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RESEARCH ARTICLE

Defining Mammalian Lifespan through Epigenetic Aging Timescales

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ABSTRACT

The lifespans of mammalian species vary across orders of magnitude, with the shortest on the order of a year and the longest more than one hundred years. These lifespans are influenced by both genetic and environmental factors. Here we asked whether we can define a molecular process that can be used to define the intrinsic molecular lifespan of a species that is largely independent of environmental factors. To address this question, we have focused on 'epigenetic clocks' - highly accurate age-predicting biomarkers based on DNA methylation. Our previous research has demonstrated that the alterations in DNA methylation related to age are non-linear, changing rapidly early in life and slowing down with advancing age. We have proposed the use of saturating exponential functions to represent these changes, which tend to stabilize at terminal methylation levels towards the end of an organism's lifespan. Our current study expands upon this by examining the exponential aging timescales across various mammalian species. We show that the DNA methylation trajectories of a broad range of species, ranging from mice to humans, adhere to a saturating exponential function. Furthermore, we find the timescale of this exponential decay to be about one third of a species' lifespan. This striking and novel observation implies that we can define an intrinsic molecular lifespan of a species that is largely unaffected by environmental factors. Although the exact mechanisms behind the variation in species-specific rates remain unclear, we hypothesize that they may be linked to the distinct metabolic rates found in each species.

Introduction

The lifespan of a species refers to the typical duration of time that individuals within a species can expect to live under natural conditions. For example, it is not uncommon for a human to have a projected lifespan of 90 years, whereas a murine natural lifespan is closer to two years. The lifespan of a species is influenced by both genetic factors and environmental conditions, and it is often difficult to disentangle these two contributors. For example, cows have an artificially short lifespan due to their management in agriculture practices. Wolves tend to have a shorter lifespan than dogs due to the rigors of life in the wild. Similarly, the lifespan of humans has increased significantly over the past centuries due to advances in sanitation and medicine. Here we ask whether it is possible to define a molecular lifespan of a species that is largely independent of environmental effects.

There are many theories that attempt to characterize the aging process and duration of a lifespan. For example, the antagonistic pleiotropy theory proposes that aging is the result of two main factors: (a) the selection of harmful alleles that have beneficial effects early in life but become detrimental with advancing age, and (b) the inability of natural selection to counteract the harmful effects of these alleles with aging. As a consequence of this theory, mutations that extend lifespan are likely to have negative consequences or lower fitness during earlier stages of life[1]. On the other hand, the disposable soma theory suggests that aging is a random process caused by the gradual buildup of molecular damage in somatic cells. This process is offset by repair mechanisms that work to preserve cellular health, albeit at an energy cost. As a result, the rate of aging, varying greatly among species, is inversely correlated with the energy allocated to somatic maintenance[1]. The information theory of aging, also known as the informational entropy theory of aging, is a concept that suggests aging results from the loss of informational integrity in the biological systems of an organism. According to this theory, aging is essentially a process of progressive loss of information necessary to maintain the organism's structure and function. One type of information that is lost is epigenetic, including the loss of DNA methylation pattern of cells that are specified early in life[2].

DNA methylation is an epigenetic regulatory mechanism that influences phenotype without altering genotype. As cells age, they accumulate epigenetic changes through both internal and external mechanisms. Internal processes, like epigenetic drift, involve random events over time, while external processes encompass environmental and stress-related influences[3,4]. These alterations gradually disrupt the cell's biological functions, leading to a subset of changes that exhibit clock-like patterns over time[5].

Consistent with this information theory of aging, cytosine methylation at specific loci in the genome has been found to be a consistently reproducible molecular mark that changes with age. This observation has enabled the development of universal aging predictors applicable to all human tissues[6,7,8]. The subsequent creation of similar epigenetic clock predictors for mice and other species indicates a shared aspect in mammals of the aging process, challenging the idea that aging is solely driven by random cellular damage accumulation over time[8]. While epigenetic clocks can be subtly influenced by environmental effects, they primarily reflect an intrinsic aging process that is occurring at very similar rates across a population.

Previous studies have investigated DNA methylation changes across species to determine whether rates of change are species specific and associated with the duration of lifespans[9].To address this question one study compared the methylation rates of conserved age-related CpG sites in blood and skin from 42 mammalian species, ranging from rats to humans. They found that the methylation rates scaled tightly with maximum lifespan in both the DNA sources, namely blood and skin[10]. The study was restricted to samples that were beyond the age of sexual maturation. Their approach was based on the assumption of a linear

relationship between epigenetic changes and chronological age. However, conclusive evidence shows that epigenetic changes occur nonlinearly, with rates of change being faster earlier in life and slowing with advancing ages[11,12].

To extend linear models of epigenetic aging, the Epigenetic Pacemaker was previously developed to model non-linear age-dependent epigenetic changes[11,13]. Rather than predicting an age based on methylation levels, which is the basis of most epigenetic clocks, the epigenetic pacemaker attempts to model time dependent changes in DNA methylation at specific sites. The underlying model is described by the following equation (equation 1):

$$m_{ij} = m^o + r_i s_j$$

Where, m_{ij} is the methylation level of position i individual j, m_0 represents the methylation level at birth, r_i represents the rate of change of methylation with respect to an underlying epigenetic state s_j. Previous studies have shown that the relationship between epigenetic state and actual age is well described by nonlinear functions such as a square root function in bovine blood samples[14] as well as in dogs[15], while a logarithmic function was used in humans[12].

