

A REVIEW OF ORIGINAL TISSUE FOSSILS AND THEIR AGE IMPLICATIONS

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ABSTRACT

The discovery and verification of original soft tissue fossils has caused perhaps the most significant paleontological excitement in recent times. Neither evolutionary nor creationary researchers anticipated these phenomena. Published discoveries of original soft tissues including some of their constituent biochemicals, like collagen, elastin, osteocalcin, hemoglobin decay products, melanin, chitin and chitin-associated protein from fossil pockets around the world are reviewed. Decay rates and longevity studies of some biochemicals estimate an outside age limit for certain original soft tissue remains, and by extension for the geologic strata associated with their fossils. Secular paleontology struggles to interpret organically-derived fossil material with suggestions that various modes of taphonomy altered or sequestered the original organics into recalcitrant products. Investigations and reports of soft tissue fossil finds are cautioned to avoid overstating the case for biblical creation, but the data currently available are more consistent with an earth history spanning thousands, not millions, of years.

INTRODUCTION

A fossil is the remains of a once living organism. Vertebrate fossil remains most often occur in sedimentary rocks as mineralized tissues, which are rocks in the shapes of bones that minerals replaced. Fossils also occur as molds, casts, fine impressions, tarry residues, molecules, and rarely mummified or desiccated tissue. The latter-most fossil forms have drawn considerable contention among paleontologists, and for good reason. Original, endogenous soft tissues are not expected to have persisted for millions of years on the basis of their measured decay rates.

The phrase "soft tissues" has been used in the literature most often to describe the remains of a vertebrate that appears to the investigators to have been altered, usually through mineralization occurring via unique taphonomic circumstances, into a form more resistant than the original biochemistry. For example, Briggs and Crowther (2003) identified anoxia, rapid burial, rapid mineralization, and sterile environments as prerequisite circumstances. In the literature, "soft tissue" can refer to a specific form of mineralization called phosphatization. In such instances, the original biomolecules have undergone chemical reactions either with exogenous chemistry, for example phosphate ions, or from endogenous chemical processes.

However, "soft tissues" can also refer to biomolecules that had been kerogenized posthumously. This first involves the spontaneous breakdown of the original biomolecules. The breakdown products naturally polymerize, forming oily or sometimes waxy kerogen, the main organic component of oil in shale oil. All mineralized tissue fossils, whether phosphatized, pyritized, or kerogenized, are distinct from "soft tissue impressions" which do not typically preserve original material, but occur as casts or molds such as in dinosaur track ways, skin and plant leaf impressions, and clam viscera.

In addition, the phrase "soft tissues" has also been applied to even rarer instances of unaltered original organic soft tissue remains, and these are the focus of this review. Thus, the phrase "original soft tissue" will serve to characterize these, distinguishing them from more common chemically altered remains or impressions. Original soft tissues do not have to be literally soft to the touch to meet this designation, as they are sometimes dry or leathery, essentially mummified, but may soften upon hydration. The decay rates of phosphatized soft tissue or kerogenized soft tissue are not as well characterized as those of original soft tissues, but as will be discussed below the original soft tissues are known to decay at rates that appear to set a maximum age of approximately hundreds of thousands of years assuming reasonable earth surface temperatures.

LABORATORY AND FIELD DISCOVERIES

Original organic pliable tissues were not expected to exist in the fossil record, but many have been found. Original soft tissue fossils occur in rare pockets in various places on most Earth continents. They include the Green River and Hell Creek formations from the Western United States (Edwards, 2011; Schweitzer, 2005), Messel Shales of Germany (Vinther, *et al*, 2008), and the Yixian Formation of China's Jehol province (Linghan-Soliar, 2008).

The most famous of these finds is probably that of a *T. rex*, meticulously documented by Mary Schweitzer. Skeptical colleagues required more rigorous proofs to justify her interpretation of original soft tissue (Buckley, *et al*, 2008). She succeeded by employing additional independent researchers to describe in detail original hadrosaur tissue (Schweitzer, *et al*, 2009). A consequent of this debate is that several biochemicals, mostly proteins, have been detected with a wide variety of methods.

These methods have included, in no particular order, synchrotron X-ray fluorescence, fourier transform infrared mapping, pyrrholysis-gas chromatography/ mass spectrometry (Edwards, 2011), protein and DNA sequencing (Schweitzer, et al, 2009; Woodward, et al, 1994), UV/vis spectroscopy, nuclear magnetic resonance, immunization reaction (Schweitzer, et al, 1997), electron microscopy (Armitage, 2001), and others. Some rigorously verified original biomolecules found in fossils published by Schweitzer and colleagues include collagen, elastin, osteocalcin, hemoglobin, laminin, histone, and hemoglobin. Other authors have reported melanin (Clarke, et al, 2010; Glass, et al, 2012), chitin and chitin-associated protein (Cody, et al, 2011), purpurin (O'Malley, et al, 2013) and keratin (Vullo, et al, 2010; Edwards, 2011). These data reveal mostly intact biomolecules, and show that not all fossil biomaterials were mineralized or kerogenized. In most or all cases, some breakdown of the original biomolecule has been directly detected or reasonably inferred. Remarkably, so many such molecules have been discovered in such a high state of organization that various techniques repeatedly and specifically identify them.

Table 1 summarizes technical and a few preliminary reports of original soft tissue fossils. The original soft tissues described in these papers represent the vertebrate taxa osteichthyes, mammalia, dinosauria, squamata, aves, and amphibia. These report that, other than having partly decayed, certain tissues have not significantly altered since capture and burial. The table was posted on the Institute for Creation Research website in July 2011. Additional discoveries since that time could be added, and the list could be significantly lengthened by including original soft tissues from ambers, or organic resinite inclusions.

