Finite ages from the Mesozoic era
Is bone collagen an open system ?

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THE COLLAGEN CONTROVERSY

Detections of original organics in fossils continue to grow.[1] Once thought of as soft parts preserved as mere impressions, new techniques reveal original biomaterials including whole tissues that persist in ancient and fossil samples of various taxa, including dinosaurs.

In 1916, Barnum Brown described a Corythosaurus casuarius with skin and tendon structures.[2] Modern techniques including mass spectrometry specify similar structures that consist partly, primarily, or entirely of original organics, consistent with preserved collagen. Examples include light micrographs of blood vessels in T. rex and Triceratops horridus,[3] protein sequence in Brachylophosaurus canadensis,[4] SR-FTIR mapping of protein signatures in a Jurassic Lufengosaurus,[5] and pliable extracellular bone matrix in the mosasaurid Prognathodon,[6] Allosaurus fragilis, and Jurassic Apatosaurus.[7] However, artificial decay of bone collagen suggests collagen should have extinguished in Pliocene strata.[8]

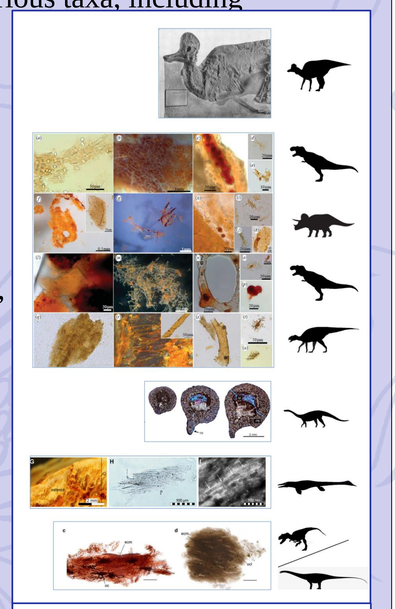
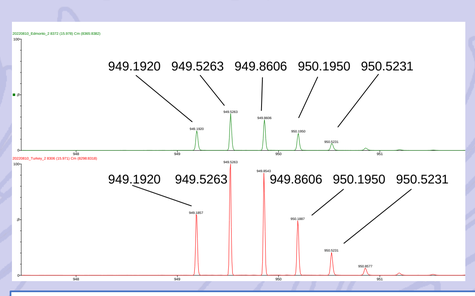


Fig. 1. Light micrographs and other images accompany dozens of molecular techniques that detect collagen remnants in fossil bone.
See text for references.

This study uses four parallel techniques to investigate the possibility of collagen preservation in fossil bone samples. Techniques included LC-MS/MS, ATR-FTIR second-harmonic generation (SHG) imaging, and accelerator mass spectrometry (AMS).

LC-MS/MS

Protein analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a standard of bone collagen identification in ancient samples. We used LC-MS/MS on trypsin digests of ~20 mg (from a total 22 kg) sacrum bone of *Edmontosaurus annectens* (UOL GEO1, pictured right) as well as modern turkey.



Waters™ Xevo G2 QToF with electron spray ionisation sample injection for MS analysis.

Fig. 2. Peaks in the range m/z 949 to 951 characteristic of collagen were found in both samples. *Edmontosaurus* in green (top). Modern turkey in red (bottom).

Edmontosaurus powder was treated[9] to perform a pre-screening check for collagen presence. HCl was used to dissolve the mineral fraction. The aliquot was centrifuged and drained, leaving any collagen behind. This was dried and analysed using Attenuated Total Reflectance (ATR) FTIR.

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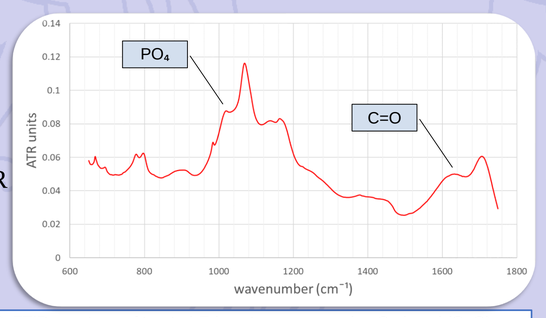

Fig. 3. FTIR spectrum of treated *Edmontosaurus* powder. Carbonyl (C=O) stretching (associated with collagen[10]) is found between ~1630 and 1650 cm^{-1}. PO_4 is normally between ~1010 and 1030 cm^{-1}. Although still visible, it is diminished compared with untreated C=O/PO_4 ratios.

