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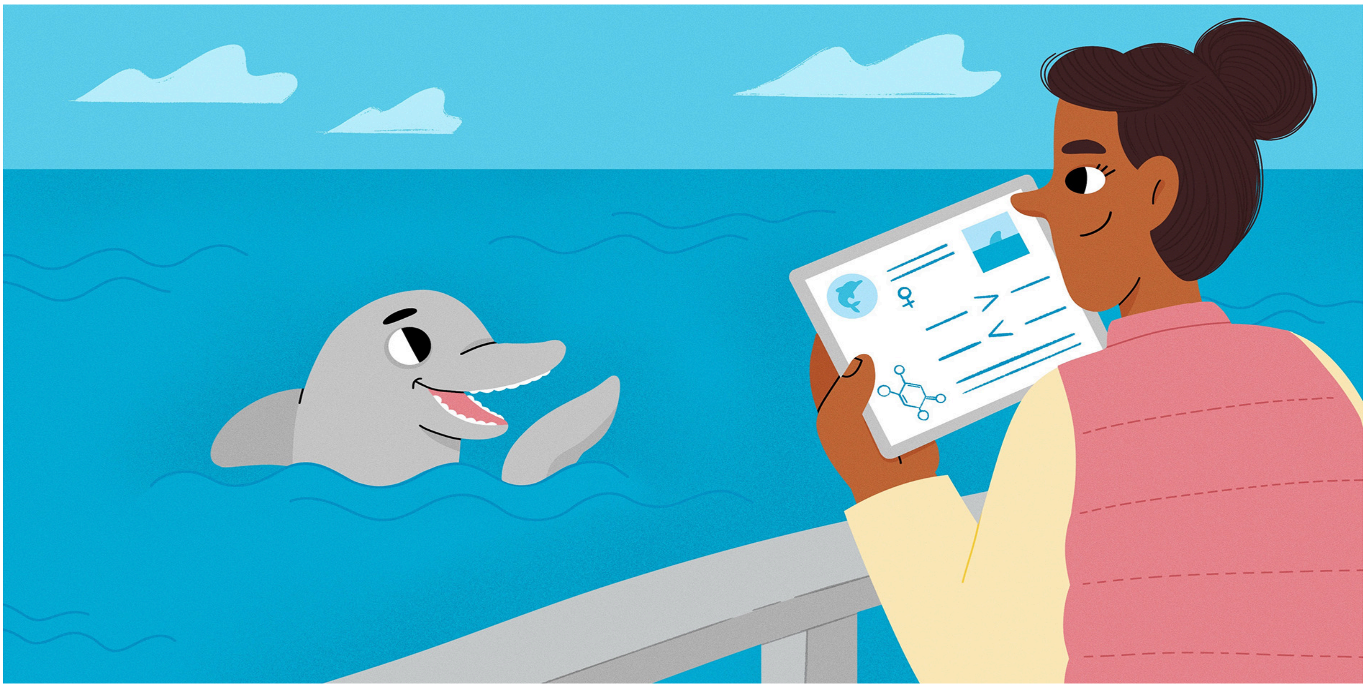
Published: 30 May 2024
doi: 10.3389/frym.2024.1342551



EPIGENETIC CLOCKS: USING DNA TO ESTIMATE THE AGE OF DOLPHINS

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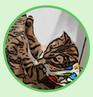
YOUNG REVIEWERS:


BENJAMIN
AGE: 11


GEMMA
AGE: 8


JACE
AGE: 10


KINABALU INTER-NATIONAL SCHOOL
AGES: 9–11


VERALYN
AGE: 12

You probably know your age and you might be good at estimating the ages of other people. However, have you ever tried to guess the age of an animal, for example a dolphin? Estimating the age of dolphins is really difficult, because their appearance does not change once they are fully grown. Fortunately, the shape of their DNA changes over time. This allowed us to develop a tool, called an epigenetic clock, to determine the age of dolphins based on their DNA. Using this clock, we can estimate the age of a dolphin with an accuracy of 2 years. Although it is not exact, this still helps us answer questions about the lives of dolphins, such as when they start their own families. Knowing dolphin ages can also tell us if a group of dolphins contains a healthy mix of older and younger individuals. Epigenetic clocks are now being developed for many other species, offering exciting new research opportunities.