Here we seek to extend previous studies that relate rates of epigenetic changes with the duration of a lifespan by asking whether the epigenetic pacemaker can be used to derive a general formulation of a rate change in DNA methylation with advancing age across species. The existence of such a model would facilitate estimation of the lifespan of a given species from DNA methylation data alone. This will likely reflect an intrinsic or biological species-specific rate that is largely independent of extrinsic environmental factors.

Methods

DNA METHYLATION DATA

The data for our study was obtained from the Mammalian array consortium[8,16]. We selected blood and skin samples for our current study. From the complete dataset available we selected blood samples from 128 cats, 742 dogs, 208 horses, 530 mice, 188 rats, 277 cows, 153 marmots[8] and 203 humans[17] along with skin samples of 183 killer whales[8].

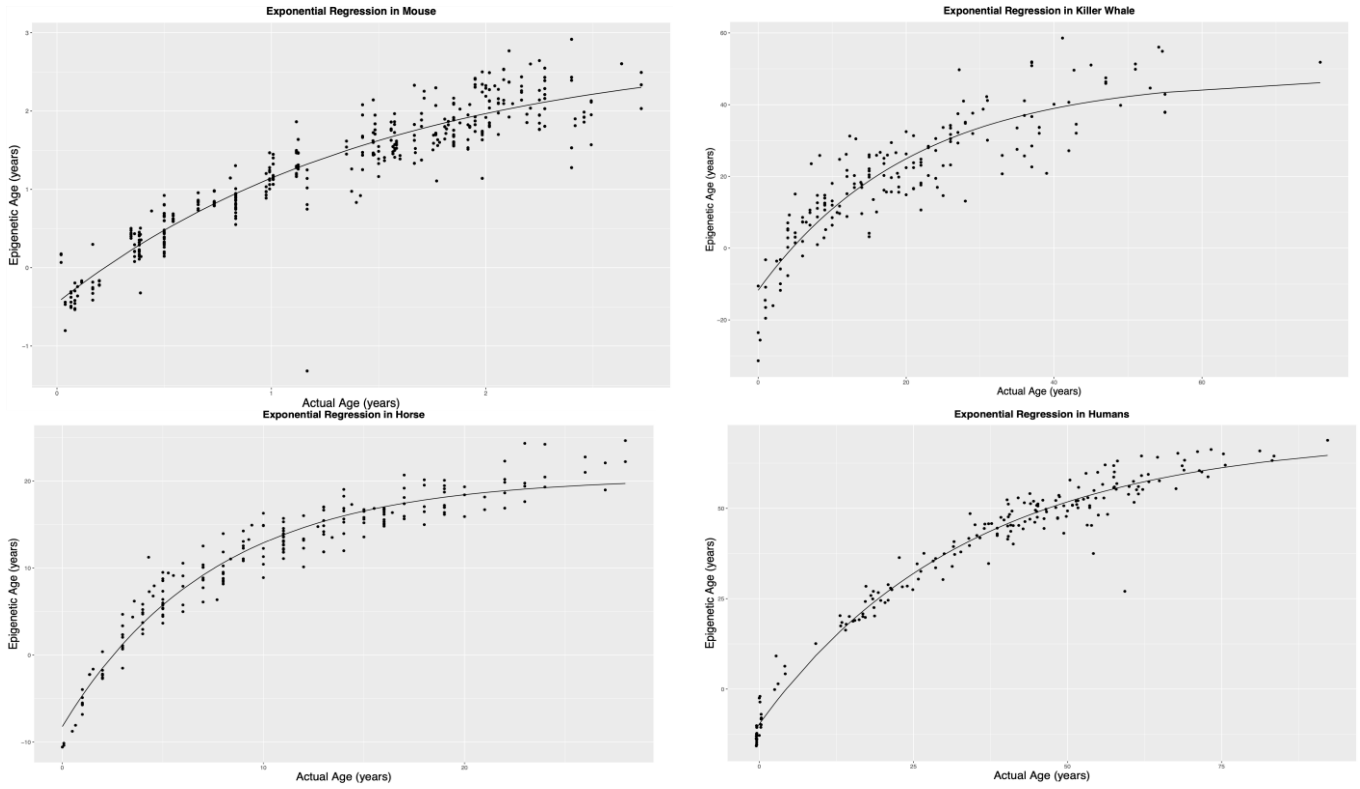
CONSTRUCTION OF EPIGENETIC PACEMAKERS

We used the epigenetic pacemaker methodology to model age associated alterations in DNA methylation for all the mammalian blood and skin samples (Figure1, Supplementary Figure 1). The epigenetic pacemaker models were created using the EPM algorithm implemented as a python package[13]. The methylation data was extracted along with the metadata for each species. Using the Spearman's rank correlation as the metric, we selected the most strongly age-associated sites in each species. The correlation threshold used to select sites was different for each species and was chosen to optimize the correlation between the epigenetic state and actual age of sample collection.

Table 1: Threshold to select sites and optimize the correlation between the epigenetic state and actual age of species.

