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Taxonomy, location of origin, and health status of proboscideans from Western Canada investigated using stable isotope analysis

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**Taxonomy, location of origin, and health status of proboscideans from Western Canada
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Running head: **Stable isotope analyses of Western Canada proboscideans**

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Abstract

We investigated the application of stable isotope analysis of proboscidean remains (collagen in bone/dentin/cementum and structural carbonate in enamel bioapatite) for genus-level identification of isolated specimens, assessment of geographic origins, and testing for nutritional stress. Mammoths (*Mammuthus* sp.) tended to have higher $\delta^{15}\text{N}_{\text{col}}$ and lower $\delta^{13}\text{C}_{\text{col}}$ than mastodons (*Mammuth americanum*), but differences were not significant in every location. Determining the genus of isolated specimens may be possible for locations and time periods with good isotopic baselines, but environmental changes can confound interpretations. For example, an Alberta proboscidean with a $\delta^{15}\text{N}_{\text{col}}$ of +1.4‰ (characteristic of mastodons) ultimately proved to be a mammoth. Its surprisingly low nitrogen isotope composition is attributable to the recently deglaciated environment it inhabited. We provided a baseline for isotopic assessment of geographic origins of isolated proboscideans in Western Canada. Bioapatite $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ can be used to distinguish specimens from Alberta, Klondike, Old Crow, Herschel Island, and further south (e.g., Arizona, Great Lakes). Finally, we found that an Alberta mammoth with morphological evidence of nutritional stress experienced a change in diet, environment, or physiology prior to death, but its isotopic compositions did not suggest a link to hypothesized starvation (catabolism of proteins or reliance on lipids).

Keywords:

Isotopes; mammoth; mastodon; collagen; carbonate

Introduction

Stable isotope compositions of fossil vertebrate remains can be used to reconstruct the behaviour (e.g., diet, migration), physiology (e.g., starvation) and paleoenvironmental context (e.g., vegetation, temperature, aridity, pCO₂, and canopy cover) of extinct organisms (Clementz, 2012; Hatch, 2012; Koch, 1998; Koch et al., 1994; Kohn and Cerling, 2002; West et al., 2006). Stable isotope compositions are also commonly used to identify or verify the origins of various modern substances (e.g., Chesson et al., 2010a, b), and can also be useful for inferring the geographical origins of humans or animals in ancient landscapes (e.g., Dupras and Schwarcz, 2001; Knudson and Price, 2007; White et al., 1998).

In this paper, we explore the use of $\delta^{13}\text{C}$ (collagen and bioapatite), $\delta^{15}\text{N}$ (collagen), and $\delta^{18}\text{O}$ (structural carbonate in bioapatite) of extinct proboscideans for genus-level identification of isolated remains, determination of location of origin, and investigation of health status, using specimens from Western Canada (Alberta, British Columbia, and Yukon). We ask: (1) Are there consistent differences in the isotopic compositions of mammoths and mastodons in each location? (2) Can skeletal fragments of proboscideans that lack morphologically diagnostic features be assigned to genus (*Mammuthus* vs. *Mammut*) based on isotopic compositions? (3) Are there consistent differences in isotopic compositions of proboscidean fossils across geographic locations (Alberta, British Columbia, and Yukon – including Old Crow, Klondike, and Herschel Island), which could allow location-of-origin determination for isolated specimens from museum collections? (4) Do the isotopic compositions of an abnormal mammoth tooth support a hypothesis of nutritional stress prior to death?

To date, studies that have used stable isotope compositions to investigate proboscidean paleobiology in western Canada have focused on food web and climatic reconstruction (Fox-Dobbs et al., 2008; Matheus et al., 2003a, b; Schwartz-Narbonne et al., 2015; Szpak et al., 2010) or nursing/weaning behaviour (Metcalf et al., 2010) within the Yukon Territory. At a fundamental level, our intent is to provide baseline isotopic data for Pleistocene proboscideans from western Canada. All subsequent paleobiological research questions require an understanding of the variation in isotopic compositions within these taxa across time and space.

Mammoth and Mastodon Ecology

The Mammutidae and Elephantidae diverged at the end of the Oligocene or the beginning of the Miocene, ca. 27-23 Ma (Agenbroad, 2005). Morphologically, the most obvious difference between mastodons (*Mammut americanum*) and mammoths (*Mammuthus* spp.) is their dentition, which is adapted to browsing in the former, and grazing in the latter. Mastodon remains are typically associated with relatively wet, forested regions, such as that present in the Great Lakes region during the terminal Pleistocene (Dreimanis, 1967, 1968; Haynes, 1991; McAndrews and Jackson, 1988; Newsom and Mithlacher, 2006; Saunders, 1996; Yansa and Adams, 2012). Studies of mastodon tooth enamel isotopic compositions, microwear, and gut/fecal contents support a browsing adaptation (Green et al., 2005; Koch et al., 1998; Lepper et al., 1991; MacFadden and Cerling, 1996; Newsom and Mithlacher, 2006), although mastodon diets did include some grasses (Gobetz and Bozarth, 2001; Koch et al., 1998) and mastodons were able to adapt to significant temporal changes in vegetation within the Great Lakes region (Metcalf and Longstaffe, 2014). Woolly mammoths (*Mammuthus primigenius*) were associated with open steppe-tundra environments (Guthrie, 2001; Haynes, 1991) and their southern cousins, the

Columbian mammoths (*Mammuthus columbi*), were most common in temperate grasslands (Agenbroad, 2005). Mammoths were predominantly grazers, as shown by their tooth enamel isotopic compositions, gut contents, fecal remains, and phytoliths in dental calculus, though they also consumed woody plants, mosses, and a variety of shrubs and forbs (Cummings and Albert, 2007; Feranec, 2004; Guthrie, 2001; Haynes, 1991; Hoppe, 2004; Koch et al., 1998; Mead et al., 1986; van Geel et al., 2008). Both mammoths and mastodons were widespread in North America during the late Pleistocene (~125 to 10 ka), occupying most of the regions south of the continental glaciers, as well as Beringia during relatively warm and wet interglacials (Agenbroad, 2005; Harington, 1990; Zazula et al., 2014). Despite their different dietary adaptations and dentitions, isolated or fragmentary postcranial elements of mammoths and mastodons are difficult to distinguish. An independent identification method for these genera would be useful and would permit re-evaluation of specimens previously restricted to higher-level taxonomic identification.

Taxonomic Identification using Stable Isotopes

Previous research has shown that some animal taxa have distinct isotopic compositions relative to others. If isotopic differences among taxa are relatively consistent at a given location and/or during a given time period, they might be useful for taxonomic identification of skeletal elements that cannot be identified on the basis of discrete morphological or morphometric data. This approach was applied by Thewissen et al. (2001), who used carbon isotopes in bioapatite to support identification of Eocene fossils as cetacean versus artiodactyl or anthracobunid, and by Clementz et al. (2009), who used $\delta^{13}\text{C}$ (collagen and bioapatite) to identify fragmentary archaeological bone samples as sea cow, baleen whale, or sperm whale. While ancient DNA or

ZooMS (“collagen fingerprinting”) analyses would provide more definitive independent identification methods (e.g., Buckley et al., 2014; Buckley and Kansa, 2011; Debruyne et al., 2008; Enk et al., 2011; Poinar et al., 1998), isotopic analysis could potentially provide an easier and less expensive methodology that can also be used to address additional paleobiological questions.

In regions with both C₃ and C₄ plants, which have very distinct carbon isotope compositions, $\delta^{13}\text{C}$ can be used to distinguish between browsers (such as mastodons) and grazers (such as mammoths) (e.g., Baumann and Crowley, 2015; Koch et al., 1998). Since cold northern regions lack C₄ plants, $\delta^{13}\text{C}$ is less useful for identification in these locations, although small differences among herbivore taxa can result from differing plant (part) selection, climate or (micro)habitat, and animal physiology (e.g., Bocherens, 2003). In general, mammoths tend to have higher $\delta^{15}\text{N}$ (and sometimes lower $\delta^{13}\text{C}_{\text{col}}$) than coexisting herbivores (Bocherens, 2003; Bocherens et al., 1994; Fox-Dobbs et al., 2008; Iacumin et al., 2000; Koch, 1991; Kuitens et al., 2012; Metcalfe et al., 2013). Schwartz-Narbonne et al. (2015) demonstrated that the high $\delta^{15}\text{N}$ of the woolly mammoth resulted from a distinct habitat or diet rather than physiology. Mammoths and mastodons may also have distinct isotopic compositions if they drank from different water sources, as shown for southwestern Ontario and western New York (Metcalfe et al., 2013). These results suggest that an isotopic approach has potential for distinguishing between mammoths and mastodons in C₃-dominated environments.

Location-of-Origin Determination using Stable Isotopes

The use of stable isotope compositions to evaluate location of origin also has precedents in the literature. Stable C, N, O, and H isotope analyses have been extensively utilized to

determine the location of origin of modern commercial animal products, including milk (Chesson et al., 2010a; Rossmann et al., 1998), cheese (Camin et al., 2012), honey (Chesson et al., 2011), and wool (Hedges et al., 2005). Isotopic compositions have been used to identify the location of origin and life history of human remains (e.g., Corr et al., 2008; Dupras and Schwarcz, 2001; Knudson and Price, 2007; Sharp et al., 2003; White et al., 1998; White et al., 2004), and are increasingly also used to aid in the identification and characterization of forensic remains (Cerling et al., 2003; Ehleringer et al., 2008; Fraser and Meier-Augenstein, 2007; Mays et al., 2011; Meier-Augenstein et al., 2013; O'Brien and Wooller, 2007).

