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Patterns in Palaeontology: Palaeoproteomics

by Caitlin Colleary*[1]

Introduction:

The fossil record is our only direct window to the history of life on Earth. The ability to find and study the remains of animals, plants and other organisms that lived millions of years ago is extraordinary, and as technology has improved over the past few decades, scientists have realized that fossils contain more information about the stories of extinct life forms than even Charles Darwin could have imagined. Biomolecules (such as DNA, proteins and lipids) that make up modern animals contain information about how their bodies work (physiology — that is, physical and chemical functions), relationships to other animals and their evolutionary histories. With the advances in analytical tools such as high-resolution mass spectroscopy, the study of biomolecules has finally been extended back in time to the fossil record (Fig. 1). However, biomolecules break down, and how they are altered affects how we interpret the organism and its environment. Nucleic acids (for example, DNA) contain the most information, but degrade the most quickly, so scientists are investigating other biomolecules that are found in fossils. The study of protein biomolecules has led to a new subfield of palaeontology called palaeoproteomics (Fig. 2), and the results of these studies have the potential to shed light on vertebrates much further back in time.

What can proteins tell us?

Bones have both organic and inorganic components. The inorganic, or mineral, component gives bone its strength and rigidity, whereas the organic component is mostly proteins. The main protein in bone is collagen; 28 different types have been identified, but the majority in vertebrates is what is known as type I collagen. In the body, collagen strengthens everything from bone to ligaments, tendons, cartilage and skin. Collagen is made up of strings of amino acids, called peptides. How it degrades is not well understood, but evidence of proteins, peptides and single amino acids that are present in collagen has been found in the fossil record. This is exciting for palaeontologists because it represents an aspect of the original animal that could not previously be studied, but that can be preserved for millions of years, giving us even more information on the original biology of animals that cannot be directly studied today. One of the techniques palaeontologists use to study collagen preserved in ancient bones is called collagen peptide fingerprinting. This works in a similar way to human fingerprinting: by attributing unknown bone fragments to an animal. This would otherwise be impossible when dealing with small, indistinguishable bone fragments, so collagen peptide fingerprinting is often the only evidence that a particular animal appeared in a certain time or place in the fossil record.