	Publication Date	Brief Description E	volutionary Age	Publication
Articles Published in Peer-Reviewed Journals				
1	5/30/1977	Catfish fatty fin in Green River	50MY	H. P. Buchheim and R. C. Surdam, Geology, 5: 198.
2	6/14/1992	Osteocalcin in a seismosaur bone	150MY	Muyzer, G. et al, <i>Geology</i> , 20: 871-874.
3	9/25/1992	DNA in amber	30MY	Morell, V. et al, Science, 257: 1860.
4	6/16/1994	Unaltered amino acids in amber insects	130 MY	Bada, J. L. et al, Geochemica et Cosmochemica Acta, 58 (14): 3131-3135.
5	6/16/1994	Dinosaur DNA from hadrosaur bone	65MY	Woodward, S. R., N. J. Weyand and M. Bunnell, Science, 266 (5188): 1229-1232.
5	5/19/1995	Live bacteria spores from amber	25-40MY	Cano, R. J. and M. K. Borucki, Science, 268 (5213): 1060 - 1064.
7	6/10/1997	Hemoglobin fragments in T. rex bone	67MY	Schweitzer, M. et al, PNAS, 94 (12): 6291-6296.
3	6/2/1999	Live bacteria from halite deposit	250MY	Vreeland, R. H. et al, American Society for Microbiology, 99th General Meeting, June 2, 1999, Chicago.
)	6/21/1999	Live bacteria from separate rock salts	250MY	Stan-Lotter, H. et al, Microbiology, 145 (12): 3565-3574.
0	6/21/1999	Ichthyosaur skin	190MY	Linghan-Soliar, T. et al, Proc. Royal Soc. B, 266 (1436): 2367-2373.
1	6/21/1999	Keratin in Madagascar Cretaceous bird	65MY	Schweitzer, M. H. et al, J. Vert. Paleo, 19 (4): 712-722 .
2	9/1/2001	T. rex collagen SEM scans	65MY	Armitage, M., Creation Research Society Quarterly, 38 (2): 61-66.
3	6/26/2004	Live (non-spore) bacteria in amber	120MY	Greenblatt, C. L. et al, Microbial Ecology, 48 (1): 120-127.
4	3/24/2005	T. rex soft tissue	68MY	Schweitzer, M. et al, Science, 307: 1952-1955.
5	7/25/2006	Soft frog, intact	10MY	McNamara, M. et al, Geology, 34: 641-644.
6	6/30/2007	T. rex collagen	68MY	Scweitzer, M. et al, Science, 316: 277-280
7	8/1/2007	Bloody frog bone marrow	10MY	McNamara, M.E. et al, Geology, 34 (8): 641-644.
8	4/7/2008	Psittacosaurus skin	125MY	Linghan-Soliar, T. et al, Proc. Royal Soc. B, 275: 775-780.
9	7/8/2008	Feather melanocytes	100MY	Vinther, J. et al, Biology Letters, 4: 522-525.
0	4/30/2009	Hadrosaur blood vessels	80MY	Schweitzer, M. et al, Science, 324 (5927): 626-631.
1	8/26/2009	Purple Messel feather nanostructure	40MY	Vinther, J. et al, Biology Letters, 6 (1): 128-131.
2	5/19/2009	Primate "Ida" soft body outline	40MY	Franzen, J. L. et al, PLoS ONE, 4 (5): e5723.
3	7/1/2009	Hadrosaur skin cell structures	66MY	Manning, P. et al, Proc. Royal Soc. B, 276: 3429-3437.
4	10/2/2009	Fungal chitin ubiquitous in Permo-triassi	c 250MY	Jin, Y. G. et al, Science, 289 (5478): 432-436.
25	8/18/2009	Squid ink	150MY	Whilby, P. R. et al, Geology Today, 24 (3): 95-98.
	11/5/2009	Salamander muscle, whole	18MY	McNamara, M. et al, Proc. Royal Soc. B, 277 (1680): 423-427.
27	2/25/2010	Sinosauropteryx melanosomes	125MY	Zhang, F. et al, <i>Nature</i> , 463: 1075-1078.
28	3/10/2010	Psittacosaurus skin color	125MY	Linghan-Soliar, T. G. and Plodowski, Naturwissenschaften, 97: 479-48 (Same sample analyzed in Proc. Royal Soc. B, 275: 775-780.)
29	5/14/2010	Mammal hair in amber	100MY	Vullo, R., Naturwissenschaften, 97 (7): 683-687.
0	5/18/2010	Archaeopteryx original tissue	150MY	Bergmann, U., PNAS, 107 (20): 9060-9065.
1	8/9/2010	Mosasaur blood, retina	968-65MY	Lindgren, J., PLoS ONE. 5(8): e11998.
32	11/12/2010	Penguin feathers	36MY	Clarke, J. A. et al, Science, 330: 954-957.
33	11/18/2010	Shrimp shell and muscle	360MY	Feldman, R. M. and C. E. Schweitzer, J. Crustacean Biology, 30 (4): 629-635.
34	2/7/2011	Chitin and chitin-associated protein	417MY	Cody, G. D. et al, Geology, 39 (3): 255-258.
35	4/1/2011	C-14 date of mosasaur (24,600 Yrs)	70MY	Lindgren, J. et al, PLoS ONE, 6 (4): e19445.
36	3/23/2011	Lizard tail skin, Green River	40MY	Edwards, N. P. et al, Proc Royal Soc B, online.
37	6/8/2011	Type I Collagen, T. rex and hadrosaur	68MY	San Antonio, J. D. et al, PLoS ONE, 6 (6): e20381.
8	6/30/2011	Bird feather pigment	120MY	Wogelius, R. A. et al, Science, online.
	Preliminary Reports Published Elsewhere			
9	8/10/2009	Live yeast in amber	45MY	Wired Science
10	4/10/2010	Australopithecus sediba brains	1.9MY	Discovery News
11	9/27/2010	Lobster shell	"millions"	Keighley News
12	10/22/2010	Mosasaur cartilage	80MY	Buchholz, C. C., Rapid City Journal
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Table 1. Published Reports of Original Soft Tissue Fossils. Papers that were excluded from the list include those with dubious verbiage, especially those which discussed "soft tissues" but failed to specify whether or not the tissues were "original" or chemically altered to a more resistant material. Those papers which specified the latter were also excluded, to the best of the author's ability to discern. The table demonstrates that original soft tissue fossils, while rare, have nevertheless been the subject of dozens of investigations.

The table does not provide the geographic information from the papers that it lists. From these papers, one learns that original soft tissue fossils have been reported from England, Germany, Spain, Gobi Desert, Eastern China, Brazil, and North America. Doubtless, more original tissue fossils await discovery, and others have gone unreported. Therefore, original tissue fossils are almost a global phenomenon.

