

CHANGES IN NEOLITHIC SUBSISTENCE PATTERNS ON FLORES,
INDONESIA INFERRED BY STABLE CARBON, NITROGEN, AND
OXYGEN ISOTOPE ANALYSES OF *SUS* FROM LIANG BUA

by

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ABSTRACT

Despite an abundance of archaeological material recovered from sites in Island Southeast Asia, the timing and route by which cultigens first arrived in Wallacea remains unclear. Many of the staple crops now grown on these islands were domesticated in mainland Asia, and were deliberately introduced by humans at an unknown point during the Holocene, through several possible routes. In this study, the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of subfossil bones and teeth attributed to *Sus celebensis* and *Sus scrofa* are analyzed. These materials, which span the last 5160 years at Liang Bua, Flores, Indonesia are used to determine if and when there was a shift towards agricultural intensification, and whether this intensification included the integration of domesticated C_4 crops. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the bone and dentin collagen samples indicate an abrupt shift towards enrichment in ^{13}C and depletion in ^{15}N at some time between 5160 and 2750 yBP. This hints at changes in human subsistence patterns that may have included the clearing of forests, and the integration of non-endemic C_4 cultigens such as foxtail millet (*Setaria italica*) onto the island. No statistically significant variation in the $\delta^{18}\text{O}$ values of the enamel carbonate samples over time is observed, suggesting that once they appeared on Flores, semi-domesticated pigs became an important part of the island ecosystem, and were bred and raised on Flores instead of being continuously imported from elsewhere.

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LIST OF MEDIA/ABBREVIATIONS/NOMENCLATURE/ACRONYMS

$\delta^{13}\text{C}$ – Delta ^{13}C ; the ratio of ^{12}C to ^{13}C

$\delta^{15}\text{N}$ – Delta ^{15}N ; the ratio of ^{14}N to ^{15}N

$\delta^{18}\text{O}$ – Delta ^{18}O ; the ratio of ^{16}O to ^{18}O

BPL – Below Present Levels

$\text{C}_2\text{H}_4\text{O}_2$ – Acetic Acid

H_3PO_4 – Phosphoric Acid

HCl – Hydrochloric Acid

ISEA – Island Southeast Asia

kyBP – Thousand Years Before Present

mTorr – millitorr; 1 millitorr = 0.133 pascals or 1.315^{-6} ATM

myBP – Million Years Before Present

NaOCl – Sodium Hypochlorite

NaOH – Sodium Hydroxide

VPDB – Vienna Pee Dee Belemnite

VSMOW – Vienna Standard Mean Ocean Water

yBP – Years Before Present

CHAPTER 1: INTRODUCTION

Problems in the Study of Past Human Activity in Island Southeast Asia

Initial Modern Human Migrations

Evidence of prehistoric human activity in the islands of Southeast Asia is rich, but its pattern, mode, and tempo resist simple archaeological narrative. Available genetic and archaeological material suggests that the first successful colonization of Southeast Asia was completed by a modern human population who first left Africa around 85 kyBP.¹ This population, according to the widely accepted “southern arc route” hypothesis, continued to spread east along the coast of the Indian Ocean, reaching the Indian subcontinent by approximately 66 kyBP (Lahr and Foley 1994; Stringer 2000; Balme et al. 2009; Rasmussen et al. 2011). From the available archaeological and genetic evidence, it appears that this population then began dispersing south into Island Southeast Asia, and arriving in Australia around 50 kyBP (Forster and Matsumura 2005; Rasmussen et al. *ibid.*). Around 32 kyBP, a second population of humans from Africa began dispersing along a similar route, but diverged, heading both north into China, and south into Island Southeast Asia (Rasmussen et al. *ibid.*). During the Last Glacial Maximum (LGM), sea levels would have been low enough that these islands, along with the Malay Peninsula and mainland Asia, would have been connected by land bridges (Figure 1). However, the dates from available archaeological material are frustratingly piecemeal, and as O’Connor (2007:523) notes, some occupation sites in Australia are

¹ There is also archaeological evidence that populations of early modern humans migrated out of Africa and started moving east around 125 kyBP. However, it appears that they spread no farther than the Levant, and disappeared by ~92 kyBP. This event is often described as a “failed migration” into Asia (Shea 2008; Dennell and Petraglia 2012; Oppenheimer 2012).

“significantly older than the oldest sites from any of the potential stepping stone islands en route”. Using U-series analysis, human skeletal remains from the Wajak site on Java have been dated to between 37 kyBP and 28 kyBP (Storm et al. 2013). The “Deep Skull”, which was excavated from the Niah Cave site on Borneo, has been reliably dated to between 45 and 39 kyBP (Barker 2007). Modern humans appear in the archaeological record on the island of Flores at 11 kyBP (Morwood et al. 2009; van den Bergh et al. 2009). However, modern human remains recovered from Timor, far east of Java and Borneo, date to approximately 42 kyBP, and burials at Lake Mungo, Australia have been dated to between 50 and 46 kyBP (Bowler et al. 2003; O’Connor 2007).

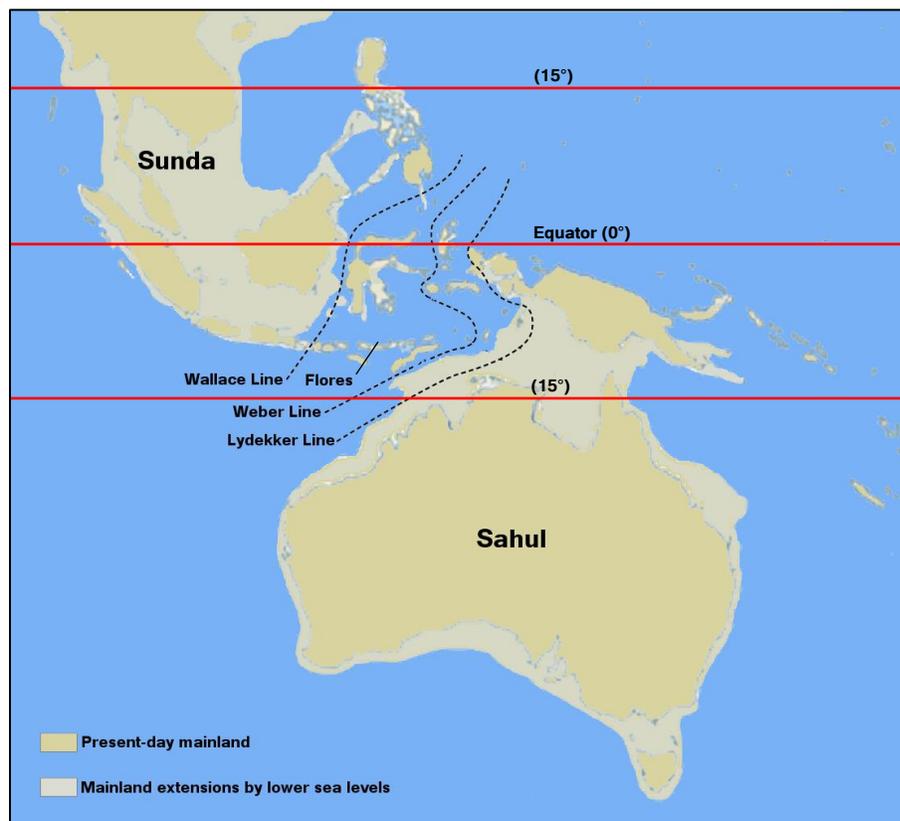


Figure 1. Map of Sunda and Sahul shelves with Wallace, Weber, Lydekker Lines, and lines of latitude shown (adapted from Maximillian Dorrbecker 2007).

The challenge of understanding early human movement into Island Southeast Asia (ISEA) is due in part to climatic changes during the Pleistocene. At different points during this epoch, sea levels were as low as 120 m below present levels (BPL), and Voris (2000) estimates that for 50% of the past 17,000 years, sea levels were at or below 30 m BPL. Therefore, many of the routes that early colonizers may have taken are now submerged beneath the waters of the Indian and Pacific oceans. As sea levels rose during the late Pleistocene and early Holocene, the lowlands slowly disappeared into the ocean, creating the 17,000+ islands now present in Indonesia alone. Further, the entire region rests on or near the boundaries of the Eurasian, Pacific, and Indo-Australian continental plates, making it geologically active and prone to rapid geographical remodeling (Birdsell 1977).

Independent of these changes, parts of Island Southeast Asia have always been isolated from the mainland by deep oceanic channels running between the periphery of the Eurasian continental plate, called the Sunda shelf, and the periphery of the Indo-Australian plate, called the Sahul shelf. The boundary between these peripheries, referred to as the Wallace Line in honor of the naturalist A.R. Wallace, marks a significant geographical border between the Asian mainland and the rest of Island Southeast Asia. West of the Wallace line, flora and fauna would have been able to move independently between the islands and mainland Asia during periods of low water levels. Fittingly, many of the plants and animals found in these islands derive from

relatively recent Eurasian clades. A similar biogeographical frontier exists off the west coast of the Australian continent. This boundary, referred to as the Lydekker Line in honor of the English naturalist Richard Lydekker, signifies the limits of the Australian shelf. Similar to the situation in the west, periods of low water levels would have allowed flora and fauna east of the Lydekker Line to move between islands and Australia. The area between these two lines, referred to as Wallacea, constitutes an important biogeographical transition area between the Asian continent and the South Pacific. The islands within Wallacea are isolated from the rest of Island Southeast Asia by no less than 60 km of open water, and even during periods of relatively low sea levels, would have remained inaccessible to humans and large animals without the use of seafaring technology (refer back to Figure 1). Despite these barriers, the islands in Wallacea have hosted a diverse mixture of flora and fauna derived from both Eurasian and Australian clades. The extent of this mixing is marked by Weber's Line, named in honor of the German zoologist Max Wilhelm Carl Weber.

Neolithic Migrations and the Spread of Agriculture

The factors encountered by researchers investigating the initial dispersal of humans into Island Southeast Asia also come into consideration when addressing later events, such as the spread of domesticated crops. In many parts of Melanesia and Island Southeast Asia, human populations were able to develop horticulture on a small-scale by domesticating indigenous plants. In Melanesia, this included the winged or "purple" yam (*Dioscorea alata*) and sugarcane (*Saccharum*), the latter of which is a C₄ plant (Harlan 1992; Daniels 1993). Some researchers have theorized that sugarcane,

along with yams, could have been carried west from Melanesia to the islands of Southeast Asia and Wallacea (Lebot 1999; Bird et al. 2004; Anderson 2011). However, there is very little indication that either of these crops was imported into Wallacea during the Neolithic (Denham 2004). The archaeological evidence instead suggests that the only plants that were domesticated or semi-domesticated within Wallacea before the introduction of those from mainland Asia were banana (*Musa sapientum*) (though it may have been introduced from New Guinea), elephant ear (*Alocasia macrorrhiza*), taro (*Colocasia esculenta*), and Kudzu (*Pueraria lobata Ohwi*), all of which are C₃ plants (Harlan 1992; Denham and Donohue 2009). At some point during the Neolithic, plants that were domesticated in mainland Asia such as millets, Chinese sugarcane (*Saccharum sinense*), and rice (*Oryza sativa*) started being introduced into Island Southeast Asia. However, the timing, route, and extent of these events are uncertain, especially in the isolated islands of Wallacea. Until very recently, there was little focus on this question, and even less evidence with which to approach it. However, in 2001 Dr. Michael Morwood and colleagues launched the *Astride the Wallace Line* project with funding from the Australian National Research Council (Morwood et al. 2009). This project sought to answer six specific questions about the deep human history of Island Southeast Asia and, including the question of “when and why...people start[ed] cultivating plants and domesticating animals” (Morwood et al. 2009: 437).

Research Objectives

This aim of this research project is to contribute directly to the resolution of the question of plant cultivation in Wallacea by analyzing the stable isotope ratios of bone

and teeth belonging to pigs (*Sus*) excavated from Liang Bua, Flores, Indonesia. To that end, this analysis involves four research questions:

1. Is there a relationship between stratigraphic depth and the preservation of *Sus* material at Liang Bua?
2. How do the isotopic values of the pig remains change over geological time, if at all?
3. What do the isotopic data reveal about the diets of domesticated pigs and the subsistence patterns of humans living on Flores during the last 5160 yBP?
4. What do the data suggest about changes in subsistence patterns in Island Southeast Asia in general and Wallacea in particular, during the Neolithic?

Stable isotope analysis of collagen from bone and dentin samples has the potential to answer these questions. Additionally, if the pattern of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the pig bone collagen samples concurs with that observed in the rat bone collagen samples, the findings of Anderson (2011) will be reinforced. If a shift is noted at an earlier point in time, or if no shift in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is observed, it may suggest that non-endemic plants reached Flores even earlier than Anderson (2011) suggests, or that rats and pigs were not consuming the same types of food. The $\delta^{18}\text{O}$ values of the enamel carbonate samples may also reveal information about the movement of the pigs during their lifetimes.

The remainder of the thesis is organized as follows. Chapter 2 reviews the considerable body of archaeological research on the ecology of Flores, and the fundamentals of stable isotope analysis and mass spectrometry. Chapter 3 introduces

the materials and methods used in this study. Chapter 4 presents the results of the isotopic analyses. Chapter 5 discusses the results within the larger archaeological and ecological context of mainland and Island Southeast Asia. Chapter 6 synthesizes the information presented in the previous chapters in a brief conclusion.

CHAPTER 2: LITERATURE REVIEW

Flores and Liang Bua

One of the sites identified for inclusion in the *Astride the Wallace Line* project is Liang Bua, a cave that is located on the island of Flores (Morwood et al. 2009). Flores is located along the eastern stretch of the Indonesian island chain, called the Lesser Sunda Islands, and lies within the area of isolation marked by the Wallace and Lydekker Lines (Figure 2). The Indonesian islands are a direct product of tectonic activity along the underlying Banda Volcanic Arc, which itself is a result of the convergence of the Eurasian and Indian continental plates (Moss and Wilson 1998; Westaway et al. 2009). This process, which began during the Cenozoic Era (65 myBP) and continues into the present, causes the slow uplift of the reefal limestone seabed, which, in concert with volcanic activity, has created the entire island chain (Moss and Wilson *ibid.*; Westaway et al. *ibid.*). Liang Bua is located in the Manggarai Regency of Western Flores (Morwood et al. 2009). The cave itself was not exposed until approximately 200 kyBP and was uninhabitable to animals until ~190 kyBP (Westaway et al. 2009). Excavations at Liang Bua suggest, however, that the cave remained unoccupied until ~95 kyBP. From this time onwards, Liang Bua provided shelter for a wide range of animal species including two species of hominins: *Homo floresiensis*, from ~ 95 kyBP to ~17 kyBP, and anatomically modern *Homo sapiens* from ~11 kyBP to present (Morwood et al. 2005; van den Bergh et al. 2009). In many of the faunal remains recovered from Liang Bua, there is evidence of both gigantism and dwarfism, which emphasizes the relative isolation of Flores (van den Bergh et al. 2009).

Suids (pigs or boar) are not apparent in the stratigraphic record at Liang Bua until well into the Holocene.² The Sulawesi warty pig (*Sus celebensis*) appears to have been introduced from Sulawesi around ~7000 yBP (Larson et al. 2007). *Sus scrofa*, the domesticated Eurasian pig, appears at ~4000 yBP, possibly as part of a large migration out of either mainland China (via Taiwan) or the Malay Peninsula (Larson et al. 2007; van den Bergh et al. 2009). Both species are represented in the stratigraphic sequence at Liang Bua, but it is not clear whether humans on Flores treated *S. celebensis* in the same manner as *S. scrofa* (discussed below) (van den Bergh et al. 2009). The arrival of *S. scrofa* on Flores around 4000 yBP is significant because this coincides with the appearance of other non-indigenous faunal species, as well as pottery on the island (Morwood et al. 2009; van den Bergh et al. 2009). This pattern appears in the archaeological record from other parts of Island Southeast Asia around the same time, suggesting that there was a single large migration of humans, plants, and animals from mainland Asia around 4000 kyBP (Storm 1995). Based on the archaeological evidence from other parts of the world, Bellwood (1996) suggests that this process probably occurred as a colonization event, where agriculturalists coming from the Asian mainland displaced or assimilated semi-sedentary foragers or hunter-gatherers. Still, the details of this event, especially on Flores, are relatively scarce (Hutterer 1983; Krigbaum 2003).

² In the literature, the species of *Sus* found on Flores are referred to interchangeably as “wild boars” and “wild pigs”. However, by the time they were brought to Flores, both species were likely semi-domesticated. For this reason, they will be referred to as “pigs” in this paper.



Figure 2. Location of Flores in Indonesian Archipelago (adapted from Google Earth 2013).

