PALEONTOLOGY

A year in the life of a giant ground sloth during the Last Glacial Maximum in Belize

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Stable isotope analysis of the first fossilized *Eremotherium laurillardi* remains from Belize offers valuable insights into the conditions within which this individual lived and its ability to adapt to the increasing aridity of the Last Glacial Maximum (LGM). Cathodoluminescence (CL) microscopy was used to identify chemical alteration of the tooth during fossilization. Results demonstrate that the inner orthodentin resists diagenesis, yielding potentially unaltered values. Using an intensive "vacuum milling" technique, the inner orthodentin produced an accelerator mass spectrometry (AMS) date of 26,975 ± 120 calibrated years before the present. The stable carbon and oxygen isotope analysis of this layer shows that the tooth recorded two wet seasons separated by one longer dry season and that this sloth was able to adapt its diet to the marked seasonality of the LGM. This study offers new insights into obtaining reliable isotope data from fossilized remains and suggests that this individual adapted to climate shifts, contributing to the conversation surrounding megafauna extinction.

INTRODUCTION

Here, we present results of stable isotope analysis of the first fossil remains of the extinct giant ground sloth, *Eremotherium laurillardi*, found in Belize (Valley of Peace Archaeology no. 2025, accession no. 10320), ultimately refining existing methods and improving our understanding of the paleoecology of this animal. Through carbon and oxygen stable isotope analysis, we reconstruct the environment of central Belize during the Late Pleistocene, approximately 30,000 years before the present (BP), and provide information on the diet and paleoecology of one of the largest members of Pleistocene megafauna in North and South America. Our analysis challenges previous analyses of megafaunal remains that do not adequately investigate the impact of diagenesis on tooth bioapatite and suggest a refinement of the application of methods used to determine the diet of extinct species and the climatic conditions during their lifetime.

Cara Blanca in central Belize (Fig. 1) is a system of 25 lakes and cenotes in a forested tropical landscape. Cenotes, or water-filled karstic sinkholes, are fed by groundwater, resulting in perennial water that typically lasts during prolonged droughts. The cenotes formed in Cretaceous limestone at the base of an escarpment (ca. 80 to 100 m high) along a fault, which may be related to the Cretaceous-Tertiary asteroid impact that created the Chicxulub crater (1). Subsequent dissolution of the limestone along fractures from the impact resulted in both cave systems and the associated cenotes that serve as water sources for fauna, playing an essential role in the seasonally arid region. Recent diving expeditions in Pool 1, the deepest of the cenotes at approximately 62 m deep, yielded megafaunal remains embedded in clay deposited on a ledge extending along the wall of the cenote. In 2014, divers recovered giant ground sloth (*E. laurillardi*) fossilized bones and teeth (2). Located within a 5.1-m-thick clay layer that begins at a depth of 17.3 m, the presence of megafaunal remains provides insight into the deeper history of this natural water source.

For ca. 25 thousand years (ka) before *E. laurillardi*'s extinction in the Late Pleistocene, the climate in Belize became increasingly arid

(2, 3). The Central American lowlands were increasingly cool and arid from Interstadial Stage 3 (~36 to 24 ka) through Glacial Stage 2 (~24 to 13 ka). Glacial Stage 2 had the lowest lake levels during this period because the environment was much cooler and drier. The lower water table would have left much of the Cara Blanca area desiccated, making all of the cenotes an important resource for megafauna seeking water and possibly exposing the ledge at 17.3 m from which the bones were recovered (2). Dry conditions persisted until the Early Holocene, when precipitation increased (3, 4).

The arid environment affected both vegetation and wildlife. Rather than the dense tropical forest present today locally, savanna and juniper scrub habitat dominated because of the drier and cooler conditions of the Last Glacial Maximum (LGM) (2, 3). *E. laurillardi* would have likely preferred this more open setting to dense forest vegetation because of its subsistence habits (2, 5, 6) and its social behavior (5), although this interpretation has been challenged recently (7). While our analysis only considers one individual, our results provide further evidence on the paleoecology of a member of this extinct species and insight into the climatic conditions under which it lived during the early LGM (27 ka) using stable carbon and oxygen isotopes obtained from a tooth of *E. laurillardi*. In addition, we present a refinement in the application of stable isotope and accelerator mass spectrometry (AMS) radiocarbon sample pretreatment methods for *E. laurillardi* and other species with similar dental structure (i.e., lacking enamel), emphasizing the need to differentiate between the different apatite layers and to examine how each is affected by diagenetic processes.