ORGANISM	NO. OF SAMPLES	SOURCE	AVG. LS	AGE RANGE	EPM LIMIT	NO. OF SITES	R^2	A	B	C
CATS	128	BLOOD	15	0.019-2.72	0.8	566	0.95	15.82	18.42	0.16
DOGS	742	BLOOD	15	0.1-17.5	0.7	739	0.92	17.12	19.00	0.10
HORSE	208	BLOOD	30	0.005-28	0.7	660	0.92	20.39	28.64	0.13
HUMANS	203	BLOOD	80	11.42-92	0.85	202	0.97	69.803	79.49	0.03
MOUSE	530	BLOOD	1.5	0.02-2.72	0.6	226	0.83	2.845	3.29	0.66
RATS	188	BLOOD	3	0.04-2.42	0.8	92	0.77	2.33	2.83	1.14
COWS	277	BLOOD	20	0.51-14.42	0.7	421	0.77	9.72	14.79	0.31
MARMOTS	153	BLOOD	15	0.01-12.04	0.8	118	0.95	11.44	13.76	0.19
KILLER WHALE	183	SKIN	70	0-76	0.7	137	0.79	47.68	59.40	0.05

Figure 1. Epigenetic Pacemaker modeling age associated alterations in DNA methylation for four mammalian blood (mouse, horse and humans) and skin samples (killer whale).



FITTING PARAMETERS OF THE SATURATING EXPONENTIAL FUNCTIONS

We fit the relationship between the epigenetic state and the actual age of each species using a saturating exponential function. The optimal values of the parameters were determined using the R package nls. The final plots were generated using the ggplot package in R studio.

Results

Our aim was to identify a universal model of methylation changes with age across species. To this end we analyzed nine species that span a large range of lifespans: mouse (1.5 years), rat (3 years), cat (15 years), dog (15 years), marmot (15 years), cow (20 years), horse (30 years), killer whale (70 years) and human (80 years). We obtained the methylation data of all these species from previously published DNA methylation datasets[16,17]. We limited the source of the samples to blood for all the species except the killer whale, where the source was skin, since blood was unavailable. The non-human data was collected using the Illumina Mammalian DNA methylation microarray which measures 37,554 CpG sites across the genome[18], whereas the human data was collected using the Illumina HumanMethylation450 (450k) microarray that measures CpG methylation across>485,000 probes[17].

To model age associated changes in DNA methylation we used the Epigenetic Pacemaker[13]. The Epigenetic Pacemaker or EPM does not assume a linear relationship between the epigenetic age and actual chronological age. The EPM approach uses a fast conditional expectation maximization algorithm to minimize the residual sum of squares between the observed and estimated DNA methylation across age associated sites[19]. The selected methylation sites for each species were chosen using the Spearman rank correlation, with a species-specific minimum correlation threshold. We selected a sufficient number of sites to obtain an optimal correlation between the epigenetic age and the actual age (see Methods). Table 1 shows the number of sites along with the correlation threshold used for each individual species. We observed in all the species that the association between methylation and age is non-linear, and could be well fit by a three-parameter saturating exponential function (Figure

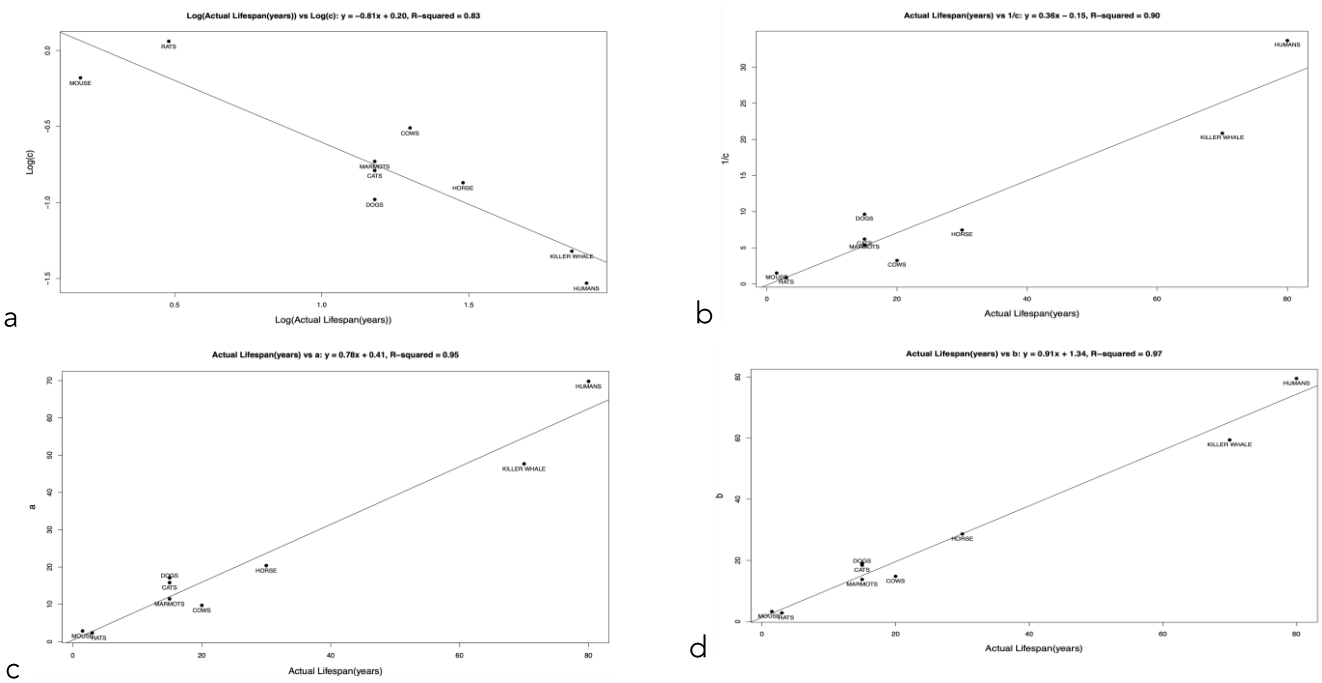
1 and Supplementary Figure 1) as shown in equation 2:

$$eA(t) = a - be^{-ct}$$

We asked whether the *c* parameter, which is the time scale of the exponential function, is related to lifespan. We found that there was a linear relationship between the logarithm of lifespan and the logarithm of *c* (Figure 2a), which implies a power law relationship between the two variables. The exponent of this power law-like relationship is close to negative one, suggesting that the lifespan is proportional to *1/c* (Figure 2b). Moreover, we found that the constant of proportionality between *1/c* and lifespan was 0.36 which we approximated as ⅓ (Figure 2b).

We next examined the relationship between the *a* and *b* parameters of equation 2 and lifespan. We find that both *a* and *b* are linearly related to lifespan, and that the ratio between *a* and *b* is close to 0.9 (Figure 2c, 2d).

Figure 2. (a). Linear relationship between the logarithm of lifespan and the logarithm of *c* implying a power relation between the two variables. (b). Plot showing that the lifespan is proportional to *1/c*. (c). Linear relationship between lifespan and the variable a. (d). Linear relationship between lifespan and variable b.



These observations lead us to propose a general relationship between epigenetic age and the duration of the lifespan of a species as shown in equation 3:

$$eA(t) = LS^*(1 - 0.8e^{\frac{-3t}{LS^*}})$$

Where, *eA* is the epigenetic age and *LS** is the inferred lifespan.

We applied this model to our DNA methylation data and computed the values of lifespan from the DNA methylation trajectories generated using the EPM. The one parameter equation showed a very similar fit with the predicted epigenetic age versus actual age with R squared values of 0.95, 0.92, 0.92, 0.97, 0.83, 0.77, 0.77, 0.95 and 0.79 for cats, dogs, horse, humans, mouse, rats, cows, marmots and killer whale respectively (Figure 3 and Supplementary Figure 3). Finally, we compared the estimated life span of each species based on this model to the reported lifespan and found a significant association between the two (Figure 4) with an R squared of 0.96 and slope of 0.85. The predicted lifespans of each species are reported in Table 2.

Table 2: Predicted lifespan for each species.

ORGANISM	NO. OF SAMPLES	SOURCE	AVG. LS	AGE RANGE	EPM LIMIT	NO. OF SITES	R^2	PREDICTED LIFESPAN
CATS	128	BLOOD	15	0.019-2.72	0.8	566	0.95	19.48
DOGS	742	BLOOD	15	0.1-17.5	0.7	739	0.92	16.53
HORSE	208	BLOOD	30	0.005-28	0.7	660	0.92	27.59
HUMANS	203	BLOOD	80	11.42-92	0.85	202	0.97	78.88
MOUSE	530	BLOOD	1.5	0.02-2.72	0.6	226	0.83	2.91
RATS	188	BLOOD	3	0.04-2.42	0.8	92	0.77	2.99
COWS	277	BLOOD	20	0.51-14.42	0.7	421	0.77	13.95
MARMOTS	153	BLOOD	15	0.01-12.04	0.8	118	0.95	13.19
KILLER WHALE	183	SKIN	70	0-76	0.7	137	0.79	58.08

Figure 3. One parameter equation plot fit with the predicted epigenetic age versus actual age for mammals - mouse, killer whale, horse and humans.