SHG

Second-Harmonic Generation Imaging

SHG confocal microscopy uses a 920 nm laser to target type I collagen fibres. Prior results demonstrated that SHG reveals decayed collagenous structures in ancient bone.[11] New results shown in Figure 4 extend SHG to three Mesozoic samples. ImageJ was used for image processing.

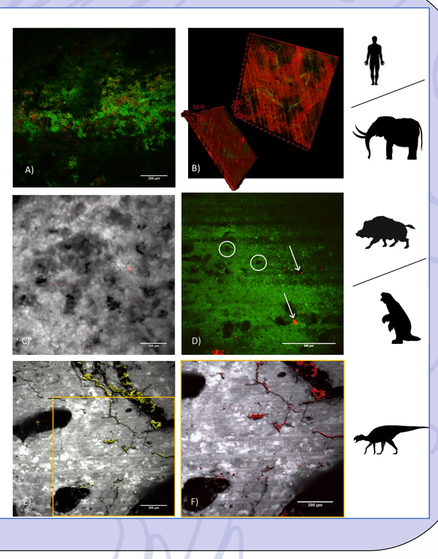

Figure 4. SHG imaging (red) shows collagen fibre remnants and autofluorescence (green) shows organic ring structures. A) Collagen remnants in medieval *H. sapiens* rib NP73_34_81. B) Collagenous osteons in *Mammuthus primegenius* femur YG130.2 C) Reflectance plus SHG of Roman Era *Sus scrofa* jaw XA102-2001-98 D) Collagen traces in pits and on surface of Ice Age *Megatherium* EHRC90002. E) SHG, autofluorescence and reflectance overlay, and F) SHG and reflectance overlay of hadrosaur femur GDFM04.001 show collagen in bone recesses.

Radiocarbon

Catalog No.	Genus	Era	Collagen (C) / Bioapatite (B)	δ13C	pMC	Lab No.
NP73_34_81	Homo sapiens	Medieval	C		92.00	Beta 425,286
XA102-2001-98	Sus scrofa	Roman	C		77.38	Poz 22846
EHRC90002	Megatherium	Ice age	B	-11.1	8.24	UGAMS 20475
GDFM04.001	Hadrosaurid	Cretaceous	C	-6.4	4.09	UGAMS 01935
GDFM04.001	Hadrosaurid	Cretaceous	C	-15.7	4.36	UGAMS 01936
GDFM04.001	Hadrosaurid	Cretaceous	C	-22.7	5.59	UGAMS 01937
GDFM04.001	Hadrosaurid	Cretaceous	C	-18.4	5.72	GX-32678
GDFM04.001	Hadrosaurid	Cretaceous	C	-16	6.17	GX-32739
CM00094	Diplodocus longus	Jurassic	B	-15.88	3.52	UGAMS 20478
YG130.2	Mammuthus primigenius	Ice age	C		1.04	UGAMS 39891

Table 1. 10 AMS results for five ancient and fossil bone samples corresponding to SHG data in Figure 4 show measurable pMC in all eras.

AMS

Bone collagen extracts are typically used for AMS radiocarbon dating, but $^{14}C/^{13}C$ ratios can also be taken from the mineral (bioapatite) fraction. Commercial labs were used to test for independent collagen extraction from bone samples including those imaged in Figure 4.[12] Table 1 summarizes the AMS results. All samples had $^{14}C/^{13}C$ ratios greater than instrument background blanks. Research focused on isotopic signatures of primary organics and not carbon ages. Thus, AMS results from bioapatite fractions were also obtained for bones with too little collagen for RC dating.

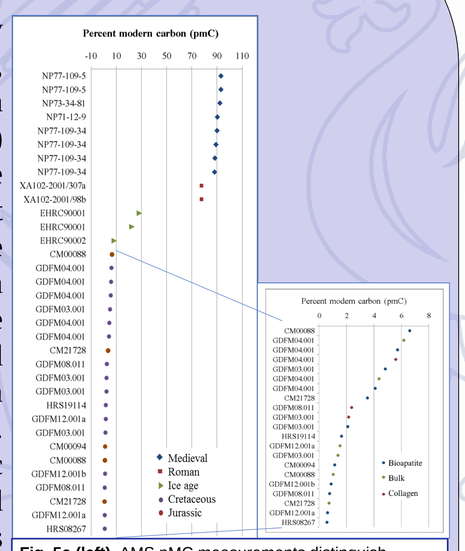

Fig. 5a (left). AMS pMC measurements distinguish between Medieval, Roman and Ice Age but not between Cretaceous and Jurassic.
Fig 5b (right). Mesozoic ^{14}C results arranged according to pMC and bone fraction.