Stable isotope methods for identifying location of origin of animal products and human remains are based on differences in diet and physiology (for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) (DeNiro and Epstein, 1978, 1981; Koch et al., 1994), and geographical variations in drinking water (for $\delta^{18}\text{O}$ and $\delta^2\text{H}$) (Bowen et al., 2005; Bowen and Wilkinson, 2002; Dansgaard, 1964; Ehleringer et al., 2008; Rozanski et al., 1993). In C_3 -dominated environments such as western Canada, regional differences in climate (e.g., temperature, rainfall) and environment (e.g., canopy cover, soil salinity, water availability, nutrient content) can cause small variations in the $\delta^{13}\text{C}$ of C_3 plants, which are passed on to consumers (Arens et al., 2000; Drucker et al., 2008; Kohn, 2010; Murphy and Bowman, 2009; Tieszen, 1991; Van Der Merwe and Medina, 1991). Herbivores occupying different ecological niches may also select plants or plant parts with different $\delta^{13}\text{C}$ values (e.g., Brooks et al., 1997; Tieszen, 1991), leading to different tissue $\delta^{13}\text{C}$. Temporal variations in the proportion and isotopic composition of atmospheric CO_2 , or in any of the factors described above, can also affect plant and consumer $\delta^{13}\text{C}$ (Arens et al., 2000; Long et al., 2005; Schmitt et al., 2012). In the present study, we expect that differences in $\delta^{13}\text{C}$ among regions will be small (e.g., around 1-3‰), with higher $\delta^{13}\text{C}$ in more arid locations (Kohn, 2010; Stewart et al., 1995).

The largest variations in nitrogen isotope compositions of animals result from trophic differences (higher $\delta^{15}\text{N}$ at higher trophic levels), which also affect juveniles consuming their mothers' milk (e.g., Fuller et al., 2006; Jenkins et al., 2001; Metcalfe et al., 2010; Minagawa and Wada, 1984; Rountrey et al., 2007). Herbivores can also have different $\delta^{15}\text{N}$ values because of environmental conditions (e.g., low rainfall amounts and high temperatures are related to high $\delta^{15}\text{N}$ in plants), plant selectivity (e.g., plants with mycorrhizal associations tend to have lower $\delta^{15}\text{N}$), or physiology (e.g., nutritional stress can increase animal $\delta^{15}\text{N}$) (e.g., Amundson et al., 2003; Craine et al., 2009; Drucker et al., 2010; Hartman, 2011; Szpak, 2014; Szpak et al., 2010). If regional differences are observed in the present study, we expect that samples from wetter locations and/or those with less-developed soils (e.g., recently deglaciated landscapes) will have lower $\delta^{15}\text{N}$ values. The magnitude of differences in $\delta^{15}\text{N}$ among regions in the present study is likely to be around 7‰ or less, based on previous isotopic studies of large mammals in glacial/postglacial Europe (Hedges et al., 2004; Richards and Hedges, 2003; Stevens and Hedges, 2004).

Oxygen isotope compositions of meteoric water decrease with increasing latitude, altitude, and distance from the sea, as a result of variations in temperature, rainfall amount, and the movement of air masses (Dansgaard, 1964; Rozanski et al., 1993; West et al., 2006). For example, modern mean annual meteoric water $\delta^{18}\text{O}$ values are approximately 3.5‰ lower in the Klondike region (Mayo, Yukon) than in Edmonton (Alberta) (IAEA/WMO, 2010). Geographic variations in modern meteoric water $\delta^{18}\text{O}$ are well-documented in global isoscape maps (Bowen, 2010; Bowen et al., 2005; Bowen and Wilkinson, 2002; West et al., 2006). Drinking water $\delta^{18}\text{O}$ values are passed on to large-bodied obligate drinkers such as proboscideans, but interpretation of ancient animal $\delta^{18}\text{O}$ can be complicated by climate change, mobility, and non-meteoric water

sources (e.g., ancient groundwater or glacial meltwater) (Ayliffe et al., 1992; Bryant and Froelich, 1995; Kohn, 1996; Kohn et al., 1996; Levin et al., 2006; Longinelli, 1984). Thus, if isotopic analyses are used to infer location of origin, it is important to establish local baselines for the ancient ecosystems and taxa of interest. Generally, we expect that samples from higher-latitude locations like Klondike and Old Crow will have lower $\delta^{18}\text{O}$ values than samples from lower-latitude or coastal locations like Alberta and British Columbia.

Based on the variations described above, an isotopic approach could be used to investigate the geographic origins of museum specimens. For example, Metcalfe et al. (2013) established a baseline for mammoth and mastodon isotopic compositions in Ontario, and examined three proboscidean specimens that lacked contextual records. A mastodon tooth they examined had isotopic compositions consistent with an origin in southwestern Ontario, whereas two mammoth teeth were extreme outliers. The authors determined that one mammoth likely lived during an interglacial period or in a more southern location, and the other was likely from Klondike, Yukon Territory. In some cases, a similar isotopic approach could also be used to investigate migration: if depositional contexts are secure and environmental change or microhabitat differences are deemed unlikely (e.g., because the same taxa are analyzed), outlying isotopic compositions can indicate migration from distant locations (Hobson and Koehler, 2015; Hoppe, 2004; Keenleyside et al., 2011; Toyne et al., 2014; Webb et al., 2013).

Nutritional Stress: Isotopic Indicators

The isotopic effects of nutritional stress are generally difficult to distinguish from other factors such as diet, climate, or geographic origins, and the relationship between food stress and isotopic compositions is not always straightforward, particularly among different species (Hatch,

2012; McCue and Pollock, 2008; Reitsema, 2013). Nevertheless, starvation, leading to catabolism of body tissues, commonly leads to an increase in $\delta^{15}\text{N}$ of $<+1$ to $+2\text{‰}$ and either no change or a $\leq 1\text{‰}$ increase in $\delta^{13}\text{C}$ (Hatch, 2012; McCue and Pollock, 2008; Reitsema, 2013). Thus, in special circumstances it may be possible to use isotopic compositions of skeletal remains to investigate health (e.g., Olsen et al., 2014). Fossil specimens that record morphological signals of poor health provide an ideal opportunity to evaluate the link between isotopic compositions and nutritional stress.

Materials and Methods

Samples

Samples of proboscidean skeletal remains (bone, tusk dentin, and molar enamel, dentin, and cementum) from known geographic localities were obtained from museum collections from British Columbia, Alberta, and Yukon (Table 1, Fig. 1). Only adult teeth and bones were selected, to avoid isotopic variations resulting from nursing (Metcalf et al., 2010; Rountrey et al., 2007). Most specimens were identified as mammoth or mastodon based on morphology, but a few were identifiable only as proboscidean. The latter were sampled as case studies for taxonomic identification. All samples were selected opportunistically, to avoid damage to museum specimens. Several grams of bone, tooth, and dentin were removed from each specimen using a Dremel tool and/or by manual breakage. Since relatively large amounts of “bulk” bone and dentin (~ 0.5 to 1.5 g) were used for collagen extraction, and because bone does not grow incrementally, collagen samples represent an average of many seasons of growth. There is some potential for seasonal bias in enamel samples, since enamel grows incrementally and smaller

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3 samples are required for analysis. To minimize bias, we sampled pieces of enamel that
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5 represented the entire enamel thickness (~1-2 mm) and typically ~10 mm or greater of tooth
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7 height, homogenizing the samples for each individual. Growth rate estimates for both mammoth
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9 and mastodon molar enamel indicate that more than 1 year is required for the full thickness of
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11 the enamel to develop (Metcalf and Longstaffe, 2012, 2014). Growth in the occlusal-basal
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13 direction of mammoth and modern elephant teeth occurs at a rate of 13 to 22 mm/a, with the
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15 faster rates only occurring near the occlusal surface (Dirks et al., 2012; Metcalf and Longstaffe,
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17 2012; Uno et al., 2013). Occlusal-basal growth rates for mastodon enamel are slower, with a
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19 maximum rate of 12 mm/a near the occlusal surface and a minimum of 2 mm/a near the cervix
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21 (Metcalf and Longstaffe, 2014). Thus, our enamel sampling method averaged seasonal
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23 variations in both directions of tooth growth.
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29 One specimen, AB5 (RAM P90.7.1) allowed a unique investigation into the influence of
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31 nutritional stress on the isotopic compositions of an extinct megaherbivore. This specimen is a
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33 morphologically-normal upper-left M6 with a malformed, supernumerary M7 united to it by
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35 hypercementosis (Fig. 2). According to Burns et al. (2003:83):
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39 “The Villeneuve mammoth [AB5 / RAM P90.7.1] was clearly in great pain due to a
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41 conspicuous bulging of the buccal alveolus, and it may be argued that it was the ultimate
42
43 cause of death.... Normal chewing action was eventually rendered impossible. The
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45 secondary occlusal facet on the buccal edge of the molar suggests that the mammoth
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47 made an effort to avoid the painful passing of the muscle across the bulge... and the
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49 animal likely died of malnutrition shortly after cutting the new facet, at age 46 to 48
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51 years, unable to process sufficient graze to survive.”
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To examine temporal changes in the collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of this unique specimen, six distinct subsamples were obtained: (1) dentin from the anterior M6, near the occlusal surface, (2) cementum from the anterior M6, near the occlusal surface, (3) M6 root (mostly dentin), (4) cementum joining the M6 and M7, (5) M7 crown cementum, and (6) M7 root (mostly dentin). These subsamples represent different formation times (Appendix S1), and could provide insight into isotopic changes in the mammoth – due to nutritional stress or other causes – prior to death.

