How reliable are genomes from ancient DNA?

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Many reports of ancient DNA (aDNA) assert recovery from specimens with age assignments that greatly exceed Scripture's age of the world. Some of these age assignments even exceed the maximum time estimated by the most accurate DNA decay rate studies. Furthermore, the quality of DNA (fragment length and degeneration) recovered in samples allegedly 50,000–300,000 years of age is similar to that recovered from sites assigned an age one or two orders of magnitude younger. Here we summarize the results from a variety of such reports, comparing the data with that expected of maximum longevity. In the process, we reveal an almost nefarious tactic that some researchers may have used to circumvent the age implications from DNA's decay rate. We also show that contamination from modern human DNA in archaic human sequences exists at levels impractical to ascertain. Some secular age assignments for aDNAs exceed those expected by DNA decay rates while others do not, emphasizing the need to investigate each instance.

After cell death, DNA steadily decays. If microbes do not consume it (biodegradation), then spontaneous chemical reactions occur to alter and break it apart. Some of these reactions transform one base into another (C-to-T and G-to-A substitutions), with the most common being cytosine to uracil (deoxyuracils arise from cytosine deamination). Other reactions cleave the molecule's phosphodiester backbone bonds either in single or multiple chemical steps.[1] "Spontaneous hydrolysis and oxidation result in double-strand breaks, abasic sites, and nucleotide modifications or miscoding lesions."[2] Of course, God equipped cellular life with a host of DNA error-detection and repair mechanisms that mitigate this damage in living cells, but *post mortem,* basic chemistry takes over. One immediate result of DNA decay is that the very long, thread-like molecule breaks down into shorter fragments over time. Like most biomolecular decay rates, this process generally occurs faster at higher temperatures.

Thanks in part to researcher Michael Collins, we have hard numbers to quantify the DNA decay rate due to the various spontaneous chemical reactions combined. Collins heads a research group at the University of York, called BioArch, that primarily investigates old proteins. He is probably the world's leading researcher on *post mortem* biomolecular decay, and he supervised a 2008 *Science* paper that critiqued Mary Schweitzer's earliest report of collagen protein from *Tyrannosaurus rex* bone.[3] Collins *et al.* argued that because their decay studies unequivocally showed that collagen cannot last longer than a million years, collagen fragments from a 68–Ma-old dinosaur was extraordinarily unlikely. In 2012, Collins worked with a group, including Morten Allentoft as lead author—an expert on characterizing biomolecules from moa egg shell fossils—that rigorously defended an experimentally derived DNA decay rate in a significant paper published in the *Proceedings of the Royal Society*.[4] However, the team used carbon dates to anchor the DNA decay measurements. Supplementary materials from the paper reveal that their 158 moa bones from New Zealand ranged from 602 to 5,791 carbon years.

Because biblical post-Flood history limits the oldest of these post-Flood materials to about 4,000 years (allowing a few generations for the birds to migrate from the ark), 5,791 is not historical, and represents an excessively old carbon age. This means that their determined half-life for DNA (specifically, the control region of more decay-resistant mitochondrial DNA) is somewhat higher than it should be. Further research might estimate just how much higher, perhaps by more closely adjusting carbon years to calendar years, then appropriately shortening the Allentoft *et al.* DNA decay rate calculation, but this is beyond the scope of this summary article. Similarly, DNA forensics data might also permit us to recalibrate the Allentoft *et al.* DNA decay rate to a more accurate, and very likely higher, number, ultimately shrinking the time expected for DNA to last *in situ*. For example, forensics analysts admit that "long PCR amplicons are difficult to amplify in degraded materials that are commonly found at crime scenes".[5] If crime scene DNA is already degraded after only a few years or decades, then how much more degraded should ancient DNA be after hundreds of thousands of years? Results like these make the Allentoft *et al*. DNA half-life seem excessively large. Nevertheless, until future research incorporates factors like DNA forensics research findings and correct calibration of carbon years into biblical calendar years, we can at present take the Allentoft *et al.* determination to represent the absolute maximum expected decay times for the sake of argument. This way we can begin to identify those claims of recovered ancient DNA that pre-date by far the oldest expected ages. Table 1 shows some of their results.