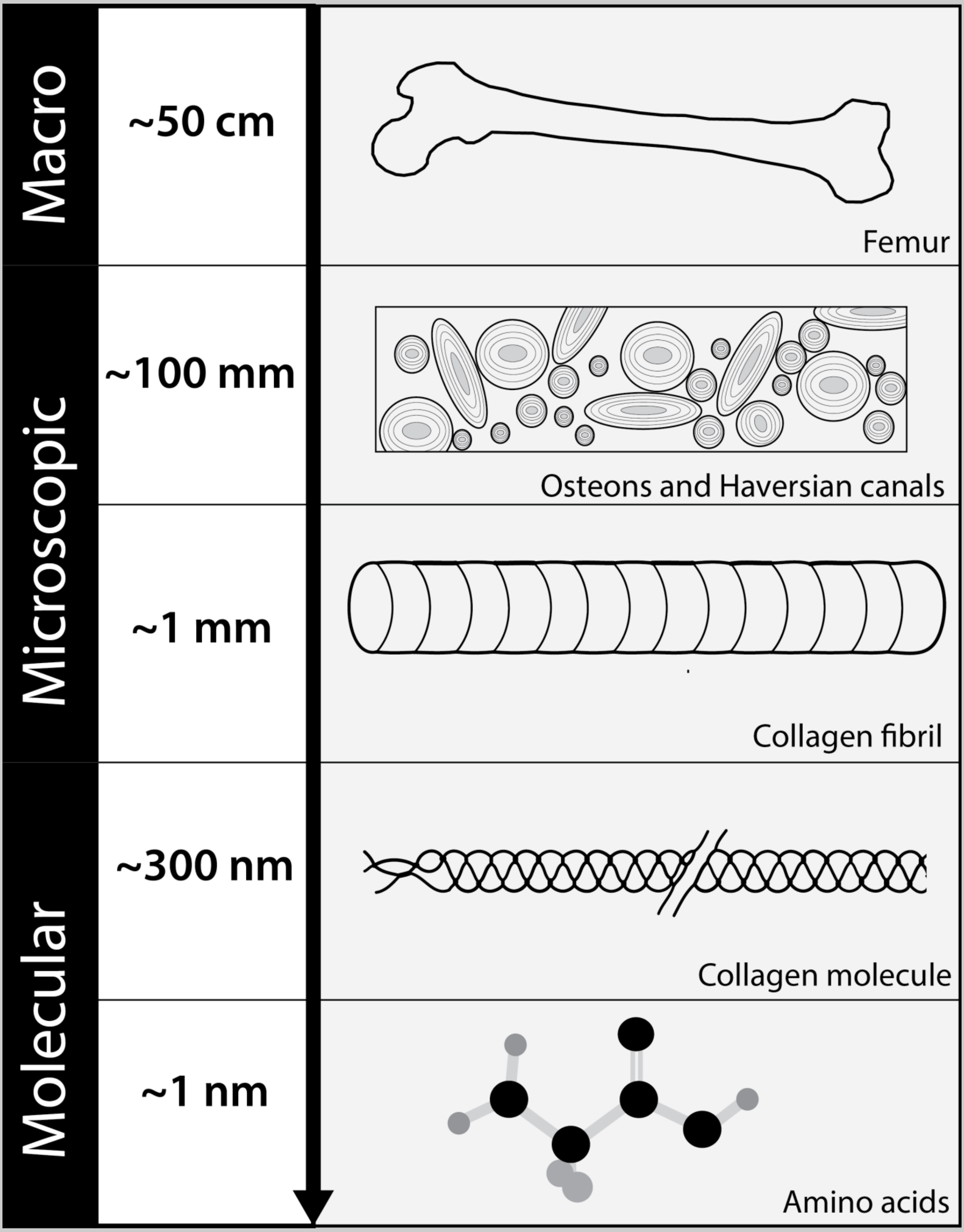

Figure 1 — Researchers study vertebrates in the fossil record in many different ways. At the macro level, they analyse features on bones that explain how ancient animals are related and have evolved. At the microscopic level, researchers can study the microstructure of bones to understand how ancient animals grew. Now, they are also beginning to study biomolecules in bone (the molecular level). One of the molecules they look at is the protein collagen, which is made up of amino acids. Credit: C. Colleary.

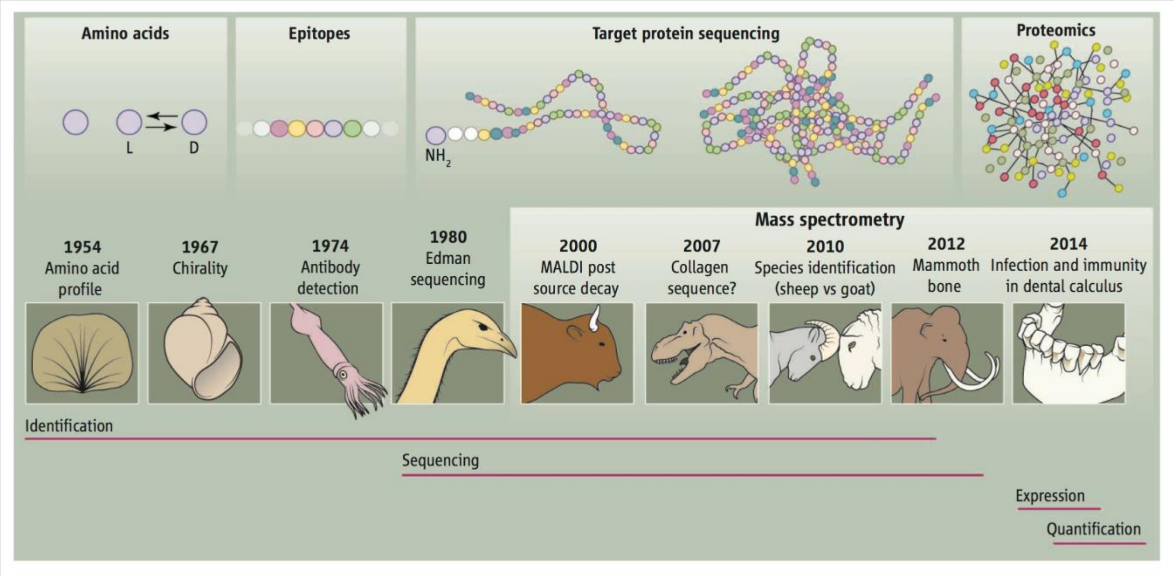

Figure 2 — History of palaeoproteomic studies. Some of the greatest advances were made after researchers started to use high-resolution mass spectrometry in 2000. MALDI is an example of one of these mass spectrometry techniques. Source: Cappellini *et al*. (2014).

Figure 3 — The toe bone of a horse found in permafrost, dating from approximately 560,000 to 780,000 years ago. This bone was sampled for protein analysis using both surface mass spectrometry and protein sequencing. Researchers identified 73 ancient bone proteins. Source: Orlando *et al*. (2013).

The majority of palaeoproteomic studies have focused on fossils from the Pleistocene epoch (approximately 2.5 million to 11,700 years ago; Fig. 3), because these have often been preserved in conditions that help biomolecules survive; for example, many have been frozen in permafrost.