Eremotherium laurillardi

E. laurillardi, sometimes referred to as the Pan-American sloth (8), had a distribution from southern Brazil to the Gulf and Atlantic coast regions of North America (2, 8). The wide latitudinal range suggests that *E. laurillardi* was capable of adapting to diverse habitats with seasonal climatic patterns and subsequently may have had a varied diet (5, 6). This idea is further supported by its unique morphology (5); this giant sloth could reach a length of 6 m, which, in an upright position, would have permitted it to feed at higher levels in trees. Its teeth are composed of three tissues—orthodentin, vasodentin, and cementum (Fig. 2)—differing from other mammals in their lack of enamel and differing from other Xenarthra species in their lack of

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Fig. 1. Location of the Cara Blanca area in central Belize. Courtesy of the Valley of Peace Archaeology project. Map by J. McMahon.

osteodentin, a fourth tissue type. In addition, at just 19% of the occlusal surface of the tooth, the orthodentin in the teeth of *E. laurillardi* is approximately half as thick as in most other Xenarthra species. As orthodentin is the hardest of the bioapatite layers, this layer must be thinner to allow the teeth of *E. laurillardi* to be self-sharpening (*9*). On the basis of its hypselodont (ever-growing, rootless) and bilophodont tooth morphology, including high crowns, and two transverse lophs, *E. laurillardi* was well suited to process food in the oral cavity, suggesting low digestive efficiency, and had a strong bite, allowing for the cutting and crushing of a variety of materials of moderate-to-soft consistency, such as leaves and some fruits (*10*). However, diet differed depending on habitat, and individuals of *E. laurillardi* have been recorded as grazers in open habitats and mixed feeders in more closed habitats (*10*).

The timing of the extinction of *E. laurillardi* is disputed (*10*), with more recent studies suggesting that these large sloths may have lived into the Late Holocene in the Brazilian Intertropical Region (*10*). The reason for their extinction is also debated; the most commonly cited causes include human predation (*11*) and changes in the climate (*12*). Our study contributes to the understanding of the diet of this animal and the climatic conditions during which this individual of *E. laurillardi* lived.

Isotopic studies

Variations in stable isotope ratios of teeth have been used often in studies of past environments and diets (*13*), including studies of other *E. laurillardi* remains (*5, 14*). Changes in the $^{18}O/^{16}O$ ($\delta^{18}O$) concentrations throughout the length of the ever-growing tooth can

Fig. 2. Structure of *E. laurillardi* tooth. Left: *E. laurillardi* teeth are composed of two cusps (i.e., bilophodont), are ever-growing (i.e., hypselodont) and self-sharpening, are made of mineralized dental tissue types that are softer than enamel. The gray infilling material at the base of this tooth is a carbonate nodule that formed after burial. This specimen is apicobasally sectioned along the mesiodistal axis. Numbers indicate the cementum (1), outer orthodentin (2), inner orthodentin (3), vasodentin (4), and carbonate concretion (5). **Right:** The location of isotope samples along the growth access of the tooth. Photo credit: J.T.L. and S.A. (University of Illinois at Urbana-Champaign)

be indicative of seasonal changes in the temperature and/or the amount of precipitation because these isotope data roughly reflect the $\delta^{18}O$ values of water consumed via drinking and/or ingested with food (*15*, *16*). These values can vary substantially with humidity and water stress, providing insight into the duration and intensity of the wet and dry seasons. In general, higher $\delta^{18}O$ values both in local water and, subsequently, in tooth enamel indicate high evaporation and/or low precipitation, often caused by warm and/or dry conditions (*15*, *17*). However, at lower latitudes, when temperatures are above 20°C and there is abundant summer rainfall, the "amount effect" becomes a dominant control of $\delta^{18}O$ and subsequently leads to lower $\delta^{18}O$ water/enamel values during the wet season (*17*, *18*). The Cara Blanca pools lie at approximately 17°N, meaning that, today, there is only one dry season (~5 months in length, typically from January to May) and one wet season (~7 months in length, typically from June to December) each year. Examining whether this pattern occurred during the Late Pleistocene will provide a greater historical context for the paleoclimatic variation in central Belize, as well as information about the conditions under which *E. laurillardi* and other Pleistocene fauna near Cara Blanca lived.

Important to understanding prehistoric seasonal patterns of the sloth's diet is identification of available vegetation. Variation in $\delta^{13}C$ values along the length of the tooth will indicate whether the sloth was eating primarily C_3, C_4, or Crassulacean acid metabolism (CAM) plants, such as epiphytic bromeliads, or whether its diet varied during the year [as has been demonstrated in horses, bison, and even marsupials; e.g., (*15*, *16*, *18*)]. If an animal is eating primarily C_3 plants, then $\delta^{13}C$ values are lower than those of animals that consume primarily C_4/CAM