Biomolecular Decay Rates and Theories

Body tissues are composed of biomaterials, mostly proteins, each of which has a shelf life due to its spontaneous decay according to the second law of thermodynamics. Many of the biochemicals that paleontologists have been detecting in the last two decades from among fossil remains had been known by experiments to naturally degrade into their constituent small, simple chemical moieties, but more rigorous characterizations of biomolecular decay permit direct comparisons, discussed below.

Although some biochemicals do decay faster than others under various conditions, all eventually decay if they are not first biodegraded by microbial action. Their spontaneous decay is exponential, usually according to first order kinetics (Sivan, *et al*, 2008) and all of them decay to the point that they are no longer recognizable or detectable in their original forms on the order of hundreds of thousands of years at most, assuming reasonable earth surface temperatures. Heat accelerates biochemical decay by increasing the number of atomic collisions, thereby increasing the likelihood of chemical reactions.

Discussing the possible tissues that could have persisted within shale and that contributed to kerogen formation, the heating of which material produces oil from oil shales, R.F. Cane noted that carbohydrates should be dismissed because they are prone to decay. He added, "Likewise, proteins are not stable enough to be considered. Enzymatic attack on the peptide linkages would be followed by further breakdown, with the ultimate production of ammonia and amines" (Cane, 1976). However, because they have been discovered in Earth materials, they should now be considered.

Similarly, noted paleontologists Briggs and Crowther (2003) discussed exceptionally preserved fossils, writing, "DNA is highly susceptible to hydrolysis and oxidation, and there are no reliable traces in fossils much more than 50,000 years old. Proteins also decay rapidly and are usually only preserved on a time scale similar to that for DNA." Thus, he concluded that "the long-term preservation of organic macrofossils involves diagenic alteration," by which he means either some form of mineralization or high temperature reactions that polymerize organic remains into tar-like kerogen (Briggs & Crowther, 2003). Kerogen is a carbonized residue that is much more stable than original labile biochemicals, so is not as unexpected in ancient remains. For example, the unique Cambrian fauna fossilized in Canada's Burgess Shale are routinely described using phrases such as "remarkable preservation," and "soft tissue preservation," but the original organics have been kerogenized or mineralized, likely when the sediments endured much greater depths and temperatures then today (Butterfield, 2003). They sometimes preserve the body outline and other information, but there are thus far no reports of original biochemistry from Burgess-type shale to this author's knowledge. However, original and unmineralized soft tissues have been found in fossils conventionally dated as millions of years, as Table 1 shows, and since chemical analyses confirm that these have not been diagenically altered, they contradict Briggs' expectation that no original biochemistry should be found in fossils since they fail to preserve "long term."

Spontaneous biomolecular degradation occurs for much the same reason that a wind-up clock eventually unwinds. Biomolecules are highly and intricately structured, and these structures constantly randomize. It is for this reason that living tissue is able to constantly recycle them, and for this reason they are rapidly biodegraded and recycled within Earth surface ecologies. If original tissues can avoid being pressed by scavengers, microbes, or chemicals, they nevertheless fall apart according to universal entropy, which describes how systems that are left to themselves spontaneously disorganize over time (Sewell, 2011).

Biochemists and others who are familiar with the labile nature of biomolecules and who are committed to deep time have offered considerable resistance to the interpretation of these soft tissue fossils as being original and essentially unaltered. Paleontologist Mary Schweitzer summarized those sentiments, saying, "The present state of knowledge holds that microbial attack, enzymatic degradation, cellular necrosis and other processes contribute to total degradation of recognizable organic materials in days to years" (Schweitzer, *et al*, 2007). As a result of this skepticism, paleontologists have endeavored to defend the veracity of their finds by characterizing fossil molecules in greater detail. The central question that emerges is, if some unique set of factors can be imagined that somehow drastically curtails microbial activity, and enzymatic and cellular breakdown, then how long could original biomaterials persist? Recent research has made strides toward answering this question as it pertains to two molecules: collagen and DNA.

Collagen Longevity

Collagen proteins are integral to connective tissues, including bone, skin, shells, and cartilage. Most of these tissues have been found in various fossils. Since all rocks are porous, over millions of years, oxygen molecules from the atmosphere would have penetrated to fossiliferous Earth materials. Oxygen reacts with biomolecules, including collagen, and accelerates their decay.

Bada, *et al* estimated the decay rate of collagen protein fibers by comparing the relative degrees of molecular integrity, as approximated by amino acid racemization, from modern and ice age bivalves. The nanostructure of clamshells consists of a mineral matrix that envelopes long, ropy collagen proteins. Assuming that partly decayed ice age bivalve collagen was deposited 10,000 years ago, he worked out a crude extrapolation of the maximum longevity for collagen in bone to be 30,000 years (Bada, *et al*, 1999).

Buckley, et al generated much more reliable results based on thorough and repeated measurements of bone collagen longevity. They identified that collagen's energy of activation $(E_a = 173 \text{ kJ/mol})$ equates to a half-life of 130 Ka at 7.5°C (Buckley, et al, 2008). Collagen decay experiments extrapolate rates from measurements of molecular integrity, detected at intervals by mass spectrometric sequence analyses of heated samples of fresh bone pieces. The measured rate at high temperature is then converted to a cooler temperature using a variant of the Arrhenius equation. This accurately relates chemical reaction rates to (usually thermal) energy of the system. For collagen at 90°C, enough data points to generate a reliable decay curve are typically gathered in a month. The technique has been used most often to produce age estimates in collagen years for archaeological specimens (Dobberstein, et al, 2009). Various factors within the micro-localized collagen settings are theorized to contribute to the variability in these measurements. However, because collagen very reliably decays within its range of rates, it clearly indicates a maximum time-to-dust estimate.