At least two features of DNA decay follow from these results. First, longer DNA segments have shorter half-lives. Their higher numbers of bonds elevate the probability of spontaneous degradation chemistry for the overall molecule.

Second, temperature significantly affects DNA decay rates. Well-frozen DNA might last over a million years—probably even after reducing DNA's half-life with corrected carbon dates.

The average annual temperature of most sites where DNA has been extracted from bone lie between five and fifteen degrees Celsius. Since this represents such a wide range of temperature-influenced times until DNA disintegration, obtaining more highly resolved information would be useful to compare with conventional age assignments for aDNAs. Therefore, Thomas applied a logarithmic trend-line to four data points from Allentoft *et al.* to plot the DNA disintegration times versus temperature (figure 1).

Accordingly, we should expect mitochondrial DNA, which Allentoft *et al.* found lasted longer than nuclear DNA, to have zero backbone bonds after approximately 350,000 years at 10˚C, as indicated by the vertical line in figure 1. However, we should also expect mtDNAs to degrade to an average length of 30 base pairs (bp) long before 350,000 years. Why would 30bp be significant? Ancient DNA re-covery experts now claim they can sequence lengths as short as 30bp. Any shorter and the PCR primers would have too small a molecular handle to attach.

Human aDNA: modern or ancient?

Some distrust the use of aDNA technology to assemble whole genomes that credibly represent real archaic humans like Neandertals and Denisovans. Author Tomkins has been analysing archaic human genomic sequences (both assembled sequence and trace reads) (figure 1) for several years and due to the poor quality of the data, finally gave up working with it. Researchers that produce and process these sequences have to do a significant amount of end trimming and error correction for degraded DNA. And a large proportion of the sequence (up to 98%) must be eliminated due to non-human microbial DNA contamination. These techniques and problems have been noted openly in many of the archaic human genome publications.

In Tomkins' estimation, the Neandertal and Denisovan sequences were not as trustworthy as DNA sequence derived from living organisms. The first public posting of data for the most recent Neandertal genome assembly contained a fairly complete Y-chromosome assembly. However, the researchers removed the Y-chromosome before publishing the genome and claimed it was from a female.[6] Clearly something was amiss. While the researchers later 'explained' that this was due to some misplaced X-chromosome sequences, the fact remains that nearly an entire human Y-chromosome was present at one point (not just a few errantly placed genes) in a genome assembly that later somehow morphed into a female. Contamination of Neandertal with modern (male) human DNA from lab workers explains the issue. In fact, a recent paper showed that non-primate DNA databases commonly contain up to 10% human DNA contamination.[7] Such contamination would be nearly impossible to accurately discern, despite the claims to the contrary, since comparison with modern human sequence—the very sequence most likely to contaminate samples—is the standard of reference for assembling the genomes.

In the most recent Neandertal genome paper, the re-searchers allege that they have solved the contamination prob-lem by targeting certain 'diagnostic sites' in mtDNA, autosomal DNA, or Y-chromosome DNA that they claim will tell them how much modern human DNA contamination exists, which they estimated at about 1 to 5% in their most recent effort.[6] These alleged diagnostic sites may simply represent lost or unknown variation in the modern human genome. Despite the recent completion of the 1,000 Genomes project, the modern human genome is only beginning to be characterized globally across all ethnic groups and our knowledge of variation in the Neandertal genome is minimal.[8] Importantly, this strategy cannot provide a reliable test for every single read produced by the sequencers prior to genome assembly because only a few reads (sequenced lengths of DNA) would overlap the diagnostic sites. Nevertheless, the 'diagnostic sites' model has been promoted as the best way to diagnose modern human DNA contamination for several years.[9] In reality, ascertaining any accurate level of contamination with modern humans is, for all practical purposes, thus far impossible (figure 2).