plants. In medium-to-large herbivorous mammals (including artiodactyls, perissodactyls, and proboscideans), $\delta^{13}C$ values of the tooth enamel reflect plants consumed plus an isotopic enrichment of ~14 per mil (‰) (*19*). However, one must also account for the decline in $\delta^{13}C$ values (~1.5‰) of atmospheric CO_2 due to the burning of fossil fuels over the past two centuries (*20*). Thus, extinct medium-to-large herbivores that consumed primarily C_3 plants have lower $\delta^{13}C$ values (≤−9‰), while those that consumed primarily C_4/CAM plants have higher $\delta^{13}C$ values (≥−2‰), with values in between suggestive of a mixed diet (*20*). However, enrichment may vary depending on the animal and is closer to 11.5 to 12.8‰ in small mammals (e.g., voles and rabbits, respectively) (*21*) and 12.8‰ in primates (*22*), while values are closer to 13.3‰ in marsupials (*23*). The isotopic enrichment between dietary food sources and sloth dentin remains unresolved, both because of the lack of modern analogs (of comparable body size) and because the teeth lack enamel. It may be close to the 14‰ of medium-to-large mammals or may be even higher due to the inferred high production of methane based on the complex chambered stomachs of extant tree sloths (*24*). In contrast, oxygen isotopes are affected by water consumption, including the degree to which animals obtain water from plants or free-water sources such as lakes and rivers (*25*). The size of *E. laurillardi* suggests that it likely obtained most of its water from drinking and thus may be a better recorder of meteoric water as compared to changes in relative aridity (similar to other large herbivores, including proboscideans) (*25*, *26*). That being said, some large herbivores including the giant wombat–like animal, *Diprotodon*, were still capable of tracking changes in climatic conditions (*27*).

RESULTS

This study examines the carbon and oxygen stable isotope data from 58 samples taken from along the growth axes of a single tooth of *E. laurillardi* (see Fig. 2) recovered from Cara Blanca Pool 1. To obtain accurate isotope results from the most diagenetically resistant apatite layer, we took the samples from three distinct tissue types—the cementum, the outer layer of the orthodentin, and the inner layer of the orthodentin (Fig. 2). In addition, to further assess the reliability of isotopic data recovered from the tooth, we conducted CL analysis.

The results of the isotopic analyses are shown in Fig. 3. Table 1 shows the range and mean of $\delta^{18}O$ and $\delta^{13}C$ recorded in each layer of the bioapatite tested. There is a marked difference in the $\delta^{13}C$ results from different layers of apatite, with the outer orthodentin exhibiting $\delta^{13}C$ values significantly lower than the inner orthodentin ($P < 0.0001$). In congruence with the results of the CL analysis (Fig. 4), the inner orthodentin exhibits the least diagenesis. The $\delta^{18}O$ values vary much less and are indistinguishable from one another ($P = 0.716$). The diagenetic carbonate concretion (Fig. 2) has a $\delta^{13}C$ value of −19.9‰, suggesting that the apatite with the most negative values, the outer orthodentin, is likely most affected by diagenesis. This conclusion is supported by the results of our CL analysis (Fig. 4), which show that the inner orthodentin exhibits the least luminescence and therefore has undergone minimal chemical alteration since deposition and fossilization. Last, using a pretreatment of vacuum milling with the material in a weak acid, we were able to obtain a date of 26,975 ± 120 calibrated years BP (cal BP) [Illinois State Geological Survey (ISGS) no. A3712] from the inner orthodentin.

Carbon isotope values of the inner orthodentin range from −5.4 to −7.4‰ with a mean of −6.8‰ (SD, *n* − 1 of 0.6‰) and a total range
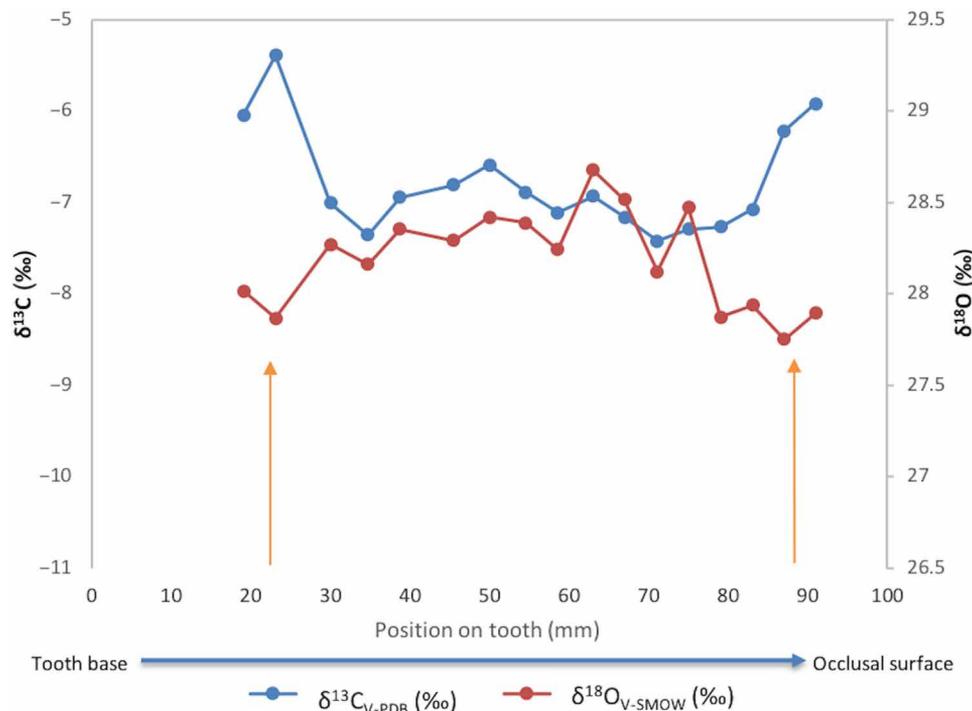
Fig. 3. Results of the inner orthodentin isotopic analysis. Results of the oxygen and carbon isotope values. Arrows indicate the two wet seasons, at ca. 2 and 9 cm. V-SMOW, Vienna Standard Mean Ocean Water; V-PDB, Vienna PeeDee Belemnite.