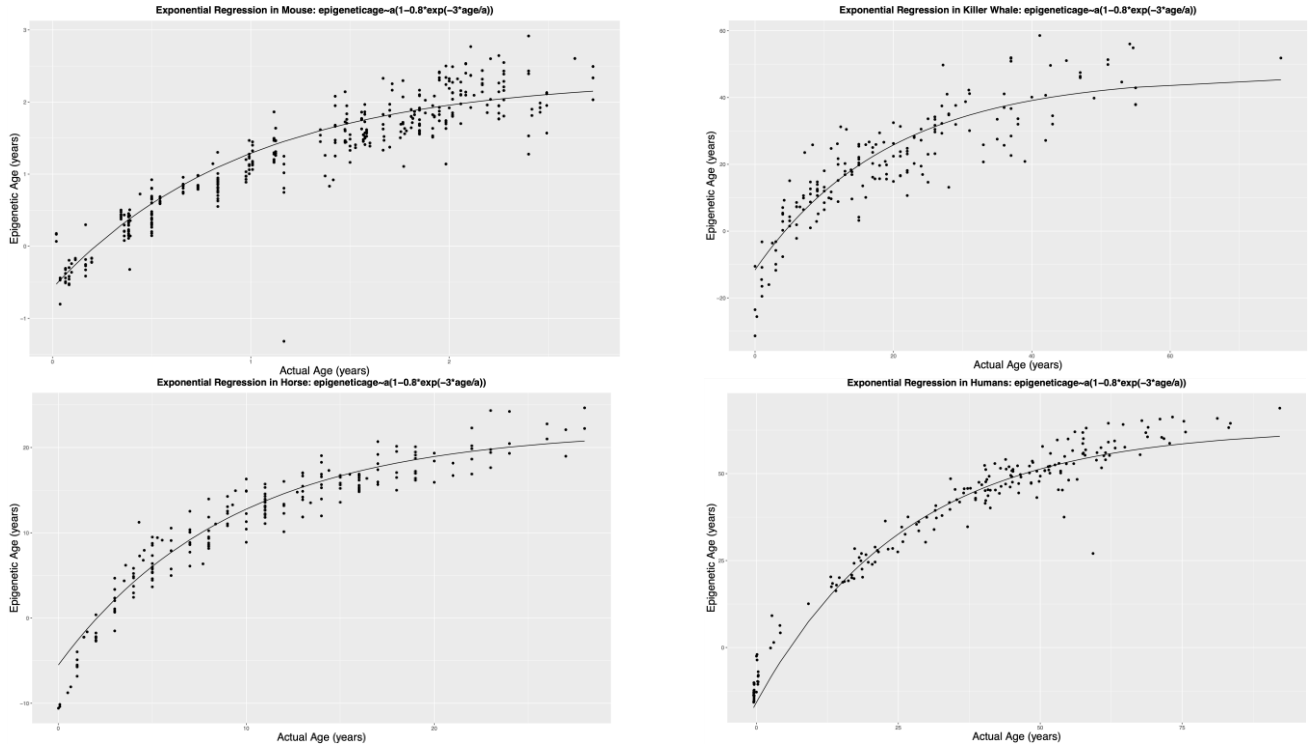
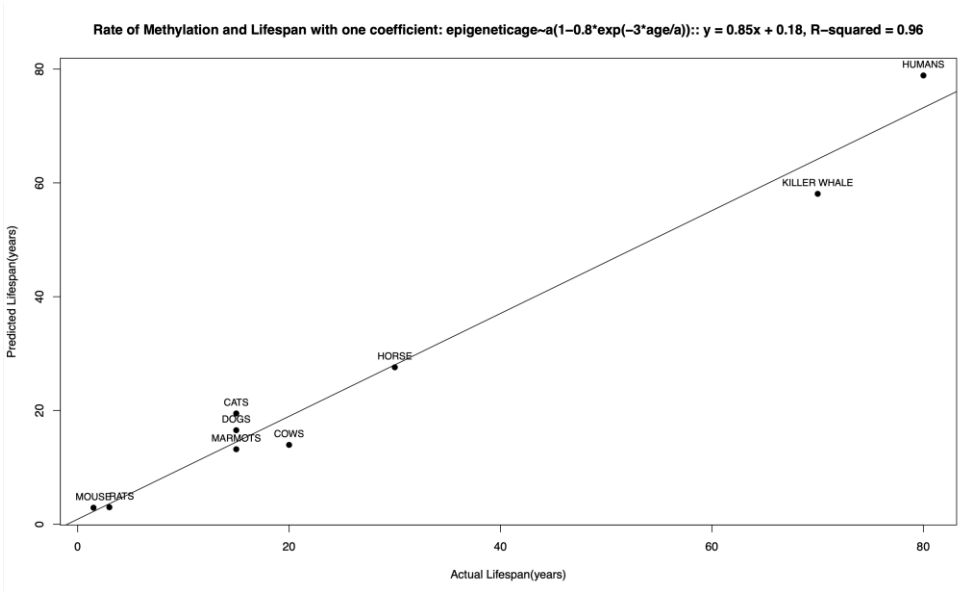
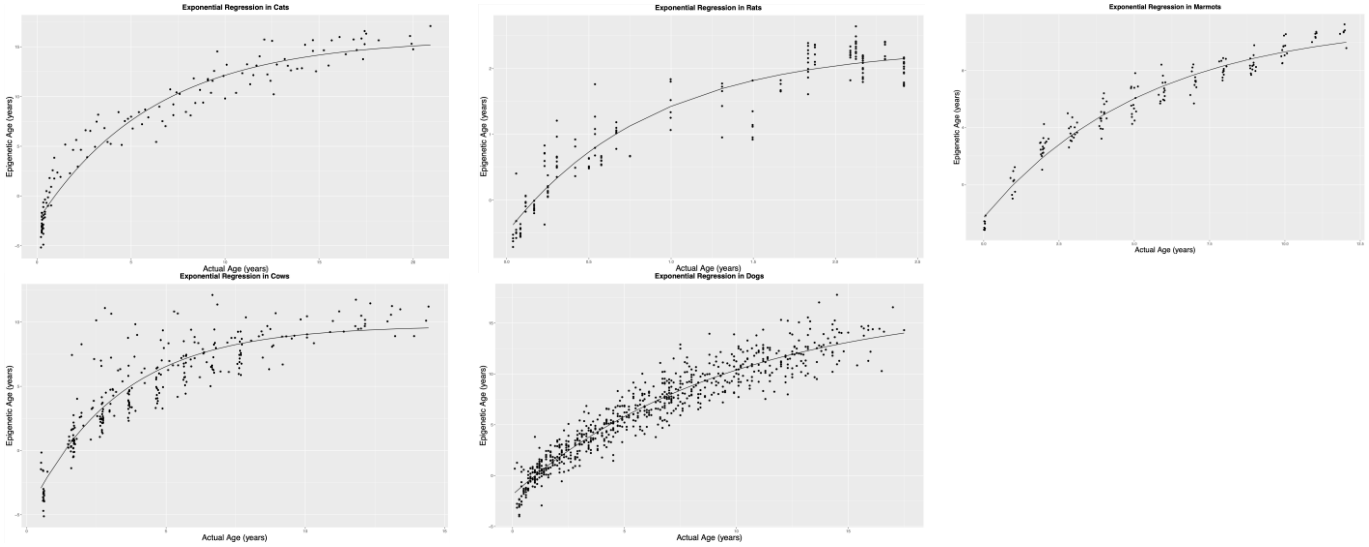