HOW TO ESTIMATE THE AGE OF DOLPHINS

When studying animals, one of the most important pieces of information to know about individual animals is their age. Scientists must know how old an animal is to understand many parts of its life history and, for example, to estimate if a population of animals can survive. But estimating an animal's age is not easy when its date of birth is unknown. Across all animal species, there are no general signs for the aging process, such as the wrinkles or gray hair seen in humans. Because there are no visible signs, it is difficult to know the age of fully grown animals, especially if they live for a very long time. For example, how would you be able to tell if a dolphin is 20 or 30 years old?

Whales and dolphins are a particular challenge because they move around a lot and are difficult or forbidden to capture [1]. A good way to estimate a dolphin's age is to pull out a tooth and look at the growth layers of its **dentine**, like you would count the rings in the trunk of a tree. This way, teeth can help scientists find a dolphin's age quite accurately. However, this method is not pleasant for the dolphins and therefore difficult to do with live animals. For some species, such as Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), a pattern of spots on their skin can tell us about their age, because they gain more spots as they grow older (Figure 1). However, individuals tend to have different amounts of spots in general, and it is often quite difficult to see enough of a dolphin's body to clearly see and count the spots. So, trying to age an animal based on its spotting pattern will only give us a rough estimate.

DENTINE

Hard, dense, bony tissue forming most of a tooth, beneath the outer coating of protective enamel.

Because of these difficulties, most studies rely on data collected over a long time, in studies where animals have been regularly observed for many years since their birth, and thus their ages are known. For species that can live for many years, it can be difficult to gather enough data in a human's lifetime.

Figure 1

Figure 1

Indo-pacific bottlenose dolphin with speckling pattern. Photo by Simon Allen.

PHOTO-IDENTIFICATION

A method of identifying individual dolphins using photos of their dorsal fins. The scars and marks on their fins make them unique, like a fingerprint in humans.

BIOPSY SAMPLING

A data collection technique where we take a small skin sample from a live dolphin using a little dart with a sharp hollow end.

DORSAL FIN

The fin on the back of a whale or dolphin.

EPIGENETICS

The study of how our genes are affected by our behaviors and the environment we are exposed to.

DNA METHYLATION

Small molecules are added to the DNA to help tell the cells in our body which parts of the DNA to read and use, like how bookmarks help us find the right page in a book. These tags can turn genes on or off, which helps control how our bodies grow and work.

SHARK BAY DOLPHINS

One of the places where dolphins have been studied for many years is Shark Bay, Western Australia. There, scientists have observed a population of Indo-Pacific bottlenose dolphins for more than 30 years [2], using techniques called **photo-identification** and **biopsy sampling**. For photo-identification, researchers take photos of the dolphins' **dorsal fins**. The shape of the fin is like a human fingerprint, and by comparing the fins of different animals, researchers can recognize individuals. Biopsy sampling means collecting a small piece of dolphin skin that can be analyzed in the lab to get the animals' DNA. Because we have been studying this dolphin population for a long time and we recognize the individual dolphins, we also know when one is born and, therefore, can calculate its age. Thanks to biopsy sampling we have small skin samples from all the dolphins we study.

WHAT IS EPIGENETICS?

Our dolphin DNA samples have opened doors to new methods of age estimation for our dolphin species, using **epigenetics**. Epigenetics is the study of how the environment and an individual's behaviors can affect the way their genes work. Epigenetic changes modify the shape of the DNA, which alters which genes are switched on or turned off. This shape change can happen by adding or removing small molecules to the DNA—a process called **DNA methylation**. As animals age, different sets of genes are switched on or "expressed", meaning that the DNA takes on different shapes throughout an animal's life (Figure 2).