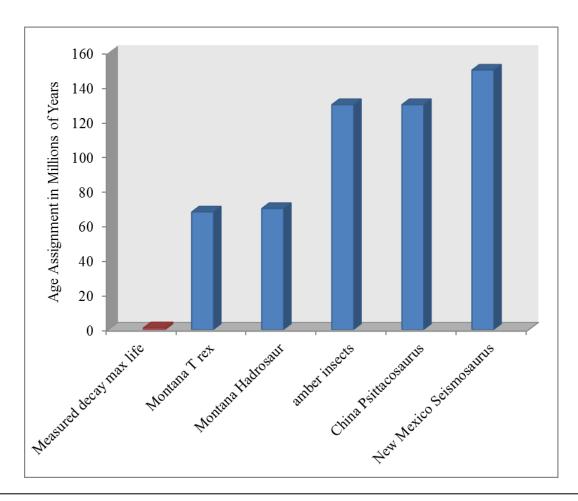


Figure 1. Maximum Collagen Longevity and Age Assignments of Collagen-containing Fossils. "Measured decay max life" is the maximum collagen time-to-dust estimate from experiment (Buckley, *et al*, 2008). Montana *T. rex*, (Schweitzer, *et al*, 2005); Montana Hadrosaur, (Schweitzer, *et al*, 2009); amber insect protein, (Wang, X.S., *et al*, 1996); China Psittacosaur, (Linghan-Soliar, 2008); New Mexico Seismosaur, (Gurley, *et al*, 1991). The high fidelity insect amino acids do not necessarily derive from collagen. The Seismosaurus protein is very likely, but not certainly, bone collagen. All collagen or unspecified protein fossils represented here carry age-dates that are orders of magnitude older than the maximum allowable from collagen half-life measurements.

Buckley, et al, (2008) applied this strategy to critique Schweitzer's interpretation of T. rex collagen as being original to the dinosaur. They converted their measured bone collagen decay to several different plausible temperatures, one of which was 7.5°C. This is an appropriate temperature from which to calculate half-life, considering that the most rigorously characterized fossil proteins are from Tyrannosaurus rex and hadrosaur from Montana, which has an average annual temperature of approximately 7°C. The fossil assemblage at that site, the Hell Creek Formation, consists of tropical flora and fauna, however, raising the possibility of higher historical temperatures that would have accelerated collagen decay. The argument that Buckley, et al, (2008) proffered, that measured bone collagen decay rates preclude the interpretation of dinosaur tissue that is millions of years old, has not yet been resolved in the literature. Schweitzer summarized the now decades-old dilemma, stating, "The idea that endogenous molecules can be preserved over geological time periods is still controversial" (Schweitzer, 2012).

England averages a few degrees warmer than Montana. Comparing British Ice Age bone collagen ages with their standard age assignments, Buckley and Collins (2011) wrote, "Extrapolation from high temperature experimental decomposition rates using this activation energy [173 kJ/mol] suggest that at a constant 10°C (the approximate mean annual air temperature in present-day Britain) it will take between 0.2 and 0.7 Ma years at 10°C for levels of collagen to fall to 1% in an optimal burial environment." The authors admitted a thus-far unexplained discrepancy between standard age assignments for the Ice Age specimens in their study and the six-fold younger ages measured in collagen years. Collins (personal communication) awaits discovery of an unknown factor that might preserve original chemistry from much earlier geologic periods through to the present.

To illustrate the age implications of collagen decay, we assume a seven ton *T. rex* was 50 percent water weight, and 70% of its dry weight was comprised of collagen, equaling 4900 pounds. At 7.5°C, that amount of collagen would decay to 0.01 percent of its original mass in ~13 2/5 half-lives, or approximately 1.7Ma. At that stage, collagen would most likely have decayed to such an extent that it would no longer be identifiable as collagen using standard techniques such as immunological assays and sequencing by mass spectrometry. So, little to no detectable bone collagen should remain after merely 2.5 percent of 67 million years, the supposed time elapsed since the dinosaur fossil-rich Hell Creek Formation was deposited.

Biomineral-encased proteins like bivalve shell collagen or bone osteocalcin are better protected from exogenous chemical exposure and water flow and thus less susceptible to oxidative attack than are other, more exposed body regions. Bone and shell collagen microfibers are also physically constrained, reducing their potential for chemical decay. Therefore, the longevity of the same proteins comprising fossilized skin, retinas, muscles, nerves and internal organs, which are not encased in protective bone or shell and are described in the literature, is expected to be even lower than that of bone collagen. Collagen is also insoluble. Therefore, bone collagen has one of the highest potentials for persistence among vertebrate proteins. Buckley and Collins (2011) mentioned that their measured half-life of bone collagen decay represents "an optional burial environment." This is significant because other known or imagined taphonomic factors merely accelerate collagen decay. Because of this, their time estimate safely represents a maximum age for collagen samples.

DNA Longevity

Using decay rate measurement techniques similar to those for collagen, Allentoft, *et al*, (2012) recently generated a half-life for DNA. They extracted mitochondrial DNA from 158 Ice Age and more recent fossil moa leg bones. These giant birds are generally considered to have been hunted to extinction by about AD 1500. Assuming that New Zealand's average annual temperature has persisted since fossil deposition, the measured spontaneous decay of the 242bp control region of moa mitochondrial DNA in bone is 521 years. Expressed in terms of a time-to-dust estimate, these bone DNA molecules would fragment into pieces too short for modern DNA detection and sequencing techniques on the order of 10,000 years. As to the question of whether or not this decay rate should apply to other organisms' DNA, there is nothing thus far revealed that is so unique about moa bone as to hint that they would preserve DNA differently than other vertebrate remains. The burden of proof lies on those who suggest that these moa results should not apply to other vertebrates to describe relevant differences between moa and non-moa bone, and demonstrate some physical mechanism to support such a claim.

This rigorously obtained DNA half-life used carbon-dates obtained for each of the moa bones to calibrate the time dimension of their DNA molecular integrity curve. As such, this DNA decay rate harbors the assumptions inherent in carbon dating techniques. However, these deep time assumptions should reduce the DNA decay rate apparent on the author's decay curve, skewing the results toward longer DNA longevity. Even though the actual half-life for this DNA is probably even shorter than 521 years, barring microbial and enzymatic degradation and air or water-based oxidation etc., these results represent a far shorter maximum time span than standard age assignments given to various fossiliferous DNA sources. Additionally, Marota, *et al*, (2002) determined a DNA half-life for DNA in papyrus to be between 19-24 years. The much greater half-life of bone DNA than of papyrus DNA likely results both from bone's protective biomineralized shielding that is absent in papyrus, discussed below, and from the bone collagen decay rate being calibrated to carbon years, which commonly exceed calendar years.