of 2.0‰, while δ¹⁸O values range from 27.8 to 28.7‰ with a mean of 28.2‰ (SD, $n − 1$ of 0.3‰) and total range of 0.9‰. On the basis of Cerling and Harris' (19) discussion of isotopic enrichment, *E. laurillardi* may have consumed both C₃ and C₄/CAM plant resources (~34% C₄/CAM plants based on a linear mixed model). However, this proportion declines to 26% with a 1‰ increase and to 19% with a 2‰ increase in the isotopic enrichment.

DISCUSSION

The sloth tooth dates to 26,975 ± 120 cal BP, contemporary with the earliest stages of the LGM. The expansion of the ice caps associated with the LGM resulted in lower sea levels and a notable drop in cenote water levels (3, 4). Because Pool 1 is more than 62 m deep, the cenote would have retained water during arid intervals when many of the surface water sources would have been completely or nearly desiccated because of the substantially lower water table. The giant sloth likely climbed down into the cenote for a drink, presumably during a dry period and became trapped by the steep-sided sinkhole, ultimately becoming buried in the clay deposits accumulating on the ledge extending from the wall of the cenote. On the basis of the fossil-laden stratum that rings the entire 100 m by 70 m cenote, it is likely that a large number of megafauna met the same fate.

The relationship between the *E. laurillardi* specimen and its environment is visible in the pattern represented in the isotope data (see Fig. 3). Previous studies have noted patterns in carbon and oxygen isotopes that provide insight into the diet of animals during different seasons (13, 15, 16). Since sloth teeth continue to grow throughout their life, changes in the isotope patterns along the length of the tooth reflect the last few years of the animal's life, with the isotope value at the base of the tooth reflecting diet and climate shortly before the time of the animal's death (the time lag may represent days,

Table 1. Mean and range of isotopic values from each layer of bioapatite.

Apatite layer	Mean δ¹³C (‰)	Range δ¹³C (‰)	Mean δ¹⁸O (‰)	Range δ¹⁸O (‰)
Orthodentin (inner)	−6.8	−5.4 to −7.4	28.2	27.8 to 28.7
Orthodentin (outer)	−10.3	−9.1 to −10.9	28.3	27.5 to 29.1
Cementum	−9.2	−8.3 to −10.0	28.3	27.5 to 29.0