Interestingly, some scientists have promoted only using sequences that explicitly show age-related damage as a diagnostic means prior to assembly into larger contiguous

Table 1. Data modified from Allentoft *et al.*[4] Half-life measurements were taken based on double-strand DNA breaks that shorten the overall strand length. 'Complete decay', shown in the fifth column, estimates the length of time at each given temperature until no phosphodiester bonds hold any nucleotides together. Allentoft *et al.* related temperature to decay rate using an Arrhenius equation, ln k = 41.2 – 15,267.6 x 1/T, where k is the experimentally determined rate constant and T is temperature in degrees Celsius. They calculated average read length as the inverse of the fraction of DNA damaged after 10,000 years of decay at each temperature's half-life.

Temperature in degrees Celsius	Half-life for a 30-base-pair seg.	Half-life for a 100-base-pair seg.	Average length after 10,000 years	Years until complete decay
15	3,000 yrs	900 yrs	13 base pairs	131,000
5	20,000 yrs	6,000 yrs	88 base pairs	882,000
–5	158,000 yrs	47,000 yrs	683 base pairs	6,830,000

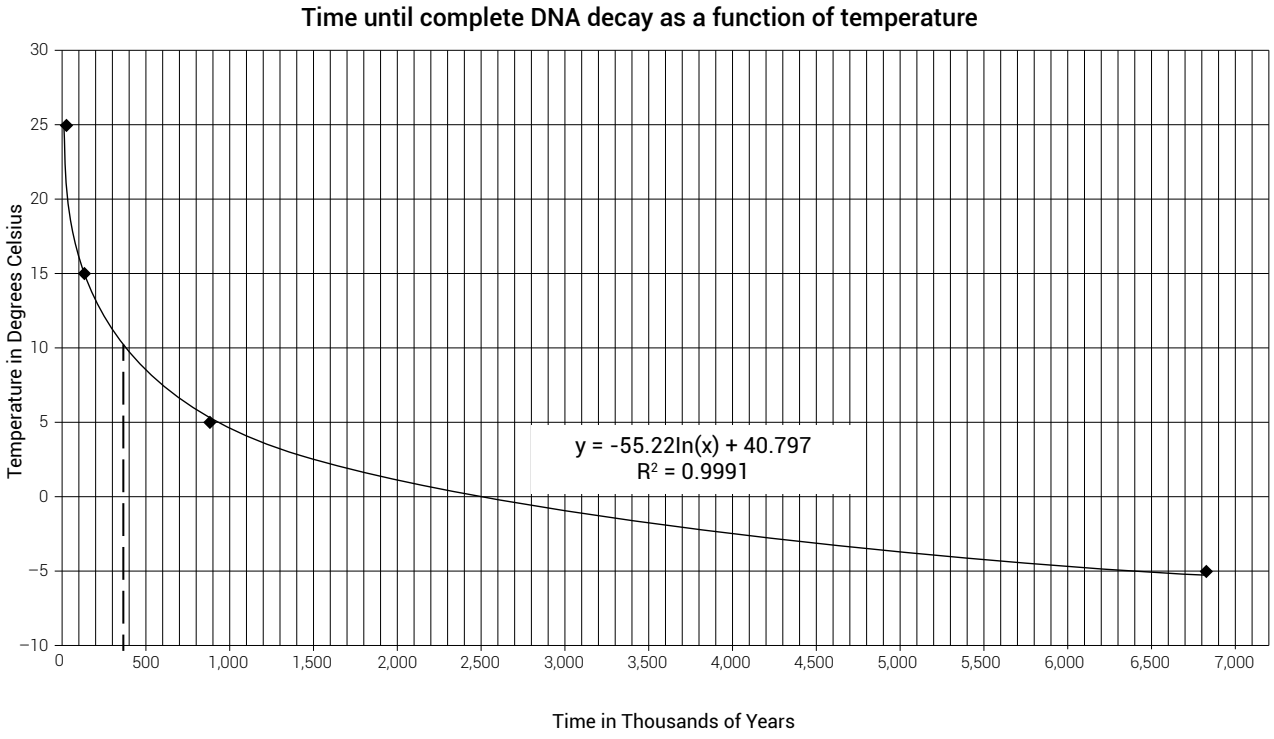

Figure 1. Time until complete DNA decay as a function of temperature using four data points in Allentoft *et al*. The Dashed line shows that at 10°C DNA completely decays after approximately 350,000 years.

