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^{14}C-AMS as a tool for archaeological investigation: Implications for Human Settlement in South America

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Abstract

In this paper, we present an overview of ^{14}C methods, Accelerator Mass Spectrometry (AMS) techniques, and their applications to radiocarbon dating of archaeological sites in South America. We describe sample preparation methods to be applied to archaeological bone samples as well as useful sample selection strategies to help achieve reliable AMS-based ^{14}C results. Application of criteria to determine bone quality (to assess lack of alteration and degradation) before and during sample preparation, and the use of a modified ultrafiltration method to extract collagen from these kinds of materials have enabled us to obtain reliable radiocarbon results. Two applications will be presented to illustrate these issues: a) Paleodietary inferences from isotopic measurements on human bone and teeth from the populations of the central mountains of Argentina, and b) the implication of new ^{14}C determinations obtained from two segments of a single mastodon bone recovered from Monte Verde, that previously differed by more than 5,000 years.

Keywords: Accelerator Mass Spectrometry, radiocarbon, bone sample preparation, South America.

1. Introduction

To better understand past civilizations and their cultures, knowledge of a timeframe to indicate the sequence of events is necessary. Due to the fact that few calendar-based chronologies exist to support comparisons between events in the New World, the most likely way to obtain a timeframe is through dating procedures on a variety of materials that somehow are related to the event of interest.

Many dating methodologies are available today to the archaeologists and geochronologists, but the most used one is still the ^{14}C technique. The ^{14}C method has some drawbacks, since it requires calibration to the conventional calendar (if comparison with real time is necessary), rigorous physical and chemical pretreatments to extract exogenous carbon contaminants, and in some cases marine reservoir corrections and anthropogenic effects must be accounted for in the final results. However, the application of this method to archaeological materials is still extremely powerful and in most cases is the only resource available. When the ^{14}C method is combined with recent advances and improvements in Accelerator Mass Spectrometry (AMS) technique (dedicated machines can now achieve 0.2% precision and can measure extra-small samples – Santos et al. 2006a, 2006b), this methodology can overcome most of these problems. However, to obtain reliable results, careful sample selection, proper chemical preparation procedures, and corrections (when required) must be applied.

In the first half of this paper we present a short overview of methods, techniques and procedures: (a) the ^{14}C dating method and principles, (b) the Accelerator Mass Spectrometry (AMS) technique and its advantages for ^{14}C measurements, (c) extraction and ultra-filtration of collagen from bone and tooth samples (important considerations and procedures to help obtain reliable ^{14}C results), and (d) stable isotope theory and interpretation of ^{13}C and ^{15}N measurements.

In the second half of this paper we will focus on some applications on South America archaeological sites, by dating freeze-dried gelatin extracted from bone and teeth samples.

2. The Radiocarbon Dating Principles

The ^{14}C dating method relies on the fact that once the living organism dies, this radioisotope will no longer be replenished and consequently will decrease with time according to its half-life (5,730 ± 40 years), as the radioisotope ^{14}C atoms decay to ^{14}N. The presence of ^{14}C in the atmosphere is due to the ongoing production by the interaction between cosmic rays and nitrogen atoms in the upper atmosphere. The ^{14}C atoms are quickly oxidized to ^{14}CO and later to ^{14}CO$_2$. The mean residence time of CO$_2$ in the atmosphere is about 5 years, sufficiently long that ^{14}CO$_2$ is mixed in almost all parts of this particular reservoir. ^{14}C can be fixed in the living tissues of plants by photosynthesis and consequently into other living organisms through the food chain.

Assuming that the production rate of the ^{14}C in the atmosphere is constant, the measurement of the remaining ^{14}C from an organism will allow the time elapsed since its death to be inferred. However, although this assumption seemed reasonable, with continued application of this method, it became clear that a series of features could affect or bias the age result. They are mentioned below:

  a) Due to the natural temporal fluctuations in ^{14}C production rate (cosmic rays fluxes and magnetic field variations), the conventional radiocarbon age must be calibrated to solar or calendar dates by an independent known-aged material and techniques. This problem became apparent very early on, and a radiocarbon calibration curve based initially on sequential dendrochronologically

aged trees (figure 1) was constructed. The current tree ring calibration datasets, that converts conventional radiocarbon ages (years BP) into calibrated years (cal yr), include the Northern Hemisphere IntCal04 and Southern Hemisphere SHCal04 (N.H. to 12,400 cal yr and S.H.: 1,000 years of data, model to 12,400 cal yr – Reimer et al. 2004; McCormac et al. 2004), and can be found on the following link http://radiocarbon.pa.qub.ac.uk/calib/ . The IntCal04 goes back to 26 cal kyr BP (Reimer et al. 2004) using mostly marine data for the period beyond the tree ring curve. Beyond this point several attempts using natural archives (such as, corals, forams, speleothems, lake sediments varves) to construct calibration curves back to ~ 50ka have not reached an agreement (figure 2). Kauri trees recovered from New Zealand swamps (Balter 2006) may help to solve this problem.

b) The exchange rate of carbon between the ocean and atmosphere has varied in the past as ocean circulation patterns have changed. Since the calculated radiocarbon age is normally obtained by the direct comparison of the activity of the unknown sample with a modern standard from the atmospheric reservoir, shells and other marine organisms show an "apparent age" older than expected (when compared with coeval organisms that sample the atmospheric reservoir). It became clear that the radiocarbon age from samples that obtained carbon from a different reservoir than the atmospheric one required a specific correction. A Global Marine Reservoir Correction Database (Marine04 - Hughen et al. 2004), compiled over several decades, is available on-line (http://radiocarbon.pa.qub.ac.uk/marine) for direct correction of marine samples. This data base allows accounting for the radiocarbon offset between the atmosphere and ocean, and for the dilution effect caused by the mixing of surface waters with upwelled ^{14}C-depleted deep waters.

c) Anthropogenic effects, such as burning of fossil fuels (Suess effect – Suess 1955) and later the nuclear bomb tests, also altered the concentration of ^{14}C in the atmosphere (figure 3). This is also why the "absolute radiocarbon standard" chosen by the radiocarbon community is an 1890 wood that grew before the fossil fuel effect became significant. The activity of this 1890 wood was than corrected for radioactive decay to 1950. The year of 1950 (chosen to honor the publication of the first radiocarbon dates calculated on December 1949) is considered the year 0 BP (Before Present) and is deemed to be the "present". For actual laboratory measurements, the "modern" radiocarbon standard used is related to the ^{14}C content of Oxalic Acid-I (OX-I; $C_2H_2O_4$) made from a crop of 1955 sugar beet. The specific activity (^{14}C/C) of this "modern" standard is defined to be 0.95 of the activity of OX-I, decay corrected to 1950. Since the original batch of OX-I is no longer commercially available, a second batch has been prepared (OX-II), which has a ^{14}C activity 1.2933 times OX-I (Taylor, 1987:97). For calibration of the post-bomb ^{14}C data, an online calibration program denominated CALIBomb can be used (http://calib.qub.ac.uk/CALIBomb/frameset.html).

d) The three naturally occurring isotopes of carbon: ^{12}C (98.89 %), ^{13}C (1.11 %), and ^{14}C (< 10^{-10} %) all take part on the same physical or chemical processes, but because they have different atomic weights they react at different rates leading to isotopic fractionation (section 5). Evaporation (that discriminates against heavy isotopes) and photosynthesis are some examples. The magnitude of these effects can be described by the measurement of the stable isotope ratio ^{13}C/^{12}C. Since the ^{14}C/^{12}C ratio is used to calculate the radiocarbon age, a mass-dependent fractionation effect expressed by the measurement of ä^{13}C values of the sample has to be taken into account to correct the final ^{14}C results.

e) The first determination of the half life of ^{14}C (5568 years) was obtained by Libby, (Libby, 1955). Later reevaluation of this half-life gave a more accurate value of 5730 ± 40 years (Godwin, 1962). However, because Libby's half-life had already become established in the radiocarbon literature it was decided to maintain it in the calculations of the conventional radiocarbon age to allow all published dates be compared on the same basis. For actual chronological use (when calibrating

the conventional radiocarbon age to the solar calendar, as was mentioned above) an automatic correction for this incorrect half-life is applied in the calibration process.

f) In addition, post-depositional carbonaceous sample materials to be dated are often degraded and altered chemically (based on environmental conditions in which the sample was exposed), requiring specific physical and chemical sample pre-treatment to remove the exogenous carbon contaminates.