weeks, or months, depending on the rate of tooth mineralization and eruption) [e.g., (28)]. Depending on the rate of tooth wear through mastication and the rate at which new tooth material is produced, the number of seasons recorded in the tooth and which ones are represented will vary based on the season of death of the individual. The pattern in this *Eremotherium* tooth shows two short wet seasons at 2 and 9 cm separated by a prolonged dry season (see Fig. 3). Because high humidity reduces evapotranspiration, leaf water δ¹⁸O is lower and the wet season can be identified by δ¹⁸O values, with large precipitation events producing lower δ¹⁸O values due to the amount effect (Fig. 5) (17, 18). As with most large mammals, giant ground sloths likely obtained most of their daily water intake from drinking rather than from plant water (29). *E. laurillardi* is the largest of the extinct sloths with an estimated body mass of up to 6550 kg (30); thus, it is likely that δ¹⁸O values from orthodentin reflect the δ¹⁸O values of meteoric water rather than of plant water from food sources, with the latter subjected to much higher rates of evaporation and hence greater fractionation and δ¹⁸O values (25, 26). Thus, these δ¹⁸O values are less likely to reflect changes in relative aridity than other taxa that acquire a greater proportion of their water from plant resources.
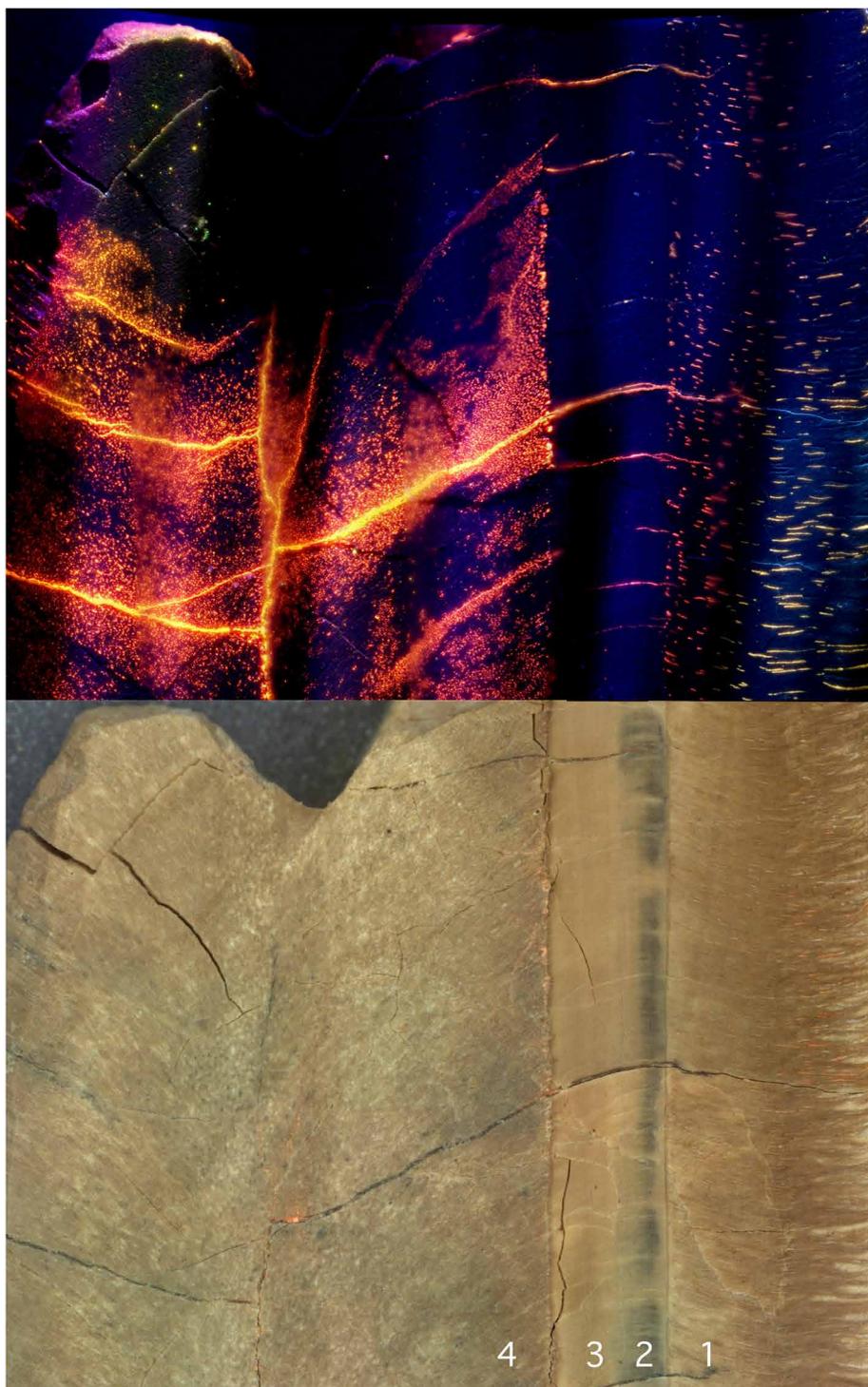
Fig. 4. CL and plain light images of sloth tooth. Bottom: A plain light image of the sloth tooth with the cementum (1), outer orthodentin (2), inner orthodentin (3), and vasodentin (4). **Top:** The CL analysis for the same portion of the tooth. CL highlights the infiltration of diagenetic material within the tooth and shows that the different layers of apatite were differentially affected by diagenesis [e.g., (*39*)]. The red and orange luminescence follows cracks in the tooth that occurred during deposition, indicating that, likely, magnesium blocky calcite infiltrated parts of the tooth while the tooth was deposited in the cenote. The cracks that do not display any coloration most likely are the result of the sawing and smoothing of the tooth surface during processing for this study. As expected and confirmed by the isotopic analysis, the orthodentin layer appears most resistant to diagenetic processes, showing almost no infiltration by diagenetic material. Although minerals were still able to move into the layer through the cracks, diagenetic calcite does not radiate out from the cracks into the hard orthodentin layer. There is a slight blue glow over the outer orthodentin layer that could be intrinsic but also might indicate that silica has diagenically altered this layer. The diagenetic alteration of the outer orthodentin indicates that the inner orthodentin is most intact. The vasodentin appears least resistant to diagenesis. The porous nature of the vasodentin (*8*) allows minerals to radiate out from cracks, contaminating the vasodentin. The cementum also shows evidence of consistent diagenesis. Photo credit: J.T.L. and S.A. (University of Illinois at Urbana-Champaign).
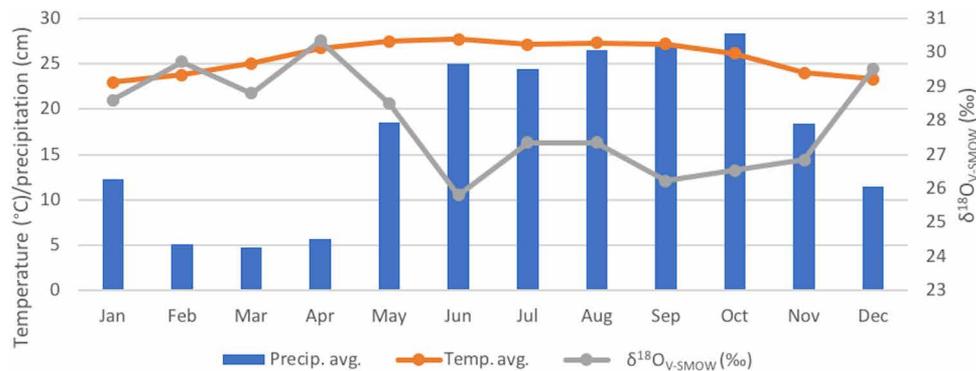
Fig. 5. Fluctuations in $\delta^{18}O$ of precipitation with average temperature and precipitation at Cara Blanca, Belize. Oxygen isotope values are calculated from the University of Utah Water Isotope Laboratory online meteoric water (rainfall) isotope calculator (*44–46*). Temperature and precipitation values are averaged for the year 2015 and were retrieved from http://sdwebx.worldbank.org/. High humidity reduces evapotranspiration, so leaf water $\delta^{18}O$ is lower and the wet/dry season can be identified with the $\delta^{18}O$ values, with large precipitation events producing lower $\delta^{18}O$ values due to the amount effect.