Figure 2. Illumina HiSeq 2500 DNA sequencer. This next-generation, ultra-high-throughput system can be found in major genome centres, including those that process ancient DNA. Systems like these allow researchers to move from sample preparation to whole genome sequence acquisition in just a few days.

regions.[9] However, other researchers have promoted fixing them using biochemical treatment. In the most recent Neandertal genome sequencing effort by Pääbo's group, the researchers treated the DNA to repair age-related damage—a technique first promoted by Pääbo's lab.[6,11] This methodology involves the treatment of extracted aDNA with uracil–DNA–glycosylase and endonuclease VIII to remove uracil residues and repair most of the resulting abasic sites—allegedly leaving undamaged parts of the DNA fragments intact. As a result, age-damaged DNA, a hallmark of aDNA, is 'fixed'—potentially frustrating the ascertainment of modern human contamination even further by removing the explicit markers of age-damaged regions.

Another (more easily resolved) problem is the elimination of non-human microbial DNA contamination. Author Tomkins' examination of millions of Neandertal and Denisovan next generation sequence reads (~75 bases long on average) revealed that non-human microbial DNA contamination typically contributes about 98% of the total

raw sequence. In a 2010 *Nature* article comparing Neandertal to a Denisovan genome, the researchers wrote, "In all Neandertal remains studied so far the relative abundance of endogenous DNA [Neandertal] is below 5%, and typically below 1%."[12] Thus, a majority of the DNA in these samples, is actually microbial and not human, which is digitally filtered out in most cases. However, in the approximately 2% of DNA that did align to modern human in the work of Tomkins, it was 99.7 to 100% identical. This means that the DNA sequences, whether derived from human contamination or endogenous aDNA, are fully human in their alignments.

Radiation effects

The highly degradative effects of radiation should be noted while discussing the veracity of aDNA sequence data. Ancient DNA reports do not take into account the degradative effects of solar/cosmic or Earth gamma radiation. The Allentoft *et al.* study, for example, assumed "that long-term DNA fragmentation happens primarily as a result of depurination", which primarily results from hydrolysis and leads to double-strand breaks.[4,13] Solar and cosmic radiation penetrates within a few feet of Earth's surface to add DNA damage to hydrolysis. The contribution of solar and cosmic radiation to the overall natural background radiation in the earth is estimated to be about 18%.[14]

Another unstudied problem is the potential for radioactive isotope radiation to disrupt aDNA sequences deeper beneath Earth's surface than cosmic radiation reaches. Specifically, Earth gamma radiation and radon contribute 20% and 50% of the total natural background radiation, respectively.[14] In caves and ancient tombs, these types of radiation would have a significant impact on DNA decay rates, whereas the effects of solar and cosmic radiation would be negligible.

DNA forensics analysts generally distrust DNAs that are only several decades old on account of the radiation problem.[15] More research is needed for forensics to properly inform genome recovery using aDNA techniques.

The DNA from Halite

The oldest claim of ancient DNA was that of halophilic bacteria from so-called evaporates in New Mexico dated up to 425 Ma. This 2002 *Nature* report was heavily criticized due to DNA's widely known labile nature, but *Discovery News* mentioned a follow-up study that allegedly vindicated the results.[16]

Finding aDNA in 425 Ma-old material would clearly demand some kind of amazing explanation if it were found loose in the environment. However, in this case, the DNA came from living bacteria. Therefore, this news from New Mexico should not have emphasized the discovery of DNA as much as the discovery of live bacteria ensconced in salt deposits—a phenomenon lying outside the scope of this review.

The ancient DNA from Spain comes mainly from this cave

The Atapuerca cave system in Spain includes the Sima de los Huesos Cave, meaning "pit of bones". The caves contain bone pits that researchers have not yet completely excavated. They include the Gran Dolina, where fossils designated *Homo antecessor* were dated to at least 780 ka.[17] The supposedly oldest human bones in the system and in all Europe were found in nearby Sima del Elefante Cave, and dated 1.1–1.2 Ma.[18] Other bones include those of bears, cats, and small mammals. Found beneath layers of guano and ancient speleothem deposits, many of the fossil fragments comprise bone breccias. A few are better preserved, as was the three-part human femur found in Sima de los Huesos, from which recent work recovered human DNA (see below). Obtaining established dates for this femur, found within a hominin site already famous for its record-setting antiquity, is of great interest.