Despite all of these problems, the radiocarbon dating method remains the most widely applied dating technique for the late Pleistocene and Holocene periods, mostly due to its half-life (which allows tracing of the history of humans on Earth for the last 50,000 years) and to its abundance in the biosphere. From the archaeology research point of view, these issues represent a series of limitations. However, they have been extremely useful for helping Earth Scientists to understand the complex path of the carbon cycle through the atmosphere, biosphere and marine reservoirs.

3. The AMS technique principles

Accelerator Mass Spectrometry (AMS) is a technique that combines the principles of conventional mass spectrometry with the use of a particle accelerator to count extremely low concentrations of rare isotopes in the sample (Kutschera 1999).

Any AMS system is composed of basic elements: 1) an ion-source; 2) magnet mass and momentum selectors; 3) an acceleration tube with a stripper canal section; and 4) a detection system. Each one of these elements will be explained on the following section using the KCCAMS compact system as an example, emphasizing how its features assist in measuring ^{14}C particles.

3.1 The KCCAMS/UCI System

The Keck Carbon Cycle Accelerator Mass Spectrometry Facility/University of California, Irvine (KCCAMS/UCI) is based on a compact AMS system built by National Electrostatics Corporation (NEC 0.5MV 1.5SDH-1 – figure 4). This system is equipped with the NEC 40-sample MC-SNICS cesium sputter ion-source that produces negative ions from solid graphite targets. These targets are bombarded individually by a cesium beam current. During the bombarding process, carbon atoms are negatively charged by free electrons, and consequently extracted off from the negative ion-source into the AMS system. The negative ion beams produced are directed towards an injection magnet, which selects the mass of interest (e.g. M=14). For ^{14}C-AMS measurements, the use of a negative ion-source is of fundamental advantage since its major isobar (^{14}N, with a mass indistinguishable from ^{14}C) does not form stable negative ions. On the other hand, molecules like $^{12}CH_2$ or ^{13}CH (stable isotopes of carbon bonded with hydrogen) will also have a total mass indistinguishable from ^{14}C and, consequently will not be discriminated by the injection magnet alone thus requiring additional stages of particle discrimination.

The next discriminator is the electrostatic accelerator (high voltage terminal coupled with gas stripper) that "pulls" the negative ions towards the accelerator terminal (charged to ~0.5MV), in which the negative ions can be "stripped" of electrons by a high density re-circulating gas stripper (2microgram/sq cm of argon) and thereby changed into positive particles. Collisions with this wall of argon gas, in the charged exchange canal in the terminal, destroy molecules by breaking them up into their constituent atoms. The positive ions are then "pushed" out of the terminal, towards the high energy analyzer system at ground potential.

Molecular fragments can also undergo charge-exchange collisions in the electrostatic accelerator thus acquiring (after still some more collisions) the same magnetic rigidity as the ^{14}C, allowing them to pass through the drift tube of the analyzing magnet towards to the detector. To definitively filter these remaining contaminants from the ^{14}C$^+$ beam an additional stage of discrimination (the electrostatic analyzer or ESA) is used, that discriminates against any particle with abnormal energy or charge state (e.g., different of the ^{14}C$^+$ particles).

In addition to the discrimination performed by the analysis system (injection magnet, stripper, analyzing magnet and ESA), a silicon solid state detector (at the end of the beam line) measures the particle energies to distinguish ^{14}C particles from the noise.

For ^{14}C-AMS dating, one must known the ^{14}C/^{12}C and ^{14}C/^{13}C ratios from samples, and consequently we have to measure the stable positive ion beam currents (^{12}C and ^{13}C) as well as the ^{14}C particles after the electrostatic accelerator. A fast beam switcher (bouncer) in the injection system is therefore used to sequentially inject ^{12}C, ^{13}C and ^{14}C into the electrostatic accelerator, cycling through the 3 isotopes ten times per second. The measurement of the ^{12}C and ^{13}C beam currents is then performed in the Faraday cup chamber (off-axis Faraday cups) after the analyzing magnet. These measurements not only help us to obtain the ^{14}C/^{12}C ratios (necessary for the calculation of age) but also to effectively correct the ^{14}C final results using their respective ä^{13}C values (obtained by the directly measured ^{13}C/^{12}C ratios). So, any "machine" or sample preparation isotopic fractionation effects in addition to the natural one (section 2d) can be effectively addressed.

The final AMS result, as mentioned before, is expressed as the ratio of the rare (^{14}C) to the abundant isotope (^{12}C or ^{13}C) measured directly on an unknown sample, relative to the corresponding ratio of a standard sample (such as, OX-I). To aid in accuracy and precision we add to each batch of unknowns selected know-age secondary standards and ^{14}C-free materials (blanks) prepared in the same fashion a the unknown samples. Secondary standards and blank materials are readily available from the international inter-comparison laboratory evaluations (such as, the TIRI, FIRI, VIRI and, IAEA series).

AMS system improvements and progress in ^{14}C-AMS sample preparation at the KCCAMS facility have enabled us to achieve high precision of 0.2% (~15years) on modern carbon samples, and backgrounds as old as 55ka BP on processed graphite samples from ^{14}C-free materials (Southon and Santos 2004).

3.2 Advantages of ^{14}C-AMS

Radiocarbon dating using AMS allows the amount of ^{14}C in the sample to be measured directly, rather than by waiting for the individual radioactive decay events to occur, as with decay counting methods. This enhances sensitivity as well as allowing us to handle small samples. The latter is in fact the crucial advantage of this method. ^{14}C-AMS samples are at least a factor of 1,000 times smaller than decay counting method samples, and they can also be measured in a very short period of time (minutes instead of days - Kutschera 1999), allowing us to:

a) Date more samples from each context in a site, as well as produce replicates from one unique sample.

b) Directly date valuable archaeological artifacts without severe damage, as well as dating individual small carbonaceous materials, such as seeds, twigs, insect remains, that may be more securely associated with the event of interest.

c) Apply more stringent pre-treatments to assure the removal of any likely contaminants, as well as date different chemical fractions from these pre-treatments to better understand the sources of contamination.

For archaeological research, however, the reliability of a radiocarbon dating chronology remains critically dependent on the relationship between the material being dated and the archaeological record. The radiocarbon method combined with the sample-size reduction of the AMS technique can help on this issue through dating smaller and more representative samples, so that dates generated can represent as closely as possible the event of interest. However, the properly selection of the samples is still in the hands of whoever is submitting the sample to the ^{14}C dating facility and, therefore, it is necessary that the sample to be submitted embody as much archaeological and contextual (e.g. geological and geomorphological) information as possible to avoid misleading dating results.

4. The ^{14}C-AMS sample preparation

Careful sampling and adequate pre-treatment (physical and chemical) are very important stages of the ^{14}C dating process. Due to environmental carbon abundances, contaminants from different ages are always expected, especially when samples are found buried. Surface layers of any carbonaceous raw materials to undergo ^{14}C sample preparation should be removed, because these are most susceptible to carbon contamination. The physical and chemical pre-treatment applied will depend on the type of sample. The carbonaceous raw materials suitable for ^{14}C measurement can be divided on two large groups: a) organics, such as charcoal, wood, macrofossils, bone, etc; and b) carbonates, such as shells, corals, speleothem, forams, etc.

Over the last twenty years, ^{14}C laboratories have being carrying on different pre-treatment procedures for these materials, also at different states of preservation. To produce reliable results processes employed at any ^{14}C laboratory, on sample cleaning and target production, must be rigorous, well tested and reproducible. Otherwise, contamination can lead to "apparent" ^{14}C ages (younger or older), where the magnitude of contamination can be impossible or extremely difficult to trace. These processes have been developed, improved and published, as part of the laboratories quality control and research programs, and can be frequently found in the proceedings of the international Radiocarbon and AMS conferences.

Similar to other labs, KCCAMS sample prep laboratory procedures include physical pre-treatment of organic and carbonate samples, simple acid-alkali-acid chemical treatment, sealed tube combustion of organics, leaching and hydrolysis of carbonates, and extraction and ultra-filtration of collagen from tooth and bone samples (described in details on section 4.1). The goal of these processes is to clean and convert the carbonaceous "raw" materials into a solid graphite targets to be loaded into the ion-source (section 3.1, figure 4) for an AMS measurement.