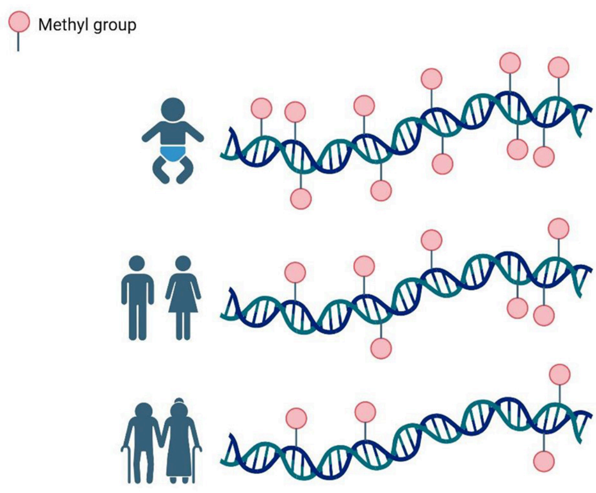


Figure 2

Figure 2

DNA (blue spiral) loses methylation as individuals age. A baby has the most methyl groups attached to its DNA, while adults have less than babies. Older people have the lowest number of methyl groups attached to their DNA. The same can be seen in dolphins and many other animals. These age-related differences in DNA methylation led us to develop our epigenetic clock to determine the ages of dolphins from the DNA found in tiny skin samples.

EPIGENETIC CLOCK

A test that looks at small chemical markers on our DNA to figure out our age. It is based on changes of these markers, called methyl groups, with age.

MACHINE LEARNING

A type of artificial intelligence that learns from data.

HOW DOES THE EPIGENETIC CLOCK WORK?

Aging changes the levels of DNA methylation. Newborn dolphin calves have the highest levels of DNA methylation, while old individuals have the lowest. This change in methylation is also referred to as the **epigenetic clock**. Using DNA from skin samples, we measured the levels of DNA methylation in dolphins of a known age, using a new tool called the HorvathMammalMethylChip40 [3], which can measure DNA methylation on up to 37,492 sites on the DNA at once. We then built an epigenetic clock using **machine learning**, which we used to estimate the age of other dolphins of the same species based on their methylation levels (Figure 3). Our clock had an average accuracy of 2.1 years, meaning if we estimated an animal to be 25 years old, it might actually be anywhere between 23–27 years old [4]. This is quite close and therefore a really good result!

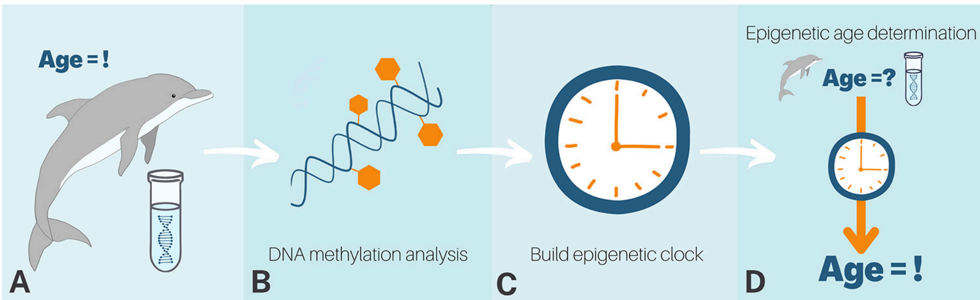


Figure 3

Figure 3

There are 4 basic steps in building/using an epigenetic clock. **(A)** Take genetic samples (for example skin) of animals for which you know the age (Age = !). **(B)** Analyze the DNA methylation of these samples. **(C)** Build the clock by connecting the age of the dolphins to the amount of DNA methylation in each sample. **(D)** Using the clock: now you can analyze a sample from a dolphin for which you do not know the age (Age = ?). Based on the amount of DNA methylation, the clock will estimate how old the dolphin is (Age = !).

We also tested how well a different clock, developed using skin samples from a related species (common bottlenose dolphins [5]), worked for our samples. Common bottlenose dolphins look almost the same as Indo-Pacific dolphins but are often a bit bigger. We found that the common bottlenose dolphin clock worked almost as well for our Indo-pacific bottlenose dolphins as for the species it was made for.

WHY IS THE EPIGENETIC CLOCK USEFUL?

Dolphins can live for more than 30 years, and in the past it has been very difficult to gather information from many animals with known ages. By using the epigenetic clock, we can now estimate the age of individual dolphins just based on their DNA, which means we only need a small skin sample from each dolphin, and we are good to go! This makes our clock extremely useful for scientists that study Indo-Pacific bottlenose dolphins but who do not have access to long-term data. The fact that the common bottlenose dolphin clock also worked really well for our species means that even researchers studying other closely related species can use our clock. Because it is expensive to build an epigenetic clock, sharing a clock can save researchers thousands of dollars, which they can then use for other interesting research.