A major assumption of this research project is that plant material possibly being cultivated and consumed by humans living on Flores was also being consumed by pigs. Though there is no direct evidence on Flores to indicate that this was the case, the archaeological record at other sites in mainland and Island Southeast Asia suggests that pigs, especially *S. Scrofa*, were an important part of the domestication package and ecology of humans (Bellwood 1987; Bellwood 2001; Barton et al. 2009; Lu et al. 2009). At many Neolithic sites in China, pigs were bred and kept, and were sustained, at least in part, by many of the same dietary resources as humans. In Island Southeast Asia, the relationship between humans and pigs may have been less intense. For instance, MacDonald (1993) surmises that on islands such as Sulawesi, indigenous pigs such as *S. celebensis* were probably left to graze freely, and were hunted when necessary,

though no direct archaeological evidence of this pattern exists. Due to their fragmentary nature, as well as general overlap in skeletal morphology, it cannot be determined whether the sample materials used here are representative of domesticated *S. scrofa*, or the indigenous Sulawesi warty pig, *S. celebensis*. If the diets of the two species varied based on differences in their relationship to humans, and the material used in this study originates from members of both, then differences in isotopic signatures could be attributed to a mixed sample of different pigs with different diets, instead of large-scale shifts in subsistence. However, Albarella et al. (2006: 211) argue that an important criterion for inferring the presence of domesticated animals is “the appearance of a species outside its natural range, or in locations where it is unlikely to have reached without the intervention of humans (*e.g., on remote islands*)” [emphasis mine]. Based on the relative isolation of Flores, even *S. celebensis* would have experienced some degree of intervention by humans before reaching the island. Their arrival around 7000 yBP was far too late to have traveled independently across a land bridge. MacDonald (1993) also notes that *S. celebensis* was probably semi-domesticated before being introduced to other islands, and may have hybridized with *S. scrofa* after their appearance in Island Southeast Asia. It is likely then, that both species of *Sus* represented on Flores were semi-domesticated, and were treated similarly by humans on the island. In this case, even if the sample material were indeed a mix of both pig species, the diets of both species should have been reasonably similar.

Stable Isotope Analysis

A promising approach for resolving questions about diet and migration is stable isotope analysis. Isotopes are atoms of a chemical element that have the same number of protons in their nucleus as all other atoms of the same element, but different numbers of neutrons, which gives them different nucleonic and mass numbers. These numbers are calculated by taking the sum of the protons and neutrons in the nucleus. All carbon atoms, for instance, have six protons in their nucleus, but a carbon atom with six neutrons in its nucleus has a mass number of 12, and is known as ^{12}C , while a carbon atom with seven neutrons in its nucleus has a mass number of 13, and is known as ^{13}C . Because of the additional neutron, ^{13}C is atomically more massive than ^{12}C . However, both of these isotopes of carbon are “stable” (nonradioactive) because the particular arrangement of protons and neutrons in their nuclei does not decay over time. In stable isotope analysis, the ratio of different stable isotopes in a given sample is the subject of interest. However, because this ratio is incredibly small, it is more convenient to measure the difference in the sample material against the difference in an international standard for which the isotopic ratio is known. This is done using Equation 1:

$$\delta = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] * 1000\text{‰}$$

(1)

Where “R” is the ratio of one stable isotope to another. The ratios are known as delta values (δ), and because they are so small, are reported as per mil (‰) values instead of percentage values.

$\delta^{13}\text{C}$ Analysis

Many plants, such as wheat, rice, legumes, tubers, fruits, and nuts preferentially absorb the lighter isotope of carbon, ^{12}C , during photosynthesis (Wickman 1952). This is due to the fact that these plants utilize the enzyme *ribulose biphosphate carboxylase* during photosynthesis to take in (fix) atmospheric CO_2 and are better able to do this with atomically lighter ^{12}C (Larsen 1997). The product of this fixation is a compound with 3 carbon atoms, and for this reason these plants are referred to as C_3 plants. Other plants including maize, teosinte, amaranth, sugarcane, sorghum, and some millets yield consistently higher ratios of the heavier carbon isotope ^{13}C (Bender 1968). Plants that take in a higher ratio of ^{13}C relative to ^{12}C do so because they evolved the ability to use the enzyme *phosphoenol pyruvate carboxylase* to fix atmospheric CO_2 around 7 myBP (Cerling et al. 1993). The product of fixation with this enzyme is a compound with four carbon atoms, and these plants are therefore referred to as C_4 plants.

International standards used in stable isotope analysis are determined by the International Union of Pure and Applied Chemistry (IUPAC). Prior to 1995, the standard for atmospheric carbon was calcium carbonate in *Belemnitella americana* fossil limestone from the Pee Dee Formation in South Carolina (DeNiro 1987). After 1995, the standard was changed to the $\delta^{13}\text{C}$ value of NBS 19 calcite, set at +1.95‰ (Bulletin of Volcanology. 1995). However, because of its widespread use, the VPDB standard will be used to report samples in this study. Most organisms, as well as their dietary resources, are less enriched in ^{13}C than the VPDB standard, and so their $\delta^{13}\text{C}$ values are usually negative. The $\delta^{13}\text{C}$ values of C_3 plants range between -33‰ and -22‰, and

average about -27‰ at present. Prior to the Industrial Revolution, the atmospheric carbon average was -26‰ (DeNiro 1987). However, due to the widespread combustion of fossil carbon as fuel, the atmospheric carbon average at present is -27‰. The $\delta^{13}\text{C}$ values of C_4 plants typically range between -16‰ and -9‰ and average about -12.5‰, with a pre-industrial average of -11.5‰. The $\delta^{13}\text{C}$ value for a given sample is calculated using Equation 2:

$$\delta^{13}\text{C}_{(PDB)} = \left[\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{Sample}}}{{}^{13}\text{C}/{}^{12}\text{C}_{PDB}} - 1 \right] \times 1000\text{‰}$$

(2)

That variation in the $\delta^{13}\text{C}$ values of plants could be used in archaeological research was first realized by Vogel and van der Merwe (1977). The authors, using human ribs from pre-horticultural and horticultural archaeological sites in New York, demonstrated that the ratio of ^{12}C to ^{13}C present in the bone collagen of inhabitants from the horticultural sites were enriched in ^{13}C relative to the bones of individuals from pre-horticultural sites. However, at each level of the food chain, isotopic values increase slightly. This is known as the trophic level effect (see Results section for discussion). The $\delta^{13}\text{C}$ values of humans, for example, are about 5‰-enriched relative the dietary (trophic) level below them.

$\delta^{15}\text{N}$ Analysis

The international standard for stable nitrogen is AIR (atmospheric N_2) which is 0‰. Because they utilize nitrogen from the atmosphere, leguminous plants have $\delta^{15}\text{N}$

values that are close to the AIR standard (0‰). Non-leguminous plants have higher $\delta^{15}\text{N}$ values that average approximately +6.5‰. Like carbon, $\delta^{15}\text{N}$ values increase per trophic level, by approximately +3‰. The $\delta^{15}\text{N}$ value for a given sample is calculated by using Equation 3:

$$\delta^{15}\text{N}_{(AIR)} = \left[\frac{{}^{15}\text{N}/{}^{14}\text{N}_{Sample}}{{}^{15}\text{N}/{}^{14}\text{N}_{AIR}} - 1 \right] \times 1000\text{‰}$$

(3)

In collagen, nitrogen isotope ratios are directly related to the isotopic composition of dietary protein, and are therefore useful for determining the trophic level, as well as the diet, of a given organism (Pate 1994). In most mammals, there is an increase of between 2‰ and 3‰ in $\delta^{15}\text{N}$ values per trophic level. Because many legumes are C_3 plants, analyzing $\delta^{15}\text{N}$ values alongside $\delta^{13}\text{C}$ ratios is helpful in determining which types of plants may have been consumed, and whether diagenetic processes have affected the carbon ratios of the sample. The theoretical concepts behind $\delta^{15}\text{N}$ analysis are similar to those of $\delta^{13}\text{C}$ analysis and divide plants into two categories: legumes and non-legumes. However, this division is not based upon differences in chemical pathways, as in carbon fixation, but on the source from which nitrogen is taken in. Legumes take in both nitrogen gas (N_2) from the atmosphere and nitrates from soil, while non-legumes cannot fix atmospheric N_2 , and only take in nitrogen from the soil (Virginia and Delwiche 1982; DeNiro 1987).

$\delta^{18}\text{O}$ Analysis

Like carbon and nitrogen, there are different stable isotopes of oxygen, ^{16}O , ^{17}O , and ^{18}O that enter the body of organisms through respiration, food, and liquids.³ As part of regular metabolism, oxygen binds with a number of chemicals to form essential compounds within the body. One such compound, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), is formed by blood bicarbonate, and comprises the largely inorganic portion of teeth (including enamel) and bones. Because tooth enamel is a “static” tissue, meaning that it is not remodeled by the body after formation, the $\delta^{18}\text{O}$ values of the carbonate incorporated during tooth crown formation are permanently recorded in the enamel (Longinelli 1984; Luz 1984; Dupras and Tocheri 2007). Therefore, the ratio of $\delta^{16}\text{O}$ to $\delta^{18}\text{O}$ in tooth enamel carbonate is a direct reflection of the $\delta^{18}\text{O}$ values of water taken in during the organism’s life. Since the specific chemical composition of water varies by geographical location, $\delta^{18}\text{O}$ values in sample material can be compared to $\delta^{18}\text{O}$ values in water samples collected from different geographical locations to determine where the organism was getting its water during the formation of its adult dentition. $\delta^{18}\text{O}$ values are measured against the ratio of ^{18}O to ^{16}O in Vienna Standard Mean Ocean Water (VSMOW) or Vienna Pee Dee Belemnite (VPDB). In VSMOW, the $\delta^{18}\text{O}$ value for a given sample is calculated by using Equation 4:

$$\delta^{18}\text{O}_{(VSMOW)} = \left[\frac{^{18}\text{O}/^{16}\text{O}_{\text{Sample}}}{^{18}\text{O}/^{16}\text{O}_{VSMOW}} - 1 \right] \times 1000\text{‰}$$

(4)

³ Because the natural abundance of ^{17}O is intermediate between that of the other two stable oxygen isotopes, it is not used in calculating oxygen isotope ratios.

Mass Spectrometry

Determining the ratio of stable carbon and nitrogen isotopes in a given sample involves analysis with a mass spectrometer, and unlike those used in standard chemical applications, requires samples to be in a gaseous state (CO₂ or N₂, respectively). Therefore, gas source isotope ratio mass spectrometers (GS-IRMS) must be used. The theory behind GS-IRMS analysis is relatively straightforward: prepared samples of collagen encapsulated in 5x3.5mm tin capsules are loaded directly into the mass spectrometer unit, which is essentially a curved, partially magnetized vacuum tube, and heated until they vaporize. Combusted along with the samples are chemical standards for which the isotope ratios are known. Combusting standards at different points during the spectrometer run allows the technician to determine if the unit is functioning correctly, and whether the isotope ratios of the samples are being reliably measured. After vaporization, the sample material is pulled by vacuum towards an ionizing filament. The filament positively charges (ionizes) the particles of the now-gaseous sample, which are then focused into a beam and pulled down the flight tube and through the curved magnetic field. As the ionized gas passes the magnet, “the magnet deflects the lighter molecules in the beam more than the heavier ones, splitting the beam into a spectrum of minor beams of ions of different mass to charge ratios”, a process analogous to the dispersion of visible light when passed through a prism. (Ambrose 1993: 68). The two beams strike electronic collectors called Faraday Cups at the end of the flight tube that record the abundance of each isotope in the sample, and the isotopic ratio is calculated. A gas analyzer interfaced with the mass spectrometer

calculates the ratio of carbon to nitrogen (C:N) in the sample. DeNiro (1985) found that samples of fresh bone collagen that have not been altered by diagenetic processes have atomic C:N ratios between 2.9 and 3.6. Collagen samples with atomic C:N ratios falling within this range are therefore considered to have isotopic values appropriate for analysis because the taphonomic environment did not significantly alter the isotopic composition of the collagen. The gas analyzer also determines the weight percentage of carbon and nitrogen (wt% C and wt% N) in the sample, which is also used to gauge its preservation. Like C:N ratios, the acceptable range of wt% C and wt% N values comes from those in fresh bone collagen. Acceptable carbon concentrations range from 15.3 to 47 percent, and nitrogen concentrations range from 5.5 to 17.3 percent (Ambrose 1990).

Unlike collagen obtained from bone and dentin, carbonate requires further processing immediately prior to mass spectrometer analysis. Following McCrea (1950) and Biasatti (2009), the samples of processed enamel carbonate are placed in glass reaction vessels, and loaded into a gas preparation and introduction interface, which is directly connected with the mass spectrometer. Once inside the gas interface, the samples are flushed with pure helium for five minutes, and injected with 100% orthophosphoric acid (H_3PO_4) for approximately 72 hours. This process converts the samples into CO_2 . The gaseous samples are then mechanically transferred to the mass spectrometer for isotopic analysis.

Stable isotope analysis is useful for understanding changes in dietary composition over time in organisms at one location, and comparing the dietary

composition of organisms at different locations. However, stable isotope analysis only provides information about the general makeup of the diet of the organisms under study, and does not directly relay information about the specific food resources that comprise diet. Therefore stable isotope analysis is most powerful when employed alongside traditional archaeological analysis and interpretation. Possible material limitations at Liang Bua include poor sample preservation in the form of collagen loss such as depolymerization via hydrolysis of peptide bonds, and diagenetic processes leading to exogenous carbon, nitrogen, and carbonate in the samples. Bone and dental collagen usually have diagenetic turnover rates of thousands and sometimes tens of thousands of years. However, environmental effects such as increased moisture in the soil matrix can speed up the degradation of collagen. In the context of Flores, the mineral-rich riverbed environment inside Liang Bua may have led to destruction of collagen and increased mineralization of the samples, as well as abnormal levels of exogenous carbonate in the material.

CHAPTER 3: MATERIALS AND METHODS

Materials

Throughout the past 900 kyBP, the nearby Wae Racang River has shifted back and forth across the local floodplain, depositing large quantities of silt into Liang Bua when seasonal rains cause it to swell (Westaway et al. 2009). The result is a complex series of uneven and often amalgamated stratigraphic layers in the cave (Figure 3).

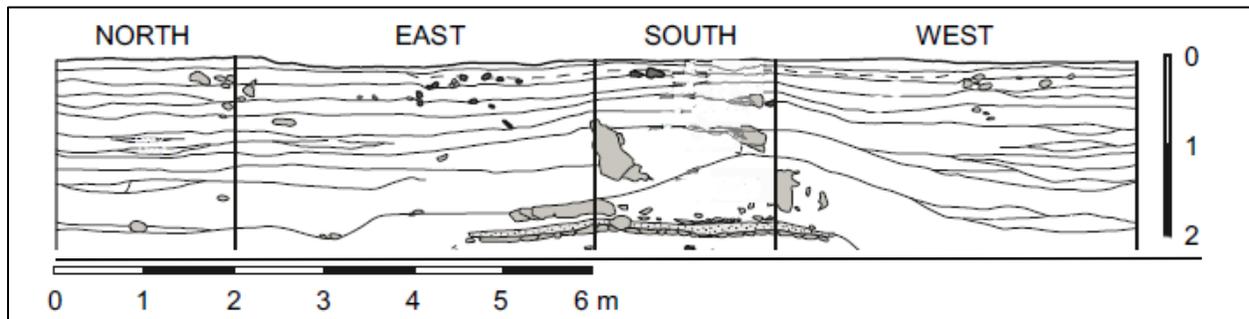


Figure 3. Stratigraphic section at Liang Bua (adapted from Roberts et al. 2009).⁴

Excavations at Liang Bua are carried out in 10-centimeter layers referred to as “spits” that follow the natural stratigraphy of the cave. All samples used in this study originate from spits 3 through 14 (30 cm to 140 cm) in sector XVI of the cave. Archaeologists from the Indonesian National Research Centre for Archaeology (ARKENAS) excavated this material over a period of eight days between August 5 and August 13, 2008. All seventeen samples are attributed to the genus *Sus*. These include nine bones (some fragmentary), and eight teeth (Table 1). None of the sample material is fossilized.

⁴ A stratigraphic section is not currently available for sector XVI. The section shown in this figure is adapted from sector VII and XI, which are adjacent to sector XVI, and is meant to demonstrate the general stratigraphic conditions near sector XVI.

Table 1. Sample identification, spit location, depth, and bone type.

Sample ID	Spit	Depth (cm)	Median Geological Age (yBP)	Element
LB-1A	3	30	140	Second lower incisor
LB-1B	3	30	140	Fragmentary long bone
LB-2A	5	50	510	Lower M3
LB-2B	5	50	510	Phalanx
LB-3A	8	80	2750	Lower M3
LB-3B	8	80	2750	Second lower incisor
LB-4A	9	90	2750	Second lower incisor
LB-4B	9	90	2750	Radius fragment
LB-5A	10	100		Second lower incisor
LB-5B	10	100		Intermediate phalanx
LB-6A	11	110		Lower M3
LB-6B	11	110		Metatarsal fragment
LB-7A	13	130		Calcaneus fragment
LB-8A	14	140	5160	Molar
LB-8B	14	140	5160	Premolar
LB-8C	14	140	5160	Distal humerus
LB-8D	14	140	5160	Radius fragment

During the time period from which these samples originate, only two species of *Sus* were likely present on Flores (van den Bergh et al. 2009). The earlier of the two is *Sus celebensis*, the Sulawesi warty pig, which was brought to the island around 7000 yBP (van den Bergh et al. *ibid.*). *Sus scrofa*, the Eurasian pig appears around 4000 yBP (Larson et al. 2005; Larson et al. 2007; Larson 2009; van den Bergh et al. 2009). This means that sample material from the two deepest spits of sector XVI (140 and 130 cm) likely represent *S. celebensis*, because *S. scrofa* was not yet on the island during

this time. However, sample material from any spit above these could be from either one of the two species. Though both species are genetically distinct from one another, they are morphologically similar, and the fact that neither species would likely have been able to reach Flores without human intervention suggests that both species were brought to the island as semi-domesticated herd animals. This means that their interactions with humans and consequently, their diets, should have been relatively similar.

