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Research paper

New single amino acid hydroxyproline radiocarbon dates for two problematic American Mastodon fossils from Alaska

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ABSTRACT

American mastodon (*Mammut americanum*) was amongst the widest ranging of Pleistocene megafaunal species, though their fossils are rare in Alaska and northwest Canada. Questions remain about their extinction chronology at high latitudes because of the limited numbers of available radiocarbon dates. New radiocarbon dates for two American mastodon fossils were generated at two separate accelerator mass spectrometry laboratories using two different approaches, dating ultrafiltered 'collagen' vs. single amino acid fractions. The bulk dates for these specimens are significantly younger than the single amino acid (hydroxyproline) dates, which are in turn close to the background threshold for radiocarbon dating. On closer study we established that contamination from consolidants used in museum conservation was not removed thoroughly despite extensive physical and chemical cleaning procedures having been applied, and this led to the anomalous ultrafiltered 'collagen' results. The new hydroxyproline dates give support to older ages for American mastodons in the Arctic.

1. Introduction

Accurate radiocarbon dating of bones remains challenging decades after the introduction of accelerator mass spectrometry in the late 1970s. Although different organic fractions can be extracted from archaeological and palaeontological bone using different methods for the purpose of radiocarbon dating, in most instances, the fraction dated is the organic fraction commonly described as 'collagen', which consists predominantly of collagen as well as other proteins (hereafter referred to as collagen).

Despite the advancement of collagen-extraction procedures over recent years, including the application of ultrafiltration, (Brown et al., 1988; Bronk Ramsey et al., 2004; Brock et al., 2007; Beaumont et al., 2010) the presence of contamination within samples remains a persistent issue. It is demonstrably difficult to achieve a contaminant-free sample that might be regarded as representing the original material, unaltered by any natural or artificial processes over the years since it ceased interaction with the biosphere. In routine radiocarbon pre-treatment methods the samples are screened based on the C:N atomic ratios of purified collagen, the collagen yield and carbon content, as well as $\delta^{13}C$ and $\delta^{15}N$ values prior to accelerator mass spectrometry (AMS) analysis (e.g. van Klinken, 1999). Fourier Transform Infrared Spectroscopy (FTIR) has also been used to screen bones and the products of collagen extraction, prior to dating, to identify the potential presence of contaminants (e.g. Gianfrate et al., 2007; Yuan et al., 2007; France et al., 2011). However, whether these techniques are employed independently or collectively, they are not always efficient in detecting trace levels of contamination that have managed to resist pre-treatment procedures. For example, an infinitely old sample with just 1% modern carbon contamination will not analytically produce an age greater than 38000 radiocarbon years (Bowman, 1995), yet the C:N atomic ratio for the contaminated bone would remain within the acceptable range. Moreover, the accuracy of these pre-treatment methods is generally assessed on their ability to precisely date a standard material of known radiocarbon activity. These standards are usually chosen because they

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are clean (or in the case of bone, very easily cleaned to produce infinite dates) and are used to assess the amount of contamination added in the laboratory. These standards are completely unsuitable to assess the ability of a certain treatment to remove a specific contaminant. Also, many times the reliability of a given radiocarbon date is judged based on the archaeological or palaeontological context from which the sample is recovered, the demonstrated purity of the material analysed, and the known accuracy and precision of the analytical method employed. Generally, the apparent reliability of a radiocarbon date comes under scrutiny only when it seriously deviates from expectations, such as the inferred age of the context in which a sample was found. However, if the context is unknown or cannot be reconstructed, an erroneous date can easily be accepted as the actual date of the material. A radiocarbon dating procedure considered to be reliable based on the selection of only certain of the criteria mentioned above cannot guarantee its universal suitability or accuracy for all samples.

Radiocarbon dating single amino acid, hydroxyproline has been demonstrated to fulfill all the basic criteria to be deemed as method of choice for dating problematic bone materials (McCullagh et al., 2010; Marom et al., 2012). Here we show how hydroxyproline radiocarbon dating can be used to resolve problems in defining the age of recalcitrant paleontological specimens.