WHAT ARE THE NEXT STEPS?

We now know the age for many dolphins in the Shark Bay population. With this knowledge, we can ask further questions, such as at what time in their lives they produce the most calves or what influences their aging. We can also study whether males and females age differently. As so often in science, an answer leads to many more questions! This is an exciting time for us, and we cannot wait to get deeper into this work!

ACKNOWLEDGMENTS

KP was supported by a Postdoc Grant from the University of Zurich. LG was supported by an Early Postdoc Mobility Grant from the Swiss National Science Foundation (P2ZHP3_200011). The A.H. Schultz Foundation and the Claraz Foundation contributed to the sequencing costs.

ORIGINAL SOURCE ARTICLE

Peters, K. J., Gerber, L., Scheu, L., Cicciarella, R., Zoller, J. A., Fei, Z., et al. 2023. An epigenetic DNA methylation clock for age estimates in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). *Evolut. Applic.* 16:126–33. doi: 10.1111/eva.13516

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SUBMITTED: 22 November 2023; **ACCEPTED:** 14 May 2024;
PUBLISHED ONLINE: 30 May 2024.

EDITOR: Emily King, Xiamen University, China

SCIENCE MENTORS: Milena Salgado-Lynn and Shu Qin Sam

CITATION: Peters KJ and Gerber L (2024) Epigenetic Clocks: Using DNA to Estimate the Age of Dolphins. Front. Young Minds 12:1342551. doi: 10.3389/frym.2024.1342551

CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author of original paper Steve Horvath is a founder of the non-profit Epigenetic Clock Development Foundation, which plans to license several of his patents from his employer UC Regents.

YOUNG REVIEWERS

BENJAMIN, AGE: 11

My name is Benjamin. I love cooking for my family and playing video games during my free time. For sports, I do archery and I have a junior black belt in taekwondo. I am very inquisitive and will ask questions to find out more about things that I do not understand. I also enjoy playing with my cats. I have 3 at home and they are a handful.

GEMMA, AGE: 8

My favorite subject is science and I love almost everything related to the ocean. I am an avid reader of science, mystery, and fantasy books. I read National Geographic Kids and enjoy playing around with their app. I am also interested in Thea and Geronimo Stilton books. I have a special liking for those with jokes and games at the end or the ones that you can help them solve the mystery with the given clues.

JACE, AGE: 10

Hi, Jace here. I am 10 years old. During my free time, I am interested in reading and I love to play soccer. I love science books the most (the cool kind, not boring!). I am currently working on my new microscope which makes tiny things HUGE and my telescope which shows things far away! Numbers are awesome, that's why I maze-ter minds. My jokes are legendary because laughing is nice. Lastly I love funny doodles, mazes, maybe some monster sketches.

KINABALU INTERNATIONAL SCHOOL, AGES: 9–11

We are a bunch of regular students who aspire to be very unique scientists one day. We are in Borneo and go to Kinabalu International School. Our aim is to try to make this world a better place through educating children like ourselves while exploring science and making new discoveries, trying to see what is possible., We want people in the world to know and love science and bring everybody's knowledge to new heights.

VERALYN, AGE: 12

I enjoy listening to music especially KPOP and Taylor Swift. I enjoy diving and singing. I love the ocean and love going to the beach. Dolphins and whales have always been a firm favorite favourite of mine.

AUTHORS

KATHARINA J. PETERS

I am a German-Australian marine ecologist and live in Australia. I have studied many animal species, but my main focus is marine mammals. My core research interest is to study the effects of humans on marine mammals and to use this information to better manage the conservation of wild populations and their environments. My projects focus on Weddell seals in Antarctica, and various species of whales and dolphins in Australia and New Zealand. *katharina_peters@uow.edu.au

LIVIA GERBER

I am a wildlife geneticist at the Australian National Wildlife Collection at CSIRO—Australia's National Science Agency. My job is to apply and develop genetic methods to learn more about animals. Using genetics, I am answering questions of why animals behave and look the way they do, live where they live, and how old they are. Over the last years, I have studied male friendships in dolphins, developed a method to age dolphins, and I am now developing epigenetic age estimators for reptiles. *livia.gerber@csiro.au