Methods

Bone Collagen

This study uses bone collagen processing methods derived from Longin (1971), Nelson (1969), and Brown et al. (1988), but modified by the Laboratory for Stable Isotope Science (LSIS) at the University of Western Ontario, Canada. Bone samples were processed in the following manner: (1) the entire bone was rinsed in distilled water, and an ultrasonicator, toothbrush, and dental pick were used to remove soil, roots, and other foreign material. (2) After being dried for 24 hours at 90° C, each sample was broken into 2 to 5 mm pieces using an agate mortar and pestle, and was (3) placed in a 2:1 chloroform methanol mixture to remove lipids. This process was repeated two more times. (4) After being rinsed with distilled water, the sample was placed in 15 mL of 0.5 M HCl until all mineral content was dissolved and a collagenous isomorph was obtained. (5) The sample was then rinsed with distilled water and placed in 15 ml of 0.10 M NaOH until humic and fulvic acids were removed. (6) The sample

was then rinsed with distilled water a minimum of six times, and placed in 15 mL of 0.25 M HCl to remove any other trace material and to bring the pH of the sample back towards neutrality. (7) The HCl was removed, and the sample was placed in 5 mL distilled water and put in an oven at 90° C for ~16 hours to gelatinize. (8) Finally, the gelatinized mixture was pipetted into a 15mL glass dram vial and placed in the oven to dry at 90° C for 24 hours. After gelatinization and drying, the percentage of collagen present in the bone, called the percentage yield of collagen (% collagen), was calculated. In conjunction with C:N ratios and atomic C and N percentages, the percentage yield of collagen is an important factor of determining whether collagen has been preserved in the sample (See Results section for more details). After the collagen extraction process was complete, between 0.60 and 0.69 micrograms (μg) of each dried collagen sample was powdered and placed in a 3x3.5mm tin capsule. As an additional measure of validity, duplicate capsules were prepared from the two samples in the series that produced the most collagen. When these duplicates are run through the mass spectrometer, they can be expected to produce data that are very similar to their original counterparts. If this does not occur, it may indicate that there are issues with the sample or the mass spectrometer. In the case of the Liang Bua bone collagen samples, duplicates were created for LB-1B and LB-2B. These duplicates are reported in this paper as LB-1A (DUP) and LB-2B (DUP). The samples were then sent to the Smithsonian Stable Isotope Mass Spectrometry Laboratory for analysis using a Thermo Scientific Delta V Advantage mass spectrometer coupled with a Costech ECS 4010

elemental analyzer via a ConFlo IV gas interface. The mass spectrometer returned data on C:N ratios, wt% C, wt% N values, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each sample.

Issues Encountered During Bone Collagen Processing

The principal segment of the collagen-processing phase is the demineralization of the sample. In bone from relatively recent archaeological contexts, this process generally takes 2 to 3 weeks with daily washes of HCl (Williams, personal communication, 2012). The demineralization segment of the Liang Bua bone samples took approximately 9 weeks to complete. Near the end of this phase, fine whitish silt was observed in the bottom of the centrifuge tube containing sample LB-7A. It was surmised that this material could be related to the conditions of the sample's immediate depositional contexts, such as tuffaceous silt derived from volcanic activity in the area. Interestingly, Anderson (2011) also reported similar diagenetic issues from rat bone samples in this spit. To accommodate this issue, as much unadulterated bone as possible was removed and placed in a new centrifuge tube, splitting LB-7A into samples LB-7A-1 and LB-7A-2. Later, additional bone material was removed from LB-7A-1, further splitting this subsample into LB-7A-1A and LB-7A-1B (See Appendix B for full descriptions).

Dentin Collagen

Dental collagen processing methods are derived in part from Wright and Schwarcz (1999), and involve a processing technique similar to that used for bone samples. Dentin samples were processed in the following manner: (1) the entire tooth

was rinsed in distilled water, and an ultrasonicator, toothbrush, and dental pick were used to remove soil, roots, and other foreign material. (2) After being dried for 24 hours at 90° C, the dentin was separated from the enamel using a Plattner's steel mortar and pestle, dental pick, and tweezers.⁵ (3) The dentin sample was then washed in 2 mL of 2% HCl daily until any trace inorganics were dissolved and a collagenous isomorph was obtained. (5) The sample was rinsed in distilled water and placed in 15 ml of 0.10 M NaOH until any humic and fulvic acids had been removed, (6) was rinsed in distilled water again and placed in an oven at 90° C for 16 hours to gelatinize. (8) Finally, the gelatinized mixture was pipetted into a 15mL glass dram vial to dry in the oven at 60° C for 24 hours. After gelatinization and drying, the percentage of collagen present in the dentin sample, called the percentage yield of collagen (% collagen), was calculated. In conjunction with C:N ratios and atomic C and N percentages, the percentage yield of collagen is an important factor of determining whether collagen has been preserved in the sample (See Results section for more details). Once processed, between 0.60 and 0.69 micrograms (μg) of each dried gelatinized collagen sample was powdered and placed in a 3x3.5mm tin capsule, and duplicates from the two most abundant collagen samples (LB-3A and LB-8B) were created. The samples were then sent to the Smithsonian Stable Isotope Mass Spectrometry Laboratory for analysis using a Thermo Scientific Delta V Advantage mass spectrometer coupled with a Costech ECS 4010 elemental analyzer via a Conflo IV gas interface. The mass spectrometer returned data on C:N ratios, wt% C, wt% N values, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each sample.

⁵ For this study, dentin and cementum were not separated. However, the inorganic phase of the cementum is dissolved in HCl.

Issues Encountered During Dentin Collagen Processing

Like the bone from Liang Bua, the dentin samples took around 9 weeks to demineralize. To accelerate the demineralization process, samples LB-1A, LB-2A, LB-3B, LB-4B and LB-6A were divided into multiple subsamples (See Appendix D for full descriptions).

Enamel Carbonate

Compared to its counterpart in bone, carbonate derived from enamel apatite is generally resistant to diagenetic processes, and is considered to produce reliable carbon and oxygen isotopic data (Lee-Thorp and van der Merwe 1987; Blondel et al. 1997). The processing of enamel carbonate was based off Garvie-Lok et al. (2004), Koch et al. (1997), Lee-Thorp et al. (1987), and Lee-Thorp et al. (1989), in was completed in the following manner: (1) the entire tooth was cleaned using an ultrasonicator, toothbrush, and distilled water. (2) After being dried for 24 hours at 90° C, the enamel was separated from the dentin using a Plattner's steel mortar and pestle, dental pick, and tweezers. (3) Trace organics, such as collagen, non-collagenous proteins, and humic acids were removed by adding 0.04 mL of 2% reagent-grade NaOCl per 1 mg of the sample material. (4) After rinsing, exogenous carbonate was removed by adding 0.04 mL of 0.1 M C₂H₄O₂ per 1 mg of sample material. (5) After a final rinse with distilled water, the sample was frozen to at least -60° C for 24 hours, and then placed in a vacuum chamber at <200 mTorr for 24 to 48 hours to undergo lyophilization (freeze drying). Once dried, the powdered samples were sent in 2 mL

glass vials to the Smithsonian Stable Isotope Mass Spectrometry Laboratory for further processing and analysis. No duplicates were created. Once at the Smithsonian, the samples were reacted with 100% H_3PO_4 , and carbon dioxide from this reaction was collected by cryogenic distillation in a vacuum line using a Thermo Gasbench II. Samples were then analyzed using a Thermo Scientific Delta V Advantage mass spectrometer coupled with a Thermo TC/EA via a ConFlo IV gas interface. The mass spectrometer returned data on $\delta^{13}\text{C}$ values, and $\delta^{18}\text{O}$ values in both VPDB and VSMOW.

In the past, stable isotope analysis was a complex and lengthy process involving the manual calculation and calibration of isotope data returned from the mass spectrometer (Katzenberg 2008). With advances in mass spectrometer technology over the last thirty years, the process is now almost completely automated, allowing information about isotopic values, carbon-to-nitrogen ratios, and carbon and nitrogen weight percentages to be collected quickly and reliably by computerized mass spectrometers and elemental analyzers. Once received, these data, along with collagen percentage yield data, are used to determine the preservation of the material. This information is presented and discussed in the next two chapters.

CHAPTER 4: RESULTS

Collagen

Validity of Bone Collagen Samples

Determining whether an adequate amount of collagen has been preserved within the organic phase of sample tissues is one of the most important steps in the process of stable isotope analysis. To accomplish this, several interrelated pieces of data collected during the sample processing stage and mass spectrometer run are analyzed. The percentage of collagen value of a sample (wt% collagen) is generally the first and most straightforward factor of preservation calculated because it requires no analysis beyond preparation of the sample. The wt% collagen value describes the amount of collagen preserved relative to the bone or dentin sample's dry weight, and is calculated using Equation 5:

$$\frac{(\text{vial with collagen (mg)}) - (\text{vial without collagen (mg)})}{(\text{sample dry weight (mg)})} * 100$$

(5)

In the adult skeleton of most mammalian species, the organic component of bone comprises about a quarter of the bones total weight (Schoeninger and Moore 1992). Of that organic component, about 90% is comprised of collagen, while the remaining 10% consists of noncollagenous proteins (Schweitzer et al. 2008). Because of this, the upper threshold for collagen yield percentages in dry bone is generally accepted to be about 20% (Schoeninger et al. 1989; Trimble 1997). Values higher than this are within the range of fresh bone, and since at least some breakdown of the collagen occurs over

time, collagen percentages above 20% generally indicate the presence of uninformative proteins, or exogenous organic contaminants. The lower threshold for acceptable collagen yields is somewhat more ambiguous. In early stable isotope studies, it was generally acknowledged that samples with collagen yield percentages of less than 5% did not contain a quantity of collagen significant enough to be considered for study (Schoeninger et al. 1989; White and Schwarcz 1989). More recent studies, however, have accepted collagen percentages as low as 3.5%, 2%, and 1% (Ambrose 1990; Ambrose 1993; van Klinken 1999; Tykot 2006). Implicit here is the nature of the depositional environment when considering collagen percentages. In temperate, stable burial contexts, bone collagen can resist diagenesis for tens of thousands of years, even if the collagen yield is extremely low. In more hostile depositional environments, the isotopic composition of collagen may be altered, even if collagen yields are high. Though the soil at Liang Bua is relatively moist, the shelter provided by the overhanging ceiling keeps the cave cool and protected from direct contact with sun and rain⁶. This suggests that even in samples with low collagen yields, the original isotopic signals will be preserved. Of the ten pig bone samples processed in this study, only three (LB-1A, -2A, and -6A) presented collagen yields above the conservative 5% threshold. However, van Klinken (1999: 689) found that, at least in bone, “large parts of the collagen macromolecule seem to be more or less structurally intact...down to values approaching the threshold of 0.5 wt%”, and that amino acid profiles also change very little within the 20 to 0.5 wt % range. Lowering the threshold to 1%, the collagen yields

⁶ Liang Bua literally translates as “cool cave” (Westaway et al. 2009).

of all ten samples fall within the range of accepted values. Still, the wet environment of Liang Bua would certainly contribute to some degree of collagen degradation, especially in bone from lower strata. As seen in Figure 4, this appears to be the case at Liang Bua: collagen percentages decrease as depth increases, $\beta = -7.956$, $R^2 = 0.8448$. However, the linearity of this relationship may also suggest that the bone samples are free of contamination from non-collagenous protein or foreign organic material, and that their collagen weight percentage values are reliable. If, for instance, some of the bone samples were contaminated or degraded, their collagen weight percentages could be expected to have no linear relationship with depth.

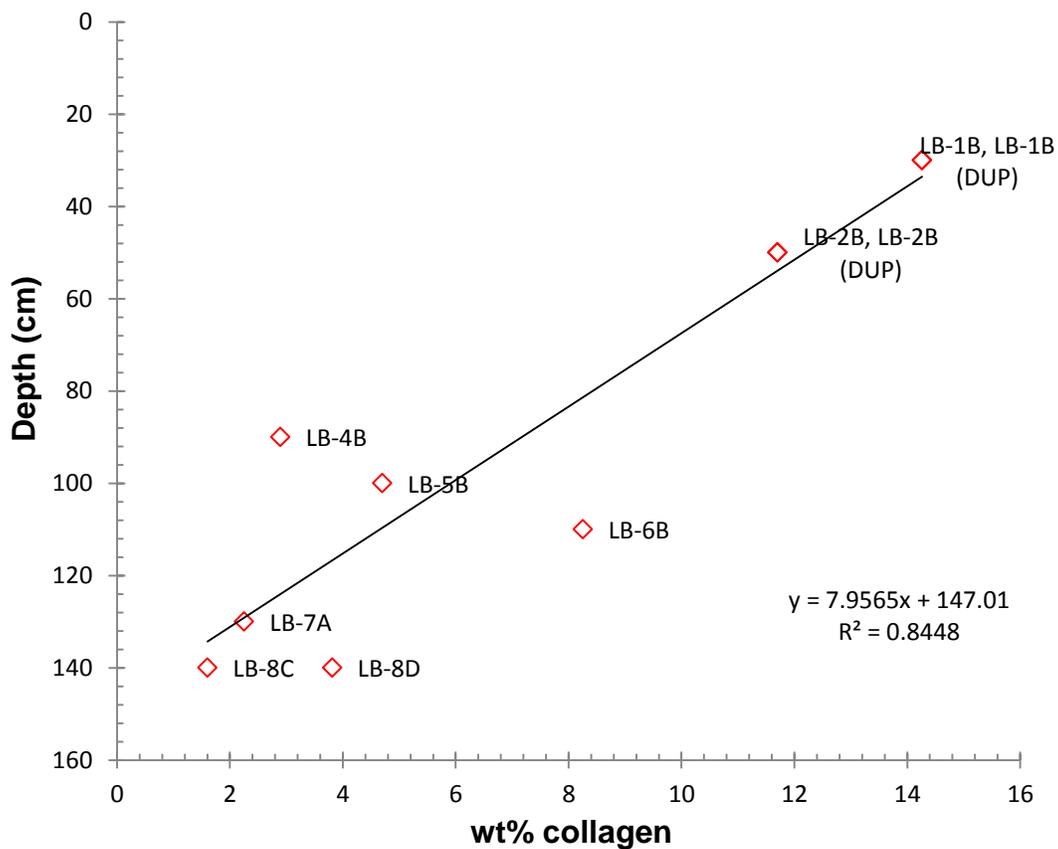


Figure 4. Bone collagen weight percentages versus depth.

Though this is encouraging, both Schoeninger et al. (1989), and Wright and Schwarcz (1999) emphasize that collagen percentage values mean little by themselves, and must be evaluated alongside other data to determine whether the collagen is suitable for analysis. These factors include the atomic carbon to nitrogen ratio (C:N ratio) of bone samples, as well as the weight percentages of carbon and nitrogen in the sample. The atomic C:N ratio of a given sample is simply the ratio of carbon to nitrogen (in moles) in the sample. DeNiro (1985) established that samples of fresh bone collagen not yet altered by diagenetic processes contained carbon and nitrogen in a ratio of between 2.9 and 3.6. Collagen samples from archaeological bone with atomic C:N ratios within this range are therefore considered to have isotopic quantities appropriate for analysis because this indicates that the taphonomic environment did not significantly alter the isotopic composition of the collagen. Values lower than 2.9 may indicate that the collagen has not been preserved well enough for analysis, while values higher than 3.6 may indicate the presence of contaminants such as lipids or humic acids (Ambrose 1993). The atomic C:N ratios of the bone collagen samples, along with other summary data, are shown in Table 2. Duplicates for LB-1B and LB-2B are reported as LB-1B (DUP) and LB-2B (DUP), respectively. Data for sample LB-7A, which was split into three different sub-samples for processing and analysis, were averaged, and are reported as a single sample (see Appendix B for complete data set). The atomic C:N ratios of all eight bone collagen samples cluster tightly within the accepted range, and the C:N ratio values of the duplicates differ from those of their parent samples by less than 1%. This suggests that even though collagen yield percentages are low in some

samples, the integrity of their collagen has been preserved. There is also limited variation (0.02) in the atomic C:N ratios of the duplicate samples from the values of their parent samples.

Additional evidence for good preservation comes from carbon and nitrogen weight percentage values (wt % C, wt % N). These values describe the amount of carbon and nitrogen, by weight, which is in the collagen sample. Like C:N values, the criteria for acceptable carbon and nitrogen weight percentages originate from those of collagen in fresh bone. Ambrose (1990) found that in fresh bone collagen, carbon concentrations range from 15.3% to 47%, and nitrogen concentrations range from 5.5% to 17.3%. Based on the statistical analysis of 2146 collagen samples from the Oxford ¹⁴C Database, van Klinken (1999) suggested that well-preserved collagen samples should yield carbon weight percentages between 26% and 43.6%, and nitrogen weight percentages between 11% and 16%. The carbon and nitrogen percentage values of the collagen samples are reported in Table 2. Using the criteria of Ambrose (1990), all carbon and nitrogen percentages are well within the acceptable range. However, the %C and %N values of several samples lie outside the range of intact collagen proposed by van Klinken (1999) by less than 2%, suggesting that in these samples there is an advanced degree of collagen degradation (see Table 2). The wt% C and wt% N values of the duplicates vary by less than 2% from the original sample.