One of three Mesozoic samples yielded collagen for carbon dating. The mammoth YG 130.2 pMC was closer to Mesozoic than other ice age results. Four results show ^{14}C in bulk extracts, which mix organic (collagen) and mineral bone fractions. A lack of correlation between pMC and fraction suggests that $^{14}C/^{13}C$ can occur in any fraction. pMCs in this data set do not resolve known eras.

Overall, measurable $^{14}C/^{13}C$ ratios, including directly from collagen, are consistent with the retention in fossil bone of original biological components.

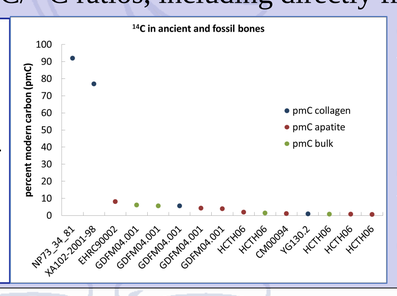

Fig. 6. 15 AMS pMC measurements in all specimens shown in Fig. 4. High collagen density with low pMC in YG130.2 is attributed to organic preservation via permafrost. One of five results for the GDFM04.001 hadrosaur came from commercial collagen extraction. See Abbreviations key for repository names.

CONCLUSIONS

Evidence for collagen presence in dinosaur bone is provided by (a) LC-MS/MS on trypsin digests of bone samples (b) ATR-FTIR after treatment with HCl (c) SHG imaging of collagen fibres. Further confirmation is provided by measurable pMC after AMS on the collagen fraction. This raises the question as to whether bone collagen is an open system and if so, to what extent. A literature search revealed previously published ^{14}C in carboniferous material including fossils from Mesozoic and earlier, showing that although unexpected, the data presented here have precedent. The ^{14}C dates obtained from AMS on the collagen fraction agree with those obtained from the apatite fraction and this is difficult to reconcile with the hypothesis of a secondary (more recent) source for the collagen. Three options present themselves as possible explanations:

(i) The biomaterial is not primary collagen.

(ii) Collagen decays at a rate orders of magnitude slower than artificial decay studies show.[8]

(iii) The collagen detected is primary but buried orders of magnitude later than prevailing age assignments for these Mesozoic fossils.

^{14}C dates on the apatite fraction to the same values is evidence *contra* (i). More research is needed to address (ii) and (iii).

Abbreviations key:

NP = Norton Priory, Runcorn, UK
XA = (Hallaton) U. of Leicester, Leicestershire, UK;
EHRC = Earth History Research Center, Keene, TX
GDFM = Glendive Dinosaur and Fossil Museum, MT
HCTH06 = See ref. 10.
CM = Carnegie Museum, Pittsburgh, PA
YG = Yukon Government

References

1. Thomas, B. and Taylor, S. Exp. Rev. Proteomics 16(11–12): 881–895, 2019.
2. Brown, B. *Bull. Amer. Mus. Nat. Hist*. 35(38): 709–716, 1916.
3. Schweitzer, M.H. et al. *Proc. R. Soc. B.* 274(1607): 183–197, 2007.
4. Schroeter, E.R. et al. *J. Prot. Res.* 16 (2): 920–932, 2017.
5. Reisz, R.R. et al. *Nat*. 496(7444): 210-214. and Lee, Y-C. et al., *Nat. Comm*. 8: 14220, 2017.
6. Lindgren, Y. et al., *PLoS ONE*. 6 (4): e19445, 2011.
7. Wiemann, J. et al. *Nat. Comm*. 9: 47412018, 2018.
8. Buckley, M., and M.J. Collins. *Antiqua*. 1: e1, 2011.
9. Yizhaq, M. et al. *Radiocarbon* 47(2): 193–206, 2005.
10. Thompson, T. et al. *J. Archaeological Science* 40(2013): 416–422, 2013.
11. Thomas, B. et al. *Bone Rep.* 7(2017): 137–144, 2017.
12. Armitage, M. et al. *Acta Hist*. 115(6): 603-608, 2013.

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