4.1 Bone Methodology

4.1.1 Issues in the ^{14}C Dating of Bone

The problems of obtaining accurate ^{14}C ages on fossil bone material have being well discussed (DeNiro 1985; Stafford et al. 1987; Ajie et al. 1990; Stafford et al. 1990; Ajie et al. 1992; Hedges and Van Klinklen 1992; Burky et al. 1998; Brown et al. 1988; Taylor et al. 2001; George et al 2005; Higham, T.F.G 2006; are some examples). These studies document significant variability in the degree to which endogenous

carbon-containing fractions in bone are retained or lost, and replaced with various amounts of exogenous organic materials. Subfossil bones in general retain 1 to 5 percent of the collagen content of modern bone and exhibit a noncollagen amino acid profile. For fossil bone with residual collagen < 1% of that of modern bone, significant anomalies in the ^{14}C values are typically encountered, with the magnitude of the age offsets tending to increase with decreasing in situ collagen concentration. [14]C sample preparation on fossil bone material showing low yield may require detailed individual attention (and consequently more complex extractions) to reach robust results. In some cases, in which bone samples are severely degraded, no reliable results can be achieved at all.

Fortunately, for bones containing sufficient quantities of collagen (the principal protein in mammalian bone) it is generally agreed that standard pre-treatment methods (such as the one reported on section 4.1.2) can effectively isolate and purify the residual collagen or collagen-derived organics materials. For the characterization of the state of preservation of bone and teeth samples, some simple initial quality tests can be performed and will be discussed on section 4.1.3.

4.1.2 ^{14}C-AMS bone sample preparation

As mentioned earlier, for bone and tooth samples (due to their high susceptibility to diagenetic changes after burial) it is essential to extract the compounds that do not contain exogenous carbon. In this case, we separate the collagen, and convert it into gelatin. We use a protein-remnants (collagen) extraction method (modified from Brown et al. 1998) that maximizes the product yield, while minimizing its incorporation of contaminants due its capability to remove low molecular weight fractions. This process can be summarized as:

a) Bone surfaces are removed with high speed dental burs and cutters.

b) Bone particulates (sizes of 0.2 to 0.5mm) are produced by crushing. For teeth samples a drilling method can be used to produce powder;

c) Acid is used to decalcify bone particulates and tooth powder for 24-36 hours;

d) Alkaline solution is used (when necessary) to remove humic acids absorbed during burial;

e) The decalcified material is than placed in weak acid and converted to gelatin by heating at 70°C for 8-12 hours;

f) The gelatin is than ultra-filtered, using Amicon[TM] Centriprep 10 concentrations to yield a >10 kD fraction and to remove low molecular weight contaminants.

g) The purified gelatin is finally freeze dried.

Before the Centriprep 10 concentrations filters can be used on samples, they are pre-cleaned using pure water through alternating sonic baths and centrifugations to remove the humectant (glycerol) used to coat the filter membrane. If the glycerol is not properly removed, it can contaminate the sample by adding "old" carbon (Higham et al. 2006).

After chemical pre-treatment, aliquots of freeze dried gelatin sample are combusted to produce CO_2. The individual CO_2 gas samples are cryogenically separated into a vacuum line and reduced to solid graphite by hydrogen into reactor vessels at 550°C over pre-baked Fe powder catalyst (Santos et al. 2004).

A small aliquot (0.5 to 0.8mg of freeze dried gelatin) is combusted into an elemental analyzer- Isotope Ratio Mass Spectrometer (IRMS) where the stable isotope ratios of carbon and nitrogen are measured (section 5). These ratios provide useful information on the purity of the sample (DeNiro 1985), climatic conditions of the environment of the living organism, and clues on diet. This data can also help distinguish between terrestrial and marine food sources - DeNiro & Epstein 1978, 1981). This can be important for the ^{14}C dating itself since some aquatic diets can affect the dates significantly (the ocean reservoir effect – section 2).

4.1.3 Criteria to help on identify bone quality

For the characterization of the state of preservation of bone and teeth samples, some simple initial quality tests can be performed:

a) Does the sample smell burned when ground? Good quality sample material, when in contact with a high-speed burr or cutter, often smells, indicating the presence of proteinaceous material.

b) Does the sample feel hard under contact with implements and tools? Much of the strength of bone is derived from the presence of the collagen that binds the crystalline component together. Soft samples may give low gelatin extraction yields and highly fractionated results (section 5).

c) Production yield is also a good indicator of bone quality (Henry et al. 1992). It can be determined by comparing the initial weight of bone or dentine with the final weight of the freeze-dried product after the gelatinization process.

d) The color of the final ultrafiltered freeze-dried extract is diagnostic: white or light tan material indicates good quality sample; dark tan to brown extracts indicates that the collagen is heavily cross linked by carbohydrates or other exogenous carbon (via the Maillard or so-called "browning" reactions).

e) In addition, stable isotope measurements of ä^{13}C, ä^{15}N, and the C and N content of bone and teeth samples can help identify the suitability of sample material for reliable radiocarbon dating. Samples with C/N ratio values > 4 indicate large amounts of exogenous carbon and/or high degree of diagenetic changes of the collagen (DeNiro 1985). Anomalous ä^{13}C values also indicate gross contamination.

Radiocarbon measurement results from samples that fail to fulfill the criteria described above should be treated with caution, or depending on the failed criterion should be rejected at once, since the material being dated has unclear chemical origins and may not reflect the event of interest.

5. Stable Isotopic Analyses of C and N

The differences in the atomic weights of the isotopes of the light elements (such as H, C, N and O) can be sufficient for many physical, chemical, and biological processes to change (or fractionate) the relative proportions between them. In nature these isotopic fractionations can have several origins (Roth 1997). A good example is the differences in the chemistry of the growth of plants and in metabolic paths of animals.

In terrestrial plants, isotopic fractionation of C happens during the uptake and conversion of CO_2 into plant carbon. Terrestrial plants fix atmospheric CO_2 by two main photosynthetic reaction pathways: the Calvin-Benson, or C3, and the Hatch-Slack, or C4. The C3 plants convert atmospheric CO_2 to a phosphoglycerate compound with three C atoms while the C4 plants convert CO_2 to dicarboxylic acid, a four-C compound. These 2 pathways fractionate differently, and as a consequence, the C4 plants have higher ä^{13}C values around -12.5‰ relative to PDB standard, whereas the C3 plants have average ä^{13}C values around -26‰ (Trimble and Macko 1997). The majority of terrestrial plants are C3 plants. The C4 plants are more frequently found in hot and arid environments, since they are more efficient at absorbing water during photosynthesis. Most of the edible C4 plants in North America today are species that have been domesticated (such as maize, sugar cane, sorghum, millet, etc). Consequently, ä^{13}C measurements from the archaeological context can provide a tracer for the introduction of these domesticates.

^{15}N/^{14}N differences in plants arise because some plants use symbiotic fixation of nitrogen. Non-nitrogen fixing plants obtained all their nitrogen from the soil whereas nitrogen-fixing plants have an alternative N source in the form of air. Atmospheric N is isotopically lighter than plant tissues, and soil. Consequently, it is expected that nitrogen fixing and non-fixing plants will differ in their ^{15}N values. Natural ^{15}N levels in plant materials typically range from approximately -5‰ to +10‰. Grazing animals show ^{15}N enrichment relative to the plants they consume, and predators show further ^{15}N enrichment relative to their prey species, so that ä^{15}N in terrestrial animal and insects tissue ranges from approximately -1‰ to +16‰.

The measurement of ^{13}C/^{12}C and ^{15}N/^{14}N in consumer tissues can provided extremely useful information on organism feeding relationships and food web structure when the ultimate sources of C and N are well elucidated and defined isotopically. DeNiro and Epstein (1978, 1981) demonstrated that these isotopic signatures are preserved after the death of the organism; thus measurements of stable isotopes of C and N of bone collagen can be used to infer diet and habitat selection. A remarkable application of this fact is the determination of the time of introduction of maize agriculture in the New World, and the rate at which it was adopted, by examining the ä^{13}C values of skeletons and carbonized deposits in cooking pots. Note that isotopes in protein reflect the dietary protein and not necessarily the whole diet of the studied individual.

5.1 Measurement of stable isotopes

In addition to the AMS system and prep-laboratory the KCCAMS facility possesses a continuous flow stable isotope ratio mass spectrometer (Finnigan Delta-Plus CFIRMS) that is interfaced with a Fisons NA1500NC elemental analyzer. Samples for ä^{13}C, ^{15}N and C/N analysis are weighed individually in small tin containers and placed inside an auto-sampler drum. The samples are dropped into a vertical quartz tube (maintained at 1020ºC) to undergo combustion. The combustion gases are carried by He_2 to a chromatographic column, where the individual components are separated and eluted as nitrogen (N_2), carbon dioxide (CO_2), and H_2O. After the separation, the isotopic compositions of the components are measured via Isotope IRMS.