How does this DNA half-life compare with fossil DNA age assignments? DNA from Cretaceous dinosaur bone has been amplified (Woodward, *et al*, 1994). However, their results were quickly denounced by the secular science establishment which argued that because DNA does not last for millions of years, the study authors must have failed to use sterile techniques and their sequence must therefore be a contaminant (Gibbons, 1994). Unfortunately, perhaps due to the strong backlash that Woodward and coauthors experienced, DNA sequencing of Mesozoic (especially dinosaur) remains has not been rigorously pursued since then. Accordingly, Schweitzer's (2013) most recent and detailed survey of Hell Creek fossil biomolecules verified the presence of DNA inside dinosaur osteocytes using two separate stains and by immunological assay, but not by sequencing. One can only speculate as to why she did not sequence the dinosaur DNA, since the technology was certainly available (Rohland and Hofreiter, 2007).

DNA age assignments from ancient human fossil bones, though much more recently deposited than amber DNA's discussed below, still exceed the maximum age for DNA longevity according to Allentoft, *et al*, (2012) results. Neandertal DNA sequence, alleged to be approximately 40Ka old, is now yielding enough reliable repeatability across different samples to reconstruct virtually his entire genome (Green, *et al*, 2010). Similarly, Reich, *et al*, (2010) reported human genomic sequence from a finger bone fragment assigned an age of approximately 50Ka from a Denisovan cave. DNA sequence for a mammoth's single-exon nuclear melanocortin type 1 receptor gene was accurate enough to predict its coat color even though assigned a typical Pleistocene age of approximately 43Ka (Römpler, 2006). Thus, even Ice Age fossil DNA finds are unexpected on the basis of DNA decay rate estimates.

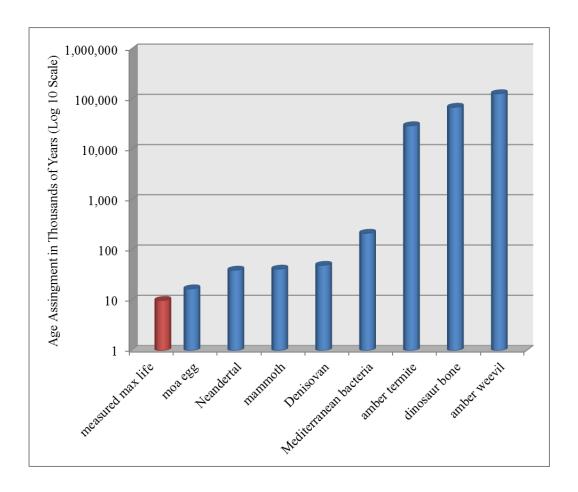


Figure 2. Maximum DNA Longevity and Age Assignments of DNA-containing Fossils. "Measured max life" is the maximum DNA time-to-dust estimate from experiment (Allentoft 2012). Moa egg shell, (Oskam, *et al*, 2010); Neandertal, (Green, *et al*, 2010); mammoth, (Römpler, 2006); Denisovan, (Reich, *et al*, 2010); Mediterranean bacteria, (Coolan and Overman, 2006); amber termite (DeSalle, *et al*, 1992); dinosaur bone, (Woodward, *et al*, 1994); amber weevil, (Cano, *et al*, 1993). Logarithmic scaling was required to visualize most of the data, which shows wide variability in fossil DNA age assignments. The greatest disparity is a four orders of magnitude age difference between "measured max life" of 0.01Ma and the 130Ma assignment for a weevil in amber. All fossil DNA sources represented here are age-dated beyond the maximum age predicted by carbon-dating calibrated half-life of bone DNA decay measurements.

Ancient DNA discoveries continue to surface, and have become a major subdiscipline within archaeological and even paleontological efforts. Many of them report ages far in excess of that expected by DNA decay rates, like Coolan and Overman (2006) who reported fossil green sulphur bacteria DNA sequences that were 540 base pairs long from 217Ka deep sea Mediterranean deposits. Others report DNA from more recently deposited remains given ages only slightly exceeding those predicted by DNA decay studies. Interestingly, one of these obtained excellent DNA quality from moa and other extinct giant bird egg shells, the oldest of which was 17Ka (Oskam, *et al*, 2010). Future experiment-based moa shell DNA decay rates could serve to check the published moa bone DNA decay rate, providing additional assessments of DNA longevity from various sources. Though archaeological DNAs present moderate challenges for uniformitarians, clearly DNA decay rate measurements most strongly confront

ancient DNAs from Mesozoic sources, which are supposed to be orders of magnitude older than the maximum measured DNA lifespan.

DNA in Resinite

DNA extracted from ambers, which are complicated mixtures of cross-linked polycommunic acids, terpenoids, and other phenolic derivates, may have a more robust representation in the literature and in the fossil record. DeSalle, *et al*, (1992) reported termite-specific DNA sequence from an amber-encased termite at 25-30Ma. Similarly, Cano, *et al*, (1993) reported weevil-specific DNA extracted from a 125-135Ma weevil encased in Lebanes amber. At present, "The exact mechanism/s involved in biological preservation by fossilized resins are unknown." (Martín-González, 2009). And being unknown, amber taphonomy thus-far does not provide sufficient means to preserve biomolecules beyond their standard decay rate estimates. In particular, older ambers become characteristically opaque and then, critically, develop cracks (Ross, 2010). At that point, encapsulated biomolecules are exposed to standard oxidation reactions leading to molecular degradation. So, again, those who wish to claim that amber could somehow confer unique longevity to encased molecules bear the burden of suggesting and testing sufficient supporting mechanisms.