The final measure used to determine the level of preservation or degradation in collagen samples is the relationship between atomic C:N ratios and wt% collagen values. Van Klinken (1999) suggests that this information may be the best judge of

preservation when other data give conflicting signals, as is the case in the collagen samples from Liang Bua. Jackes et al. (2001) also note that in well preserved samples, the percentage of collagen is slightly inversely proportional to its atomic C:N values. This appears to hold true for the bones from Liang Bua, suggesting that even though the collagen percentages are low in some of the samples, collagen has been preserved. As seen in Figure 5, atomic C:N values are slightly higher in samples with lower collagen yields. This correlation is relatively weak, and the slope of the line created by plotting the data is very close to zero, $\beta = -0.004$, $R^2 = 0.2301$.

Table 2. Collagen yields, C:N ratios, wt% C and wt% N values of bone samples.⁷

Sample	wt% Collagen	C:N ratio (atomic)	wt% C	wt% N
LB-1B	14.26	3.25	45.50*	16.31*
LB-1B(DUP)	14.26	3.27	45.25*	16.13*
LB-2B	11.70	3.26	43.60	15.63
LB-2B(DUP)	11.70	3.26	44.18*	15.80
LB-4B	2.89	3.28	33.21	11.79
LB-5B	4.70	3.21	42.37	15.40
LB-6B	8.25	3.32	31.07	10.93*
LB-7A	2.25	3.30	35.90	12.71
LB-8C	1.60	3.38	36.42	12.59
LB-8D	3.81	3.31	30.59	10.77*

⁷ Values marked with an asterisk (*) fall outside the range accepted by van Klinken (1999), suggesting that some degradation of the collagen has occurred.

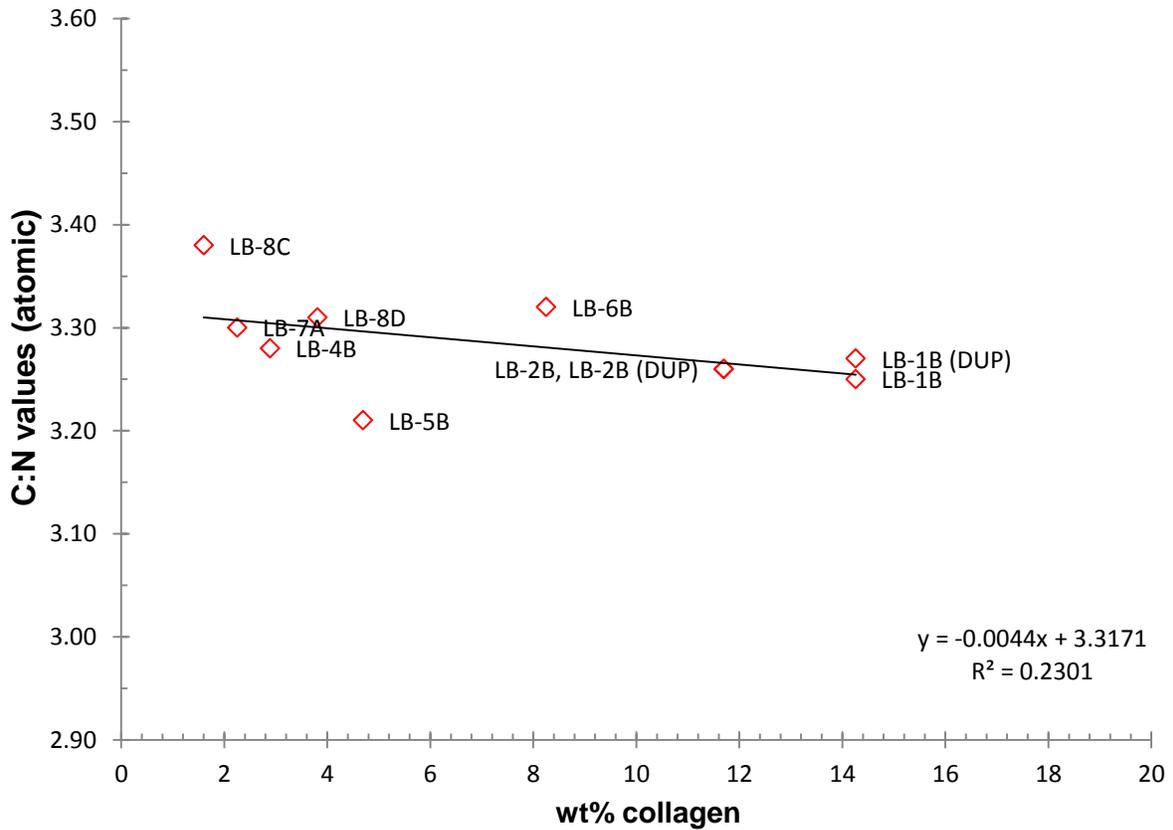


Figure 5. Bone collagen weight percentages versus C:N ratios.

Based on the evidence presented, as well as the fact that all of the bone collagen data fall within acceptable ranges, all eight bone collagen samples, as well as the two duplicate samples are considered valid.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values of Bone Collagen Samples

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the bone collagen samples are plotted in Figure 6. Isotopic values for all samples are within the range that would be expected from terrestrial opportunistic omnivores such as pigs (red box, Figure 6), though there are

clear differences in ^{13}C enrichment throughout the sample series (DeNiro 1987; Tykot 2006).

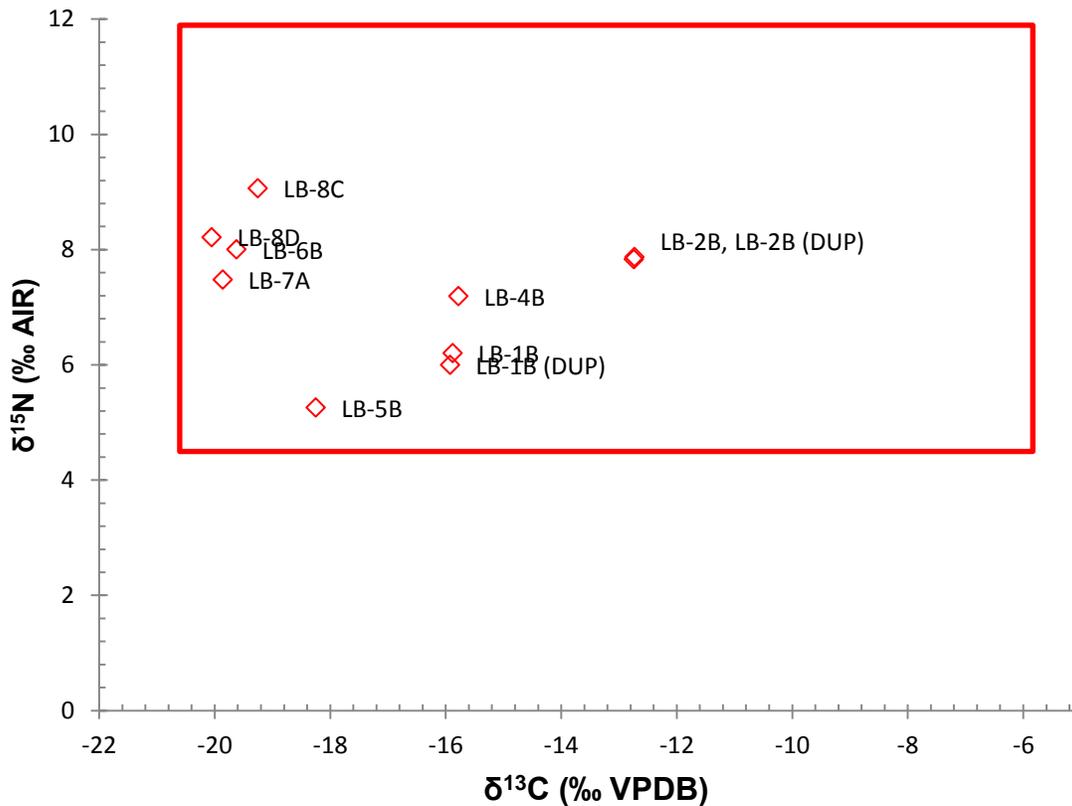


Figure 6. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen – red box indicates range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values typically observed in terrestrial omnivores.

$\delta^{13}\text{C}$ Values of Bone Collagen Samples

The $\delta^{13}\text{C}$ values for the bone collagen samples are found in Table 3. The isotope ratio values of both duplicate samples are within 1% of the isotope ratio values of their parent samples. Typically, C_3 plants have $\delta^{13}\text{C}$ values that average around -26‰, while C_4 plants have $\delta^{13}\text{C}$ values averaging -12.5‰. Because the formation of collagen in the body involves enrichment in ^{13}C of about 5‰, organisms feeding

exclusively on ^{13}C -discriminant C_3 plants have ^{13}C values around -21.5‰ , while organisms feeding exclusively on C_4 plants have ^{13}C values around -7.5‰ (Vogel and van der Merwe 1977). Mixed feeders have ^{13}C values intermediate between these two extremes.

Table 3. $\delta^{13}\text{C}$ values of bone collagen samples.

Sample	$\delta^{13}\text{C}$ (‰)
LB-1B	-15.88
LB-1B(DUP)	-15.92
LB-2B	-12.73
LB-2B(DUP)	-12.74
LB-4B	-15.78
LB-5B	-18.25
LB-6B	-19.62
LB-7A	-19.86
LB-8C	-19.25
LB-8D	-20.05

As seen in Figure 7, bone collagen samples from deeper strata are less enriched in ^{13}C than samples from strata closer to the ground surface. There is no evidence that this pattern is the result of diagenesis or contamination, suggesting that the values reflect a shift towards enrichment in ^{13}C caused by changes in the dietary behavior of Liang Bua pigs over time.

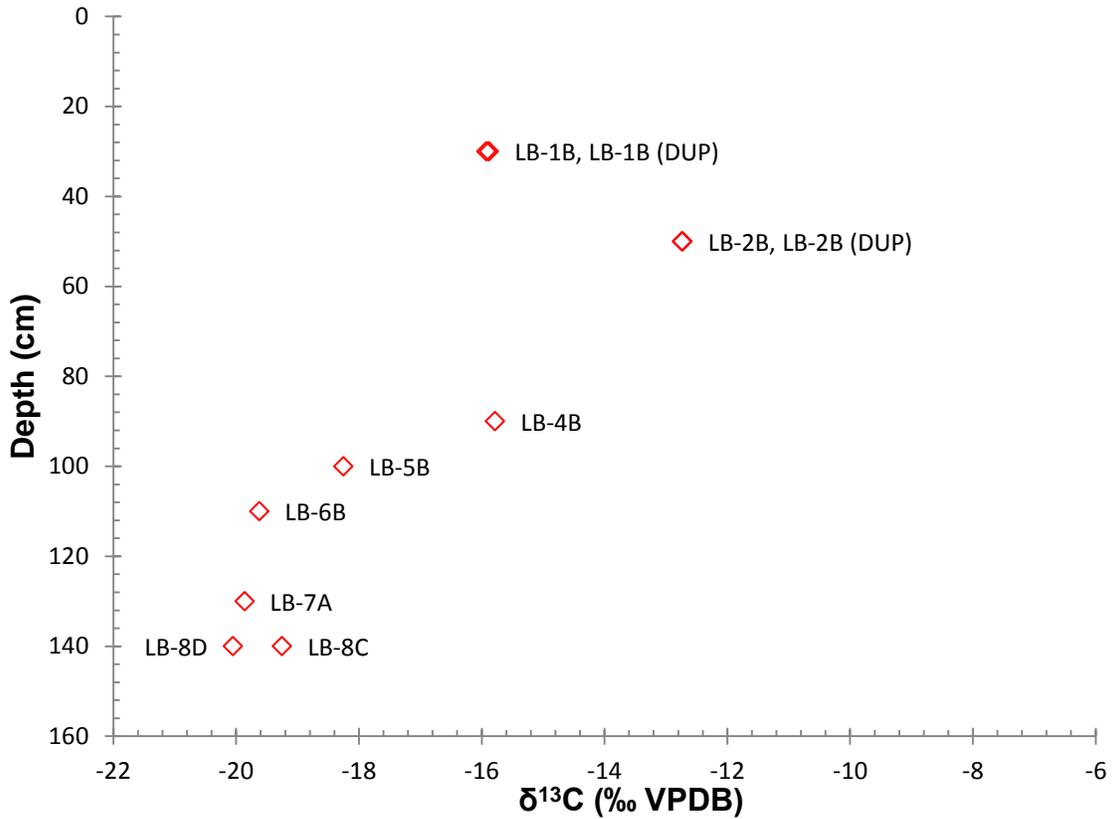


Figure 7. $\delta^{13}\text{C}$ values of bone collagen samples versus depth.

Assuming that it is the result of the incorporation of C_4 plants into the diets of the pigs, the degree of change in $\delta^{13}\text{C}$ values can be quantified using Equation 6, which was first implemented by White and Schwarcz (1989):

$$PC4 = \frac{(\delta_c - \delta_3 + \Delta_{dc})}{\delta_4 - \delta_3} * 100$$

(6)

where “PC4” is the percentage of C_4 plants in the diet, “ δ_c ” is the carbon isotopic value of the collagen sample, “ Δ_{dc} ” is the degree of enrichment between diet and organism,

“ δ_3 ” is the average ^{13}C value of C_3 plants, and “ δ_4 ” is the average ^{13}C value of C_4 plants.

The PC4 values of the bone collagen samples are reported in Table 4.

Table 4. Depth and PC4 values of bone collagen samples.

Sample	Depth (cm)	PC4 (%)
LB-1B	30	40.14
LB-1B(DUP)	30	39.86
LB-2B	50	62.64
LB-2B(DUP)	50	62.57
LB-4B	90	40.86
LB-5B	100	23.21
LB-6B	110	13.43
LB-7A	130	11.71
LB-8C	140	16.07
LB-8D	140	10.36

This change occurs rather abruptly between the strata at 100 and 90 cm (between samples LB-6B, LB-5B, and LB-4B (Table 4)). The geological age range of the strata from which these samples originate is between 5160 and 2750 yBP (Roberts et al. 2009). The trend line calculated from the depth and PC4 data does show a tendency towards abrupt integration of C_4 plants, with the most of the data clustering into two distinctive groups, but with LB-5B intermediate between the two $\beta = -1.803$, $R^2 = 0.6789$ (Figure 8).

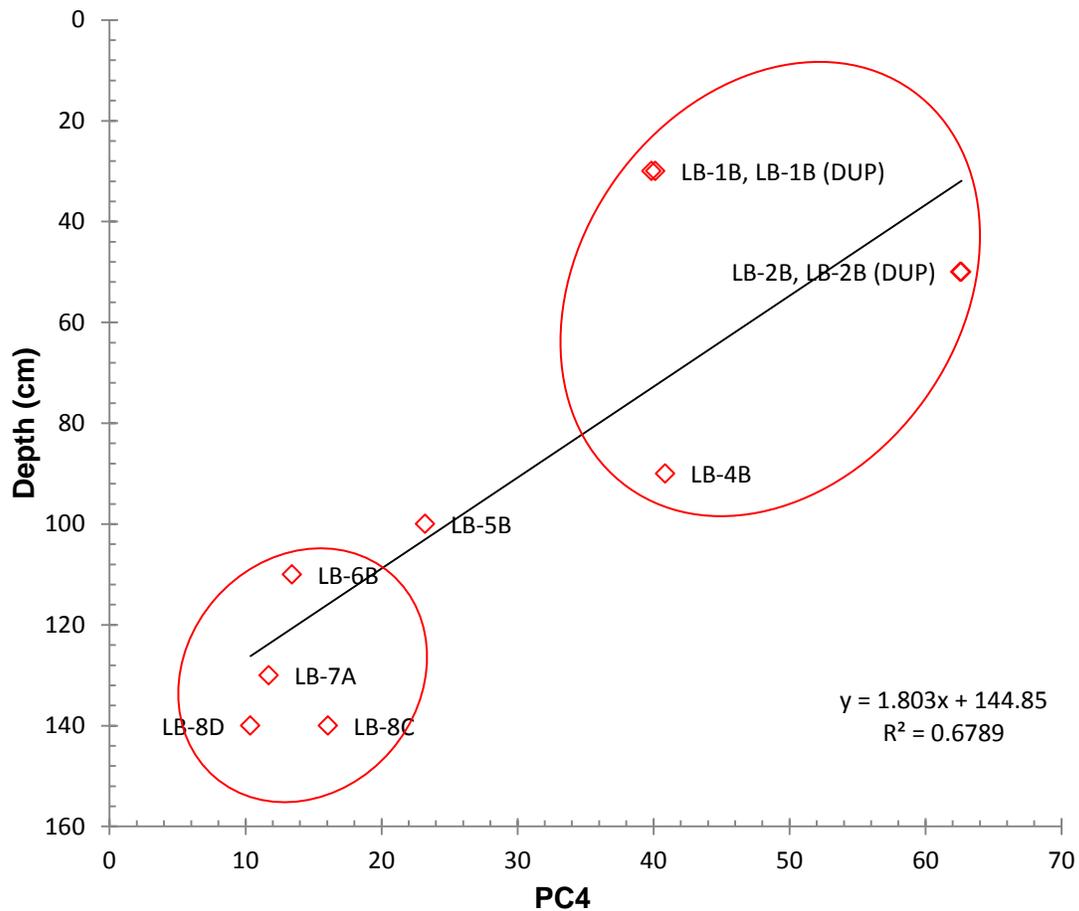


Figure 8. PC4 values of bone collagen samples versus depth – red circles indicate clustered values.