Results are expressed in standard ä notation relative to the international standards carbonate PDB and atmospheric nitrogen (N_2), where

$$ä^{13}C \text{ or } ä^{15}N = [[(R_{sample})/(R_{standard})]-1] \times 1000$$

and R = (^{13}C/^{12}C or ^{15}N/^{14}N), respectively. A substance with an isotope ratio less than that of the standard will have a negative ä value, and is said to be depleted in the heavy isotope relative to the standard. A substance that is enriched relative to the standard will have a positive ä value. Secondary standards of known C, N, ^{13}C and ^{15}N content are used routinely for cross-calibration checks. In addition, empty tin capsules are also measured in order to determinate the background signals that may influence the final results.

This equipment can provide very precise measurements of the isotope ratios and the concentration of the individual components of the mixture (typically 0.1‰), allowing us to infer important information on the C and N sources and pathways taken from samples through terrestrial and ocean reservoirs to the samples.

6. Some applications on human settlement in South America

6.1 Paleodietary inferences on the human populations of the Central Mountains (*Sierras Centrales*) of Argentina.

As mentioned on section 5, the characteristic isotope-ratio "signatures" of food species can be passed on to consumers. Although further fractionation may occur during metabolic processing of food, the mean ä^{13}C and ä^{15}N values of primary producers can remain visible through many trophic levels to the top of the food chain. If the isotopic signatures of C and N from a particular region are well defined, it is possible to infer changes on consumption products if consumers move from a C3 to a C4 base diet or vice-verse or, when they are ascending or descending the food chain, respectively.

Although the native cultures of Argentina and the Southernmost region of South America have been extensively studied in the last decade, many questions of these extinct populations remain unanswered. The Central Mountains or *Sierras Centrales* are located in the southern region of the Pampean Hills, between 30-33º (S) latitude and 62-65º (W) longitude, occupying the current territory of Córdoba and part of the San Luis province (Figure 5 - map). The area is characterized by three mountain chains, the *Sierras Grandes*, *Sierras Chicas*, and *Sierras Occidentales*, which are separated by longitudinal valleys and high-altitude plains, or pampas. The *Sierras Centrales* region has a crucial importance for the understanding of the population processes of the southernmost region of South America, given its crossroad geographic location. A possible common origin of the ancient inhabitants of this region and the groups that colonized the southern extreme of the Americas has been suggested from both archeological and morphological evidence (Laguens et al., 2003; Fabra et al., 2005).

Since the end of the 19th century to present, archaeologists have found evidence for the presence of human populations in central mountains of Argentina for approximately the last 10,000 years (Ameghino, 1885, 1889; Castellanos 1943; Montes, 1960; González, 1960, D'Andrea et.al., 1997). During this period, these human populations had adapted to the variability of the environmental conditions, and developed subsistence strategies based on hunting (mainly guanacos and deer) and harvesting of wild fruits (such as the carob tree). In the middle of the first millennium, maize agriculture was added to the hunter-gatherer strategy, complementing its economy of subsistence (Laguens 1999). The faunal and archaeological record of these populations have been used to study and interpret the diet of these populations; and computer models of sustainable capacities have being used to simulate optimum dietary solutions for this environmental context (Laguens and Bonnin 1987, Langues 1999, Bonnin and Laguens 2000, Laguens and Bonnin 2006). A common element that arises in the regional models is that the dietary

resource replacement (agriculture) did not surpass 50% of the overall diet, being in fact complemented with 17% of hunting and 33% of harvesting (Laguens 1999).

The aim of this study was to infer the spatial and temporal paleodiet diversification through isotopic measurements ($ä^{13}C$ and $ä^{15}N$) on human bone and teeth from individuals who lived in this area during the Holocene. A complete description on this work can be found elsewhere (Laguens et al., 2006).

6.1.1 Sample selection and processing

The archaeological sites (figura 5), relevant to this study, are located in Cordoba's Hills (both oriental and westerner sides), the oriental plain and the Mar Chiquita Lagoon (in the Northeast).

Samples were sent to KCCAMS/University of California, Irvine for stable isotope analyses and ^{14}C-AMS age determinations. The isotopic measurements were performed on 5 samples of bone and 5 of teeth from 10 human skeletons. The samples belong to one juvenile and nine male and female adult. Five of the samples are from burial sites excavated during archaeological "rescues". These samples were storage at the *Museo de Antropología*. The remaining samples were from different public and private museums of Cordoba. In those cases in which samples were excavated during archaeological rescues (Agua de Oro, Guasmara and La Granja sites) we had information about mortuary practices and archaeological materials associated, that allow us to infer a relative antiquity, previous ^{14}C-AMS analysis. The remaining samples (Amboy, East Coast, Rincon II, Ayampitin and Miramar sites) were not from systematic archaeological surveys, there was no information about archaeological contexts.

Overall visual inspection of the samples indicated that they were very well preserved, except the teeth samples UCIAMS# 22279 [61B, Agua de Oro site ("I2,CB")] and 22286 [64B, Agua de Oro Site, ("I1, CD")], in which roots were no longer attached (leaving the dentine exposed – table 1).

For bone samples, the surface was scraped off using stainless steel cutters attached to a Dremel tool (Figure 6,a) to help remove any extraneous material attached to the sample. After the surface was removed, the sample was broken into small fragments between 0.5mm to 2mm (Figure 6,b) to speed up the demineralization process as mentioned on section 4.1.2. Amounts of 100 to 160mg of material were used depending on the initial inspection of bone.

For the teeth samples, a more complex extraction process was required to allow the preservation of the tooth enamel for the preparation of molds for producing replicas of the samples. To avoid any contamination by extraneous carbon, the stable isotope analyses and ^{14}C dating measurements were done before any contact of teeth samples with molding materials. To reach the dentine (the organic dating material) and leave the enamel intact, the crown and roots were separated using a thin flexible diamond wheel (Figure 6,c). Dentine was then extracted from the inner part of the teeth using a diamond burr (Figure 6,d). Diamond wheels and burs were fully cleaned between samples to avoid any cross-contamination. It was not possible to keep the enamel from sample UCIAMS# 22286 intact. When this sample was held to be drilled it shattered at once. In this case, dentine was taken from the broken fragments. Overall amounts of 63 to 122mg of material were used from the tooth samples depending on the amount of dentine available.

Clean aliquots of samples (bone and teeth) were converted to collagen by decalcification, gelatinization, and ultrafiltration (as described on section 4.1.2). For ^{14}C measurements, the chemically extracted freeze-dried ultra-filtered collagen was combusted and graphitized (Santos et al. 2004) to produce 1mgC samples

of graphite. Samples of ^{14}C-free bone and a secondary standard of known-age whale bone (for background correction and accuracy checks, respectively) accompany the unknown samples through the entire sample preparation process. An extra tooth sample obtained in a local dentist's office was also added to the batch to be used as modern quality control sample. Complete results are shown in table 1, corrected for isotopic fractionation by on line AMS-ä^{13}C measurements. Radiocarbon concentrations are given as conventional radiocarbon age (years BP), following the conventions of Stuiver and Polach 1977.

Aliquots of 0.5 to 0.6mg of freeze-dried ultrafiltered collagen were sent to the elemental analyzer-IRMS (section 5) for ä^{13}C, ä^{15}N and C/N ratio measurements. Samples were measured to a precision of <0.1‰ based on scatter of several standards. The ratio C/N for all samples for which we have data was close to 2.8 (table 1) indicating that they were well preserved (DeNiro1985, Ambrose 1990). The yield for sample UCIAMS# 22286 was low, and it did not produce enough ultrafiltered collagen for an EA/IRMS determination in addition to ^{14}C-AMS measurement. A ä^{13}C of -13.2±0.4‰ was obtained on prepared graphite using the AMS spectrometer, but AMS-measured ä^{13}C values can differ from 1 to 3‰ from those of the original material. Due to the fact that this sample did not fulfill the selected criteria for quality control (section 4.1.3.), the final age result should be treated with caution.

6.1.2 Discussions about the paleodiet inferences on the human populations of the *Sierras Centrales*.

Figure 7 shows measured ä^{13}C and ä^{15}N values from the sample analyzed and also gives literature values for food resources from the region studied. The overall carbon stable isotope results showed that the local inhabitants consumed mixed diets (C3, CAM and C4), as was expected. The diamonds symbolize samples from sites of hunter-gatherer archaeological contexts (**H-G**). The triangles symbolize samples from agricultural ones (**A**). We see that both cases are distributed between the areas of the vegetables C4 plants and the herbivores. Two of the samples the hunter-gatherer sites are located fully in the highest field of the trophic chain (herbivores), while the third one is in the superimposed fields of herbivores and the C4 plants. A similar situation occurred with the individuals of the Mar Chiquita Lagoon (Miramar site) and Traslasierra Valley (Guasmara site). In the case of Guasmara site, the sample is in the superimposed fields of herbivores and the CAM plants (e.g., between the C3 and C4 fields). From these results we can make the following deductions: a) The differences observed between the hunter-gatherer diet sites (diamonds) can be associated with possible differing emphasis in animal resources (at the extreme of the local trophic chain); b) The agricultural diet sites (triangles) could be interpreted as more omnivorous, with greater emphasis on vegetable resources, as expected from the models.