Regardless, analysis of the *E. laurillardi* tooth suggests that the wet season is substantially shorter than the dry season, indicating that the sloth endured a period of prolonged aridity annually. Wet season peaks in this *E. laurillardi* tooth are separated by ca. 70 mm; therefore, the growth rate of this tooth was ca. 70 mm/year. Approximately 50 mm of this tooth formed during the dry season. If tooth growth rate was constant, then the dry season spanned 8 to 9 months during the early LGM. This is nearly twice the duration of the modern ~5-month dry season in Belize (Fig. 5).

Although *E. laurillardi* has been considered a grazer in some environments (*10*) and a browser in others (*6*), the results of this study show that they were mixed feeders, using both browsing and grazing (*5*), and likely relied more on C_4 or CAM vegetation during the wet season, and C_3 plants during the dry season, consistent with their hypothesized adaptive flexibility (*5, 6, 10*). This diverse diet would have increased the ability of this species to inhabit varied landscapes and adapt to variable climatic conditions, thus enabling its wide latitudinal distribution (from ca. 30°S to 40.3°N) (*31*). With an increased canopy density, $\delta^{13}C$ values of plants decrease, and this ^{13}C depletion is passed to the consumer (*32*). While it is challenging to place a fixed $\delta^{13}C$ value for a closed canopy forest, some suggest that the depletion value is ~13‰ in Quaternary sites in South America (*32*). As all $\delta^{13}C$ values are $\geq -7.4‰$, there is no evidence that these giant ground sloths were consuming vegetation in dense forests similar to that found at Cara Blanca today. Instead, *E. laurillardi* from Cara Blanca consumed food in a more open environment, corroborating McDonald's (*2*) discussion of a scrub and juniper forest during the Late Pleistocene.

On the basis of skeletal morphology, it has been suggested that this species was a browser, rather than a mixed feeder (*6*); yet, the higher average $\delta^{13}C$ value of −6.8‰ (intermediate between −9 and −2‰) indicates more of a mixed diet and is consistent with studies of other Xenarthra species (*5*). While there is compelling evidence for a mixed diet, the degree to which C_4 (typically warm-season grasses and shrubs in this region) or CAM plants (such as bromeliads) were consumed varies depending on the physiology of *E. laurillardi* and, subsequently, on the isotopic enrichment between dietary resources and tooth orthodentin. Although C_4 resources are typically warm-season grasses at low latitudes (*33*), the consumption of CAM plants cannot be ruled out. It has previously

been suggested that CAM plants do not make up a large portion of mammal diets (*34*); however, some CAM vegetation, such as epiphytic bromeliads, was likely common in the neotropical environment and should be considered as potential contributors to the diet of *E. laurillardi*. On the basis of the range of stable isotope values (and an enrichment of 14‰), C_4/CAM plants could have comprised 29 to 44% of their diet.

The lower CO_2 levels in the atmosphere that accompanied the LGM (*35*) may have encouraged C_4 vegetation growth (although the opposite pattern has been observed in Florida during glacial conditions) (*15*). Instead, lower CO_2 and pronounced aridity likely contributed to an increase in C_4 vegetation, based on modern analogs (*33*). C_4 grasses are more palatable during the wet season, making it more likely that C_4 vegetation (potentially grasses, C_4 shrubs such as *Atriplex*, and/or CAM plants) comprised a large portion of the sloth's diet during the wet season, which may explain higher $\delta^{13}C$ values when $\delta^{18}O$ values are the lowest (the inferred wet seasons). During the dry season, when C_4 grasses and shrubs are less palatable, they contributed less to the overall diet. Consequently, it is possible that, during the dry season, these sloths instead relied more heavily on C_3 plants, as supported by the lower $\delta^{13}C$ signatures during the prolonged dry season (as inferred from elevated $\delta^{18}O$ values). Our data suggest that their diet was seasonally variable and that they were likely opportunistic feeders. The ability to shift between C_3 and C_4, and possibly CAM vegetation, may have increased their dietary adaptability to changing environmental conditions.