On the other hand, the ignorance concerning biomolecular preservation in resinite also means that it is not known if other factors might explain apparent DNA persistence. For example, Beimforde and Schmidt (2011) suggest that most claims of ancient DNA from ambers actually find their origin in modern microbe contaminants, including resin-loving fungi and bacteria that invade microscopic cracks in amber structure. According to these authors it is very difficult, if not impossible, to extract clean DNA from deep within amber. They cited a failed attempt to sterilize microbes in amber fissures, and reasoned that because the PCR DNA amplification technique favors long DNA strands, that it preferentially reports the longer DNA lengths from modern sources over the ancient DNA, which in their time scale should have fragmented beyond all recognition. This explanation needs further investigation, but two preliminary rebuttals present themselves. First, the study authors' resistance to the idea of original DNA in amber might come from their understanding that "today it is widely assumed that DNA does generally not persist for more than thousands of years as amplifiable [by PCR] molecules" (Beimforde and Schmidt, 2011). Similarly, they cited other works arguing that because the characteristics of live microbes extracted from ambers (of which there are many dozens, championed in the 1990's by Raul Cano) were perfectly identifiable with modern microbes both morphologically and genetically, that these "supposed microfossils showed that they were much younger than the amber itself' (Beimforde and Schmidt, 2011). In other words, since the microbes from within amber were virtually identical to modern microbes, and since the ambers are millions of years old, the microbes must be of recent origin and therefore surface or subsurface contaminants. This is clearly a circular argument that merely assumes the evolutionary time scale.

This knife cuts both ways—through bacteria as well as arthropods. Ambers are renowned for preserving insects that are virtually identical to modern taxa, but evolutionists do not reason from this that the insects are modern contaminants of resinite. In any case, the amplification of insect-specific, not microbial, DNA sequence from within resinites does not match the microbes-in-the-cracks contamination hypothesis, an issue that Beimforde and Schmidt did not address. The data from within resinites, including high fidelity microbial DNA sequences, live microbes, intact insect chitinous exoskeleton and internal organ arrangements, etc., are more consistent with the hypothesis that the resinites and their inclusions are thousands of years old.

Comparison with C-14 Dates

Clearly, biomolecular decay rates measured in laboratory settings challenge deep time age assignments for fossil material. A separate naturalistic dating technique applied to ancient earth materials would help inform this discrepancy. Baumgardner, *et al*, (2003) worked toward this by carbon dating ten coal samples and listing 90 additional carbon ages reported in secular literature, mostly compiled by Paul Giem (2001), from such sources as marble, graphite, calcite, natural gas, and wood. The precise carbon ages that they obtained are not critical to the present study, but merely the fact that C-14 is routinely detected in materials designated millions of years old. The maximum age limit for endogenous C-14 that its measured half-life permits, analogous to "time-to-dust" estimates from biomolecular decay rate measurments, is under 90,000 years.

Though endogenous C-14 in various earth materials is established, similar results for fossil tissues is meager. Snelling (2008) reported carbon ages from between 36,400±350 and 48,710±930 years for lower Cretaceous California ammonite fossil shells. Lindgren, *et al*, (2010) reported a carbon age for the fossil mosasaur from Kansas chalk of 24,600 years before present (ybp). Like most reports of C-14 in ancient earth materials, Lindgren, *et al* attributed their surprising C-14 results to contamination. Giem's (2001) refutations of contamination apply just as aptly to this mosasaur as they did to the earth materials that he had in view, leaving open the possibility that the C-14 was original to the mosasaur tissue.

In addition to these reports, Dahmer, *et al*, (1990) provided two carbon dates, one each from a different dating facility, for an *Acrocanthosaurus* excavated from Glen Rose, Texas. The preliminary lab results matched measured carbon ratios to ages of 23,760±270 and 25,±750 ybp. The same brief report listed an age of 16,120±220 ybp for a Colorado *Allosaurus* fossil. Their ongoing efforts have continued to yield concordant carbon ages for four more dinosaurs and an array of other carbonaceous fossils including wood and amber (personal correspondence). Thus far, the fact that fossil carbon ages have been obtained from Mesozoic sources including fossils is consistent with the interpretation of original soft tissue in similar fossils. However, further work is needed to more firmly establish that the radioactive carbon is endogenous to the fossils and not the result of recent contamination.

INTERPRETING ORIGINAL TISSUE FOSSILS

Both creationary and evolutionary research seeks to interpret both the lab bench and original tissue fossil data. Young age assignments for fossil material resolve the current controversies over collagen and DNA decay. However, more research is needed to ascertain detailed models by which animal carcasses might have avoided the dramatic and rapid degradation that occurs on earth's surface, and to provide a more robust data set to confirm the time constraints of biblical creation. The secular community is entitled to attempt interpreting original tissue finds from a deep time perspective, and their efforts promise to add to our understanding of which possible scenarios might or might not explain some of the data.

Taphonomy with high preservation potential

Whereas the exponential decay rates of some biomolecules are better known than others, and some of the rates vary, maximum time-to-dust estimates can be made by assuming the slowest

possible chemical decay rates, and the set of burial conditions most likely to facilitate maximum tissue and therefor biomolecular longevity.

A best-case scenario would see the creature rapidly inundated and covered by significant overburden to prevent scavenging, with the water draining from the entire area soon after burial. This characterizes catastrophes. The water must drain quickly because its presence dramatically accelerates biodegradation both by directly reacting with biochemical and by sustaining tissue-degrading microbes. One taphonomy reconstruction study found that entire fish bodies completely biodegrade in weeks even when submerged several feet below muddy anoxic lacustrine beds (Donovan, 1991). This and similar experiments contrasts assertions common in the literature that an anoxic environment helps preserve organic remains.

Next, at least partial sterilization of the fossil environment must be considered as key to maximizing molecular preservation. A host of microbes have been equipped by their Creator with enzymes specified to degrade even the toughest organic compounds, including bone bioapatite, ropy proteins including collagen, keratin, and even chitin and lipids. Being hydrophobic, often saturated carbon chains, lipids do not have as much chemical reaction potential. Therefore, lipids like waxes typically have the highest post-mortem longevity of the biochemical types. Partial or total sterilization of fossilized organisms may have been achieved by heating, as probably occurred in the case of an original soft tissue whole frog fossil from Spain. Its exterior was carbonized by heat from the nearby volcanic explosion that buried it (McNamara, *et al*, 2007). The investigators found bacterial traces on the exterior of the fossil, but not inside the bones, where marrow had been essentially unaltered.