When the ä^{13}C results are investigated chronologically (figure 8), we notice different isotopic values for the beginning and the end of the Holocene. Samples with older ^{14}C ages show more negative ä^{13}C values, suggesting mixed diets with emphases on C3 consumption. For younger or more recent ^{14}C ages the average ä^{13}C values suggest a tendency to more C4 consumption. The most likely explanation is that the isotopic shift observed in the diet of these populations (from the Southern portion of the *Sierras Pampeanas*) reflected the introduction of maize in the Late Holocene. A similar conclusion was reported for the Western region of the country (Novellino et. al., 2004). The use of a combination of hunter-gatherer and agricultural strategies implies a detailed knowledge of local resources and their regional and seasonal variations.

Regarding the spatial variation, the ä¹³C results suggest that late Holocene diets were similar across the region, except for the sample from Traslasierra Valley (Guasmara site) and one sample from Sierra Chicas (La Granja), which show ä¹³C values similar to the mid Holocene values from some of other sites.

These results, though preliminary, suggest that the adoption of agriculture was complementary of hunter-gatherer strategies. However, this cultigen incorporation seems to have been carried out differently in the different regions, e.g. was less complete at some sites (such as Traslasierra Valley) than at others (oriental plains, Northwest). These isotopic results confirm the mixed character of the economy, previously advanced from indirect estimates and projections of similar anthropological cases (Laguens, 1999). To strengthen these conclusions, it will be necessary to perform analyses on a greater number of human samples, and on the available regional resources to better evaluate its isotopic variability.

6.2. Resolving an anomalous radiocarbon determination on Mastodon bone from Monte Verde, Chile.

Beginning in 1976, excavations at the Monte Verde site in the forested Lake District of south-central Chile recovered a wide-ranging assemblage of materials from stratified contexts (Dillehay 1989, Dillehay and Pino 1997). Several 14C determinations on various organics interpreted as artifacts and others characterized as culturally affiliated average approximately 12,500 years BP (Dillehay and Pino 1997; Nagle and Wilcox 1982). These 14C results suggest that human occupation in southwestern coastal South America occurred about 1,000 earlier than the evidences reported from Clovis sites from North America (Taylor et al. 1996). In the absence of any geochronological marker to help place this site in a temporal context, the evidence for this 1,000-year offset was resting solely on these 14C results.

Previous 14C determinations obtained on two segments of a single mastodon bone recovered from this site were highly discordant, differing by more than 5,000 years (Dillehay and Pino 1997). A mastodon bone fragment that had been eroded out onto the surface in a modern creek bed, yielded a 14C age of 6,550 160 yrs BP (BETA-7824). A second segment excavated from the upper layer of a stratigraphic unit designated as MV-6 was date to 11,990 260 yrs BP (TX-3760). The bone segments, however fit together, and were therefore determined to belong to a single femur of a mastodon (Dillehay and Pino 1989:136).

Two possible explanations for this offset can be summarize as: a) a serious and highly unusual 14C contamination problem in at least some of the Monte Verde samples or, b) very poor preservation of the collagen contained in one or both of these two bone segments. Regarding the contamination issue, no contaminants have been reported by radiocarbon laboratories that processed the initial 14C determinations (Dillehay and Pino 1997). Further investigations indicated that no humics were present on these bone samples to explain the gap between the 14C results (Tuross 1997). A lab contamination was also unlikely. To produce the age offset, a large percentage of the organic components (at least 30% of it) would have to be contaminated by modern carbon and, if so, such massive contamination would have been removed by almost any standard bone chemical pretreatment procedure. On the other hand, based on experiences with bone 14C dating, the large offset could be explained by contamination, if only trace amounts of protein (primarily collagen) were retained in one or both of the two segments.

Due to the high significance of this archaeological site, additional radiocarbon and stable isotope measurements were than performed from the organic fractions isolated from both bone segments to

elucidate the early ^{14}C disagreement (George et al, 2005). Table 2 shows the previously published ages for the two bone fragments (Dillehay and Pino 1997) as well as the new ^{14}C results (George et al. 2005).

6.2.1 New ^{14}C determinations

Dr. Thomas D. Dillehay kindly provided both segments (figure 9) of the mastodon bone. To confirm the absence of humic acid products, Dr. Taylor and collaborators repeated a test carried out previously by Dr. Tuross (1997). Only minimal coloration was observed during repeated treatment of bone powder with 1N NaOH, and subsequent treatment with concentrated HCl failed to precipitate any humics from the base solution.

For each bone segment, two different organic fractions - total amino acids (UCR-4014 and -4015) and ultrafiltered gelatin (UCIAMS-10737 and -10738) - were chemically isolated, and ^{14}C and ä[13]C values were measured (table 2). At the Radiocarbon Laboratory (University of California, Riverside), the bone was physically cleaned, and total amino acid fractions were isolated using an ion-exchange chromatography procedure described in detail by Burky (1998). To evaluate the preservation of samples, profiles of the constituent amino acids of total hydrolysates of both bones (obtained by ion-exchange chromatography) was compared against standardized amounts of total amino acids obtained from a modern bone. The total amino acid residues isolated from the aliquots produced (UCR-4014 and -4015) were 20 and 31% that of modern bone, respectively. Furthermore, the ratios of the amino acids aspartic acid, glycine, and alanine to glutamic acid in the total hydrolysates from fossil bones were in the typical range of modern values, indicating that both bone segments retain appreciable remnants of intact collagen.

At the KCCAMS lab (at the University of California, Irvine), ultrafiltered gelatin fractions were prepared from cleaned bone segments using the approach described on section 4.1.2. The >10 kD fraction yields from both bones were 13-14%, and the final products were a very light tan color, indicative of very good preservation (section 4.1.3).

These chemically extracted freeze-dried ultra-filtered gelatin samples as well as the isolated fractions obtained at UCR were than combusted to CO_2, and the CO_2 was converted to graphite following established protocols (Santos et al. 2004). The ^{14}C content from all graphite samples produced was measured using the AMS system (figure 4).

6.2.2 Discussions about the new ^{14}C determinations obtained on the two segments of the single mastodon bone

During the new ^{14}C determinations, it was found that the preservation of the collagen in both segments of bone was excellent. Two different organic extracts (total amino acids and ultrafiltered gelatin) yielded four statistically identical ^{14}C results, corroborating that the two segments belong to the same bone. These results indicate that for undetermined reasons, the original age assigned to the segment of the mastodon bone recovered from the surface of the site (BETA-7834) was incorrect. No conclusive explanation for this anomaly can be established, due to the lack of data, such as percentage yield of collagen extraction, ä[13]C and C/N measurements, etc, associated with this initial ^{14}C determination. However, the ^{14}C age of this Monte Verde mastodon is now solidly established and is concordant with ^{14}C values obtained on other organics excavated from the MV-II level.

7. Summary

The crucial advantage of the ^{14}C-AMS methodology is that only milligram sized samples are required for dating. When this advantage is combined with the recent developments of high-precision measurements (up to ±0.2% or ±15 yr), and with proper corrections for isotopic fractionation and (where appropriate) marine reservoir effects, the ^{14}C technique is enormously powerful. The radiocarbon calibration curve beyond 26,000 cal yrs still an issue, however, promising Kauri trees recovered from New Zealand swamps may help to resolve the disagreements between existing calibration data sets.

Regarding on the paleodietary studies from the *Sierras Centrales* samples: Although they were the first samples isotopically analysed from this particular region, the results obtained are concordant with other archaeological data. They suggest that maize agriculture was introduced in the late Holocene as an extra subsistence strategy (e.g. complementary to the preceding hunter-gatherer lifestyle). The maize agriculture incorporation may have helped to optimize the use of the different resources available and to achieve a stable annual food supply. Further analyses will be required to confirm this conclusion.

Regarding on the anomalous ^{14}C determinations on Mastodon bone from Monte Verde, Chile: Four new ^{14}C-AMS results obtained on total amino acids and ultra filtered gelatin fractions yield an average age of 12,460 ± 30 yrs BP. This value is concordant with ^{14}C values obtained on other culturally affiliated organics associated with the MV-II levels at this site (Dillarey and Pino 1997), what indicates that at least one of the previous ^{14}C determinations obtained from the same bone fragments was incorrect (possibly associated with poor preservation of the sample dated).