CONCLUSIONS

Although only a single *E. laurillardi* tooth was analyzed, this study provides a protocol for isotopic analyses of xenarthran teeth. Because members of this order do not have enamel, which is most often used for stable isotope studies, understanding the robusticity of dentin to preserve unmodified stable isotope signatures is necessary. Our study has confirmed that dentin layers, and more specifically orthodentin layers, are differentially affected by diagenesis and that the inner layer of the orthodentin is most resistant. If isotopic analyses are conducted on any other layer with diagenetic alteration, then they are likely to produce inaccurate results. While other

studies have conducted isotope and AMS analyses on dentin layers of *E. laurillardi* teeth, none have clarified which layer of dentin was tested. The implications of this study might alter the conclusions generated from previous analyses. In addition, it offers a refinement in the application of methods that results in more reliable analyses on other Xenarthra specimens because the orthodentin layer of other Xenathra species is twice as thick as that in *E. laurillardi* teeth and thus provides more material for analysis. Because collagen is most frequently absent within fossilized remains from the tropics, the apatite carbonate portion from the inner orthodentin was used in this study. The results of this study suggest that the pretreatment method used here—vacuum milling of inner orthodentin in a weak acid—is adequate to obtain reliable dates in lieu of preserved collagen. Recently, De Iuliis and colleagues (*36*) reported the presence of the smaller sloth, *Nothrotheriops shastensis* from Actun Lak in central Belize, roughly 40 km south of Cara Blanca. Although a molariform tooth was recovered, it was not dated because collagen was absent. Reliable dates might be obtained from the inner orthodentin of this specimen using the combination of CL analysis and vacuum milling technique presented here.

The examined sloth remains date to the Late Pleistocene, a period of increasing aridity in Central America, and the isotopic analyses shed light on how one member of the species *E. laurillardi* was adapting to seasonally variable climates. Accessing the watering hole during severe dry periods might have been difficult because of the steep sides of the sinkhole, but it may have been one of the few watering holes in the region. During the brief wet seasons, these sloths may have taken advantage of relatively more palatable C_4 vegetation that was likely more abundant due to both low CO_2 levels during the LGM and more arid conditions locally. *E. laurillardi* did not consume foliage from a dense canopy forest and appears to have been adapted to more open habitats. Additional detailed analyses of giant ground sloth teeth, including more specimens of *E. laurillardi*, can help elucidate whether this behavior is typical of the species. Better understanding of the dietary ecology of giant ground sloths via proxy evidence including stable isotopes is important, and now possible, using the methods proposed here.

MATERIALS AND METHODS
Experimental design
This study examines the carbon and oxygen stable isotope data from 58 samples taken from along the growth axes of a single tooth of *E. laurillardi* recovered from Cara Blanca Pool 1. To obtain accurate isotope results from the most diagenetically resistant apatite layer, samples were taken from three distinct apatite layers within the tooth for comparison: the cementum, the outer layer of the orthodentin, and the inner layer of the orthodentin, as indicated by the darker and lighter layers within the orthodentin. The difference in orthodentin coloration is caused by dentinal tubules in the bioapatite changing direction, differentially affecting the permeability of the orthodentin to diagenetic material (*9*). Since the orthodentin is the hardest layer of apatite, it was expected to be least subjected to diagenesis (*9*), with the inner and outer orthodentin layers differentially affected by postdepositional processes. After the sloths' death, the tubules can act as channels for diagenetic material during the fossilization process (*9*); thus, the positioning of the tubules would affect tooth resistance to diagenesis. It has been asserted that dentin might be a reliable replacement for enamel for geochemical analysis,

depending upon the extent of diagenesis (*37*). However, there are no specific data as to which layer of dentin should be targeted; in the past, the dentin and orthodentin layers appear to have been treated as a single layer (*38*). The internal structure of the specific dentin will likely affect the reliability of the results. In this study, both the inner and outer layers of orthodentin were tested separately. To further assess the reliability of isotopic data recovered from the tooth, we conducted CL analysis. Last, we used a vacuum milling technique to sample the orthodentin for an AMS date.

CL analysis
To determine which layer of apatite is most resistant to diagenesis, we used CL [e.g., (*39*)]. CL uses electron beam bombardment to stimulate visible light emission from the minerals comprising the fossilized tooth based on their major and minor element compositions. This analysis allows us to identify differences in diagenetic mineral recrystallization and replacement of the three distinct tissues, or to determine which tissue is most intact and most altered. After sawing and smoothing a portion of the tooth, we mounted it on a slide and bombarded it with an electron ray accelerated up to 9 keV within a vacuum-sealed microscope stage. CL produces high-resolution images of luminescent material within the tooth, including contaminant elements that might reflect diagenesis. CL analysis allows us to ensure that we take into account only the isotopic analyses of bioapatite material that is unaltered, in the process elucidating which layer of bioapatite produces results least affected by post-depositional processes. The CL analysis (Fig. 4) shows the relative diagenesis between the inner and outer orthodentin layers. We determined that the inner orthodentin is the most resistant layer of apatite in enamel-less teeth and will therefore provide the most reliable results (see Results). Subsequently, we compared the stable isotope signatures of tissues that experienced more and less diagenesis.