Collagen

Bone, dentin (crown, root, tusk), and cementum were used for collagen analysis. Collagen extraction was modified from Longin (1971), and included demineralization with 0.25–0.5 M HCl (at room temperature), rinsing to neutrality, humic removal with 0.1 M NaOH (at room temperature), rinsing to neutrality, and solubilization in 10^{-3} M HCl at 75–90°C. Carbon and nitrogen isotope ratios were obtained using a Costech elemental combustion system (ECS4010) coupled to a Thermo-Finnigan Delta^{Plus}XL stable isotope ratio mass spectrometer (IRMS) operated in continuous-flow mode. The $\delta^{13}\text{C}_{\text{col}}$ values were calibrated to VPDB using ANU-Sucrose (IAEA-CH-6) and NBS-22 (accepted values = -10.50‰ and -30.03‰ , respectively), yielding the accepted value of $+1.95\text{‰}$ for NBS-19 (Coplen, 1994; Coplen et al., 2006). The $\delta^{15}\text{N}_{\text{col}}$ values were calibrated relative to AIR using IAEA N-1 and IAEA N-2 (accepted values = $+0.4$ and $+20.3\text{‰}$, respectively). Carbon and nitrogen contents were also calibrated using the accepted values for the above standards. An internal keratin standard was included in each analytical session to monitor accuracy and precision (mean $\pm$ 1 SD: $\delta^{13}\text{C}$ measured = $-24.03 \pm 0.06\text{‰}$ (n=77), accepted = -24.04‰ ; $\delta^{15}\text{N}_{\text{col}}$ measured = $+6.29 \pm 0.10\text{‰}$ (n=77), accepted = $+6.36\text{‰}$). The average difference between duplicate analyses of collagen

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3 samples was 0.06‰ for $\delta^{13}\text{C}$ and 0.11‰ for $\delta^{15}\text{N}_{\text{col}}$ (n=16). For six individuals, collagen was
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5 extracted from multiple tissues (e.g., bone, dentin, cementum) from the same individual. The
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7 means of all measurements obtained for each individual are presented in Table 2. The differences
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9 between tissues from the same individuals ranged from 0.3 to 0.7‰ for $\delta^{13}\text{C}_{\text{col}}$ and 0.6 to 1.5‰
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11 for $\delta^{15}\text{N}_{\text{col}}$ (Table 3, Table S1).
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18 **Bioapatite**

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20 Only enamel was used for bioapatite analysis, because it is much more resistant to
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22 alteration than bone (Ayliffe et al., 1994). Pieces of enamel were mechanically cleaned of
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24 adhering dirt, dentin and cementum, sonicated in distilled water 2-3 times (minimum), and
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26 examined under an optical microscope prior to being ground to a powder using a mortar and
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28 pestle. Aliquots of enamel powder were treated with ~2.5% NaOCl at room temperature for ~21
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30 hours, rinsed five times with de-ionized water, reacted with 0.1M acetic acid for 4 hours, rinsed
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32 as above, and freeze-dried (Garvie-Lok et al., 2004; Koch et al., 1997). Carbon and oxygen
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34 isotope compositions were obtained using a MultiPrep automated sampling device (reaction with
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36 ortho-phosphoric acid at 90°C) coupled with a VG Optima IRMS in dual-inlet mode. The $\delta^{13}\text{C}$
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38 values were calibrated relative to VPDB using NBS-19 (+1.95‰) and Suprapur (−35.28‰), and
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40 the $\delta^{18}\text{O}$ values were calibrated relative to VSMOW using NBS-19 (+28.6‰) and NBS-18
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42 (+7.2‰). Accuracy and precision were assessed using standards not included in the calibration
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44 curve: NBS-18 ($\delta^{13}\text{C}$ measured = -4.94 ± 0.10 ‰, n=16, accepted = -5.01 ‰), internal laboratory
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46 standard WS-1 calcite ($\delta^{13}\text{C}$ measured = $+0.76 \pm 0.15$ ‰, n=5, accepted = $+0.76$ ‰; $\delta^{18}\text{O}$
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48 measured = $+26.36 \pm 0.18$ ‰, n=5, accepted = $+26.23$ ‰), and Suprapur ($\delta^{18}\text{O}$ measured =
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50 $+13.15 \pm 0.12$ ‰, n=15, accepted = $+13.25$ ‰). The average difference between duplicate
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analyses of enamel samples was 0.07‰ for $\delta^{13}\text{C}$ and 0.19‰ for $\delta^{18}\text{O}$ (n=6). Carbonate contents were determined using a calibration curve based on the reading of a pressure transducer in the mass spectrometer and the known carbonate contents of calcite standards. Replicate measurements of tooth enamel CO_3 contents were within 0.4% on average (range of offsets = 0.1 to 0.9%).

Statistics

For isotopic comparisons of mammoths and mastodons within a region (Alberta, Klondike, or Old Crow), an F-test was used to determine whether variances of the two groups were equal, followed by a t-test assuming either equal or unequal variance, as appropriate. For isotopic comparisons among locations (Alberta, Klondike, Old Crow, Herschel Island, and British Columbia), a Kruskal-Wallis non-parametric analysis of variance was used to determine whether there were differences among groups, and a Dunn's multiple comparison test was used to determine which groups differed. For comparisons among locations, British Columbia (BC) and Herschel Island proboscideans specimens that were not identified to genus were compared separately to mammoths and mastodons from other locations. If the unknowns had isotopic compositions more similar to either mammoths or mastodons from the other locations, a case could be made for their inclusion in that genus. An alpha of 0.05 was used for all statistical tests.

Results

Diagenesis

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The C/N ratios, carbon and nitrogen contents, and extraction yields indicate that the collagen samples were well-preserved (Table 2) (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). Three samples [dentin from AB5 (P90.7.1), AB11 (P00.2.1), and AB6 (P94.4.3)] had C and N contents (ca. 25-30% and 10%, respectively) that were slightly lower than is usual for well-preserved collagen, but their C/N ratios were normal. Powder X-ray diffraction (pXRD) analysis of the collagen showed that inorganic salts (brushite and halite) were present in these (but not other) samples. Since brushite and halite contain no carbon or nitrogen, the low C and N contents were likely caused by the presence of these salts. We note that a variation in the collagen extraction procedure for these samples (i.e., demineralization of large intact chunks of dentin rather than small pieces) rather than poor preservation was likely responsible for the formation of the salts, since the salts only formed in samples extracted using the former method. Based on the results above, the $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ results obtained for these specimens are included in the discussion below.

The carbonate contents of enamel samples ($4.9 \pm 0.8\%$, Table 2) are similar to those of mature enamel (2.7 to 5.0%) (Hillson, 1996). Fourier-transform infrared spectroscopy and pXRD analysis of selected pretreated samples indicated no secondary mineral contamination. Thin section analysis of one enamel specimen (AB6 / P94.4.3) revealed clearly visible incremental growth features (e.g., Striae of Retzius, cross-striations). All indicators suggest good preservation of enamel bioapatite.

Taxonomic Identification (Mammoth vs. Mastodon)

The mean mammoth $\delta^{15}\text{N}_{\text{col}}$ was higher than that of mastodons in all regions (Table 2, Fig. 3). The difference was significant for Klondike (mean $\delta^{15}\text{N}_{\text{col}} = +7.5$ vs $+4.2\text{‰}$; $t = 7.8$, $\text{df} = 24$, $p < 0.001$) and Old Crow (mean $\delta^{15}\text{N}_{\text{col}} = +9.1$ vs $+3.1\text{‰}$; $t = 9.9$, $\text{df} = 13$, $p < 0.001$) but not for Alberta (mean $\delta^{15}\text{N}_{\text{col}} = +7.6$ vs $+4.7\text{‰}$; $t = 1.7$, $\text{df} = 5$, $p = 0.15$). The mean mammoth $\delta^{13}\text{C}_{\text{col}}$ was lower than that of mastodons in all regions, but the difference was only significant for Old Crow (mean $\delta^{13}\text{C}_{\text{col}} = -21.4$ vs -20.1‰ ; $t = 6.6$, $\text{df} = 13$, $p < 0.001$). Neither $\delta^{13}\text{C}_{\text{sc}}$ nor $\delta^{18}\text{O}_{\text{sc}}$ differed for mammoths and mastodons (note: only Alberta and Klondike had sufficient samples for comparison).

The $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ of BC proboscideans (unidentified to genus) were intermediate between those of mammoths and mastodons from other locations, and did not differ significantly from any other group. Herschel Island unknown proboscideans (unidentified to genus) had significantly different $\delta^{13}\text{C}_{\text{col}}$ and/or $\delta^{15}\text{N}_{\text{col}}$ from Yukon mastodons (Old Crow and Klondike) (Kruskall-Wallis and Dunn's tests, $p < 0.05$ and $p < 0.001$, for $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$, respectively) but did not differ significantly from Yukon mammoths.

Location of Origin

Collagen Analyses

Because mammoths and mastodons had significantly different $\delta^{15}\text{N}_{\text{col}}$ and $\delta^{13}\text{C}_{\text{col}}$ in Old Crow and Klondike, statistical analyses considered the taxa separately for these locations. Mammoth, mastodon, and unknown proboscidean collagen data from Alberta were combined because they did not have significantly different means. BC and Herschel Island samples that

were not identified to genus were included as separate groups in the statistical analyses, and compared to both mammoths and mastodons from other regions, as described above.

For mastodons, there were no significant differences among locations for $\delta^{13}\text{C}_{\text{col}}$ or $\delta^{15}\text{N}_{\text{col}}$. For mammoths, there were clear differences among locations for $\delta^{13}\text{C}_{\text{col}}$ (Kruskall-Wallis test, $p<0.001$). Dunn's multiple comparison tests showed that differences in mammoth $\delta^{13}\text{C}_{\text{col}}$ existed between Alberta and Old Crow (means of -20.5 vs. -21.4‰), Alberta and Herschel Island (means of -20.5 vs. -21.6‰), and Klondike and Old Crow (means of -20.7 vs. -21.4‰). The Kruskal-Wallis test suggested differences for mammoth $\delta^{15}\text{N}_{\text{col}}$ in different locations ($p<0.05$), but Dunn's multiple comparison tests showed no differences among groups. To be conservative, we assume this contradictory result indicates no reliable significant differences.