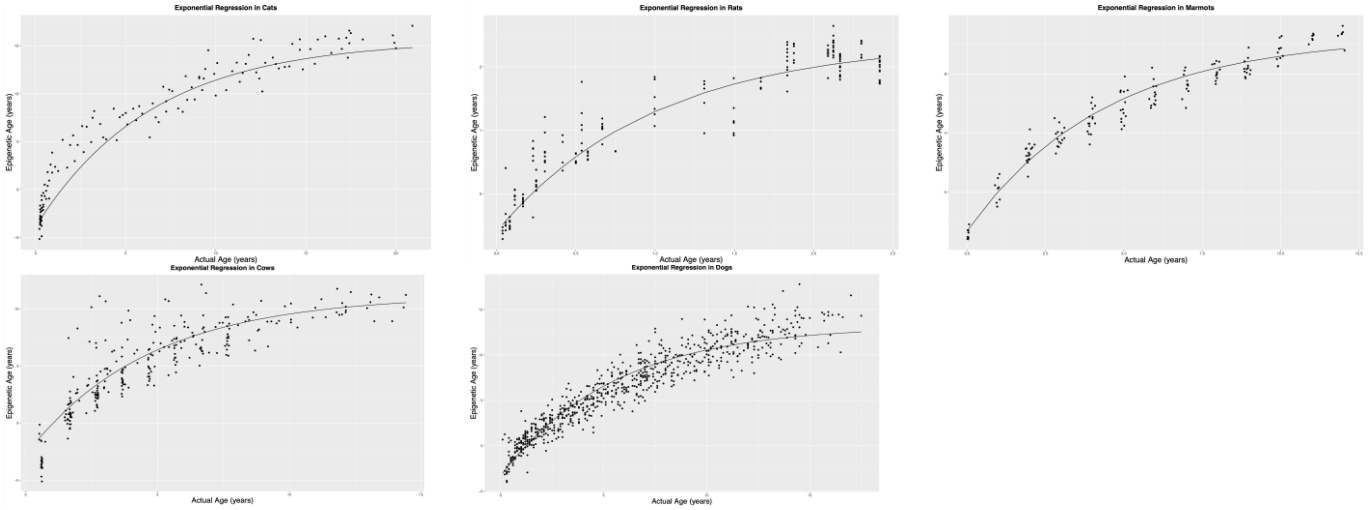
Figure 4. Comparison between the estimated life span of each species based to the reported lifespan.



Supplementary Figure 1. Epigenetic Pacemaker models for the remaining species – Cats, Rats, Marmots, Cows and Dogs.



Supplementary Figure 2. One parameter equation plot fit with the predicted epigenetic age versus actual age for the remaining mammals - Cats, Rats, Marmots, Cows and Dogs.



Discussion

Our study suggests that saturating exponential functions can be used to model DNA methylation dynamics over a lifespan. This suggests that the epigenetic age of a species converges to stable methylation levels as an organism approaches the end of its lifespan. We have previously shown that a simple model of DNA methylation as a chemical reaction with on and off rates will lead to these types of saturating exponential dynamics. This is true even if we average the properties of multiple CpG sites with different underlying kinetics[20].

Building on this view, our present investigation delves into the exponential aging dynamics across diverse species. Our analysis reveals that the patterns of DNA methylation follow saturating exponential dynamics across a wide array of species, spanning from mice to humans. We find that these dynamics can be well approximated by a universal saturating exponential function with a single species-specific parameter. We observe that the timescale parameter of the exponential dynamics is approximately one third of a species' lifespan. We hypothesize that the lifespan derived from these trajectories represents an intrinsic molecular lifespan of a species that is largely independent of environmental effects, and should not be significantly affected even when lifespans are curtailed by unanticipated accidents, predation or disease. Therefore, we would predict that this molecular lifespan should remain largely

unchanged when genetically similar domesticated and wild species are compared (such as dogs and wolves) even though their actual average lifespan may be quite different due to risks associated with life in the wild for the latter. We find that under this model, the molecular lifespan of humans is approximately 80 years, which is remarkably close to the current global lifespan that has emerged over the past few decades as disease risks have been minimized across the globe[21]. Moreover, we find that our predicted molecular lifespan is quite close to the typical lifespan of many captive animals, such as that of dogs and cats.