Dating the cave's remains

Several secular studies have under-taken the task of dating the cave remains. Most seem to rely on biostratigraphy-bolstered (rubber-stamped?) U-series dates. In 1997, Bischoff *et al.* obtained "U-series dates for 25 bear bones (154 +/– 66 ka) and for 16 human bones (148 +/– 34 ka)", despite the fact that the human bones occur below (and should thus have been deposited prior to) the bear bones.[19] Parés *et al.* reported, in 2000, ages for Sima de los Huesos human remains between 325 ka to 205 ka by correlating magnetostratigraphic clues to established (i.e. by consensus, not by science) magnetic polarity chrons, also aligning some of the cave's fossil fauna to published fossil 'ages'.[20] They also assigned an age of at least 780 ka to the non-fossiliferous sand and silts underlying the human bones. Later still, Bischoff redated the layers, this time with coauthor Shamp, obtaining Uranium-series 'ages' for each of the 14 cm of muddy sediments that contain human remains.[21] They cited Parés *et al*.'s 780 ka as "another estimate" to set the oldest, lower-age boundary. They then asserted their new, older date of "perhaps 400–500 ka" without directly refuting the arguments used to support much younger ages published earlier by Parés *et al.* and Bischoff *et al.*

This more recently published older Bischoff and Shamp age assignment invoked its own unique set of fossil correlations, including lion fossils for which 600 ka is "the oldest currently accepted age for this species".[21] Overall, age assignments for Sima de los Huesos human bones have ranged from 148 ka to 500 ka, and seem to depend on which

evidence researchers subjectively choose to include. An 'age' for the human bones at Sima de los Huesos of at least 400 ka is currently in vogue, setting a chronological stage for its DNA to be investigated (figure 3).

Sequencing the cave's aDNA

Svaante Pääbo runs a large aDNA operation and enjoys academic rock star status in the field, with the Sima de los Huesos Cave's fame plainly attracting financial backing for his lab to investigate mtDNA from a Sima de los Huesos human femur.[21] By citing the alleged 400 ka, headlines were able to advertise the data as the oldest human DNA sequences successfully recovered. For example, *Nature News* wrote, "Analysis of oldest sequence from a human ancestor".[22] We suspect human DNA sequences advertised at only 150 ka would have garnered less attraction for those in the race to find the oldest human DNA.

Pääbo was senior author of the eventual 2014 *Nature* report, which was based on the sequence of a mitochondrial genome.[23] It revealed similarities between the Sima de los Huesos human bone aDNA and Denisovan human sequence. Denisovan DNA came from a finger bone in Siberia and its sequence was similar to Neandertal and modern human—assuming the study authors were able to accurately reconstruct the degraded DNA. Pääbo was also senior author on the headline-grabbing Denisovan report.[12]

Dating human aDNA by circular reasoning

The preceding discussion brings us to this crux: if 100% of mtDNAs decay down to lengths of 1bp (i.e. the DNA is gone) after 350 years at 10°C, then recovery of 30 bp lengths of mtDNA from 400 ka bones should be impossible. At 10.6°C, remains within Sima de los Huesos should not even last 350 ka based on the measured DNA half-life as calibrated by carbon ages; less still if the carbon years were adjusted downward to calendar years, and less still if radiation was factored in. What tactics might one use to insulate exceedingly old age assignments from DNA decay rate implications? One could obscure the issue in a paper trail. Worse, one could mask a circular argument beneath a paper trail.

The first step on this trail follows a reference in the *Nature* paper describing Sima de los Huesos human bone aDNA to a *PNAS* paper describing optimized aDNA recovery techniques.[24] In this work, they recovered and digitally filtered "ultrashort DNA fragments" 35 to 150 bp in length from a cave bear that was estimated at >300 ka. The *PNAS* authors, including Pääbo, brushed aside Allentoft *et al*.'s DNA rate implications when they wrote:

> "This correlation has, in fact, been established in a recent study that analyzed samples of different ages from the same archeological sites [i.e. the half-life of DNA by Allentoft *et al*.], but the correlation vanishes in comparisons across different sites."