Bioapatite Carbonate Analyses

Bioapatite isotopic compositions for mammoths and mastodons did not differ significantly in any of the regions studied, and were combined within each region in the following comparisons. Results for tooth enamel are available only for Alberta, Klondike, Old Crow, and Herschel Island. The Kruskal-Wallis test showed significant differences among locations for $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ ($p<0.001$ in both cases). Dunn's multiple comparison tests showed that differences for $\delta^{13}\text{C}_{\text{sc}}$ existed between Old Crow and Klondike (means of -9.9 vs. -11.5‰), and Old Crow and Herschel Island (means of -9.9 vs. -13.7‰), whereas differences for $\delta^{18}\text{O}$ existed between Alberta and Old Crow (means of $+14.2$ vs. $+10.7\text{‰}$), and Alberta and Klondike (means of $+14.2$ vs. $+11.0\text{‰}$).

Health Status

Two of the AB5 (P90.7.1) mammoth subsamples lacked collagen (M6-M7 cementum and M7 root), but data were obtained for the remaining four subsamples (Table 3). Overall, AB5 had the second-highest mean $\delta^{15}\text{N}_{\text{col}}$ and the lowest $\delta^{13}\text{C}_{\text{col}}$ of any Alberta proboscidean (Table 2). The $\delta^{13}\text{C}_{\text{col}}$ values of all AB5 subsamples were similar, with a range of only 0.4‰ (Table 3). The $\delta^{15}\text{N}_{\text{col}}$ values of the three M6 subsamples were very similar, but that of the M7 was considerably lower (by 1.2 to 1.5 ‰) (Table 3). Enamel was obtained only from the M6 crown. Its $\delta^{13}\text{C}_{\text{sc}}$ was the lowest of any Alberta proboscidean, but its $\delta^{18}\text{O}$ was not an outlier (Table 2).

Discussion

Taxonomic Identification

The data suggest that $\delta^{15}\text{N}_{\text{col}}$ might be useful for assigning preliminary taxonomic designations (mammoth vs. mastodon) to isolated postcranial remains. Differences in mammoth and mastodon $\delta^{15}\text{N}_{\text{col}}$ were generally large, as shown previously for other regions (e.g., France et al., 2007; Koch, 1991; Metcalfe et al., 2013), and there were no significant differences among locations in the $\delta^{15}\text{N}_{\text{col}}$ of either mammoths or mastodons.

Case Study 1: Alberta (individual specimens)

The unidentified proboscidean bones from Alberta had a range for $\delta^{15}\text{N}_{\text{col}}$ that exceeded the range for the securely-designated mammoths and mastodons from the same region (Table 2, Fig. 3). Despite the lack of statistical significance for the Alberta mammoth vs. mastodon $\delta^{15}\text{N}_{\text{col}}$ comparison, we might reasonably hypothesize taxonomic designations for the unknown

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3 proboscidean bones with the highest and lowest $\delta^{15}\text{N}_{\text{col}}$ values. We therefore hypothesized that
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5 AB3 (P95.1.427) (+10.9‰) and AB7 (P91.11.9) (+9.0‰) were mammoth, and AB12 (P96.12.1)
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7 (+1.4‰) was mastodon.
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10 To test our isotopically-derived taxonomic prediction for AB12 (P96.12.1), which had the
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12 lowest $\delta^{15}\text{N}_{\text{col}}$ of any specimen in the present study, we submitted a segment of unprocessed bone
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14 (a remainder of the same piece used for isotopic analysis) for ancient DNA analysis. Contrary to
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16 expectations, the DNA results suggested that the bone belongs to *Mammuthus* (Jake Enk and
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18 Hendrik Poinar, personal communication, Jan. 30, 2014). To our knowledge, such a low $\delta^{15}\text{N}_{\text{col}}$
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20 has never been previously reported for *Mammuthus*. Interestingly, AB12 (P96.12.1) was the only
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22 post-glacial specimen from Alberta (directly dated to $10,743 \pm 100$ ^{14}C a BP, BGS 2131) (Burns,
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24 2010), and was recovered further south than the other Alberta individuals analyzed in this study
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26 (Table 1, Fig. 1). Thus, it may have inhabited a very different environment than the other (more
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28 northern, glacial-aged) specimens. The very low $\delta^{15}\text{N}_{\text{col}}$ of AB12 (P96.12.1), as well as the
29
30 greater variability in Alberta mammoth $\delta^{15}\text{N}_{\text{col}}$ in general (SD = 2.4 for Alberta vs. 1.2 and 1.1‰
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32 for Klondike and Old Crow, respectively) is likely related to Alberta's geographical position at
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34 the point of convergence of the Cordilleran and Laurentide Ice Sheets, whose waxing and
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36 waning would have had significant effects on the local nitrogen cycle. Both the Alberta and
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38 Yukon proboscidean remains were from time-averaged deposits, but only Alberta was repeatedly
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40 covered by glacial ice. The so-called ice-free corridor in Alberta not only facilitated gene flow
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42 between southern and northern faunal populations (Burns, 1996; Shapiro et al., 2004; Wilson,
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44 1996), but also underwent significant environmental transformations as the ice sheets advanced
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46 and receded (Beaudoin and Oetelaar, 2003; Jass et al., 2011), drastically altering the soil-plant
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48 nitrogen cycle. Plants and animals in recently deglaciated areas tend to have low $\delta^{15}\text{N}$ as a result
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of low temperatures, soil immaturity (low nitrogen availability), plant type, and mycorrhizal associations (Drucker et al., 2003; Drucker et al., 2012; Drucker et al., 2011; Hobbie et al., 1999; Hobbie et al., 2000; Szpak, 2014). The first proboscideans to enter Alberta after deglaciation (e.g., AB12 / P96.12.1) would therefore be expected to have had low $\delta^{15}\text{N}$ values. In contrast, the Yukon locations were never covered by glacial ice and may not have experienced such dramatic environmental shifts, though we note that very low $\delta^{15}\text{N}$ values have been observed for herbivores in unglaciated northwestern Europe during the Last Glacial Maximum (Dyke et al., 2002; Gautney and Holliday, 2015; Hedges et al., 2004; Richards and Hedges, 2003; Stevens and Hedges, 2004).

Recent research supports the idea that the environmental niches of various proboscidean taxa were broader than traditionally envisioned (Baumann and Crowley, 2015; Metcalfe and Longstaffe, 2014; Saunders et al., 2010). The example of AB12 (P96.12.1) demonstrates that mammoths in Alberta occupied a range of environments, and suggests that taxonomic designations based on stable isotope analyses should ideally be attempted for specimens from known locations and time periods, where baseline isotopic compositions for the taxa in question have already been determined.

Case Study 2: British Columbia and Herschel Island (groups of specimens)

Unfortunately, the paleontological record is incomplete and it is sometimes not possible to develop baseline isotopic data for all taxa at any given location, such as British Columbia and Herschel Island. BC proboscideans had $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ that were intermediate between those of mammoths and mastodons from other locations, and therefore did not differ significantly from either taxon. Although it is possible that the BC group includes both mammoths and mastodons, the very small range of $\delta^{15}\text{N}_{\text{col}}$ for BC specimens (difference of 1.1‰ between maximum and

minimum values) suggests, rather, that the animals were all of the same taxon. In support of this inference, ancient DNA analysis of BC1 (EH2009.008.0001), BC2 (EH1994.003.0041), BC4, and BC5 (EH1994.003.0040) showed that nine genus-discriminating sites within two species-delineable regions of the mitochondrial genome of these individuals matched those of *Mammuthus* (Jake Enk and Hendrik Poinar, personal communication, Jan. 30, 2014).

Herschel Island specimens not identified to genus had $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ that differed significantly from Old Crow and Klondike mastodons, but were indistinguishable from Old Crow or Klondike mammoths. This suggests that the specimens from Herschel Island were predominantly *Mammuthus*, an interpretation consistent with the preponderance of mammoth remains, and rarity of mastodon remains, in Alaska and Yukon (Harington, 2003; Zazula et al., 2014).

Location of Origin

Bioapatite $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ could be useful for determining locations of origin, as they are quite distinct among several locations (Fig. 4). The oxygen isotope compositions of Old Crow and Klondike specimens are lower than those of Alberta specimens, as expected based on latitudinal variations in meteoric water $\delta^{18}\text{O}$ (Dansgaard, 1964; Rozanski et al., 1993). Contrary to latitude-based expectations, the highest-latitude location (Herschel Island) had relatively high $\delta^{18}\text{O}_{\text{sc}}$ values. This is likely a result of Herschel’s close proximity to the sea, where local evaporation of seawater would result in precipitation with a relatively high $\delta^{18}\text{O}$ (Dansgaard, 1964; Rozanski et al., 1993). The similarity in $\delta^{18}\text{O}_{\text{sc}}$ for Old Crow and Klondike specimens is unexpected since they are at different latitudes, but could reflect different atmospheric moisture