Biomolecules are likely to have broken down if the organism has been preserved in less suitable conditions, or if it died much longer ago than the Pleistocene, but to what degree they have broken down and what sort of information we can still gather from them is a focus of current studies.

One of the most recognizable Pleistocene animals is the mammoth; proteins in mammoth bones have been studied extensively (Fig. 4), and scientists have discovered differences between the sequences of these proteins and those found in modern elephants. These differences can give us information on how mammoths were adapted to their environment and how closely they are related to elephants and other animals, living or extinct. For example, mammoths were found to have changes in their blood proteins indicative of adaptations for cold weather. The differences between protein sequences in mammoths and elephants can also tell us about how proteins have degraded since the animal died. Distinguishing between changes that occurred while the animal was alive (which are important for understanding the physiology of an animal) and changes that came after death (which are important for understanding how proteins degrade and preserve) is essential in how we interpret the proteins we find and what information we can gain about the animal.

A 2015 study was able to tell the difference between protein changes that occurred during the life of a moa — a giant bird that went extinct around 600 years ago (Fig. 5) — and those that happened after death. The researchers were able to do this by comparing the information preserved in the bones with what is currently known about how proteins behave in the body and how they change during the life cycle of an animal. It may now be possible to begin compiling protein databases for fossilized animals (similar to those that exist for living animals), including the proteins that changed in the animals' lifetime.

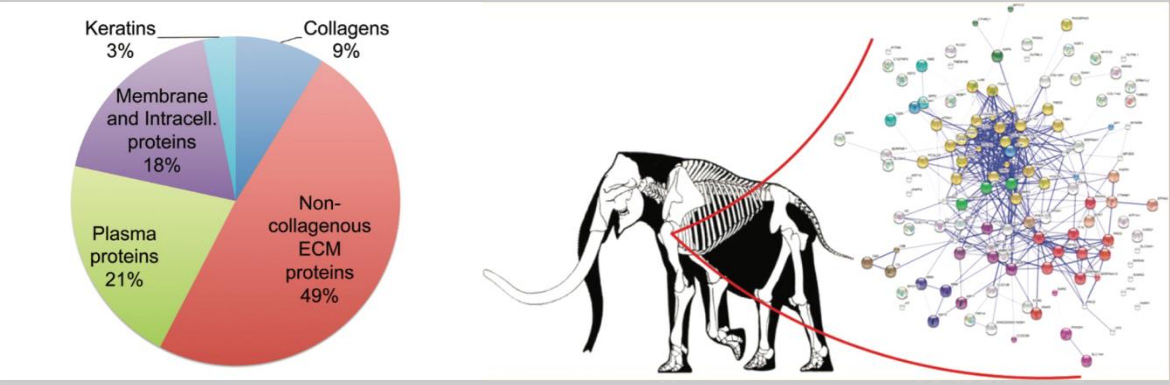

Figure 4 — Of the proteins sequenced from a 43,000-year-old mammoth preserved in permafrost, only 9% were from collagen. The majority were much less common proteins, which may indicate that these are preserved more easily than collagen. Source: Cappellini *et al*. (2012).

How long can proteins preserve in fossils?

Palaeontologists have discovered proteins in 3-million-year-old ostrich eggshells, which will help us understand even more about how proteins might be preserved. In the eggshells, they found that the proteins actually bind to the original mineral surface. This would potentially allow them to be preserved for a very long time, perhaps even as far back as the origin of dinosaurs.

Figure 5 — Moa painting by German artist Heinrich Harder (1858–1935). Source: WikiCommons

Identifying proteins as far back in time as the non-bird dinosaurs, which lived approximately 230 million to 66 million years ago, is extremely complicated. Palaeontologists are still trying to understand exactly how these biomolecules degrade, how they might be preserved and how much is still present in the fossil. They also have to account for potential sources of organic contamination, which only increases with time.

But that doesn't mean palaeontologists aren't going to try.

New studies are coming out all the time, reporting evidence of peptides in dinosaur fossils from the Jurassic and Cretaceous periods and even amino acids from the Triassic Period. One of the most well known studies is from the Cretaceous period, from a *Tyrannosaurus rex* fossil from the Hell Creek Formation in Montana (approximately 68 million years old). This fossil was the subject of multiple analyses, including mass spectrometry, and the results showed the presence of amino acids such as glycine and alanine. These amino acids are present in collagen and were found in the bone, but they were not found in the surrounding sediment.

Although not all scientists yet agree that proteins can be preserved on such long time scales, over the past few decades (as technology has improved), we've been able to uncover more from the fossil record than we could have ever imagined. More and more studies are trying to push our ability to detect proteins and other biomolecules further back in time, but we still need to have a better understanding of how they degrade and how they can be preserved in vertebrate fossils to really understand how this avenue of research can tell us about animals that have long been extinct.

Suggestions for further reading:

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