The applications showed in this paper highlights the importance of ensuring that bone material selected for ^{14}C dating is not degraded, and that contamination is effectively removed before dating. This can be achieved by screening bone samples using simple criteria that can identify possibly degraded and non-reliable material for dating, as well as through the application of rigorous preparation methodology such as the ultrafiltration of gelatin, a procedure that produces results comparable with the more complex total amino acid extraction technique.

List of figures:

Figure 1: Matching growth rings to build radiocarbon calibration curve (adapted from http://www.ncdc.noaa.gov/paleo/treering.html and http://www.14c.uni-erlangen.de/).
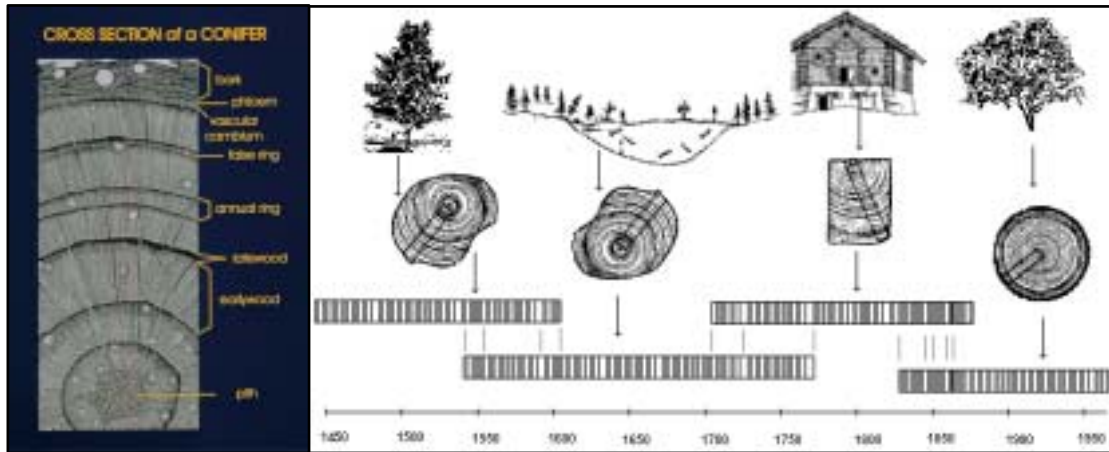

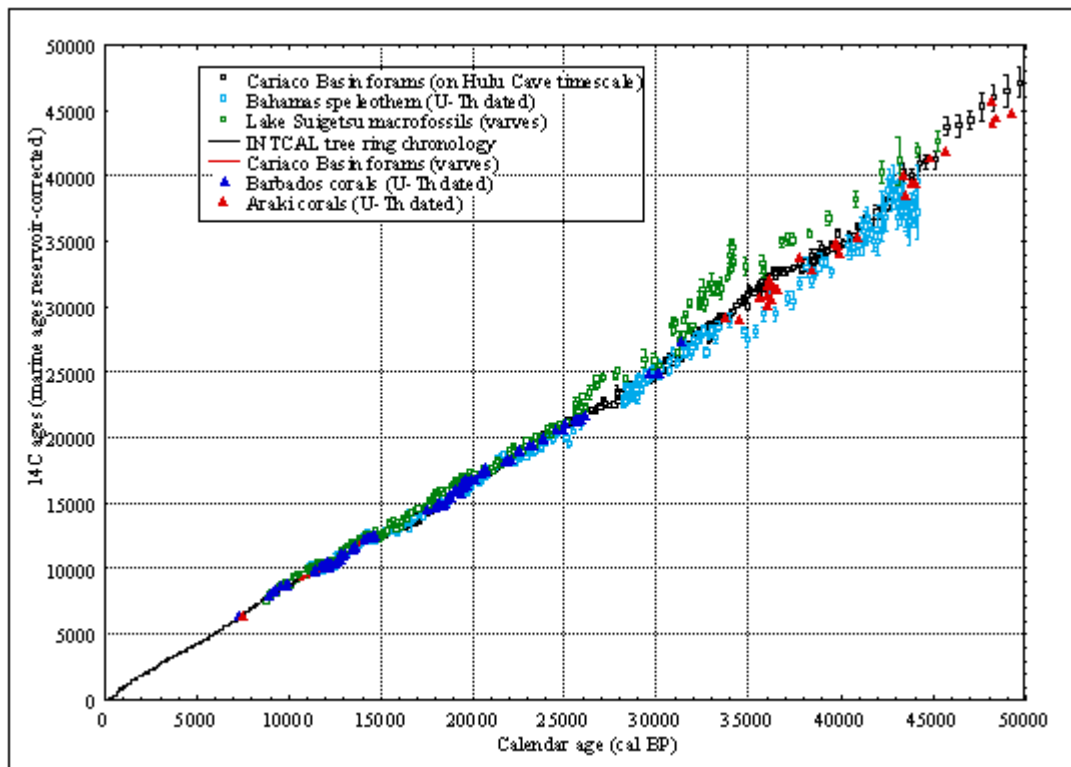Figure 2: Radiocarbon calibration data.

Figure 3: The bomb peak curve (left - nuclear weapon testing in the atmosphere in the late 1950's and early 1960's cause a near-doubling of the ^{14}C activity; right - the mushroom cloud from the WW II bombing over Nagasaki, Japan).
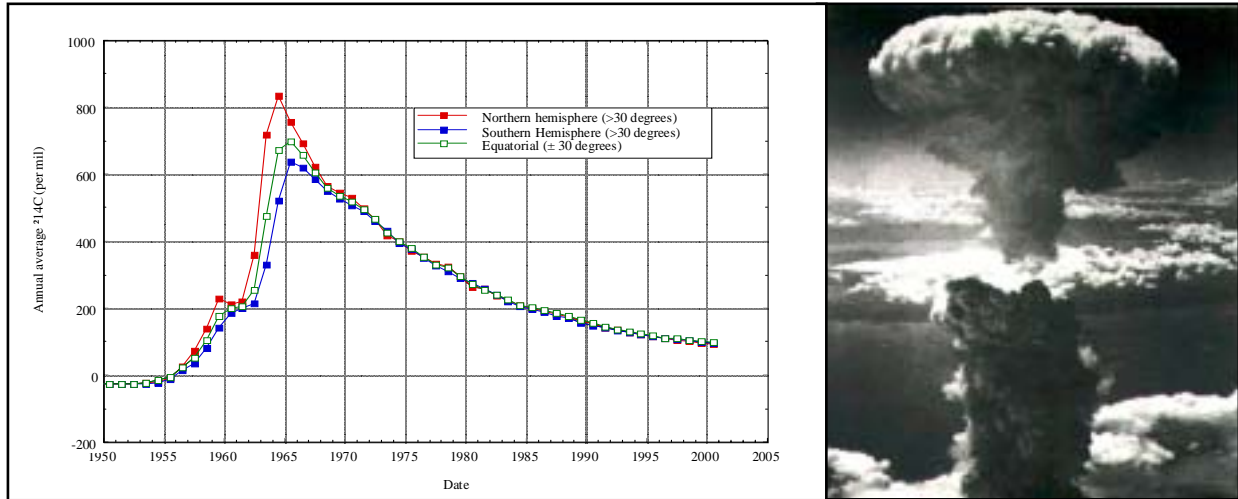