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3 or surface water source regions, and/or temporal differences among the specimens. The carbon
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5 isotope compositions of the Herschel Island specimens are strikingly low, which may reflect
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7 significantly greater water availability in proximity to the coast (Kohn, 2010). Likewise, the
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9 highest $\delta^{13}\text{C}$ values in Old Crow may indicate that the driest conditions were present in that
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11 location.
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15 Regardless of the causes of variation, the data presented here provide a baseline for
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17 investigating the origins of museum specimens lacking collection data. Alberta specimens can
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19 clearly be distinguished from Yukon specimens using $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ (Fig. 4). Specimens from
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21 different regions of Yukon (Klondike, Old Crow, Herschel Island) may also be distinguishable
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23 using a combination of $\delta^{18}\text{O}_{\text{sc}}$ and $\delta^{13}\text{C}_{\text{sc}}$ (and possibly $\delta^{13}\text{C}_{\text{col}}$), though there is some overlap
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25 between Old Crow and Klondike specimens (Fig. 4). Further, because of the predictable change
26
27 in meteoric water $\delta^{18}\text{O}$ with latitude, $\delta^{18}\text{O}_{\text{sc}}$ could be used to distinguish between regions
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29 included in the present study and areas further south. For example, mammoths and mastodons
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31 from Ontario and western New York had $\delta^{18}\text{O}_{\text{sc}}$ values between $\sim +19$ and $+23\text{‰}$ (Metcalf et
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33 al., 2013), and mammoths from Arizona had $\delta^{18}\text{O}_{\text{sc}}$ values between $\sim +22$ and $+28\text{‰}$ (Metcalf
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35 et al., 2011), in contrast to values of $\sim +10$ to $+15\text{‰}$ presented here (all locations combined). Of
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37 course, temporal differences among regions can complicate such comparisons, since animals
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39 living in colder intervals would have lower $\delta^{18}\text{O}_{\text{sc}}$ than those living in warmer intervals, all else
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41 being equal (Ayliffe et al., 1992; Koch, 1998).
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48 Unlike the situation presented here, mammoth and mastodon $\delta^{18}\text{O}$ values may not be the
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50 same in every location. For example, in the Great Lakes region there was no overlap between
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52 $\delta^{18}\text{O}_{\text{sc}}$ of mammoths and mastodons (Metcalf et al., 2013). This demonstrates the need for
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54 baseline studies in the particular areas and time periods of interest, to determine the range of
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values and degree of overlap among taxa. Only after these are established can comparisons with other regions and inferences about geographic origins be made.

Since knowing the proboscidean taxon was deemed unnecessary for $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ comparisons, these proxies could be applied to isolated postcranial elements that lack taxonomic designation. However, bone bioapatite is much more susceptible to diagenetic alteration of its isotopic composition, and its analysis (not attempted here) is not recommended (Ayliffe et al., 1994; Koch et al., 1997).

Collagen isotopic compositions were not useful for distinguishing location of origin for mastodons, and were of limited use for mammoths. Although the $\delta^{13}\text{C}_{\text{col}}$ of mammoths in several locations differed significantly, the magnitude of the differences was very small ($\sim 1\text{‰}$ or less). Compared to these differences among means, variability within a single location was relatively large ($\sim 1\text{-}2\text{‰}$). As such, it would not be possible to match an isolated specimen to any particular location based on $\delta^{13}\text{C}_{\text{col}}$ alone. However, $\delta^{13}\text{C}_{\text{col}}$ and/or $\Delta^{13}\text{C}_{\text{sc-col}}$ (the difference between $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{13}\text{C}_{\text{col}}$, which can be calculated for specimens with isotopic data for both collagen and bioapatite) might provide secondary lines of evidence for location of origin in certain cases. For example, Old Crow mammoths had $\Delta^{13}\text{C}_{\text{sc-col}}$ values between 10.1 and 13.1 ‰ , whereas mammoths and mastodons from other locations had $\Delta^{13}\text{C}_{\text{sc-col}}$ between 8.0 and 10.1 ‰ .

Health Status

The abnormal M6/M7 (AB5 / P90.7.1) mammoth specimen provides a clear example of a skeletal anomaly that directly impacted the health of the individual, and a unique opportunity for investigating the links between isotopic compositions and nutritional stress in an individual

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2
3 extinct animal. The subsamples of the AB5 specimen likely developed in the following order: (1)
4 anterior M6 dentin, (2) anterior M6 cementum, (3) M6 root dentin, (4) M7 crown cementum, (5)
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6 cementum between the M6 and M7, (6) M7 root dentin (Appendix S1) (Haynes, 1991; Hillson,
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8 2005; Laws, 1966; Maschenko, 2002; Metcalfe et al., 2010). Unfortunately the two latest-
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10 developing subsamples lacked collagen, depriving us of data that would have represented the
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12 time closest to death. The fact that the M6 root and M7 crown had very distinct $\delta^{15}\text{N}_{\text{col}}$ values
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14 suggests that they formed at different times, with the M7 forming after the M6 was fully
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16 developed (Appendix S1). This is in agreement with Burns et al.'s (2003:82) inference that "the
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18 M6 was fully erupted when the M7 was forced into place." The lack of space for the M7 caused
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20 the still-developing lamellae to deform as they impacted against the fully-formed M6. Based on
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22 this evidence, in combination with elephant tooth development times and the estimated age-at-
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24 death for this specimen, we can surmise that the M7 formed primarily during the last 4-5 years of
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26 the animal's life (Appendix S1). It is currently not possible to make a more precise estimate of
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28 how much time elapsed between formation of the M7 crown cementum (our latest-developing
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30 sample that yielded results) and the animal's death (Appendix S1).
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38 The $\delta^{15}\text{N}_{\text{col}}$ values of AB5 (P90.7.1) are relatively high, as expected for nutritional stress
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40 leading to the catabolism of body tissues, which tends to cause increases of $<+1$ to $+2\text{‰}$ (Hatch,
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42 2012; McCue and Pollock, 2008; Reitsema, 2013). However, contrary to expectations for
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44 starvation later in life, the M7 had a lower $\delta^{15}\text{N}_{\text{col}}$ than the M6 tissues (Table 3). This suggests
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46 that body proteins were not being catabolized late in life, during the development of the M7
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48 crown. Nutritional stress leading to reliance on body fats (which would not affect $\delta^{15}\text{N}$) remains
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50 a possibility (Hatch, 2012). It is also possible, however, that the relatively high $\delta^{15}\text{N}$ values
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52 reflect the environment in which the animal was living, and are unrelated to nutritional stress.
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The low $\delta^{13}\text{C}$ values of AB5 (P90.7.1) relative to other Alberta mammoths also do not support expectations for severe starvation and fasting (which tend to have either no effect on tissue $\delta^{13}\text{C}$, or cause a slight increase) (Hatch, 2012; McCue and Pollock, 2008). Although the low $\delta^{13}\text{C}$ values could reflect increased fat metabolism (Bocherens, 2003; Bocherens et al., 1996; Hatch, 2012; Polischuk et al., 2001), there is no difference in the $\delta^{13}\text{C}_{\text{col}}$ of the M6 root and M7 crown, suggesting no change in the last several years prior to death. Together, the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among subsamples of AB5 suggest a change in diet, environment, or physiology in the years leading up to death, but there is no isotopic evidence for starvation (neither catabolism of body tissues, nor increased reliance on body fats). We emphasize that our data represent some period within approximately 4-5 years of death, but not necessarily the final months/years of the animal's life.

Conclusions

Stable isotopes can be of use in designating isolated proboscidean skeletal remains as mammoth or mastodon, but such designations remain tentative in the absence of morphological or molecular data. Mammoths tend to have higher $\delta^{15}\text{N}_{\text{col}}$ and lower $\delta^{13}\text{C}_{\text{col}}$ than mastodons, but the specific range of isotopic compositions differs among geographic locations and time periods, and may not be significantly different in every region. Mammoths, in particular, can have a very large range of $\delta^{15}\text{N}$ values, as shown for specimens from Alberta. Ideally, taxonomic designations based on stable isotopes should only be attempted for specimens from known locations and time periods, where baseline (preferably non-overlapping) isotopic compositions for the taxa in question have been determined previously.

We have shown that extraordinary exceptions to general trends in taxonomic differences can exist (i.e., a mammoth with a $\delta^{15}\text{N}_{\text{col}}$ of +1.4‰). This extremely low value may be typical for fauna inhabiting recently-deglaciated late Pleistocene landscapes of Alberta. Further research into the isotopic compositions of plants and animals present at that time and location would be useful to support this hypothesis. This example shows that isotopic analyses may be useful in determining outliers that may be selected for additional research (e.g., DNA, morphology) and may inspire broader research questions. These data also demonstrate the environmental adaptability of *Mammuthus*.

Our results also demonstrate that stable isotope analysis can be helpful in assigning geographical provenance to isolated museum samples that lack collection data. Bioapatite $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ are most useful for determining locations of origin, and could be used to distinguish proboscideans from Alberta, Old Crow, Klondike, Herschel Island, and locations further south (e.g., Arizona and the Great Lakes). Collagen $\delta^{13}\text{C}$ could provide a secondary line of evidence for determining the location of origin of mammoths, but $\delta^{15}\text{N}_{\text{col}}$ was not useful for this application. The isotopic data we have provided can be used as a baseline for future studies of specimens lacking provenance, and are particularly useful since the Yukon is the most prolific source of proboscidean fossils in Canada. It is highly probable that many skeletal remains without provenance in local museum collections are from Yukon, and isotopic determination of their location of origin may be possible. Furthermore, the isotopic baseline we have provided could be used to support inferences of migration, but only if climate-related variations can be ruled out.

The AB5 (P90.7.1) mammoth possessed a dental anomaly that caused impaired chewing, which was hypothesized to have caused nutritional stress and ultimately the animal's death

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(Burns et al., 2003). Our data indicate that in the years prior to its death, the animal experienced a change in diet, environment, or physiology – but not one consistent with nutritional stress. This does not necessarily contradict the interpretation that this animal starved to death (Burns et al., 2003), since the precise amount of time that elapsed between the formation of the analyzed tissues and death remains unknown.

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Table 2. The isotopic compositions of collagen (various tissues) and structural carbonate in bioapatite (enamel) for mammoths, mastodons, and unknown proboscideans analyzed in this study and by Zazula et al. (2014).