American mastodons (*Mammut americanum*) were widespread members of the Pleistocene megafauna in North America, ranging from the tropics of Central America in Honduras to near the Arctic coast of Alaska (Kurtén and Anderson, 1980; Faunmap Working Group, 1996). As tracked by radiocarbon dating, mastodons living near the Great Lakes region of the American Midwest were amongst the latest surviving members of the Pleistocene megafauna, lasting until ~10000 ^{14}C yr BP (Woodman and Athfield, 2009). However, questions remain regarding the chronology of this species at high latitudes and their possible persistence in the cold, dry steppe-tundra environment that characterized glacial periods in Alaska (Guthrie, 1990). Many researchers have assumed that American mastodons only lived at high latitudes during Pleistocene interglacial periods when boreal forest habitats recolonized the arctic and subarctic landscape (Harington, 1990). The radiocarbon dates reported in this paper suggest how lack of available samples for dating and a reliable radiocarbon chronology for mastodons in Alaska has prevented a robust understanding of how this large mammal responded to late Pleistocene environmental change, and the timing of their extinction on a continental scale.

American mastodon fossils have been collected in Alaska for nearly a century, though they are relatively rare in comparison to remains of the other extinct proboscideans that inhabited North America in the latter part of the Pleistocene, *Mammuthus primigenius* and *Mammuthus columbi* (the woolly and Columbian mammoths, respectively). Harington (1978) estimates that less than 5% of proboscidean fossil remains in eastern Beringia represent American mastodons (c.f. Guthrie, 1968).

Glues, varnishes, and other preservatives have been used since the earliest days of paleontology to protect fossils from damage or deterioration. Such materials are usually carbon based, and may affect the radiocarbon date. Therefore, best practice is to collect a sample for dating before the application of any reagents. However, a record of consolidant application is usually lacking for fossils conserved in the early twentieth century.

We encountered this last problem whilst conducting a study of high latitude American mastodon fossils. Among our specimens were two samples that yielded ultrafiltered 'collagen' radiocarbon dates from as late as the Last Glacial Maximum, and which were significantly younger than those recovered for other samples. Because mastodon survival this late in northwestern North America seemed improbable, we questioned whether the presence of undetected consolidants in the samples might have skewed our radiocarbon dating results. We tested this idea using a novel approach developed at the Oxford Radiocarbon Accelerator Unit (ORAU) that utilizes radiocarbon dating of a single amino acid, hydroxyproline. In brief, we found that the single amino acid dates were close to background ^{14}C levels, and thus are in better agreement with ages derived for other high latitude mastodon samples.

2. Samples

Specimen 1, UAMES 7663 from the University of Alaska Museum Earth Sciences Collection is an American mastodon cranium (Fig. 1) recovered at a placer gold mine at Livengood, Alaska, approximately 80 km (50 mi) north of Fairbanks, in 1941 and donated to the University of Alaska Museum of the North in the early 1980s. Exposed surfaces of this cranium are covered with a thick coating of an unknown consolidant. Specimen 2, AMNH 103277 is registered in the American Museum of Natural History, vertebrate paleontology catalog as a mandible recovered at Little Eldorado Creek, near Fairbanks, Alaska, in 1940, but no further information is available. We are of course aware that these samples may also contain carbon-containing contaminants other than those derived from consolidants, but FTIR analysis (see below) indicates that the latter were certainly present and most probably the major, if not the exclusive, source of error. Sample AMNH 103277 showed clear signs of the presence of surface consolidants. Presence of consolidants was also suspected on sample UAMES 7663 although not visible to the naked eye.

3. Methods

3.1. UCIAMS [University of California Irvine accelerator mass spectrometer] ultrafiltered 'collagen' extraction method

After removal of the bone surface with a Dremel™ grinding tool, ~150 mg of bone was crushed to mm-sized chips. Since the presence of consolidants was known or suspected, the crushed bone samples were sonicated in acetone, methanol and water (for 30 min each) before decalcification. The 2nd specimen (the mandible) produced lots of flocculent material and so the solvent wash

Fig. 1. *Mammut americanum* cranium, UAMES 7663, at the University of Alaska Museum.