This simple formulation of the dynamics of epigenetic changes across species leads us to ask what factors may be regulating the species-specific timescales (and hence lifespans). To date it is still not clear what molecular processes determine the lifespan of a species. Many prior studies have demonstrated that lifespan is associated with metabolic rates, with longer living species having slower metabolic rates than shorter living species[22]. Under this model, the timescales of the exponential functions we observe may be a manifestation of the underlying metabolic rates of species.

This hypothesis generated from our present study, leads us to speculate about the factors that serve as regulators of species-specific metabolic rates. One explanation that has emerged in recent years is based on investigations related to the rate of development, rate of biochemical reactions, and the dynamics of epigenetic changes across species[23]. This hypothesis is that the mitochondria, the powerhouse of the energy metabolism, may also act as a chronometer of metabolism. Under this model the mitochondria may regulate the cadence of various developmental and biochemical processes that are essential for the kinetics of development[23]. One could therefore speculate that the species-specific timescales of epigenetic aging may be a manifestation of cellular metabolic rates regulated by the mitochondria.

In the future we may use these models to understand whether these species-specific timescales can be altered by environmental or pharmaceutical interventions. The same could also be conducted on organisms other than mammals to examine whether similar trends hold. It is well established that caloric restriction can alter metabolic rates and lifespans in short lived mammals[24]. Therefore, there may also be interventions that slow the intrinsic epigenetic aging in longer-living mammals as well, although these are largely yet to be discovered.

Conclusion

Our study demonstrates that DNA methylation dynamics can be universally modeled using saturating exponential functions, revealing that the timescale parameter is approximately one-third of a species' lifespan. This suggests an intrinsic molecular lifespan largely unaffected by environmental factors. Our findings indicate that the molecular lifespan of humans is around 80 years, aligning with current global averages and reinforcing the accuracy of our model across diverse species, including domesticated and wild animals. We speculate that metabolic rates, possibly regulated by mitochondrial activity, may influence these timescales, opening avenues for interventions that could extend lifespans by modifying intrinsic epigenetic aging processes.

Conflict of Interest:

None

Funding Statement:

None.

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None.

References:

1. Taormina, Giusi, Federica Ferrante, Salvatore Vieni, Nello Grassi, Antonio Russo, and Mario G. Mirisola. "Longevity: Lesson from Model Organisms." *Genes* 10, no. 7 (July 9, 2019): 518. https://doi.org/10.3390/genes10070518.

2. Yang Jae-Hyun, Motoshi Hayano, Patrick T. Griffin, João A. Amorim, Michael S. Bonkowski, John K. Apostolides, Elias L. Salfati, et al. "Loss of Epigenetic Information as a Cause of Mammalian Aging." *Cell* 186, no. 2 (January 19, 2023): 305-326.e27. https://doi.org/10.1016/j.cell.2022.12.027.

3. Declerck Ken, and Wim Vanden Berghe. "Back to the Future: Epigenetic Clock Plasticity towards Healthy Aging." *Mechanisms of Ageing and Development*, Epigenetics in Ageing and Development, 174 (September 1, 2018): 18–29. https://doi.org/10.1016/j.mad.2018.01.002.

4. Duan Ran, Qiaoyu Fu, Yu Sun, and Qingfeng Li. "Epigenetic Clock: A Promising Biomarker and Practical Tool in Aging." *Ageing Research Reviews* 81 (November 1, 2022): 101743. https://doi.org/10.1016/j.arr.2022.101743.

5. Yu Ming, William D. Hazelton, Georg E. Luebeck, and William M. Grady. "Epigenetic Aging: More Than Just a Clock When It Comes to Cancer." *Cancer Research* 80, no. 3 (February 1, 2020): 367–74. https://doi.org/10.1158/0008-5472.CAN-19-0924.

6. Horvath Steve, Joseph A. Zoller, Amin Haghani, Anna J. Jasinska, Ken Raj, Charles E. Breeze, Jason Ernst, Kelli L. Vaughan, and Julie A. Mattison. "Epigenetic Clock and Methylation Studies in the Rhesus Macaque." *GeroScience* 43, no. 5 (September 6, 2021): 2441–53. https://doi.org/10.1007/s11357-021-00429-8.

7. Jasinska Anna J., Amin Haghani, Joseph A. Zoller, Caesar Z. Li, Adriana Arneson, Jason Ernst, Kylie Kavanagh, et al. "Epigenetic Clock and Methylation Studies in Vervet Monkeys." *GeroScience* 44, no. 2 (September 30, 2021): 699–717. https://doi.org/10.1007/s11357-021-00466-3.

8. Lu A. T., Z. Fei, A. Haghani, T. R. Robeck, J. A. Zoller, C. Z. Li, R. Lowe, et al. "Universal DNA Methylation Age across Mammalian Tissues." *Nature Aging* 3, no. 9 (2023): 1144–66. https://doi.org/10.1038/s43587-023-00462-6.

9. Li, Caesar Z., Amin Haghani, Qi Yan, Ake T. Lu, Joshua Zhang, Zhe Fei, Jason Ernst, et al. "Epigenetic Predictors of Species Maximum Life Span and Other Life-History Traits in Mammals." *Science Advances* 10, no. 23 (June 7, 2024): eadm7273. https://doi.org/10.1126/sciadv.adm7273.