Table 3. Isotopic results for collagen extracted from different areas on AB5 / P90.7.1 (mammoth M6 and M7).

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Figure 1. Locations mentioned in the text. Red dots indicate provenance for specimens used to establish baseline data for location of origin studies. VI = Vancouver Island, C = Cochrane, E/V = Edmonton/Villeneuve, B = Bezanson, K = Klondike, OC = Old Crow, H = Herschel Island.

Adapted from <http://jan.ucc.nau.edu>

Figure 2. (A) Lingual view of the sampled left M6/M7 *Mammuthus* teeth (AB5 / P90.7.1) as compared to (B) the normal condition in a left M6 *Mammuthus* tooth (P84.5.1). Scale bars = 10 cm.

Figure 3. Carbon and nitrogen isotope compositions of collagen for (A) *Mammut*, (B) unknown proboscideans, and (C) *Mammuthus* from Western Canada locations.

Figure 4. Carbon and oxygen isotope results obtained for structural carbonate in enamel bioapatite from *Mammuthus* (open symbols and stars) and *Mammut* (closed symbols). Polygons enclose specimens from the different regions. AB = Alberta, OC = Old Crow, K = Klondike, H = Herschel Island.

Supplementary Information

Table S1. Isotopic results for collagen extracted from different tissues within the same individuals (excluding AB5).

Appendix S1. Developmental timing of the AB5 (P90.7.1) mammoth M6/M7

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Table 1 Geographic and temporal context and sampling information for specimens used in this study

Specimen									Radiocarbon			Ref
LSIS #	Source	Museum #	Taxon	Area	Site	Tissues	Bone element	Tooth	Date (¹⁴ C a BP)	Lab #		
British Columbia												
BC1	RBCM	EH2009.008.0001	Proboscidea	Vanc. Island	Island View Beach	T		tusk				1
BC2	RBCM	EH1994.003.0041	Proboscidea	Vanc. Island	Cordova Bay	T*		tusk				1
BC3-1	RBCM	EH1994.003.0039	Proboscidea	Vanc. Island	Cordova Bay	T		tusk				
BC4	UWO		Proboscidea	Northern BC		T		tusk				
BC5	RBCM	EH1994.003.0040	Proboscidea	Vanc. Island	Cordova Bay	B	humerus		17,000 ± 240	GSC-2829		1
Alberta												
AB1	RAM	P97.11.1B	<i>Mammuthus</i>	Edmonton	Riverview Pit	B, D, C, E	mandibular symphysis	LLM6	43,300 ± 3000			2
AB2	RAM	P94.1.698	<i>Mammuthus</i>	Edmonton	Consolidated Concrete Pit 48	D, C, E		URM5	>37,700	AA84996		2, 3
AB3	RAM	P94.1.427	Proboscidea	Edmonton	Consolidated Concrete Pit 48	B	left innominate					2, 3
AB4	RAM	P94.16.1B	<i>Mammut</i>	Edmonton	Apex Galloway Pit	B, D, E	mandible (ascending ramus)	LRM5				3
AB5	RAM	P90.7.1	<i>Mammuthus</i>	Villeneuve	Alberta Conceret Products Ltd.	D, C, E		ULM6+M7				4
AB6	RAM	P94.4.3	<i>Mammuthus</i>	Villeneuve	Consolidated Concrete Pit 46	D, C*, E		LLM6				3
AB7	RAM	P91.11.9	Proboscidea	Villeneuve	Consolidated Concrete Pit 46	B	cortical					3
AB8	RAM	P94.4.1	Proboscidea	Villeneuve	Consolidated Concrete Pit 46	B	right scapula					3
AB9	RAM	P94.4.2	<i>Mammuthus</i>	Villeneuve	Consolidated Concrete Pit 46	C*		URM6				3
AB10	RAM	P97.7.1	<i>Mammut</i>	Villeneuve	Consolidated Concrete Pit 46	D, E		LLM6	>41,100	AA84997		3
AB11	RAM	P00.2.1	<i>Mammuthus</i>	Bezanson	Smoky River	D, C, E		LRM4/M5	40,000 ± 3500	AA84979		
AB12	RAM	P96.12.1	Proboscidea	Cochrane	Burnco Pit	B	humerus (deltoid tuberosity)		10,743 ± 100	BGS 2141		5
Yukon												
YT1	YG	291.1	<i>Mammuthus</i>	Old Crow	Old Crow River	RD, E		LRM6	>41,100	AA84987		6
YT2	YG	122.2	<i>Mammuthus</i>	Old Crow	CRH 94	RD, E		ULM6	>41,100	AA84992		6
YT3	YG	173.5	<i>Mammuthus</i>	Old Crow	Ch'jee's Bluff	D, E		U M6				6
YT4	YG	285.1	<i>Mammuthus</i>	Old Crow	OCR, REM 78-1	B, E		LRM6	>39,100	AA85002		6
YT5	YG	60.2	<i>Mammuthus</i>	Old Crow	OCR, Bluffs-R bank	RD, E		LRM5/M6				6
YT6	YG	57.1	<i>Mammuthus</i>	Old Crow	OCR, Bluffs-R bank	C, E		LRM6				6
YT7	YG	325.22	<i>Mammuthus</i>	Old Crow	HH-68-10	B, E		ULM6	>40,100	AA84984		6
YT8	YG	357.1	<i>Mammut</i>	Old Crow	Old Crow River	D, E		M5/M6	>41,100	AA84995		
YT9	YG	284.4	<i>Mammuthus</i>	Old Crow	CRH 11	C, E		ULM6				6
YT10	YG	252.2	<i>Mammuthus</i>	Old Crow	CRH 20	RD, E		LLM6	>40,000	AA85001		6
YT11	YG	173.1	<i>Mammuthus</i>	Old Crow	Ch'jee's Bluff	RD, C, E		U M6				6
YT24	YG	78.1	<i>Mammuthus</i>	Klondike	Last Chance Creek	RD, E		M6	>48,800	UCIAMS41491		7
YT25	YG	2.16	<i>Mammuthus</i>	Klondike	Hester Creek	C, E		adult				
YT26	YG	2.14	<i>Mammuthus</i>	Klondike	Hester Creek	C, E		adult	35,500 ± 2000	AA84986		
YT27	YG	29.248	<i>Mammuthus</i>	Klondike	Hunker Creek	B	mandible		43,500 ± 1900	UCIAMS41493		7
YT28a,b	YG	6.51, 6.49	<i>Mammuthus</i>	Klondike	Gold Run Creek	B, E	mandible	LLM6,LRM6	17,950 ± 120	Beta 70099		
YT29	YG	358.1	<i>Mammuthus</i>	Klondike		B, E	mandible	M6				
YT30	YG	302.26	<i>Mammuthus</i>	Klondike	Skookum Gulch	B, E	mandible	M5/M6	>39,200	AA84983		
YT31	YG	2.4	<i>Mammuthus</i>	Klondike	Hester Creek	B, E	mandible	M6?	27,380 ± 730	AA85000		
YT32	YG	84.1	<i>Mammuthus</i>	Klondike	Hunker Creek	C, E		adult				
YT33	YG	159.83	<i>Mammut</i>	Klondike	Quartz Creek	B	fibula					
YT34	YG	43.2	<i>Mammut</i>	Klondike	Upper Gold Run	RD, E		M6	>49,200	UCIAMS75320		8
YT35	YG	26.1	<i>Mammut</i>	Klondike	Gold Run Creek	RD, E		M6	>50,300; >51,700	UCIAMS 78705, 78704		8
YT36	YG	50.1	<i>Mammut</i>	Klondike	Thistle Creek	RD, E		M5?	>41,100	AA84994		
YT37	YG	139.5	<i>Mammut</i>	Klondike	Unknown?	B, E	mandible	M6	>41,100	AA84985		
YT38	YG	33.3	<i>Mammut</i>	Herschel	Simpson Point	B	cuboid		>45 130	Beta 189291		9
YT39	YG	68.2	<i>Mammuthus</i>	Herschel	Simpson Point	D, E		fragment				
YT40	YG	68.4	<i>Mammuthus</i>	Herschel	Simpson Point	C, E		fragment	>35,900	AA84988		
YT41	YG	154.2	Proboscidea	Herschel	South Shore	T		tusk				
YT42	YG	12.9	Proboscidea	Herschel	Simpson Point	T		tusk	34,100 ± 1,700	AA84999		
YT43	YG	206.29	Proboscidea	Herschel	Simpson Point	T		tusk				
YT44	YG	33.7	Proboscidea	Herschel	Simpson Point	T		tusk				
YT45	YG	68.1	Proboscidea	Herschel	Simpson Point	T		tusk	>39,400	AA84982		
YT46	YG	271.93	<i>Mammuthus</i>	Herschel	Simpson Point	B	thoracic vertebra					
YT47	YG	2.7	<i>Mammuthus</i>	Klondike	Hester Creek	B	fibula		27,540 ± 270	UCIAMS 41488		7
YT48	YG	133.21	<i>Mammuthus</i>	Klondike	Whitman Gulch	B	right tibia		34,180 ± 590	UCIAMS41489		7
YT49	YG	5.46	<i>Mammuthus</i>	Klondike	Hunker Creek	B	rib		22,430 ± 140	UCIAMS41487		7
YT50	YG	219.3	<i>Mammuthus</i>	Stewart River	Ash Bend	B	long bone fragments					
YT51	YG	317.51	<i>Mammuthus</i>	Old Crow	CRH 11	T		tusk	MIS5, ~140,000			

LSIS #: Sample ID used for Laboratory for Stable Isotope Science analyses, and referred to in this study;
Source of skeletal materials: RBCM = Royal British Columbia Museum, RAM = Royal Alberta Museum, YG = Yukon Government, CMN = Canadian Museum of Nature
Tissues: T = tusk, B = bone, D = dentin (crown), RD = root dentin, C = cementum, E = enamel
Radiocarbon dates in bold were obtained as part of the present study
* Insufficient collagen preservation; no data

References

1 Keddie, 1979
2 Burns and Young, 1994
3 Young et al., 1994
4 Burns et al., 2003
5 Burns, 2010
6 Metcalfe et al., 2010
7 Debruyne et al., 2008
8 Zazula et al., 2014
9 Zazula et al., 2009

Table 2. The isotopic compositions of collagen (various tissues) and structural carbonate in bioapatite (enamel) for mammoths, mastodons, and unknown proboscideans analyzed in this study and by Zazula et al. (2014).