Figure 4 : The schematic of a modern ^{14}C-AMS system (http://www.pelletron.com).
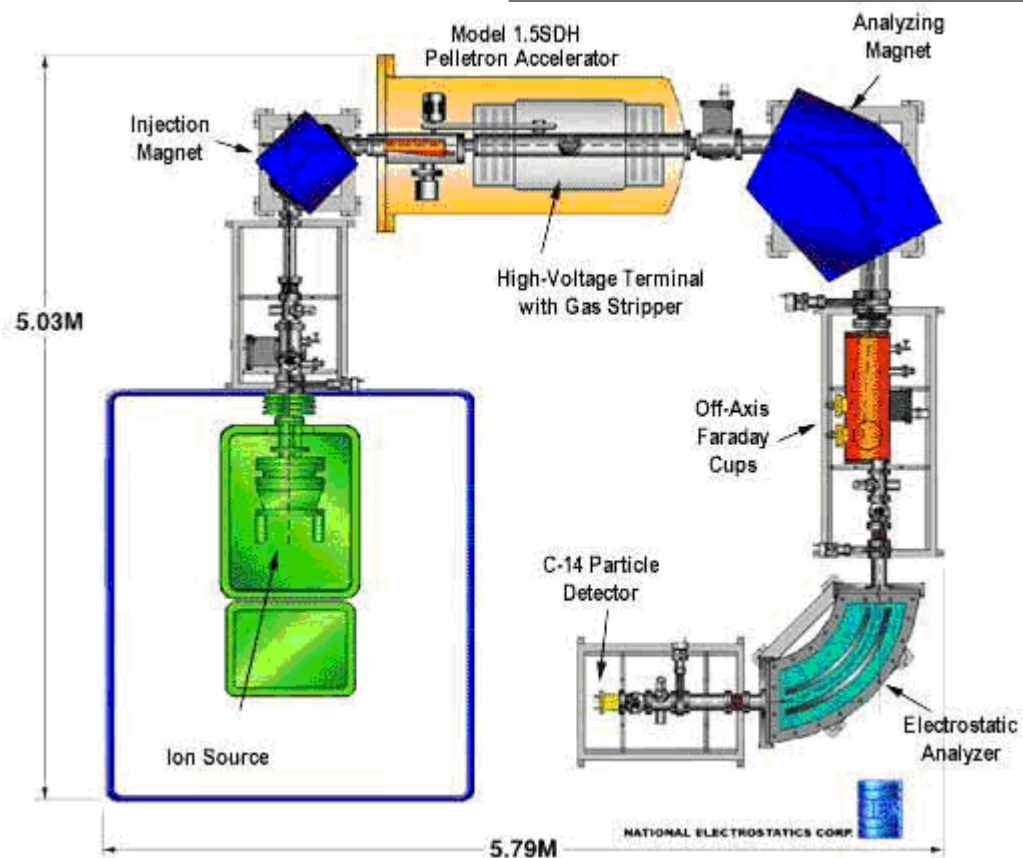
Figure 5: The territory of Córdoba and part of the San Luis province.

● Late initial Holocene (cal 3500-1000 BP)

■ Late final Holocene (cal 1000-400 BP)
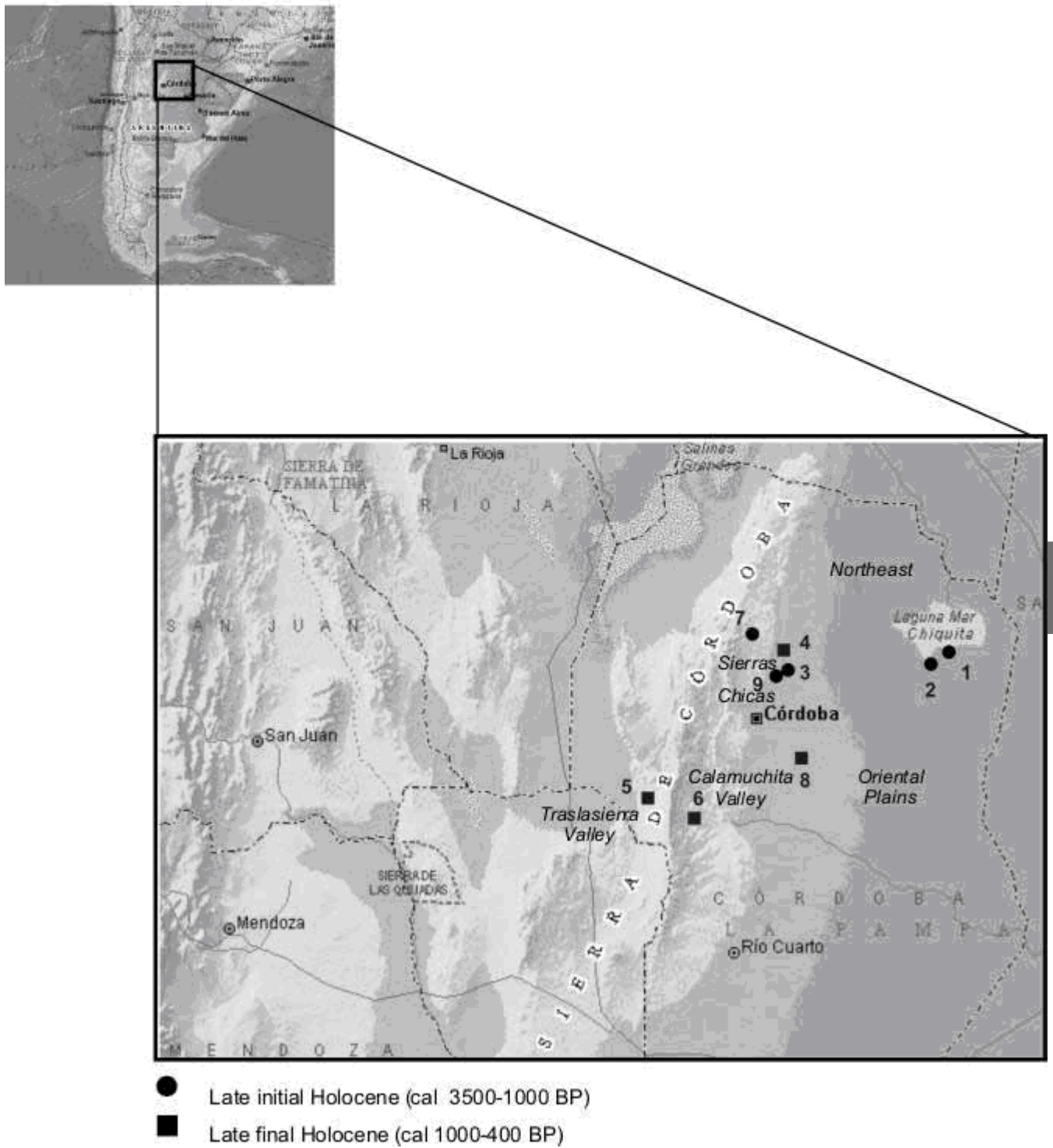
Figure 6: ^{14}C-AMS bone and tooth sample preparation: (a) surface cleaning of bone; (b) fragments of cleaned bone; (c) preparation of teeth – separating the crown from the roots, and (d) extraction of dentine by drilling.
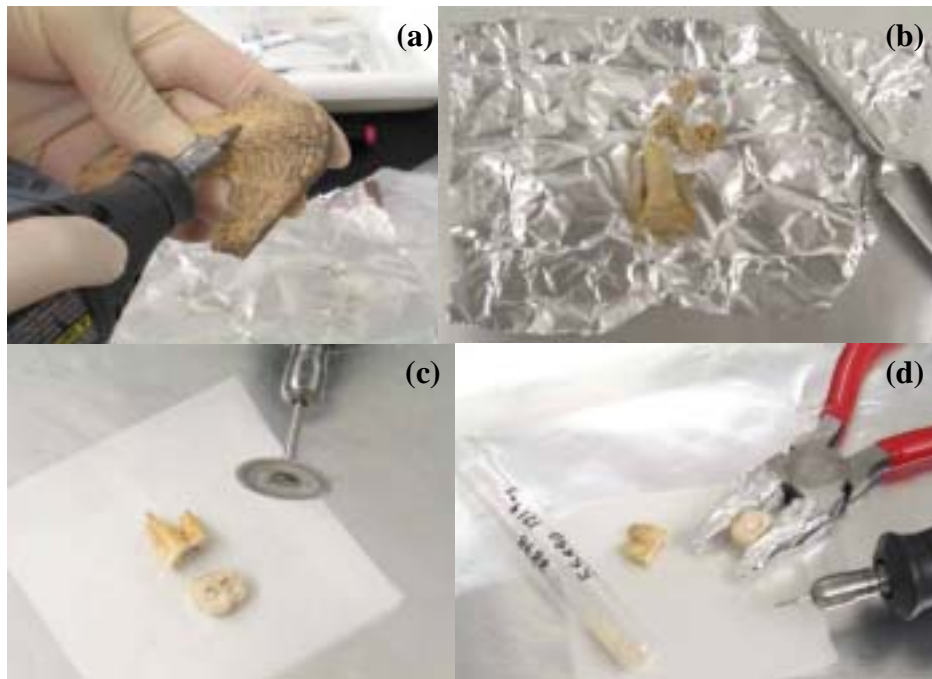