We recall that Allentoft *et al*. correlated mtDNA structural integrity measurements to 158 carbon-dated bone fossils. On what basis does the correlation supposedly vanish?

That very sentence references a *PLoS ONE* paper written before the Allentoft results, the next step on the trail. In *PLoS ONE*, Pääbo's group were reported to have examined mitochondrial DNA sequences from a variety of animals, including various primate, horse, and cow remains. The source bones varied in conventional ages between 18 and 60,000 years.[25] Their study showed, "despite a very large variation in DNA amounts, a significant negative correlation between amounts of endogenous DNA and age". This means that whether young or old, bone fossils from various sources still retained similar amounts of endogenous DNA, on average.

Since DNA does decay over time, shorter segments should populate older sites. So, the varying factor was not the condition of the aDNA, but sample age assignments. Their 'ages' were not anchored by carbon dates or other more reliable inferences. They merely cited conventional geologic age assignments as though they were accurate, objectively measured data points. However, as shown above and in many other places, secular age-dating is not objective, and consistently relies on circular reasoning. The argument in this *PLoS ONE* paper thus reduces to the same circular reasoning so often invoked to support deep time.[26] Let us summarize the contrast:

1. From Allentoft *et al*.: Based on correlating DNA decay to carbon ages, DNA at 10°C totally disintegrates after 350 ka.[4]
2. From Pääbo *et al*.: Based on correlating DNA decay to fossil ages, DNA decays at variable rates, permitting its survival even at 10°C for perhaps over 700 ka.[25]

The first premise relies on direct measurements, but the second premise relies on cherry-picked age assignments. One correlates DNA damage to carbon ages, and the other correlates DNA damage to fossil ages—these are not the same age sources. The two groups did not trace the same correlation, totally undermining the *PNAS* contention that "the correlation vanishes".

At its core, their logic resorts to asserting that because fossils are 700,000 years old, and because the DNA inside them is therefore also that old, DNA can last longer than its measured decay rate suggests. Well, of course it can last that long if we assume fossils are that old in the first place. But one must follow the paper trail to discover this circular argument, and to reveal that it invokes the fallacy of equivocation, where one definition for "the correlation" is subtly swapped for another.

Are these researchers playing an elaborate shell game where they deliberately overlook critical distinctions and

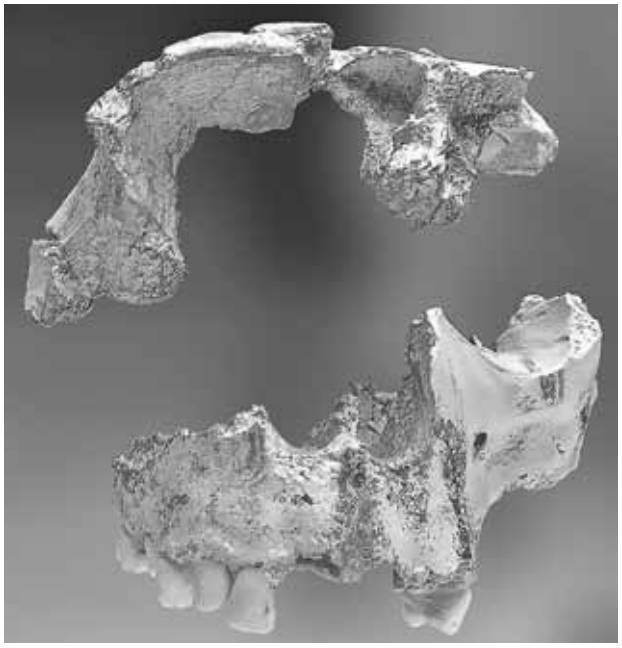

Figure 3. Skull remains of *Homo antecessor* from Sima de los Huesos in Atapuerca, Spain, a World Heritage cave. Its description in Science identified it as the oldest human remains in Europe at the time of publication in 1995. These results established this cave as a premier site for the search for the oldest European human genome.

hide faulty reasoning behind multiple publications? Reich *et al*.'s claim in *Nature* of sequencing ~400 ka DNA violates the highest quality DNA decay rate measurements, and reducing the ages of Sima de los Huesos fossiliferous strata resolves the issue.