Region	LSIS #	Collagen							Lab*	Structural carbonate			
		Tissue used	C (%)	$\delta^{13}\text{C}$ (‰)	N (%)	$\delta^{15}\text{N}$ (‰)	C/N (Atomic)	Yield (%)		Tissue used	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)	CO ₃ (%)
British Columbia													
	Proboscidea												
	BC1	T	38.4	−19.9	14.2	+7.0	3.2	4.3	LSIS				
	BC3-1	T	42.4	−20.7	15.8	+6.9	3.1	6.3	LSIS				
	BC4	T	47.0	−21.3	17.4	+6.9	3.1	22.9	LSIS				
	BC5	B	41.3	−21.3	15.2	+5.9	3.2	11.0	LSIS				
	Mean		42.3	−20.8	15.7	+6.7	3.1	11.1					
	SD		3.6	0.7	1.3	0.5	0.0	8.3					
Alberta													
	Proboscidea												
	AB3	B	42.0	−20.5	15.8	+10.9	3.1	11.0	LSIS				
	AB7	B	41.5	−20.4	16.3	+9.0	3.0	10.9	LSIS				
	AB8	B	39.9	−19.7	14.8	+4.4	3.1	7.5	LSIS				
	AB12	B	32.8	−21.0	12.1	+1.4	3.2	4.3	LSIS				
	Mean		39.0	−20.4	14.8	+6.4	3.1	8.5					
	SD		4.3	0.5	1.8	4.3	0.1	3.2					
	Mammuthus												
	AB1	B,C,D	40.9	−20.6	15.6	+7.8	3.1	>5.9 to 16.0	LSIS	E	−10.7	+14.2	4.0
	AB2	C, D	36.1	−21.1	13.3	+9.7	3.2	14.4 to >15.5	AMS/LSIS	E	−11.4	+15.0	6.8
	AB5	C, D	36.1	−21.4	13.0	+10.0	3.2	5.3	LSIS	E	−11.5	+13.6	4.9
	AB6	D	28.0	−20.1	10.2	+5.1	3.2	>14.7	LSIS	E	−10.7	+15.2	5.6
	AB11	C, D	34.9	−20.6	13.0	+5.5	3.1	>4.3 to 16.1	AMS/LSIS	E	−10.5	+13.1	5.9
	Mean		35.2	−20.8	13.0	+7.6	3.2				−10.9	+14.2	5.4
	SD		4.6	0.5	1.9	2.3	0.1				0.5	0.9	1.1
	Mammut												
	AB4	B, D	40.3	−20.8	15.0	+4.5	3.1	>11.9 to 13.6	LSIS	E	−11.6	+14.9	3.6
	AB10	D	41.1	−19.5	15.4	+5.0	3.1	12.4	AMS	E	−10.3	+13.5	4.0
	Mean		40.7	−20.1	15.2	+4.7	3.1				−10.9	+14.2	3.8

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SD		0.6	0.9	0.3	0.4	0.0				0.9	1.0	0.3
Yukon - Klondike												
Mammuthus												
YT24	RD	45.7	-20.8	16.9	+8.2	3.2	20.2	LSIS	E	-11.7	+10.1	5.4
YT25	C	42.0	-21.2	15.5	+8.0	3.2	7.3	LSIS	E	-11.8	+11.4	2.8
YT26	C	43.3	-20.5	16.0	+7.4	3.2	15.3	AMS	E	-12.5	+12.3	5.3
YT27	B	44.4	-21.1	16.3	+7.0	3.2	13.5	LSIS				
YT28	B	47.5	-20.7	18.0	+10.5	3.1	19.1	LSIS	E	-11.5	+9.9	5.6
YT29	B	42.0	-20.6	15.2	+7.2	3.2	12.7	LSIS	E	-11.47	+12.0	5.1
YT30	B	41.5	-20.3	15.6	+5.8	3.1	18.0	AMS	E	-12.1	+10.5	5.1
YT31	B	37.8	-20.5	14.1	+7.0	3.1	13.0	AMS				
YT32	C	45.9	-20.7	16.9	+7.7	3.2	12.4	LSIS	E	-11.1	+11.7	5.0
YT47	B	46.8	-20.6	17.6	+6.5	3.1	16.8	LSIS				
YT48	B	46.6	-20.7	17.3	+6.8	3.1	21.0	LSIS				
YT49	B	45.0	-21.3	16.3	+8.7	3.2	20.6	LSIS				
YT50	B	35.9	-20.8	12.9	+7.2	3.3	1.2	LSIS				
Mean		43.4	-20.7	16.0	+7.5	3.2	14.7			-11.7	+11.1	4.9
SD		3.5	0.3	1.4	1.2	0.0	5.7			0.4	0.9	1.0
Mammut												
YT33	B	43.2	-20.9	15.8	+6.9	3.2	18.5	LSIS				
YT34	RD	45.7	-20.0	16.9	+4.5	3.2	18.4	LSIS	E	-11.34	+10.8	4.9
YT35	RD	42.4	-20.6	16.0	+4.4	3.1	19.9	AMS	E	-11.3	+10.4	4.0
YT36	RD	41.7	-21.2	15.6	+4.3	3.1	12.6	AMS	E	-11.3	+10.9	4.4
YT37	B	42.0	-20.6	15.0	+3.5	3.3	14.5	AMS	E	-10.6	+11.4	3.7
¹ CMN333	D/RD	43.6	-20.6	15.4	+4.1	3.3	6.1	UCI				
¹ CMN33897	D/RD	42.7	-19.9	15.7	+4.9	3.2	7.6	UCI				
¹ CMN 8707	D/RD	42.9	-20.8	15.6	+4.3	3.2	8.4	UCI				
¹ CMN 11697	D/RD	42.2	-20.5	15.4	+4.6	3.2	8.5	UCI				
¹ CMN 42551	D/RD	42.2	-20.1	15.1	+3.0	3.4	4.0	UCI				
¹ CMN 42552	D/RD	39.1	-19.9	14.0	0.0	3.3	0.6	UCI				
¹ F:AM: 104842	?	43.5	-21.4	15.3	+3.6	3.3	4.1	UCI				
¹ YG 361.9	?	41	-20.7	14.5	+3.2	3.3	8.9	UCI				
Mean		42.5	-20.6	15.4	4.2	3.2	10.2			-11.1	+10.9	4.2
SD		1.5	0.5	0.7	1.0	0.1	6.2			0.4	0.4	0.5
Yukon- Old Crow												
Mammuthus												

YT1	RD	41.6	-21.4	15.6	+8.7	3.1	14.4	AMS	E	-11.3	+11.4	n.d.
YT2	RD	39.2	-20.9	14.6	+8.4	3.1	11.0	AMS	E	-8.3	+9.9	5.3
YT3	D	44.0	-21.8	16.4	+9.7	3.1	>6.7	LSIS	E	-10.1	+10.0	5.5
YT4	B	41.1	-21.4	15.2	+9.5	3.1	13.1	AMS	E	-9.7	+11.5	5.1
YT5	RD	42.9	-21.5	15.5	+9.8	3.2	11.2	LSIS	E	-8.4	+10.5	5.1
YT6	C	42.5	-21.7	15.2	+7.7	3.3	10.1	LSIS	E	-9.7	+9.8	4.6
YT7	B	42.3	-21.4	15.8	+7.6	3.1	15.7	AMS	E	-9.1	+10.2	5.3
YT9	C	41.4	-21.5	15.0	+8.2	3.2	7.1	LSIS	E	-9.9	+11.9	5.0
YT10	RD	38.9	-21.5	14.4	+9.8	3.2	11.4	AMS	E	-10.9	+10.7	5.3
YT11	C, RD	45.6	-215	16.7	+8.9	3.2	16.3	LSIS	E	-10.6	+10.5	5.1
YT51	T	37.9	-20.7	13.9	+11.3	3.2	18.6	LSIS				
Mean		41.6	-21.4	15.3	+9.0	3.2	12.9			-9.8	+10.6	5.1
SD		2.3	0.3	0.8	1.1	0.0	3.4			1.0	0.7	0.3
<i>Mammut</i>												
YT8	D	42.3	-20.6	16.0	+3.5	3.1	17.8	AMS	E	-11.1	+11.6	4.4
¹ CMN 15352	D/RD	43.4	-19.6	14.8	+4.1	3.3	1.3	UCI				
¹ CMN 31898	D/RD	42.6	-19.9	15.2	+2.4	3.2	2.1	UCI				
¹ CMN 33066	D/RD	43.3	-20.1	15.3	+2.5	3.2	3.9	UCI				
Mean		42.9	-20.1	15.3	+3.1	3.2						
SD		0.5	0.4	0.5	0.8	0.1						
Yukon- Herschel Island												
<i>Proboscidea</i>												
YT41	T	45.0	-22.1	16.7	+10.7	3.1	17.8	LSIS				
YT42	T	42.7	-20.3	15.7	+8.2	3.2	23.3	AMS				
YT43	T	42.5	-21.9	15.5	+8.0	3.2	15.1	LSIS				
YT44	T	44.9	-20.7	16.4	+8.8	3.2	18.5	LSIS				
YT45	T	43.6	-21.6	16.2	+15.2	3.1	21.5	AMS				
Mean		43.7	-21.3	16.1	+10.2	3.2	19.2					
SD		1.2	0.8	0.5	3.0	0.0	3.2					
<i>Mammuthus</i>												
YT39	D	44.4	-21.7	16.2	9.7	3.2	9.0	LSIS	E	-13.8	+12.2	5.0
YT40	C	42.4	-21.5	15.9	+6.8	3.1	14.1	AMS	E	-137	+13.3	5.5
YT46	B	41.7	-21.6	15.5	+5.5	3.1	14.8	LSIS				
Mean		42.8	-21.6	15.9	+7.4	3.1	12.6			-13.7	+12.7	5.2
SD		1.4	0.1	0.3	2.2	0.0	3.2			0.1	0.8	0.4
<i>Mammut</i>												
YT38	B	41.8	-21.8	15.1	+5.8	3.2	13.9	LSIS				