Humidity is another consideration in high preservation potential taphonomy, but as with other factors, increased humidity generally speed decay and low humidities are not known to extend decay rates. Microbes might be present, perhaps as spores, but they do not grow and consume the soft tissues without water. Thus, significant desiccation is essential to the preservation of original soft tissues for more than a dozen or so years. As far as is known, taphonomic conditions do not specially preserve original tissue from its own spontaneous decay. The most favorable experimental conditions merely maximize potential for molecules to last as long as their biochemical decay rates permit.

Mineral Sequestering as a Rescuing Device

James San Antonio and colleagues (2011) recently argued that certain functional groups within collagen protein molecules could be preferentially preserved across geologic time by spontaneously associating via hydrogen or covalent bonding with nearby bioapatite mineral. This represents an attempt from within the evolutionary community to generate a plausible mechanism to explain the extreme disparity between maximum collagen longevity from measured bone collagen decay rates and the much older age assignments for now well-characterized Mesozoic fossil bone collagen (See Figure 1). However, this speculative hypothesis ignores theory and experiment. Even if portions of the molecule are sequestered, other portions are not, and are thus exposed to oxidative and other chemical breakdown. The study authors also suggested that the local bioapatite constrains the embedded collagen, providing mechanical resistance to collagen degradation, which occurs more rapidly where there is space for the collagen to more easily unwind, shed chemical breakdown products, and accommodate potential exogenous reactants. However, their discussion ignored the technique used to measure collagen decay. Its decay rate measurements were taken from bone collagen and

therefore incorporate the very chemical and microphysical factors that San Antonio, *et al*, (2011) describe. In other words, if any feature intrinsic to the bone collagen microenvironment caused its own longevity through geologic time, then they would be reflected in the measured half-life of bone collagen decay experiments showing decay rates that permit geologic time. They do not.

Similar to San Antonio, *et al's* suggestions, Edwards, *et al*, (2011) argued for a complicated combination of original tissue stabilization by chemical interactions with adjacent minerals common to clay and to the shale matrix encasing a lizard leg fossil from the Green River Formation. As noted above, they characterized original lizard skin keratin protein. Similar to collagen, keratin is insoluble in water and resists abrasion in lizard skin, mammal hair, and bird feathers. The study detected protein-specific amide bond signatures within the scales but not between them nor in the surrounding matrix. Keratin's resilience, as easily identified by the sulfurous smell from burning hair, is partly due to disulfide linkage between adjacent sulfurcontaining cysteine amino acids, which they also mapped within the thickened scale portions of the fossil. In addition, they inferred melanin, which chelates metal ions, using non-destructive metal detection techniques that generated metal distribution maps. The metal distributions overlie the plainly visible lizard skin scale structure, amide bonding, and organic sulfur distribution maps, indicating that they are all linked, consistent with the hypothesis that they are all sourced from the original lizard skin.

Edwards, et al, (2011) effectively ruled out exogenous keratin sourcing, as well as mineralization by phosphatization, sulphurization, or pyritization, which are far more common within "soft tissue" fossil finds than are original tissues. Their task was therefore to explain how original keratin could persist over geologic time (50Ma). They suggested first that because organic sulfur is very soluble in water, the fossil would have to have remained dry during virtually its entire geologic time. However, given the climate, tectonic and other continually changing and operating earth processes, continuous dessication for 50 million years is extraordinarily unlikely even within uniformitarian aversions to catastrophism.

Next, they suggested that phyllosilicate minerals may have complexed with the lizard skin protein chemical moieties that they identified. For example, broken cystine bonds expose cysteine's –SH groups that might subsequently adsorb to a class of clay minerals called smectite. Perhaps original biochemistry can be preserved indefinitely if the exposed ends of the amino acids adsorb to adjacent minerals and the basal planes of the same amino acids adsorb to more minerals, extending across the whole skin. In short, "Chelate complex formation may act to stabilize these organic compounds over time" (Edwards, *et al*, 2011).

Whereas further modeling is necessary to more fully characterize the potential for various minerals to interact with biomolecules, the theoretical potential for biochemical recalcitrance over geologic time via mineral complexation has more shortcomings than just the requirement of continual dessication through deep time. First, this unique lizard leg fossil skin records not only the presence of these biochemical constituents, but it also records the overall distribution of those original biochemicals across the surface, showing the lizard skin scale pattern. Thus, the postulated mineral complexes would be required not only to shelter the biochemicals from decay, but also to faithfully preserve the gross morphology of scales. The authors did not discuss this question, but presumably the minerals would have to be first distributed across the surface of the fossil in order to interact with the skin. This might be achieved by fluid transport within the relevant bedding plane. However, fluid transport introduces water to the system, and as noted above, water would have dissolved the original organic sulfur that they detected. Subsequently,

the minerals would have to cross-link parallel to the bedding plane in order to tightly hold the gross skin structure in place. The authors did not discuss ways in which these minerals could move into place but not move out, nor ways they might cross-stabilize.

By making the anhydrous condition prevail for 50 million years, Edwards, *et al*, (2011) assume an extremely unlikely scenario whereby the sedimentary micrite of the Green River Formation has virtually no effective porosity or permeability. These authors imagine a "lizard skin tomb" with its own remarkable properties. Are the properties of the supposed tomb more extraordinary than the biomolecular taphonomy preserved on the bedding surface within the rock? Like other characterizations of molecular taphonomy, this one asks us to swallow a generous helping of speculation with just a dash of fact.

Another factor that Edwards, et al, (2011) left unattended was that the very phyllosilicate minerals proposed to sequester biochemistry are renowned for adsorbing and desorbing water, as has been demonstrated by investigating clay soils associated with modern landslides. Indeed, smectite is commercially available as a product used to absorb chemical spills in laboratories or mechanic shop floors. So, in these authors' taphonomic scenario, some of the very water ostensibly required to evenly and completely distribute the minerals across the skin's surface would be absorbed by those minerals, causing competition with the skin biochemistry for the mineral's water-filled molecular pockets and tubes. Further studies would be needed to ascertain how this water would affect the mineral's potential to form the alleged sequestering bonds with nearby biochemistry. Thus, the very taphonomic scenario intended to favor preservation across deep time presents thus-far unexplained difficulties for original tissue preservation.