A new argument against old ages

The *PNAS* report of ancient cave bear DNA disclosed that "the age of the Sima de los Huesos fossils is currently being re-evaluated using additional geological data, work that will be important especially for interpreting the human fossil record". This is consistent with an inherent subjectivity in the secular age assignment process.

In the *PLoS ONE* paper discussed above, the authors reported a wide variety of DNA integrity across sites bearing similar 'ages'. These varying DNA lengths further expose subjectivity in secular methods. The authors explained their data by reasoning that DNA decays at variable rates due to differences in local environments, ignoring the chemistry behind molecular decay over time. While it is true that DNA decay can be accelerated by different environmental factors, this does not justify either the assertion that aDNA can last longer than its ideal decay rate or the idea that shorter aDNA lengths do not correlate to longer exposure times. A few years of warm temperature would accelerate DNA decay, whereas a stretch of cold years would have a nearly negligible effect.

These data suggest an alternative explanation. Similar amounts of DNA within variably age-dated sources probably expose incorrect age assignments. Basic chemistry predicts that amounts of DNA diminish with time. Ergo, if one measures similar DNA amounts from various sources, then those sources may share similar ages. These secular researchers glibly dismissed the aDNA-length data in favour of their conventional ages, but there is no scientific reason to instead dismiss conventional ages in favour of the aDNA lengths. More research is needed to substantiate these arguments.

Oldest DNA ever sequenced: horse

In July of 2013, a large team published the new record for oldest DNA in *Nature,* sampled in 2003 from a 700 ka horse bone in Canadian permafrost.[27] Lead author Ludovic Orlando also presented the results at the Royal Society in London. He explained that the team initially thought the endeavour would be impossible, given the half-life of DNA at 521 years. Orlando told Western Digs, "The fact that the remains were frozen helped slow the rate of decay."[28] According to Allentoft *et al*.'s data plotted in figure 1, some permafrost DNAs might retain sufficient length for modern detection even after a million years, since it is not predicted to completely decay until after about 2.5 Ma. This diminishes arguments suggesting that 700 ka is too old an age assignment because DNA could not possibly last that long, since it probably can. Thus, challenges to the conventional age assignment of this horse fossil would have to come from other evidence, for example carbon dating.

Conclusions

Ancient DNA is difficult to work with and challenging to interpret. Allentoft *et al*. published a solid benchmark for DNA's half-life at 521 years, though calibrated by carbon dates. When used to predict DNA's decay rate at different temperatures (using a version of the Arrhenius equation), their measured rate predicts that no DNA backbone bonds remain after about 350 ka at 10°C, and after about 2.4 Ma at 0°C. The real maximum time would be much shorter after correcting for inflated carbon ages and for natural background radiation.

Technology has advanced to the point that contamination can be effectively eliminated by comparing aDNA data sets to the reference genome of a modern counterpart animal. For example, cave bear aDNA sequence is readily discernable from microbial or human DNA by comparing it to the modern bear genome. However, human aDNA cannot be reliably distinguished by this means since the contamination is human-based. Each case needs to be carefully investigated before claiming that DNA cannot last as long as claimed,

since, at sufficiently cold temperatures, DNA in bone can last a surprisingly long time.

Once contamination can be confidently ruled out, comparisons between an aDNA age assignment and the DNA decay rate can begin. In addition, reports of long ages for fossils contributing to aDNA sequencing have revealed an apparent attempt to insulate deep time's circular reasoning behind a trail of references. These comparisons also show how similar levels of aDNA degradation across different sites refute widely varying 'ages' for those sites. Some age assignments for aDNA sequence sources (e.g. a 700-ka permafrost horse) do not exceed DNA's maximum predicted longevity, emphasizing the need to carefully sift each publication. Other reports do exceed DNA's maximum 'shelf life' (e.g. a 400-ka human from Sima de los Huesos), exposing the error of their excessive age assignments.

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