Isotopic analysis
The samples were prepared for apatite $\delta^{13}C$ and $\delta^{18}O$ analysis from 58 distinct locations taken along the growth axes of the tooth (see Fig. 2), from the occlusal surface to the base of the sloth tooth. Preparation procedures followed those outlined by Balasse and colleagues (*13*). Using a 0.9-mm diamond burr microdrill tip, at each of the 58 locations, 5 to 15 mg of sample were removed from the tooth and collected in a microcentrifuge tube. During drilling, all cracks in the surface of the tooth were avoided to prevent contamination. To remove organics, 1.5 ml of 2.6% NaOCl was added to each sample and left uncapped overnight. After 24 hours, the NaOCL was rinsed from the sample with three washes in distilled water. Next, 0.1 M acetic acid was added to remove secondary carbonates. All samples were left for precisely 4 hours before they were rinsed clean with three washes of distilled water. The samples were then placed in the freezer for 45 min at the ISGS Stable Isotope Laboratory. Samples weighing 550 to 700 µg were placed in glass tube reaction vessels for phosphoric acid reactions. Samples were run on the Finnigan MAT 252 Isotope Ratio Mass Spectrometer with an attached KIEL III carbonate device. The precision values are 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively. All results (table S2) are reported using δ notation, $\delta = [(R\text{sample}/R\text{standard} - 1) \times 1000]$, where oxygen isotope values $R = {}^{18}O/{}^{16}O$ and all values are reported against V-SMOW (*40*). For carbon isotope values, $R = {}^{13}C/{}^{12}C$ and all values are reported against V-PDB (*41*).

AMS dating

A single sample from the vasodentin layer was tested for collagen content, and results showed that no collagen was preserved in the tooth. As is common in tropical environments, postdepositional diagenetic processes affected the collagen within the tooth, a fact further complicated by the specimen's deposition in a cenote where it was submerged for thousands of years, causing the leaching of collagen. In this case, because of the lack of collagen, dentin was used as a replacement, as has been done in previous studies (28, 41). Although this has not been specified in previous studies, to ensure that the results were accurate, we only sampled the inner orthodentin layer, the portion of bioapatite expected to be most resistant to diagenesis. All cracks in the tooth, where contaminants could infiltrate the inner orthodentin layer, were avoided. Approximately 200 mg of inner orthodentin was drilled from the tooth, and 25 ml of 0.1 M acetic acid was added using a modified procedure designed to minimize isotopic exchange between apatite structural carbonate and modern air CO_2 and diagenetic carbonate during acid treatment. During reaction under vacuum, small CO_2 bubbles expand rapidly and are evacuated. Repressurization with N_2 from a liquid N_2 vessel guarantees exclusion of ^{14}C-enriched air CO_2 while forcing the acid deeper into the orthodentin microstructure. Cycling between vacuum for ca. 15 to 20 min and brief repressurization with N_2 continued until the sample ceased to produce CO_2 bubbles under vacuum (2 to 3 hours). The sample was rinsed four times with distilled water and freeze-dried. In the ISGS radiocarbon Laboratory, the sample was reacted with phosphoric acid to release apatite carbonate CO_2 and was cryogenically distilled. Radiocarbon dating was performed at the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory, University of California, Irvine. The dates were calibrated using IntCal13 (42).

Statistical analysis

Linear mixed models were used to estimate the percent of C_3 and C_4 plants consumed, with mean $\delta^{13}C$ values of −27.0 and −13.0‰ used to represent modern C_3 and C_4 plants, respectively. However, after taking into consideration atmospheric changes in CO_2 due to fossil fuel burning (43) and a discrimination of +14.0‰ for medium-to-large–bodied herbivores (19), mean values for sources of C_3 and C_4 plants of −11.5 and 2.5‰ were used, respectively. As isotopic enrichment in giant ground sloths is not well understood and may be larger than +14.0‰ due to the inferred digestive physiology (specifically, increased methane production), we also ran the models again after changing the sources (i.e., enriching values by 1 and 2‰). Mean stable carbon and oxygen isotope values were also compared between tissue types using parametric tests (Student's t tests) for oxygen isotopes, which were all normally distributed, and nonparametric tests (Mann-Whitney tests) for both inner and outer orthodentin carbon isotopes, which were nonnormally distributed.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/2/eaau1200/DC1

Table S1. Raw data from isotopic analysis of all three dental tissues.

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Science Advances

A year in the life of a giant ground sloth during the Last Glacial Maximum in Belize

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