$\delta^{15}N$ Values of Bone Collagen Samples

The $\delta^{15}N$ values of the bone collagen samples are displayed in Table 5. The isotope ratio values of both duplicate samples are within 1% of the isotope ratio values of their parent samples. The relationship between dietary $\delta^{15}N$ and the collagenic $\delta^{15}N$ values of an organism is somewhat more complex than the relationship between dietary and collagen $\delta^{13}C$ values. In bone collagen, nitrogen isotope values directly reflect the

composition of dietary protein sources, and like dietary carbon, there is an increase in $\delta^{15}\text{N}$ of $\sim 3\text{‰}$ at each trophic level. In general, the collagenic ^{15}N values of terrestrial fauna range between 2‰ and 10‰, with an average around $5.7 \pm 2.2\text{‰}$ (Schoeninger and DeNiro 1984; Pate 1994). Because the marine food chain is much longer than the terrestrial food chain, organisms that utilize marine resources in their diet are generally more enriched in ^{15}N than terrestrial organisms. Though marine resources may have been used by humans on Flores, Liang Bua is approximately 50 km from the ocean, and the terrain between is challenging. Tocheri (personal communication, 2013) suggests that it would have taken about a week to get from Liang Bua to the ocean. Based on the $\delta^{15}\text{N}$ values of the bone collagen samples, it appears that marine resources did not play a role in the diet of pigs at Liang Bua (Table 5).

Table 5. $\delta^{15}\text{N}$ values of bone collagen samples.

Sample	$\delta^{15}\text{N}$ (‰)
LB-1B	6.20
LB-1B(DUP)	6.00
LB-2B	7.87
LB-2B(DUP)	7.83
LB-4B	7.19
LB-5B	5.26
LB-6B	8.00
LB-7A	7.48
LB-8C	9.06
LB-8D	8.21

As seen in Figure 9, $\delta^{15}\text{N}$ values appear to decrease over geological time, such that $\delta^{15}\text{N}$ values of bone collagen samples from older strata appear to be more enriched

in than samples from younger ones $\beta = 19.943$, $R^2 = 0.2335$. Pate (1994) notes that sample diagenesis is directly related to loss of ^{14}N , and enrichment in ^{15}N , meaning that collagen samples from greater depths may be enriched in ^{15}N by up to 10‰ relative to younger samples. However, the relationship between sample depth, collagen degradation, and ^{15}N enrichment has not been quantified, so it is not possible to determine whether the shift in the $\delta^{15}\text{N}$ values of the bone collagen samples from Flores can be attributed to degradation. Instead, it is likely that this pattern is related to changes in the diets of the pigs over time (discussed in the Discussion section).

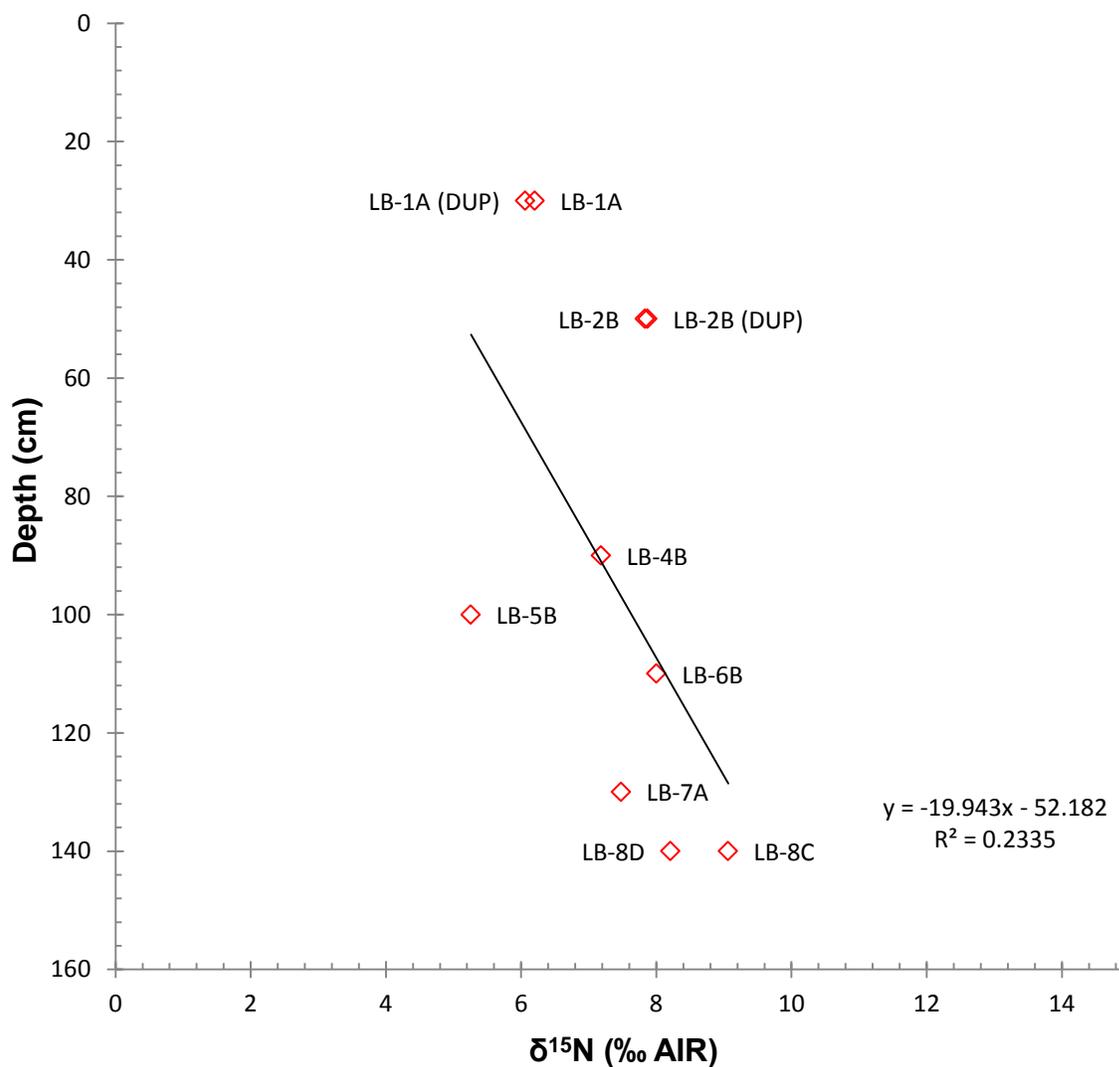


Figure 9. $\delta^{15}\text{N}$ values of bone collagen samples versus depth.

Validity of Dentin Collagen Samples

Methods used to assess the level of preservation in collagen extracted from dentin are similar to those used for collagen extracted from bone, and the acceptable range of values for wt% collagen values, atomic C:N ratio values, wt% C values, and wt% N values are all the same for dentin as they are for bone (Wright and Schwarcz

1999). These data are reported for all nine dentin collagen samples, including duplicates in Table 5. The wt% C and wt% N values of the duplicates vary by less than 1% from those of their parent samples. Samples that were split up into different vials during processing were averaged, and are reported as single samples (See Appendix D for complete dataset). Overall, the collagen percentages are greater across the dentin collagen samples than the bone collagen samples. This is expected given the fact that dentin, being partially encapsulated by the tooth enamel, may be more resistant to physical damage and contamination than dry bone. Still, collagen percentages appear to share the same linear relationship with sample depth as the bones collagen percentages do, and samples from older strata have smaller collagen percentage values than those from younger ones (Figure 10). Atomic C:N ratios are all well within the range of accepted values, with the exception of LB-6A, which exceeds the upper C:N threshold (Table 6). Carbon and nitrogen weight percentages for this sample are also below the acceptable threshold. Given the significance of atomic C:N ratios, as well as carbon and nitrogen weight percentages in determining the quality of the sample, LB-6A will be excluded from further analysis. The C:N ratio values of the duplicate samples differ from those of their parent samples by less than 1%. Like the bone collagen samples, atomic C:N values are slightly higher in dentin collagen samples that have lower collagen yields (Figure 11). Again, this correlation is relatively weak, and the slope of the line created by plotting the data is, like the bone collagen samples, very close to zero, $\beta = -0.0076$, $R^2 = 0.0067$.

Table 6. Collagen yields, C:N ratios, wt% C and wt% N values of dentin samples.⁸

Sample	wt% collagen	C:N ratio (atomic)	wt% C	wt% N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
LB-1A	9.49	3.26	44.93	16.06	-20.04	6.78
LB-2A	7.20	3.29	42.49	15.10	-15.73	7.93
LB-3A	5.23	3.28	36.55	12.99	-19.42	7.76
LB-3A DUP	5.23	3.30	36.26	12.83	-19.46	7.76
LB-3B	15.40	3.37	38.47	13.34	-18.92	7.62
LB-4A	14.47	3.60	32.89	10.57	-19.69	7.68
LB-5A	6.41	3.30	43.75	15.45	-16.83	8.24
<i>LB-6A</i>	3.65	<i>4.75</i>	<i>18.41</i>	<i>3.98</i>	-20.89	8.06
LB-8A	4.75	3.33	36.49	12.80	-20.29	9.38
LB-8B	1.13	3.30	35.84	12.67	-20.34	8.99
LB-8B DUP	1.13	3.30	36.21	12.79	-20.33	9.10

⁸ Unacceptable values are bolded and italicized.

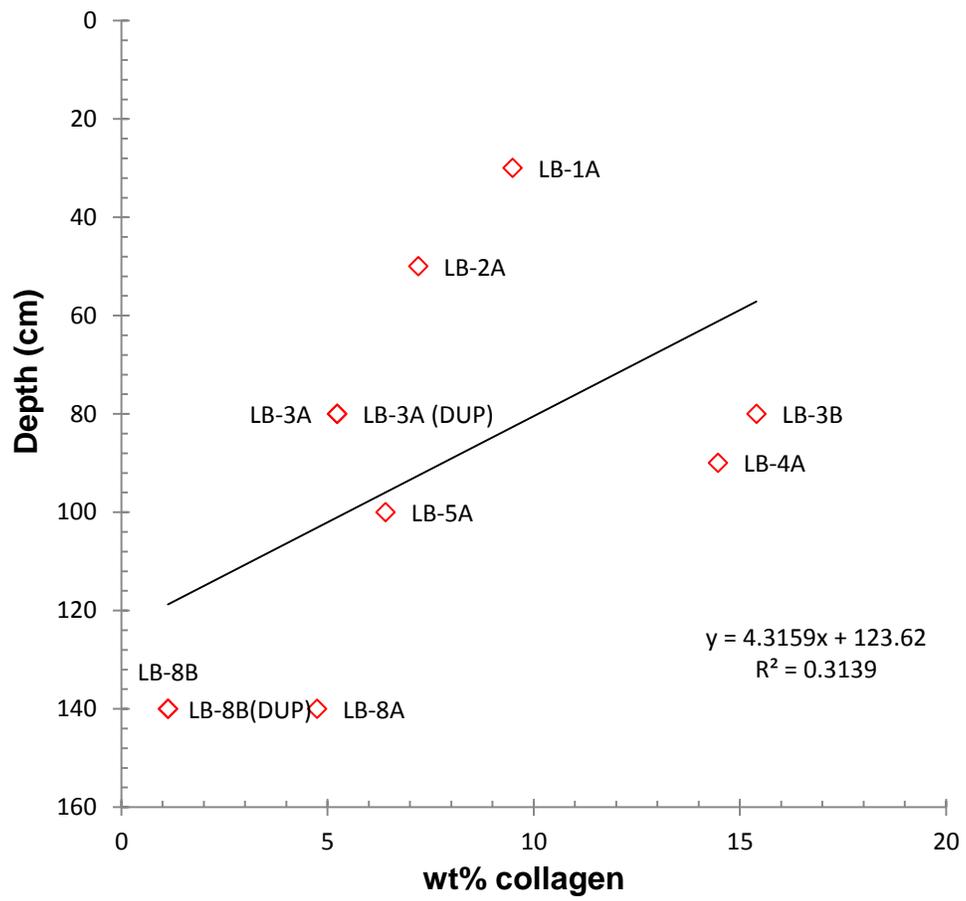


Figure 10. Dentin collagen weight percentages versus depth.

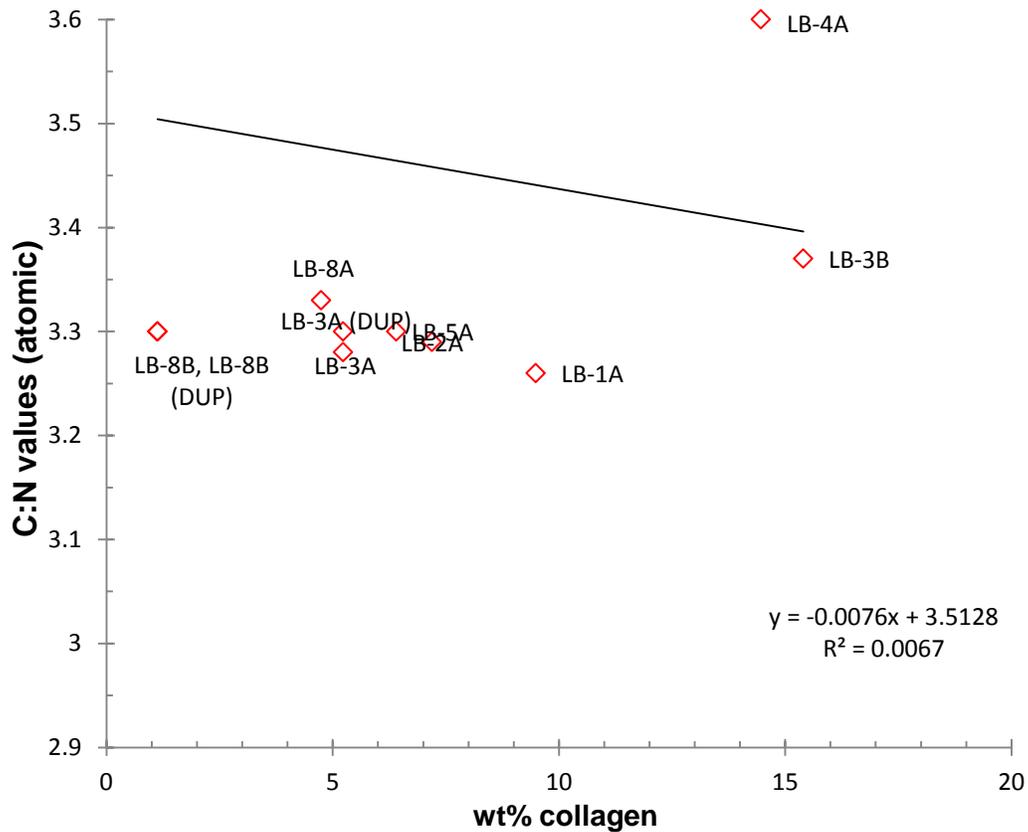


Figure 11. Dentin collagen weight percentages versus C:N ratios.

With the exception of sample LB-6A, the isotopic data from the dentin collagen samples are considered valid.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values of Dentin Collagen Samples

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the dentin collagen samples are plotted in Figure 12. Like the bone collagen samples, these values are all within the range that would be expected of terrestrial opportunistic omnivores such as pigs (red lines, Figure 12) (DeNiro 1987; Tykot 2006).