10. Crofts, Samuel J. C., Eric Latorre-Crespo, and Tamir Chandra. "DNA Methylation Rates Scale with Maximum Lifespan across Mammals." *Nature Aging* 4, no. 1 (2024): 27–32. https://doi.org/10.1038/s43587-023-00535-6.

11. Snir Sagi, Colin Farrell, and Matteo Pellegrini. "Human Epigenetic Ageing Is Logarithmic with Time across the Entire Lifespan." *Epigenetics* 14, no. 9 (June 6, 2019): 912–26. https://doi.org/10.1080/15592294.2019.1623634.

12. Dufek Grant, Guy Katriel, Sagi Snir, and Matteo Pellegrini. "Exponential Dynamics of DNA Methylation with Age." *Journal of Theoretical Biology* 579 (February 21, 2024): 111697. https://doi.org/10.1016/j.jtbi.2023.111697.

13. Farrell Colin, Sagi Snir, and Matteo Pellegrini. "The Epigenetic Pacemaker: Modeling Epigenetic States under an Evolutionary Framework." *Bioinformatics* 36, no. 17 (June 23, 2020): 4662–63. https://doi.org/10.1093/bioinformatics/btaa585.

14. Ratan Prashansa, Liudmilla Rubbi, Michael Thompson, Kaushik Naresh, Jolena Waddell, Barbara Jones, and Matteo Pellegrini. "Epigenetic Aging in Cows Is Accelerated by Milk Production." *Epigenetics* 18, no. 1 (n.d.): 2240188. https://doi.org/10.1080/15592294.2023.2240188.

15. Rubbi Liudmilla, Haoxuan Zhang, Junxi Feng, Christopher He, Patrick Kurnia, Prashansa Ratan, Aakash Tammana, et al. "The Effects of Age, Sex, Weight, and Breed on Canid Methylomes." *Epigenetics* 17, no. 11 (n.d.): 1497–1512. https://doi.org/10.1080/15592294.2022.2069385.

16. Haghani Amin, Caesar Z. Li, Todd R. Robeck, Joshua Zhang, Ake T. Lu, Julia Ablaeva, Victoria A. Acosta-Rodríguez, et al. "DNA Methylation Networks Underlying Mammalian Traits." *Science (New York, N.Y.)* 381, no. 6658 (August 11, 2023): eabq5693. https://doi.org/10.1126/science.abq5693.

17. Jaffe Andrew E., Yuan Gao, Amy Deep-Soboslay, Ran Tao, Thomas M. Hyde, Daniel R. Weinberger, and Joel E. Kleinman. "Mapping DNA Methylation across Development, Genotype and Schizophrenia in the Human Frontal Cortex." *Nature Neuroscience* 19, no. 1 (January 2016): 40–47. https://doi.org/10.1038/nn.4181.

18. Arneson, Adriana, Amin Haghani, Michael J. Thompson, Matteo Pellegrini, Soo Bin Kwon, Ha Vu, Emily Maciejewski, et al. "A Mammalian Methylation Array for Profiling Methylation Levels at Conserved Sequences." *Nature Communications* 13, no. 1 (February 10, 2022): 1–13. https://doi.org/10.1038/s41467-022-28355-z.

19. Snir, Sagi, Bridgett M. vonHoldt, and Matteo Pellegrini. "A Statistical Framework to Identify Deviation from Time Linearity in Epigenetic Aging." Edited by Kevin Chen. *PLOS Computational Biology* 12, no. 11 (November 11, 2016): e1005183. https://doi.org/10.1371/journal.pcbi.1005183.

20. Dufek Grant, Guy Katriel, Sagi Snir, and Matteo Pellegrini. "Exponential Dynamics of DNA Methylation with Age." *Journal of Theoretical Biology* 579 (February 21, 2024): 111697. https://doi.org/10.1016/j.jtbi.2023.111697.

21. Garmany, Armin, Satsuki Yamada, and Andre Terzic. "Longevity Leap: Mind the Healthspan Gap." *NPJ Regenerative Medicine* 6 (September 23, 2021): 57. https://doi.org/10.1038/s41536-021-00169-5.

22. Bartke Andrzej, Savannah Brannan, Erin Hascup, Kevin Hascup, and Justin Darcy. "Energy Metabolism and Aging." *The World Journal of Men's Health* 39, no. 2 (April 2021): 222–32. https://doi.org/10.5534/wjmh.200112.

23. Lázaro Jorge, Maria Costanzo, Marina Sanaki-Matsumiya, Charles Girardot, Masafumi Hayashi, Katsuhiko Hayashi, Sebastian Diecke, et al. "A Stem Cell Zoo Uncovers Intracellular Scaling of Developmental Tempo across Mammals." *Cell Stem Cell* 30, no. 7 (July 6, 2023): 938-949.e7. https://doi.org/10.1016/j.stem.2023.05.014.

24. Pifferi, Fabien, Jérémy Terrien, Julia Marchal, Alexandre Dal-Pan, Fathia Djelti, Isabelle Hardy, Sabine Chahory, et al. "Caloric Restriction Increases Lifespan but Affects Brain Integrity in Grey Mouse Lemur Primates." *Communications Biology* 1, no. 1 (April 5, 2018): 1–8. https://doi.org/10.1038/s42003-018-0024-8.