Further demonstration of the potential for minerals to adhere to original biochemistry, form a broad sheet to preserve those proteins, melanins, and other organics in their original gross structure, and to persist in this state for millions of years is in order. For example, protein decay could be measured over time in experimental setups of the proposed mineral-organic complexation, similar to the now well-established techniques used to measure bone collagen decay rates. Without such studies, the mineral-sequestering taphonomic scenarios remain just-so stories that present insufficient rescuing devices.

Bacterial Biofilm as a Rescuing Device

Thomas Kaye, et al, (2008) used scanning electron microscopy and infrared spectroscopy of fossil bone coatings to support their claim that "dinosaurian soft tissues" are not original to the creatures, but instead result from more recently derived bacterial biofilms. This interpretation appears to have relied on techniques that may have been hand-picked for their potential to more easily fit their hypothesis, and it failed to face the most relevant fossil data. For example, the study did not address the previously published characterizations by immunological assay (Schweitzer, et al, 2005) except to assert that others' claims of original dinosaur tissue can be dismissed simply because their SEM images are consistent with biofilms and more consistent with the constraints of biomolecular decay rates.

In response to the bacterial biofilm suggestion, Schweitzer, *et al*, (2009) enlisted Harvard colleagues to separately sequence vertebrate-specific collagen protein from hadrosaur. The results reported antibody reactivity to elastin, laminin, and hemoglobin from the demineralized hadrosaur bone. These three additional proteins, like collagen, are produced by vertebrates and

not by microbes. Prior reports had already characterized the same proteins from *T. rex* (Schweitzer, 2007).

Bacterial biofilms also fail to match whole-cell and tissue morphology from Hell Creek dinosaur bones and other original organic fossil material. For example, bacterial biofilms are not known to conform to the peculiar osteocyte morphology clearly represented in the literature (Schweitzer, *et al*, 2009, Armitage & Anderson, 2013). And although Kaye, *et al*, (2008) reported bacterial slime trails, these trails are not known to form branching, hollow tubes with red-colored erythrocyte-like inclusions.

Schweitzer even more thoroughly refuted the bacterial biofilm hypothesis by reporting (2013) immunological reactivity in dinosaur bone tissue to additional vertebrate-specific proteins PHEX, actin, tubulin, and Histone H4. In sum, "It has been proposed...that the 'vessels' and 'cells' arise as a result of biofilm infiltration; but no data exist to support this hypothesis" (Schweitzer, *et al*, 2013).

ORIGINAL TISSUES AND CREATION RESEARCH

The current status of soft tissue fossil research offers several cautions to creationary attempts to interpret and communicate them. First is the often opaque or absent chemical identification offered in many paleontology reports. For example, the popular phrase, "remarkable preservation" most often does not refer to original biochemistry, but to such details as whole-body outlines or mineralized tracings of organs or other body parts.

Second, fossil biochemicals are more often than not altered by mineralization and in particular keratinization, and cannot therefore be considered to be original. A problem for creationary interpretation is that many secular study authors do not report detailed fossil chemistry. Instead, molecular characterization often takes a back seat to phylogenetic comparisons. But unless chemical analyses are performed, for example by sequencing, tissue-specific staining, immunological reactivity, synchrotron X-ray, or GC mass spectrometry, to verify original tissues, then claims of "soft tissue preservation" cannot be reliably inferred to indicate a contrast to deep time age assignments.

Last, the longevity of certain biomolecules is either unknown or poorly characterized. In particular, hemoglobin, chitins, melanins and keratins are reported in fossil dinosaur and mosasaur, insect and lobster, dinosaur skin and squid ink, bird feathers and reptile skin, respectively. But without more intimate knowledge of their decay rates, care must be taken to avoid overstating its case for creation. At best, characterization of these fossil molecules from Mesozoic or Paleozoic strata are considered unexpected by uniformitarians. However, their attempts at explaining the data from their perspective should not be ignored because they might advance our understanding of what could or could not lengthen molecular longevity. In any case, young-world arguments offered from original tissue fossil finds should be limited to those finds that characterize biochemicals with known decay rates. Reporting other molecular finds should be appropriately cautious and accompanied by reasonable caveats.

CONCLUSIONS

Paleontology continues to unearth original tissue fossils from unique pockets found on several continents. Work to identify and characterize their biochemistry continues to reveal new fossil

biochemicals and more information on fossil biomolecular integrity. Work is also ongoing, albeit more slowly, to characterize the decay rates for specific biochemicals in certain settings. Half-life measurements for two molecules in particular—bone collagen and bone mitochondrial DNA—are now rigorously established, and suggest maximum time-to-dust age estimates given assumed temperatures. At reasonable earth surface temperatures, these rates predict near-total molecular degradation on the order of hundreds of thousands of years for collagen, and approximately ten thousand years for DNA. These maximum age estimates are incongruent with fossil age assignments in general, and incongruent by orders of magnitude with Mesozoic original biochemistry discoveries.

Evolutionary research continues to explore possible modes by which biochemistry can endure across geologic time despite the counter-indications from decay measurements. Currently, the most relevant of these modes involve proposed mineral-organic complexes. While these ideas await further investigation, thus far they remain untested hypotheses with logical and empirical shortcomings, and are therefore unconvincing.

In addition, a few studies of carbon-14 ratios from fossils corroborate the time indications inferred from biomolecular decay data. Additional carbon dates for fossils would be a relevant pursuit for future studies. Unlike most uniformitarian methods of fossil dating, the carbon-14, collagen, and DNA integrity analyses derive directly from the fossils. These more direct methods so far confute uniformitarian age assignments. Nevertheless, creation research and communication of original tissue fossil finds is cautioned to carefully scrutinize each report in order to avoid the potentially embarrassing pitfalls of incorrectly identifying mineralized or impressed tissues as original biochemistry and of succumbing to the temptation to sensationalize results that appear to favor biblical creation but may yet be explained, at least in part, by unknown natural processes instead of exclusively by a thousands-of-years time scale. At present, certain original tissue fossils strongly challenge deep time fossil age assignments. Further research into biomolecular decay rates, in particular for arthropod chitin, fungal chitin, melanins, and keratin, all of which persist in fossils, may add more such challenges.

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