
Wong, P. B. Y. (2017). Conservation and biological senescence in polar bears: telomeres and inuit traditional knowledge. Doctoral Thesis: University of Toronto, Toronto. https://hdl.handle.net/1807/79501.
Wright, P. G. R., Mathews, F., Schofield, H., Morris, C., Burrage, J., Smith, A., Dempster, E. L. E. L. & Hamilton, P. B. P. B. (2018). Application of a novel molecular method to age free-living wild Bechstein's bats. *Molecular Ecology Resources* **18**, 1374–1380.
Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W. & Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. *Developmental Genetics* **18**, 173–179.
Wu, P., Takai, H. & De Lange, T. (2012). Telomeric 3′ overhangs derive from resection by Exo1 and apollo and fill-in by POT1b-associated CST. *Cell* **150**, 39–52.
*Yoneda, M. (1982). Growth layers in dental cementum of Saguinus monkeys in South America. *Primates* **23**, 460–464.
Yuan, X., Ishibashi, S., Hatakeyama, S., Saito, M., Nakayama, J. I., Nikaido, R., Haruyama, T., Watanabe, Y., Iwata, H., Iida, M., Sugimura, H., Yamada, N. & Ishikawa, F. (1999). Presence of telomeric G-strand tails in the telomerase catalytic subunit TERT knockout mice. *Genes to Cells* **4**, 563–572.
*Zapata, S. C., Perea, R. G., Beltran, J. F., Ferreras, P. & Delibes, M. (1997). Age determination of Iberian lynx (*Lynx pardinus*) using canine radiograph and cementum annuli enumeration. *Zeitschrift fur Saugetierkunde* **62**, 119–123.
Zapico, S. C. & Ubelaker, D. H. (2016). Relationship between mitochondrial DNA mutations and aging. Estimation of age-at-death. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **71**, 445–450.
Zhang, G. (2015). Bird sequencing project takes off. *Nature* **522**, 34.
Zhang, Q., Han, X., Hao, X., Ma, L., Li, S., Wang, Y. & Du, W. (2018). A simulated heat wave shortens the telomere length and lifespan of a desert lizard. *Journal of Thermal Biology* **72**, 94–100.
Zhang, W., Klinkebiel, D., Barger, C. J., Pandey, S., Guda, C., Miller, A., Akers, S. N., Odunsi, K. & Karpf, A. R. (2020). Global DNA hypomethylation in epithelial ovarian cancer: passive demethylation and association with genomic instability. *Cancers* **12**, 764.
Zhao, Z., Pan, X., Liu, L. & Liu, N. (2014). Telomere length maintenance, shortening, and lengthening. *Journal of Cellular Physiology* **229**, 1323–1329.
Zhdanova, N. S., Minina, J. M., Karamysheva, T. V., Rubtsov, N. B. & Londono-Vallejo, J. A. (2010). The structure of long telomeres in chromosomes of the Iberian shrew. *Russian Journal of Genetics* **46**, 1084–1086.
*Zhu, X.-M., Xiao, H. & Wu, J.-Y. (1992). Age determination of Goitred gazelle (*Gazella subgutturosa*). *Zoological Research* **13**, 95–99.
Zoller, J. A., Parasyraki, E., Lu, A. T., Haghani, A., Niehrs, C. & Horvath, S. (2022). DNA methylation clocks for clawed frogs reveal evolutionary conservation of epigenetic ageing. *bioRxiv* 2022.08.02.502561. https://doi.org/10.1101/2022.08.02.502561.

X. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary of all species within the class Mammalia for which tooth cementum annulation (TCA) has been used as biological clock method for age determination.

Fig. S1. PRISMA flow diagram for search, screening, and inclusion of methylation studies for age determination in animals.

Fig. S2. PRISMA flow diagram for search, screening, and inclusion of telomere studies for age determination in animals.

Fig. S3. Relationship between power and sample size for different effect sizes in a multiple linear regression with a minimum of three predictors at a significance of 95% ($\alpha = 0.05$).
Fig. S4. Forest plot and results for the meta-analysis of studies using methylation to estimate animal age with subgrouping based on the defined groups.
Fig. S5. Forest plot and results for the meta-analysis of studies using methylation to estimate animal age with subgrouping based on tissue type.
Fig. S6. Forest plot and results for the meta-analysis of studies using methylation to estimate animal age with subgrouping based on study method.
Fig. S7. Forest plot and results for the meta-analysis of studies using telomere length to estimate animal age with subgrouping based on defined groups.
Fig. S8. Forest plot and results for the meta-analysis of studies using telomere length to estimate animal age with subgrouping based on tissue type.
Fig. S9. Forest plot and results for the meta-analysis of studies using telomere length to estimate animal age with subgrouping based on study method.
Fig. S10. Forest plot indicating and comparing the results obtained from independent meta-analyses of studies using methylation *versus* telomere length to model the relationship with ageing in animals.

(*Received 21 October 2022; revised 4 June 2023; accepted 8 June 2023*)