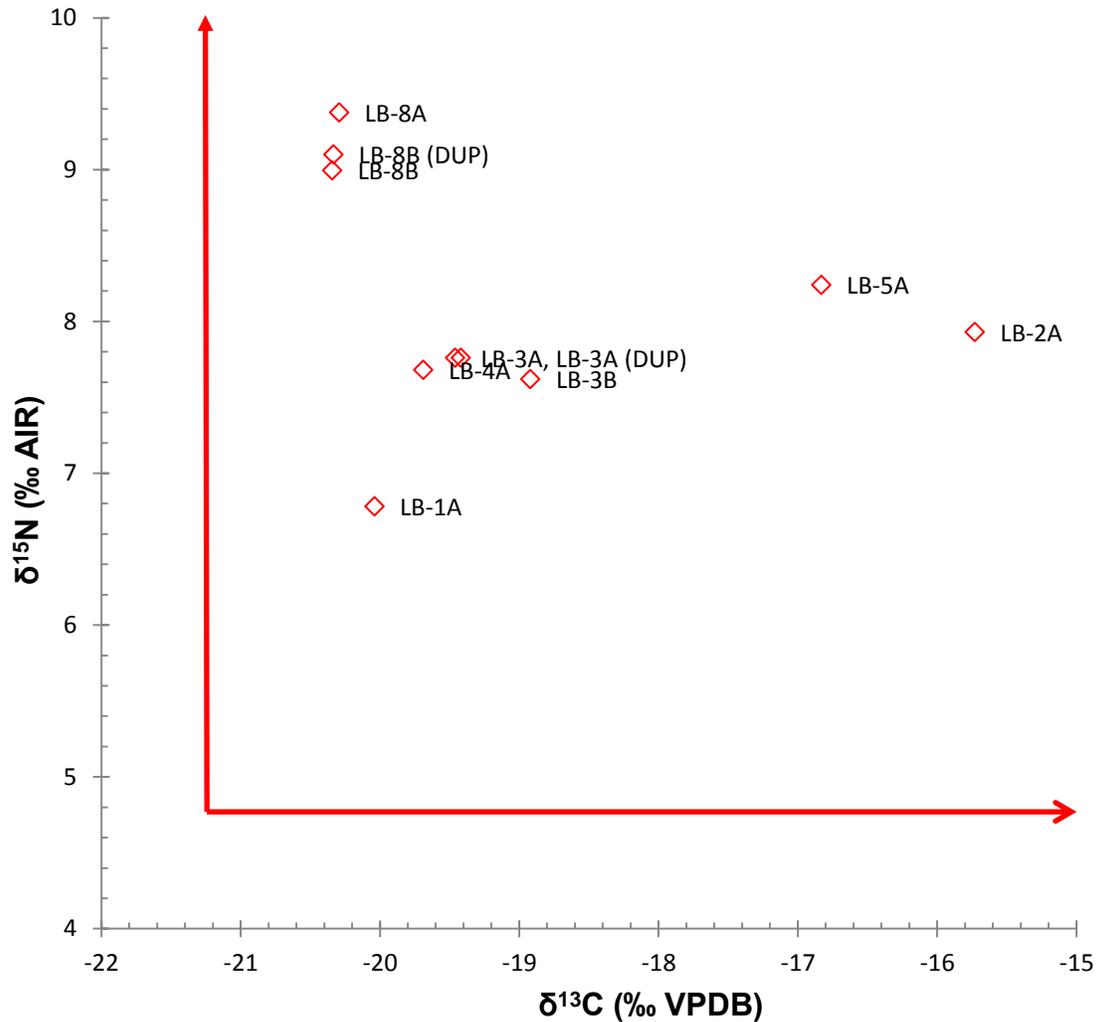


Figure 12. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of dentin collagen samples – red lines indicate minimum $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values typically observed in terrestrial omnivores.

$\delta^{13}\text{C}$ Values of Dentin Collagen Samples

The $\delta^{13}\text{C}$ values of the dentin collagen samples are reported in Table 7, and are plotted against depth in Figure 13. Like those of the bone collagen samples, these values also reflect a tendency towards ^{13}C enrichment over geological time, along with depletion in ^{15}N .

Table 7. $\delta^{13}\text{C}$ values of dentin collagen samples.

Sample	$\delta^{13}\text{C}$ (‰)
LB-1A	-20.04
LB-2A	-15.73
LB-3A	-19.42
LB-3A DUP	-19.46
LB-3B	-18.92
LB-4A	-19.69
LB-5A	-16.83
LB-8A	-20.29
LB-8B	-20.34
LB-8B DUP	-20.33

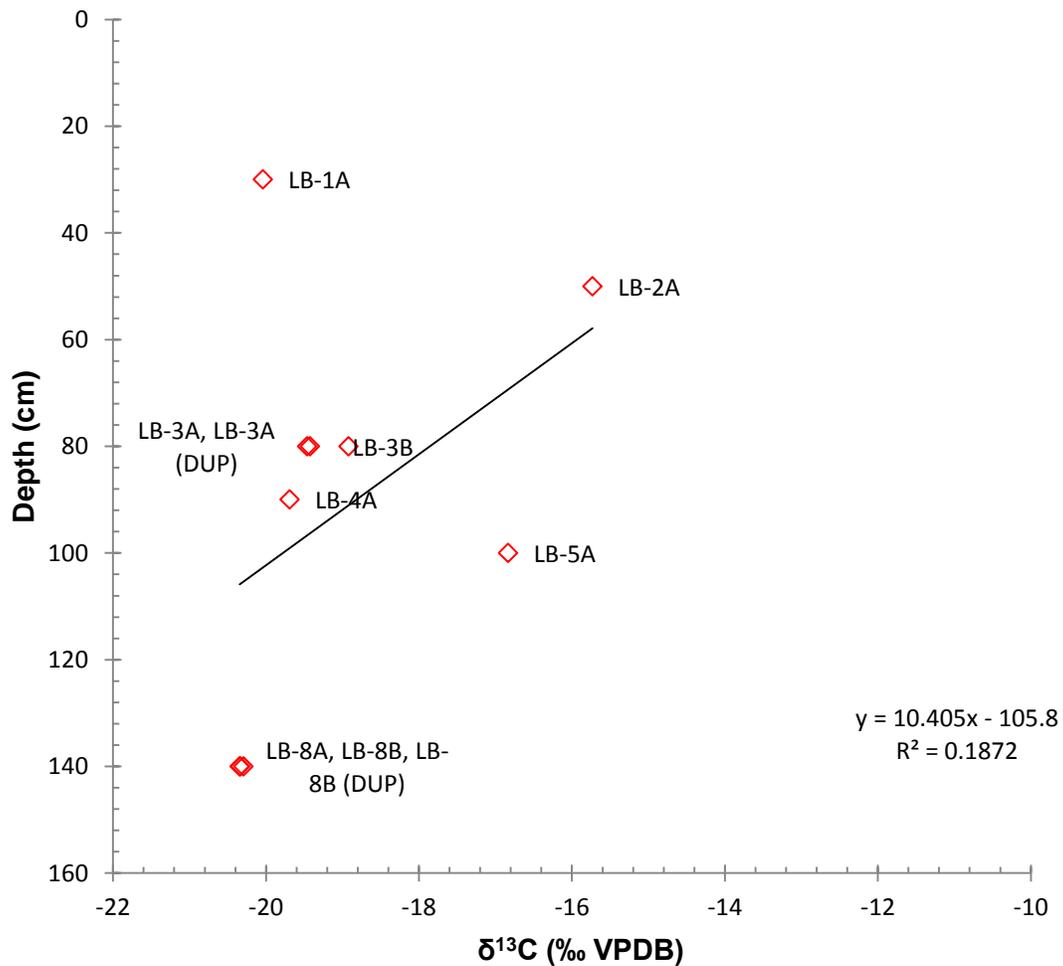


Figure 13. $\delta^{13}\text{C}$ values of dentin collagen samples versus depth.

The $\delta^{13}\text{C}$ values of the bone collagen samples are plotted against those of the dentin collagen samples in Figure 14. Though it is not known whether bones and teeth from the same spit come from the same individual, the general pattern of enrichment over time is reiterated in the $\delta^{13}\text{C}$ values of both materials. No studies have been completed on the spacing between bone and dentin collagen values in pigs, but these data suggest, with the exception of the bone and dentin collagen samples at 100 cm depth (LB-5B and LB-4A, respectively), some degree of fractionation. This is most likely

attributed to differences in the timing of bone and tooth formation, along with differential rates of ^{13}C enrichment and turnover in bone and dentin. The two samples at 100 cm depth likely originate from different pigs (hence the different sample ID numbers), which suggests that LB-4A may have been taking in more C_4 plant material than LB-5B.

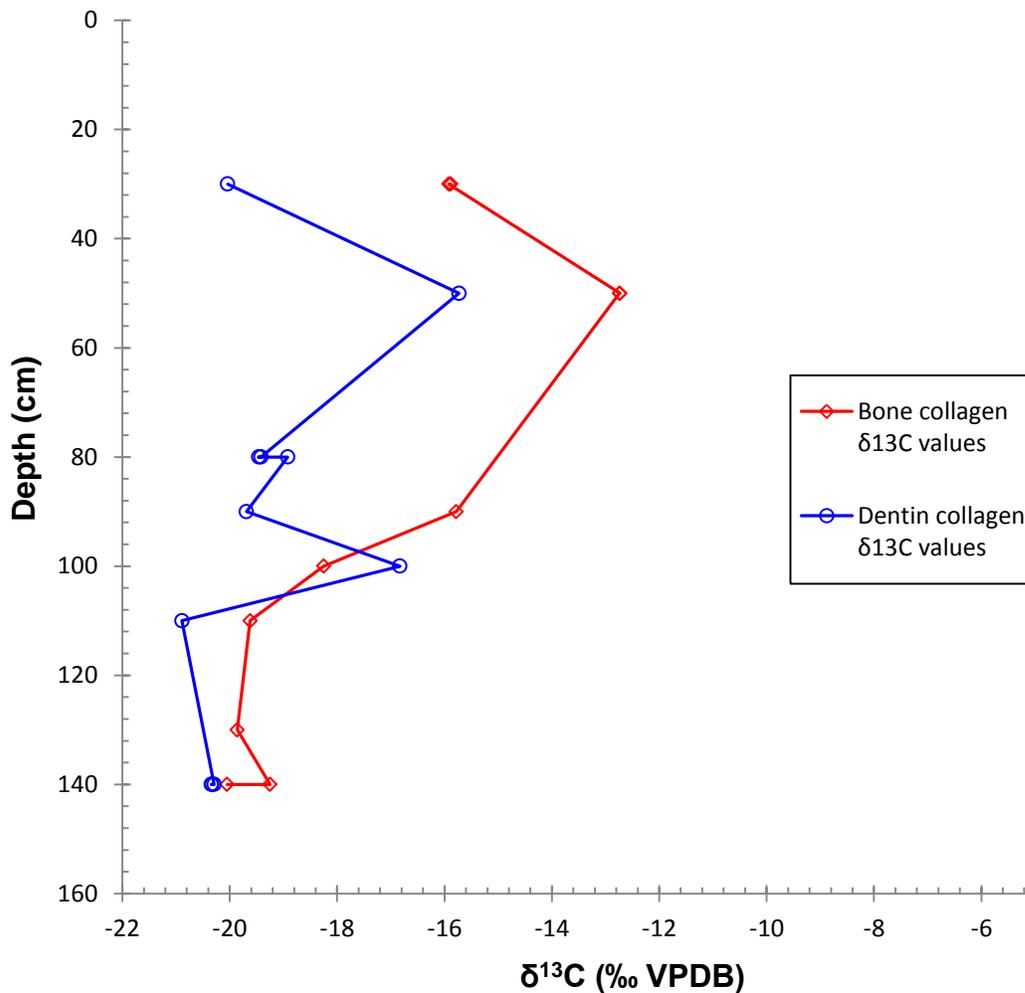


Figure 14. $\delta^{13}\text{C}$ values of bone collagen and dentin collagen samples versus depth.⁹

⁹ It is not known if the bones and teeth from the same depth are from the same individual.

$\delta^{15}\text{N}$ Values of Dentin Collagen Samples

The $\delta^{15}\text{N}$ values of the dentin collagen samples are reported in Table 8, and are plotted against depth in Figure 15. $\delta^{15}\text{N}$ values appear to decrease over geological time, such that values of bone collagen samples from older strata appear to be more enriched in ^{15}N than samples from younger ones $\beta = 43.894$, $R^2 = 0.8662$. The fact that this pattern is observed in collagen samples from both bone and dentin may suggest that soil conditions or dietary protein sources were changing over time, or that the pigs increasingly experienced disease-related dehydration as a result of being penned or kept in close quarters to one another.

Table 8. $\delta^{15}\text{N}$ values of Liang Bua dentin collagen samples, including duplicates.

Sample	$\delta^{15}\text{N}$ (‰)
LB-1A	6.78
LB-2A	7.93
LB-3A	7.76
LB-3A DUP	7.76
LB-3B	7.62
LB-4A	7.68
LB-5A	8.24
LB-8A	9.38
LB-8B	8.99
LB-8B DUP	9.10

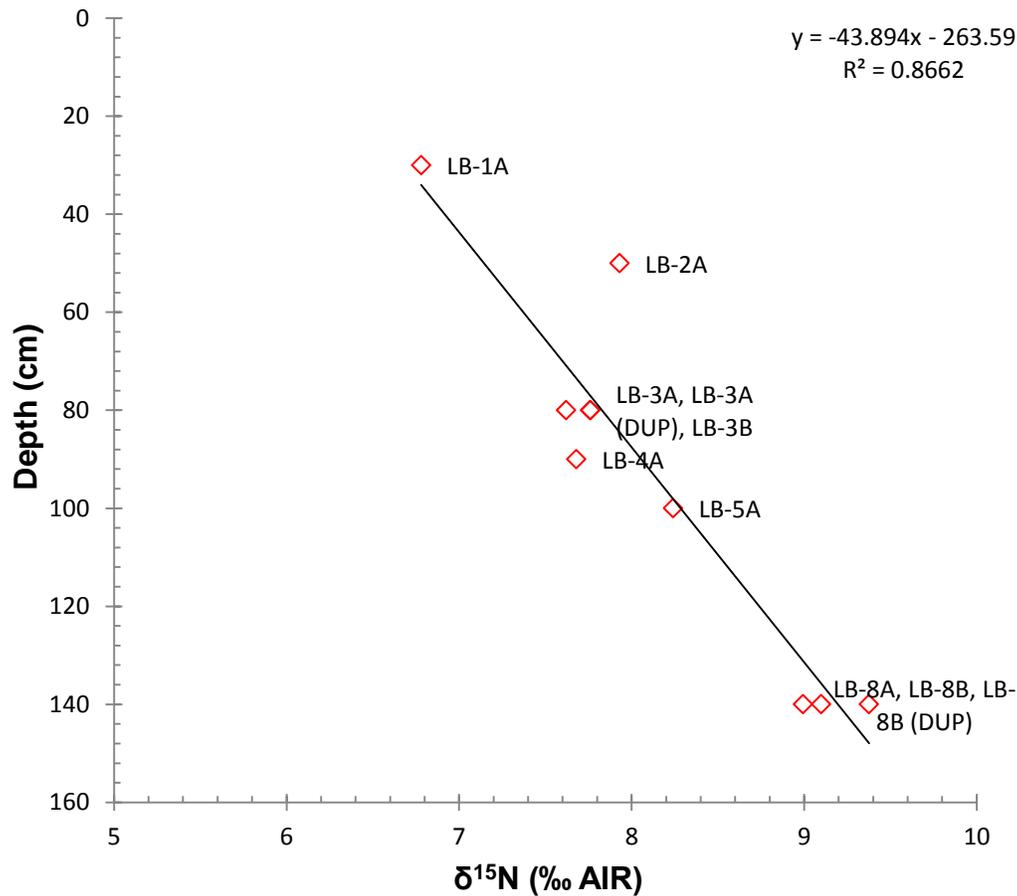


Figure 15. $\delta^{15}\text{N}$ values of dentin collagen samples versus depth.

Plotting the $\delta^{15}\text{N}$ values of the dentin collagen against the $\delta^{15}\text{N}$ values of the bone collagen reveals an interesting relationship (Figure 16). Richards et al. (2002: 208) demonstrated that in juvenile humans, the isotopic values of tooth dentin are generally enriched relative to those of bone collagen due to breastfeeding. In dentin, this enrichment becomes “diluted” as additional dentin is deposited, and breastfeeding ceases. Though no studies have been completed on the intra-individual variation of isotope signals in pigs, it appears that a process similar to that in humans occurs. In Figure 16, bone collagen $\delta^{15}\text{N}$ values are plotted against the dentin collagen $\delta^{15}\text{N}$

values. While the dentin collagen $\delta^{15}\text{N}$ values appear to be slightly more enriched than those of the bone collagen $\delta^{15}\text{N}$ values, the relationship is not consistent. This likely has to do with the fact that not all bone and teeth in the same spit are from the same individual, though it might also have to do with variation in the timing of tooth formation.

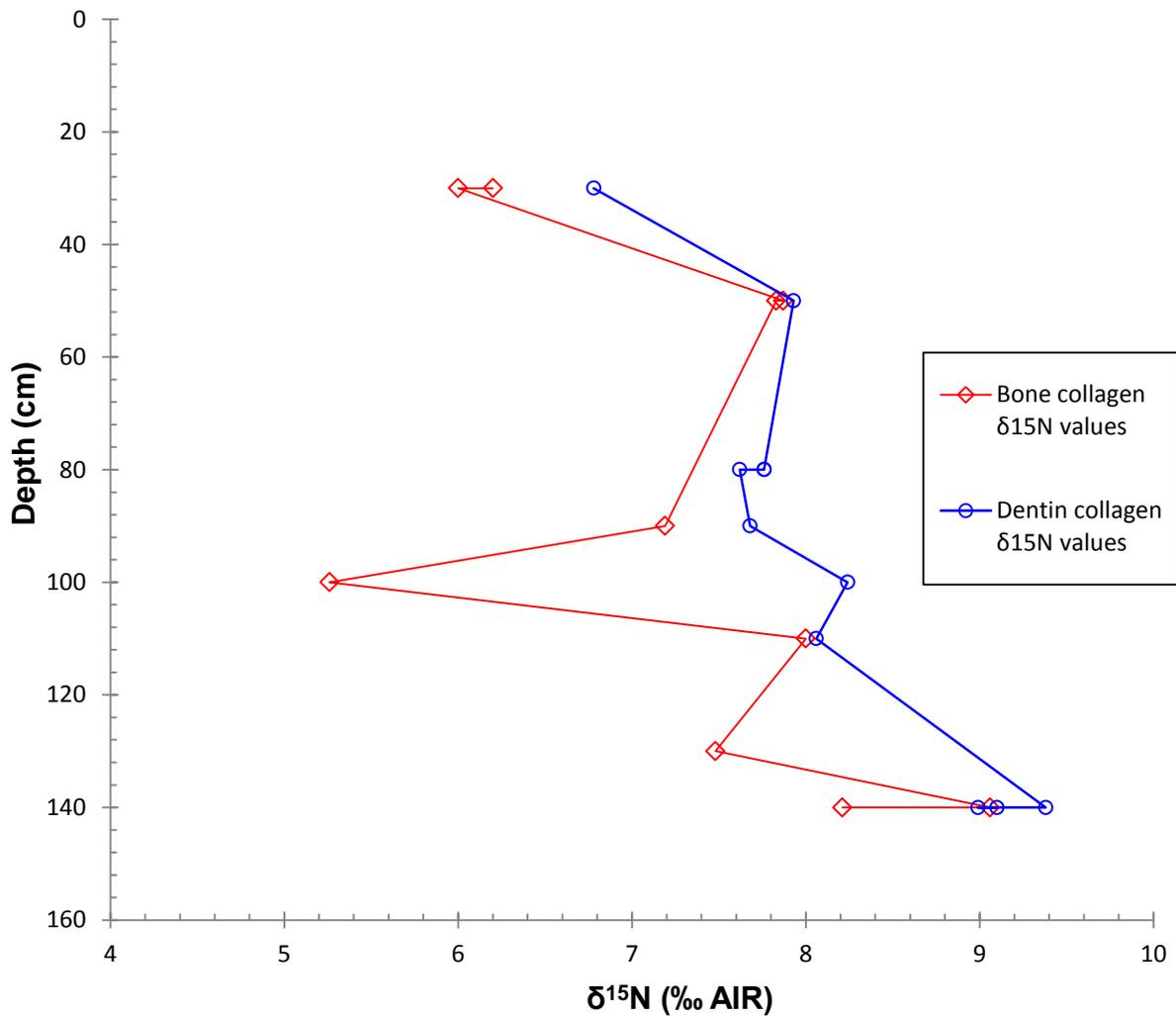


Figure 16. $\delta^{15}\text{N}$ values of bone collagen and dentin collagen samples versus depth.¹⁰

¹⁰ It is not known bones and teeth from the same depth are from the same individual.