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Values in **bold** are the means of duplicate analyses or analyses of different tissues from the same individual (see Supplementary Table S1).
Tissue: RD = root dentin, D = crown dentin, T = tusk dentin, B = bone, C = cementum, E = enamel
* Lab in which collagen extraction was performed: LSIS (University of Western Ontario), AMS (University of Arizona), UCI (University of California, Irvine)
1 Data are from Zazula et al. (2014). Yields are >30kDa fraction of collagen.

Table 3. Isotopic results for collagen extracted from different areas on AB5 / P90.7.1 (mammoth M6 and M7), in order of inferred development time (top is earliest, bottom is latest).

LSIS #	Tooth	Location	Tissue	Collagen						
				C (%)	$\delta^{13}\text{C}$ (‰)	N (%)	$\delta^{15}\text{N}$ (‰)	C/N (Atomic)	Yield (%)	Lab*
AB5	M6	anterior crown	D	29.2	-21.6	10.6	+10.2	3.2	3.6	LSIS
AB5	M6	anterior crown	C	41.9	-21.4	15.3	+10.4	3.2	8.6	LSIS
AB5	M6	root	D	36.5	-21.2	13.2	+10.5	3.2	4.4	LSIS
AB5	M7	crown	C	37.0	-21.2	13.4	+9.0	3.2	4.7	LSIS
AB5	M6-M7	between the 2 teeth	C	<i>not preserved</i>						
AB5	M7	root	D	<i>not preserved</i>						
<i>AB5 mean</i>	<i>M6, M7</i>	<i>all</i>	<i>C, D</i>	<i>36.1</i>	<i>-21.4</i>	<i>13.1</i>	<i>+10.0</i>	<i>3.2</i>	<i>5.3</i>	<i>LSIS</i>

Tissue: D = dentin, B = bone, C = cementum

* Laboratory in which collagen extraction was performed: LSIS (University of Western Ontario)

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a. Mastodons

b. Unknown proboscideans

c. Mammoths

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Table S1. Isotopic results for collagen extracted from different tissues within the same individuals (excluding AB5).

Collagen								
LSIS #	Tissue	C	$\delta^{13}\text{C}$	N	$\delta^{15}\text{N}$	C/N	Yield	Lab*
		(%)	(‰)	(%)	(‰)	(Atomic)	(%)	
AB1	B	44.4	-20.8	16.7	+7.3	3.1	16.0	LSIS
AB1	C	39.7	-20.6	14.9	+8.0	3.1	>11.0	LSIS
AB1	D	38.6	-20.5	15.1	+8.2	3.0	>5.9	LSIS
AB1 mean	B,C,D	40.9	-20.6	15.6	+7.8	3.1	>5.9 to 16.0	LSIS
AB2	C	42.1	-21.1	15.6	+9.9	3.1	14.4	AMS
AB2	D	30.1	-21.1	10.9	+9.5	3.2	>15.5	LSIS
AB2 mean	C, D	36.1	-21.1	13.3	+9.7	3.2	14.4 to >15.5	AMS&LSIS
AB4	B	43.5	-20.9	16.3	+4.1	3.1	13.6	LSIS
AB4	D	37.1	-20.7	13.8	+4.8	3.1	>11.9	LSIS
AB4 mean	B, D	40.3	-20.8	15.0	+4.5	3.1	>11.9 to 13.6	LSIS
AB11	C	43.8	-20.3	16.3	+5.9	3.1	16.1	AMS
AB11	D	25.9	-21.0	9.7	+5.1	3.1	>4.3	LSIS
AB11 mean	C, D	34.9	-20.6	13.0	+5.5	3.1	>4.3 to 16.1	AMS & LSIS
YT 11	C	44.1	-21.5	15.9	+8.3	3.2	18.2	LSIS
YT 11	RD	47.2	-21.5	17.6	+9.5	3.1	14.3	LSIS
YT 11 mean	C, RD	45.6	-21.5	16.7	+8.9	3.2	16.3	LSIS

Appendix S1. Developmental timing of the AB5 (P90.7.1) mammoth M6/M7

Interpretation of the isotopic results for the AB5 (P90.7.1) mammoth depends in part on knowledge of mammoth tooth development, which is not yet complete. Although growth rates of proboscidean molar crowns and the timing of eruption and wear have been estimated (Dirks et al., 2012; Haynes, 1991; Laws, 1966; Maschenko, 2002; Metcalfe and Longstaffe, 2012; Metcalfe et al., 2010; Uno et al., 2013), we lack complete knowledge of (1) the time required for a full M6 or M7 to develop, (2) the relative timing (i.e., degree of overlap) of the development of these teeth, and (3) the amount of time that elapsed between the development of the M6/M7 and the AB5 (P90.7.1) mammoth's death. These issues are explained more fully below, and inferences about the developmental timing of the AB5 (P90.7.1) specimen are made based on our best estimates for the timing of mammoth tooth development and the age-at-death of the AB5 (P90.7.1) mammoth.

In proboscidean teeth, lamellae (plates) are formed in sequence from anterior to posterior. Anterior crown dentin forms first, followed by anterior enamel and cementum (Hillson, 2005; Maschenko, 2002). Root formation begins after the lamellae of the crown are present, but before the cementum is completely formed (Maschenko, 2002). In general, as one tooth is in wear another is developing posterior to it, but there can be overlap in the development time of successive teeth (Laws, 1966). Growth of mammoth and modern elephant teeth crowns in the occlusal-basal direction occurs at a rate of 13 to 22 mm/a (Dirks et al., 2012; Metcalfe and Longstaffe, 2012; Uno et al., 2013). Dirks et al. (2012) estimated that in *Mammuthus columbi* it took about 10.6 years for an enamel plate of 180.9 mm height to develop, but this does not necessarily include the full mineralization time, and is an estimate for only one lamella. The full

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development time for a single tooth also depends on the number of lamellae present, the height of each lamella, and the developmental timing of the crown dentin, roots, and cementum, which remains unknown. Since the anterior of an M6 is in wear while the posterior is still developing, it is not possible to physically measure the “full” tooth at any given time. The rate of development of the M7, which may differ from that of the M6, also remains unknown. Based on the general development of proboscidean teeth, we can infer the following relative timing of development for our subsamples: (1) anterior M6 dentin, (2) anterior M6 cementum, (3) M6 root dentin, (4) M7 crown cementum, (5) cementum between the M6 and M7, (6) M7 root dentin. The potential for overlap in the developmental timing of the M6 and M7 is discussed below.

We lack any general knowledge of the developmental timing of M7 relative to that of M6, since the former is not normally present in elephant dentition. Based on the development and wear of elephant and mammoth teeth (Haynes, 1991; Laws, 1966), full crown formation of a *Mammuthus* M6 likely occurred occur over a period of about 17 years, between ages 26 and 43 (Metcalf et al., 2010). Burns et al. (2003) estimated the AB5 mammoth to be about 47-48 years old at the time of death. If these inferences are correct, then only about 4-5 years elapsed between complete formation of the M6 crown and the animal’s death. Burns et al. (2003:82) infer that the M7 “was undoubtedly deformed from a very early stage of its development because it is so thoroughly distorted... virtually all lamellae were disengaged from their neighbours and later cemented at odd angles.” They further argue that “the M6 was fully erupted when the M7 was forced into place, but that the M6 functioned for some years before the M7 came to rest” (Burns et al., 2003:82). Thus, the morphological evidence indicates that deformation of the M7 occurred after formation of the M6 was complete. However, the M7 lamellae may have begun to form either before or after the posterior/basal portions of the M6 were still developing. As

described in the main text, the isotopic compositions of the teeth can provide additional insight into this problem. Similar isotopic compositions for the two teeth would support the idea that there was significant overlap in their development times, whereas different isotopic compositions would suggest that they developed largely at different times. The latter proved to be the case: different $\delta^{15}\text{N}_{\text{col}}$ values of the M7 crown cementum and the M6 tissues (including the M6 root, the last part of the M6 to develop) support the hypothesis that the M6 was fully formed when the M7 began to develop. Thus, if all of the above inferences are correct, the isotopic data we obtained for the M7 thus represents some portion of the last 4-5 years of the animal's life, whereas the M6 developed earlier, well in advance of the animal's death. Unfortunately, the M7 root and the cementum linking the two teeth, which likely represented the time closest to death, did not contain well-preserved collagen (see main text). Crown cementum develops after crown enamel and dentin, so the M7 crown cementum isotopic results could still represent a period of time close to death. It is currently not possible to determine whether development of the M7 crown cementum would overlap the period of impaired chewing and acute nutritional stress represented by the secondary wear facet on the M7, which apparently developed over a "relatively short time" (Burns et al., 2003: 83).

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