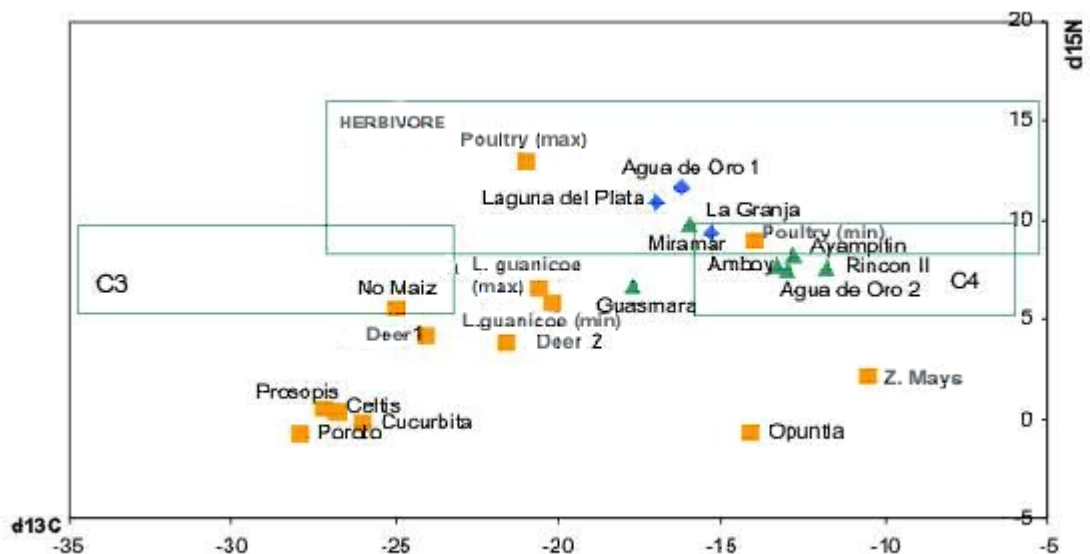Figure 7: ä[13]C and ä[15]N values from the samples analyzed and from food resources from the region studied.

Figure 8: Distribution of ä^{13}C values during Late Holocene.

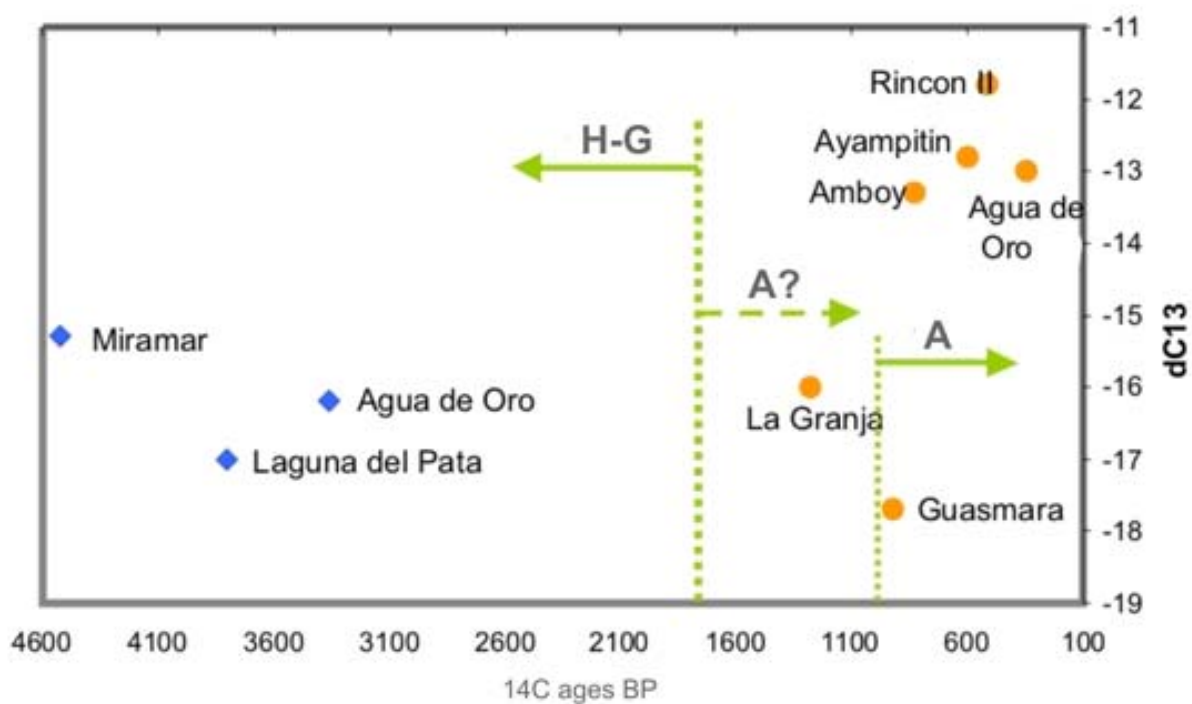Figure 9: Segments of the mastodon bone from Monte Verde, Chile.

Table 1:

UCIAMS #	Sample name	Archaeological Site #	Sample type	Hardness and smell	Initial weight (mg)	Gelatin coloration	δ15N (‰)	δ13C (‰)	C/N	14C age (BP)	±
22279	61B, Agua de Oro site ("I2,CB")	3	Tooth; lower second premolar	Dentine / no smell	160	pure white	11.6	-16.2	2.9	3360	20
22280	62B, Agua de Oro site, ("I1, CC")	9	bone; postcranial - fragment of scapula	Hard bone / smell	105	light tan	7.5	-13	2.7	345	20
22281	66B, Guasmara Site, ("I2")	5	bone; postcranial - Proximal epifisis left rib	Hard bone / smell	137	light tan	6.7	-17.7	2.7	920	20
22282	67, La Granja Site, ("I1")	4	bone; postcranial - right foot medial phalange	Hard bone / smell	100	light tan	9.8	-16	2.8	1280	20
22283	14B, Amboy Site, ("I2")	6	bone; postcranial	Avoid spongy area / no smell	158	light tan	7.7	-13.3	2.9	830	20
22284	72A, East Coast Site, (I1)	2	bone; cranial fragments –sphenoid	Avoid spongy area / no smell	152	tan	10.9	-17	2.8	3805	20
22285	1B, Rincon II Site (SI, I2607)	8	Tooth; lower right first premolar	Dentine / smell	63	pure white	7.6	-11.8	2.9	520	15
22286*	64B, Agua de Oro Site, ("I1, CD")	3	Tooth; upper right canine	Dentine / no smell	110	light tan	-	-	-	2980	30
22287	17B, Ayampitin Site, (I1)	7	Tooth; upper left second molar	Dentine / smell	122	light tan	8.2	-12.8	3	600	20
22288	56B, Miramar Site, (MIR5 ZS2)	1	Tooth; lower right second molar	Dentine/ smell	83	pure white	9.3	-15.3	2.8	4525	20
22289	Local dentist office	N/A	Modern Tooth	Dentine/ smell	63	pure white	10.7	-16.1	2.8	modern	-

*This sample did not fulfill the selected criteria for quality control (section 4.1.3.). The final 14C result should be treated with caution

Table 2: ^{14}C and ä^{13}C data on two segments of a single mastodon bone form Monte Verde.

Laboratory number[a]	Context	Fraction	δ^{15}N (‰)[b]	δ^{13}C (‰)[b]	C/N[b]	^{14}C (Age BP)
BETA-7824[c]	surface	Collagen[d]	not reported	not reported	not reported	6,550 ±160
TX-3760[c]	Area B. Column 4. Unit 9.	Collagen[d]	not reported	not reported	not reported	11,990 ± 260
UCR-4014/UCIAMS-2765[e]	surface	total amino acids	not reported	-25.5[f]	not reported[g]	12,510 ± 60
UCIAMS-10737[e]	surface	ultrafiltered gelatin	0.6	-22.5	2.8	12,450 ± 40
UCR-4015/UCIAMS-2766[e]	Area B. Column 4. Unit 9.	total amino acids	not reported	-25.7[f]	not reported[g]	12,450 ± 60
UCIAMS-10738[e]	Area B. Column 4. Unit 9.	ultrafiltered gelatin	0.5	-22.7	2.8	12,455 ± 40

[a] Laboratory measurement identifiers:

BETA - Beta Analytic, Inc., FL, USA

TX - Radiocarbon Laboratory, University of Texas at Austin, Austin, TX, USA

UCR – Department of Anthropology, University of California, Riverside, CA, USA

UCIAMS – KCCAMS Facility, University of California, Irvine, CA, USA

[b] ä^{13}C values continuous flow stable isotope ratio mass spectrometer (Finnigan Delta-Plus CFIRMS) that is interfaced with a Fisons NA1500NC elemental analyzer

[c] Previously published in Dillahay and Pino 1997.

[d] Characterized as "collagen" by Dillahay and Pino 1997.

[e] New ^{14}C determinations published in George et al. 2005.

[f] ä^{13}C values were obtained on total organics (demineralized/acid insoluble) bone fraction.

[g] Quality of samples were evaluated through profiles of the constituent amino acids of total hydrolysates and compared against standardized amounts of total amino acids obtained from a modern bone.

Acknowledgments

Footnotes

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