Carbonate

Validity of Enamel Carbonate Samples

The enamel rods that make up tooth enamel consist of extremely dense, tightly structured hydroxyapatite crystals. Because of this, tooth enamel is generally more resistant to diagenesis and contamination than both bone and dentin (Tuross et al. 1989; Bell 1991). Nevertheless, there are at least six methods typically used to assess the level of diagenesis in enamel carbonate (Kohn and Cerling 2002). These include examination of changes in enamel crystal structure using X-ray crystallography, comparison of the isotopic values of the enamel samples and the sediments in their immediate depositional environment, and comparison of carbonate and phosphate values. In recent years, Fourier transform infrared spectroscopy (FTIR) has also been used to evaluate samples by analyzing their chemical composition. Unfortunately, none of these methods is available for use in this analysis. However, numerous studies have demonstrated that enamel carbonate can successfully resist diagenesis for as long as 34 million years (Ericson et al. 1981; Ayliffe et al. 1994; Bryant et al. 1998; Blondel et al. 1997; Barrick 1998; Fricke and Rogers 2000). Based on this fact, the visually observed condition of the teeth before processing, and consistency in their carbon and oxygen isotopic values, data from all tooth enamel samples are considered valid.

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ Values of Enamel Carbonate Samples

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the enamel carbonate samples are plotted in Figure 17. Carbon and oxygen isotope ratio values are both reported in VPDB unless otherwise noted.

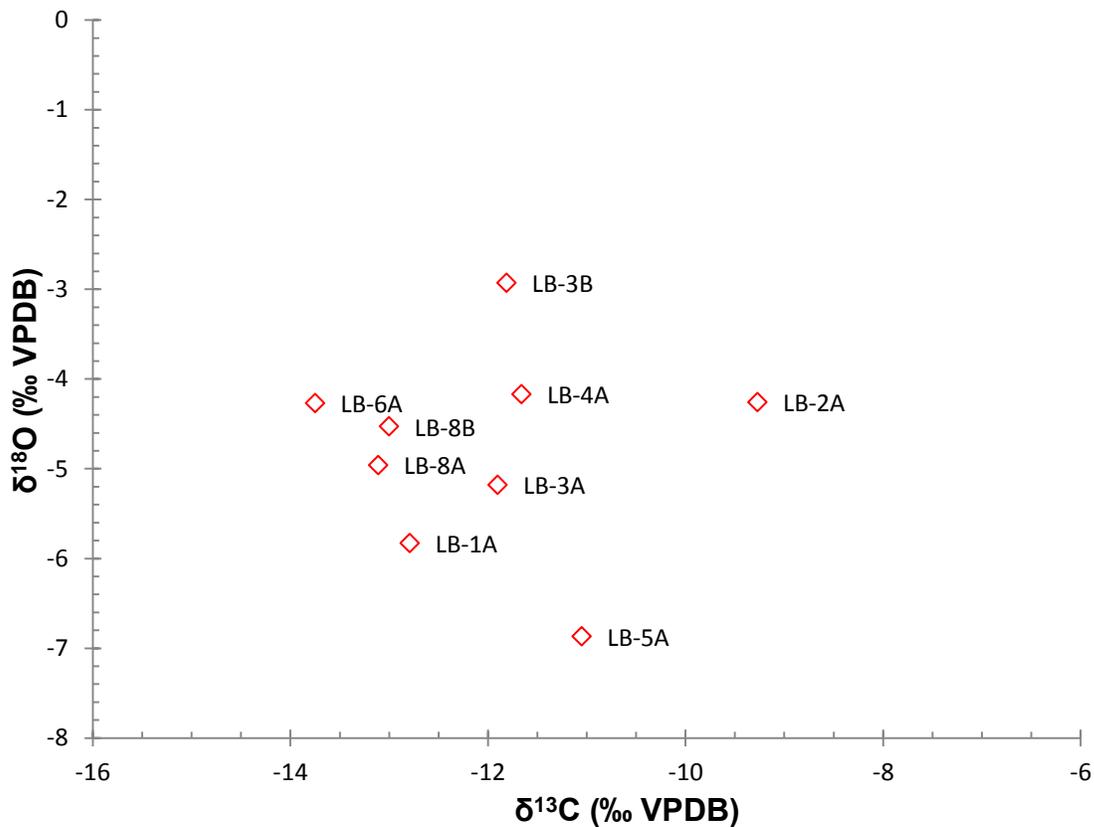


Figure 17. ^{13}C and ^{18}O values of enamel carbonate samples.

$\delta^{13}\text{C}$ Values of Enamel Carbonate Samples

$\delta^{13}\text{C}$ values for the enamel carbonate samples are listed in Table 9 and are plotted against depth in Figure 18. The values of all carbonate samples are within the range of organisms that would be subsisting on a mixed diet of C_3 and C_4 plants (red

line, Figure 18 (upper bound of 0.5‰ not pictured)) (Tykot 2006). However, throughout the series, samples do become increasingly enriched in ^{13}C , reiterating the pattern of $\delta^{13}\text{C}$ values observed in both the bone and dentin collagen samples, $\beta = 13.542$, $R^2 = 0.2429$. In humans, apatites are enriched in ^{13}C by approximately 7‰ relative to collagen due to the fractionation effect: carbonate preferentially incorporates ^{13}C over ^{12}C to a greater degree than collagen (Schurr 1998). The $\delta^{13}\text{C}$ values of enamel carbonate samples from adult organisms may also reflect a higher level of enrichment in ^{13}C at the time of tooth formation due to breastfeeding, while $\delta^{13}\text{C}$ values of bone collagen reflect dietary protein sources over the last several years of their adult life (Ambrose and Norr 1993). While the collagen-carbonate spacing of pigs is not known, this pattern is apparent in the tooth samples from Liang Bua, though it decreases through the series (Figures 19, 20 and 21). The spacing between the dentin and enamel samples is much larger, but varies less (Figure 20). There appears to be no significant change in the spacing of dentin and enamel $\delta^{13}\text{C}$ values over time (Figure 21).

Table 9. $\delta^{13}\text{C}$ values of enamel carbonate samples.

Sample	$\delta^{13}\text{C}$ (‰)
LB-1A	-12.79
LB-2A	-9.27
LB-3A	-11.90
LB-3B	-11.81
LB-4A	-11.66
LB-5A	-11.05
LB-6A	-13.75
LB-8A	-13.11
LB-8B	-13.00

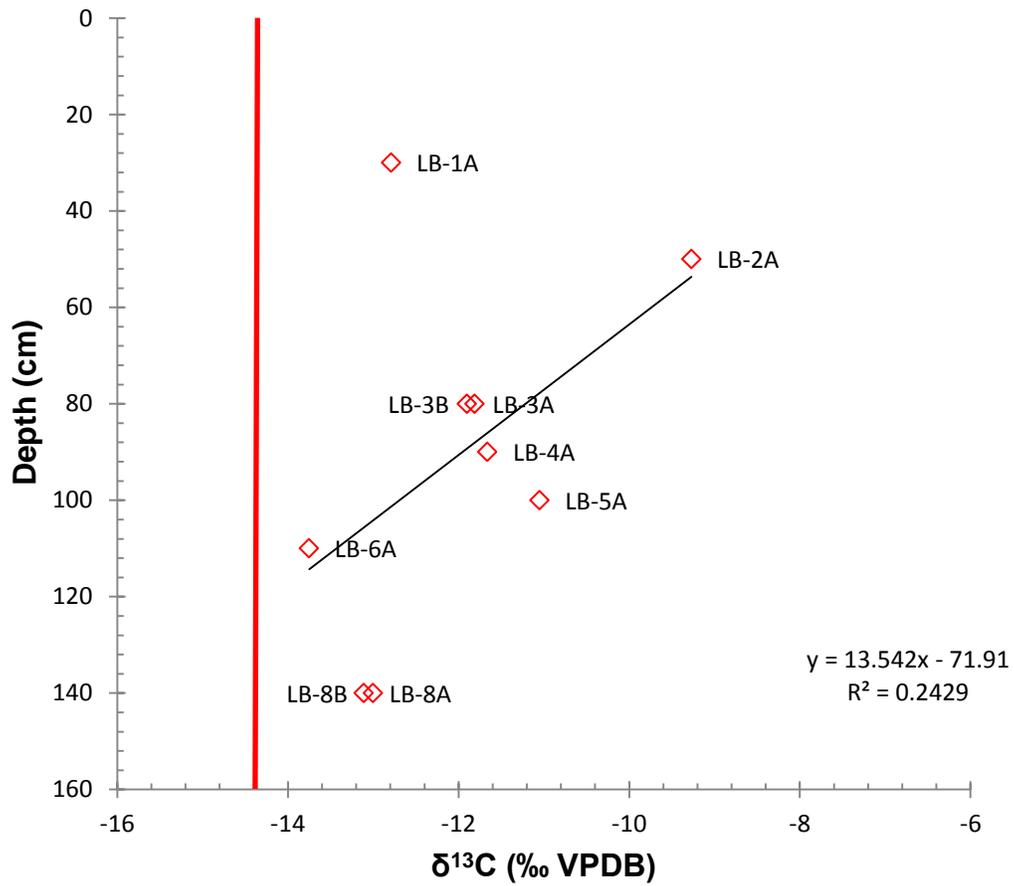


Figure 18. $\delta^{13}\text{C}$ values of enamel carbonate samples versus depth - red line indicates minimum $\delta^{13}\text{C}$ value typically observed in terrestrial omnivores.

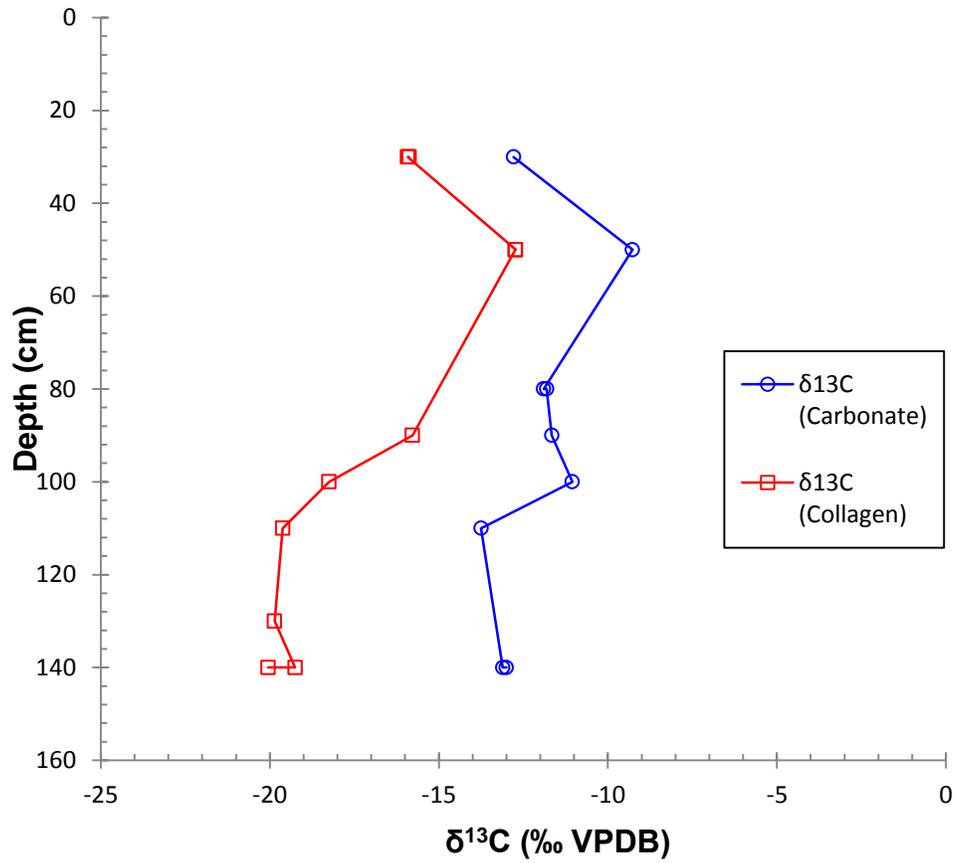


Figure 19. $\delta^{13}\text{C}$ values of bone collagen and enamel carbonate samples versus depth.¹¹

¹¹ It is not known if the bones and teeth from the same depth are from the same individual. However, the chart demonstrates the general trend over geological time in both materials towards enrichment in ^{13}C .

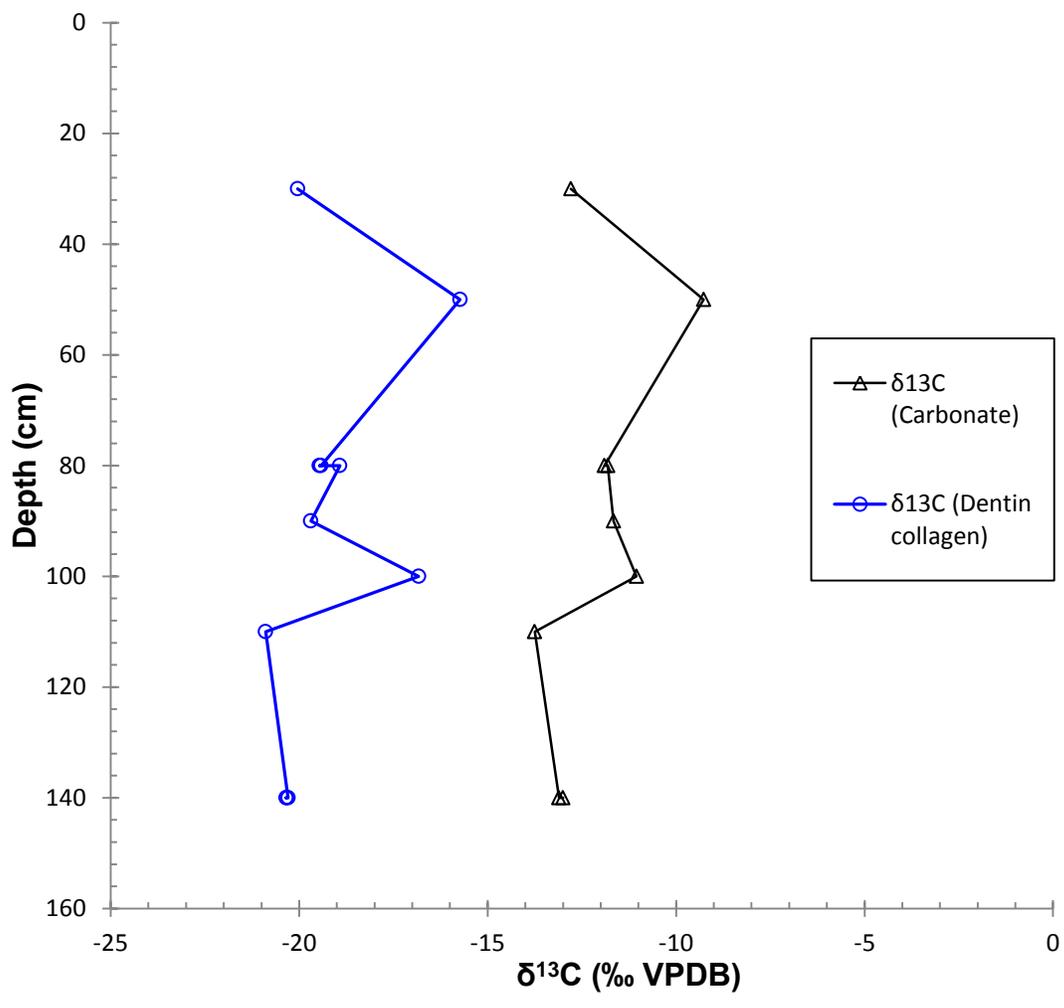


Figure 20. $\delta^{13}\text{C}$ values of Liang Bua dentin collagen and enamel carbonate samples versus depth.

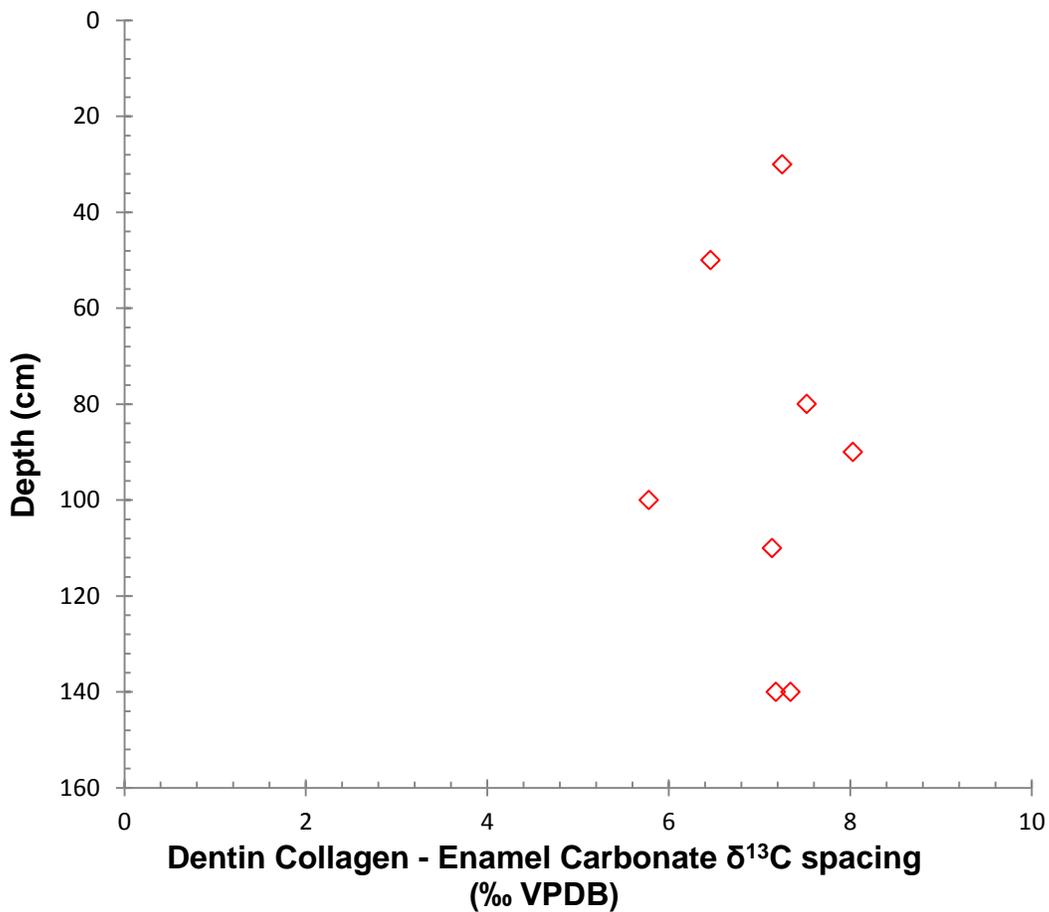


Figure 21. Spacing of dentin collagen and enamel carbonate $\delta^{13}\text{C}$ values versus depth.

Once again, assuming that it is the result of the incorporation of C_4 plants into the diets of the pigs, the degree of change in $\delta^{13}\text{C}$ values can be quantified using Equation 6. However, to account for the $+7\text{‰}$ increase between diet and biological carbonates, the “ δ_{dc} ” value is adjusted from 5‰ to 12‰ (Schwarcz 2013; personal communication). The PC4 values of the enamel carbonate samples are reported in Table 10.

Table 10. Depth and PC4 values of enamel carbonate samples.

Sample	Depth (cm)	PC4 (%)
LB-1A	30	12.21
LB-2A	50	37.36
LB-3A	80	18.57
LB-3B	80	19.21
LB-4A	90	20.29
LB-5A	100	24.64
LB-6A	110	5.36
LB-8A	140	9.93
LB-8B	140	10.71

Again, an abrupt change in PC4 values is observed at 110 and 100 cm, and between samples LB-6A and LB-5A (Table 10). Plotted against sample depth, the data do show a strong tendency towards increased C_4 integration, with the majority of the data points clustering into two distinctive groups and LB-1A intermediate, $\beta = 1.8959$, $R^2 = 0.2429$ (Figure 22).

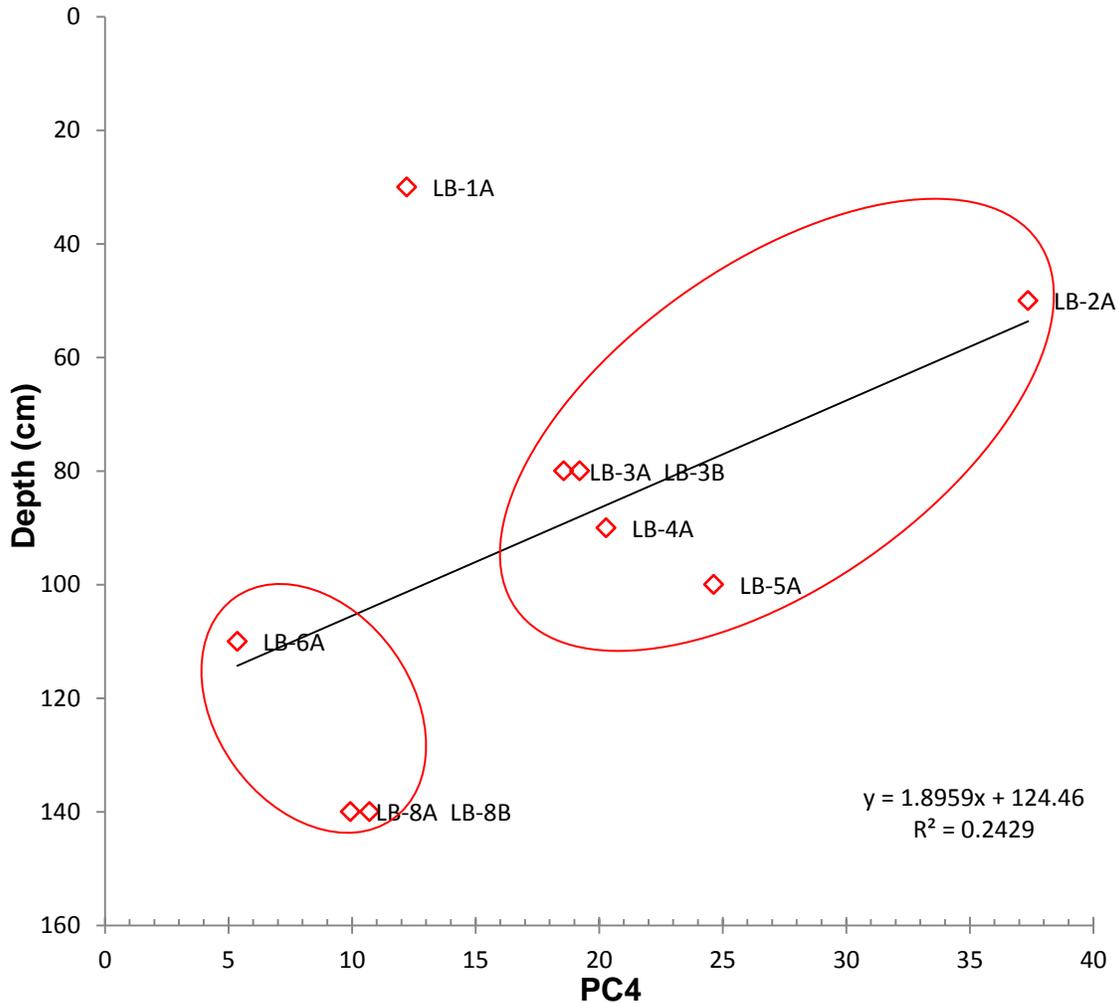


Figure 22. PC4 values of enamel carbonate samples versus depth – red circles indicate clustered values.

$\delta^{18}\text{O}$ Values of Enamel Carbonate Samples

$\delta^{18}\text{O}$ values of organically derived carbonate are reported using the VPDB standard. When these values are compared with the oxygen isotopic values of carbonate from meteoric water sources, they are reported in the Vienna Standard Mean Ocean Water (V-SMOW) standard. $\delta^{18}\text{O}$ values of the enamel carbonate samples are

reported in both VPDB and V-SMOW, along with the group mean and standard deviation, in Table 10. Unfortunately, no oxygen isotope standards exist yet for Flores or the surrounding islands, so no direct inferences can be made about possible differences in the sources of water that pigs were taking in. However, variation in the distribution of $\delta^{18}\text{O}$ values over geological time can suggest differences in location, as well as elevation, during the development of the adult dentition, even if the $\delta^{18}\text{O}$ values cannot be compared to oxygen isotope standards. Oxygen isotope values are plotted against depth in Figure 23. The oxygen values as a whole were distributed normally and most values clustered closely around the mean ($M = -4.77\text{‰}$, $SD = 1.12\text{‰}$), indicating that throughout time, the majority of the pigs in the sample were probably taking in water from sources in the same area. However, LB-1A and LB-5A seem to be possible outliers. Several studies have established that $\delta^{18}\text{O}$ values decrease as elevation increases (Garzzone et al. 2000; Poage and Chamberlain 2001; Kohn and Dettmann 2007). This may indicate that these two pigs were brought from areas of higher elevation on Flores, such as the nearby mountains, or from elsewhere.

Table 11. $\delta^{18}\text{O}$ values of enamel carbonate samples in VPDB and V-SMOW, mean, standard deviation.

Sample	$\delta^{18}\text{O}$ (‰ VPDB)	$\delta^{18}\text{O}$ (‰ V-SMOW)	Mean	Standard Deviation
LB-1A	-5.83	24.92		
LB-2A	-4.26	26.53		
LB-3A	-5.18	25.59		
LB-3B	-2.93	27.89		
LB-4A	-4.17	26.62	-4.77	1.12
LB-5A	-6.87	23.86		
LB-6A	-4.27	26.52		
LB-8A	-4.96	25.81		
LB-8B	-4.53	26.26		

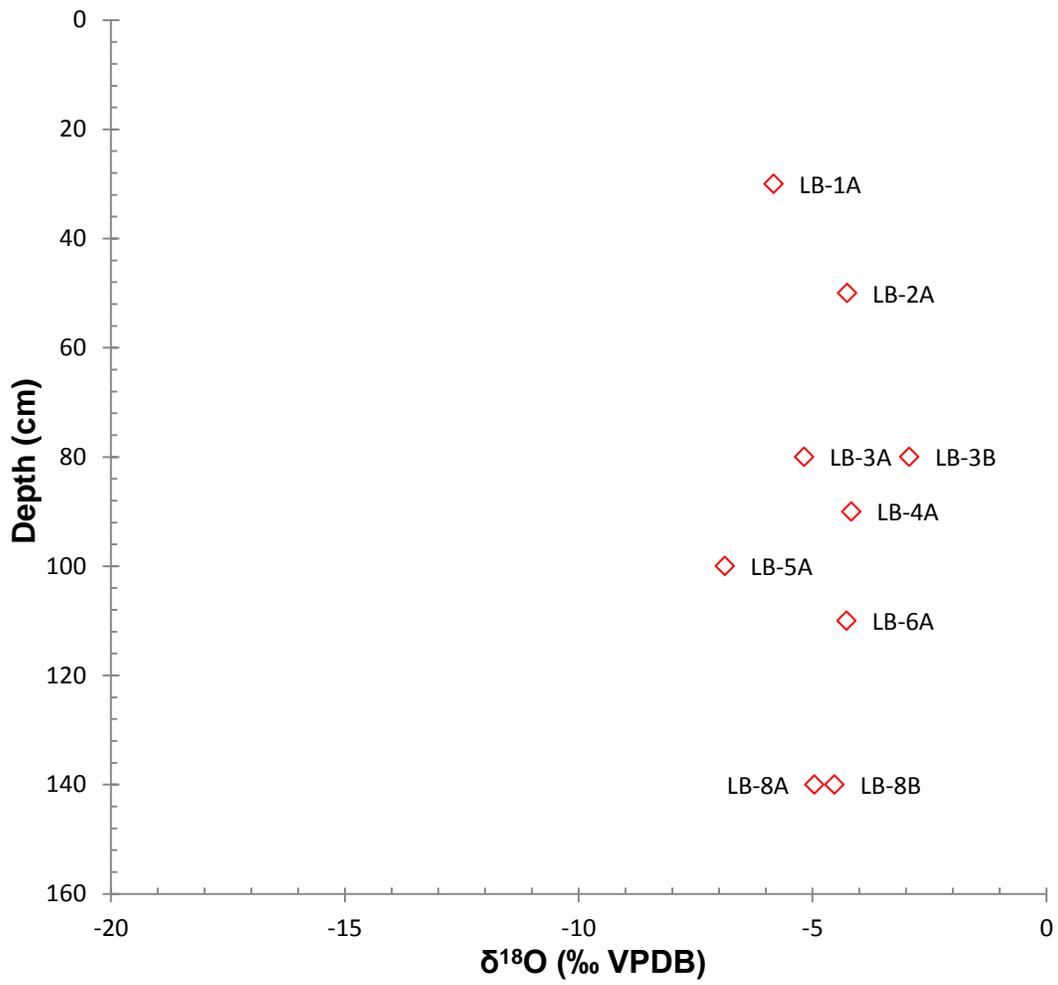


Figure 23. $\delta^{18}\text{O}$ values of enamel carbonate samples versus depth.

CHAPTER 5: DISCUSSION

The Timing and Route of Domestication into Island Southeast Asia

In general, the study of the origins and spread of agriculture has focused on the relationship between agricultural *centers* and *noncenters*. As originally conceptualized by the Russian botanist Nikolai Vavilov (1951) and expounded upon by American botanist Jack Harlan (1971), agricultural *centers* are areas where a number of wild plant species originated or were intensively domesticated before being introduced to agricultural *noncenters*. *Noncenters* are areas where these species did not previously grow, or where limited or no plant domestication previously took place. In many regions, this distinction becomes unwieldy because the agricultural center is spread over such a large geographical range that the dichotomy between *center* and *noncenter* breaks down. However, this does not seem to be the case in Eastern Asia. The archaeological and archaeobotanical record suggests that specific regions in China, Japan and Korea were all major centers of plant domestication in Asia during the early Holocene, and that limited plant domestication took place in the islands of Southeast Asia before crops were introduced from the mainland (Crawford 1992, Higham 1995; Zohary and Hopf 2000; Bellwood and Hiscock 2005). There is also archaeological evidence for the emergence of a domestication package in China between 10 kyBP and 7000 yBP that involved the close association of semi-domesticated Eurasian pigs (*Sus scrofa*), broomcorn millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), and later, Asian rice (*Oryza sativa*) (Bellwood 1987; Bellwood 1992; Bellwood 2001; Barton et al. 2009; Lu et al. 2009). It seems likely that this complex spread into the islands of

Southeast Asia over the next several millennia, though its terminus is a point of debate that will be explored later in this section (Bellwood 2011).

Currently, it is supposed that broomcorn and foxtail millet were both originally domesticated at the location of the Cishan archaeological site in the Loess Plateau region of China between 10 kyBP and 8700 yBP (Lu et al. 2009) (Figure 24). The archaeological data also suggest that within the next two to three thousand years, both millet species along with *S. scrofa* begin to appear at multiple sites along the Yellow River including Dadiwan, Peiligang, Xinglonggou, and Yuezhuang (Lu et al. *ibid*) (Figure 24). By analyzing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of human, dog (*Canis familiaris*), and pig (*S. scrofa*) remains at the Dadiwan archaeological site in Northwestern China, Barton et al. (2009) established that hunter-gatherer populations began experimenting with the small scale cultivation of broomcorn millet around 7500 yBP to supplement their hunting practices. Though this first phase appears to have ended by 7200 yBP, Barton et al. (*ibid.*) found that Dadiwan was reoccupied around 6000 yBP by full-time agriculturalists of the Yangshao culture. These occupants bred and kept herds of *S. scrofa*, and began intensively cultivating both broomcorn and foxtail millet. The results of the study by Barton et al. (*ibid.*) also demonstrate that foxtail millet made up an important part of the diets of both the human occupants and pigs at Dadiwan. Some researchers have suggested that this agricultural flourishing was encouraged by a period of warmer temperatures between 8000 and 5000 yBP.¹² Though paleoclimatic data indicate that

¹² Most studies attribute this warm period, often referred to as the *Holocene climatic optimum*, to a combination of predictable eccentricities in the orbit of the Earth called Milankovitch cycles that reoccur over many thousands of years.

the effects of this warm period were greatest in the northern hemisphere, palynological studies have suggested that the southern hemisphere was warmer during this period as well (Crawford 1992). During this time, Asian rice (*Oryza sativa*) also begins appearing in the archaeological record while broomcorn millet slowly falls out of use in regions south of Japan (Bellwood 1987; Castillo and Fuller 2010).

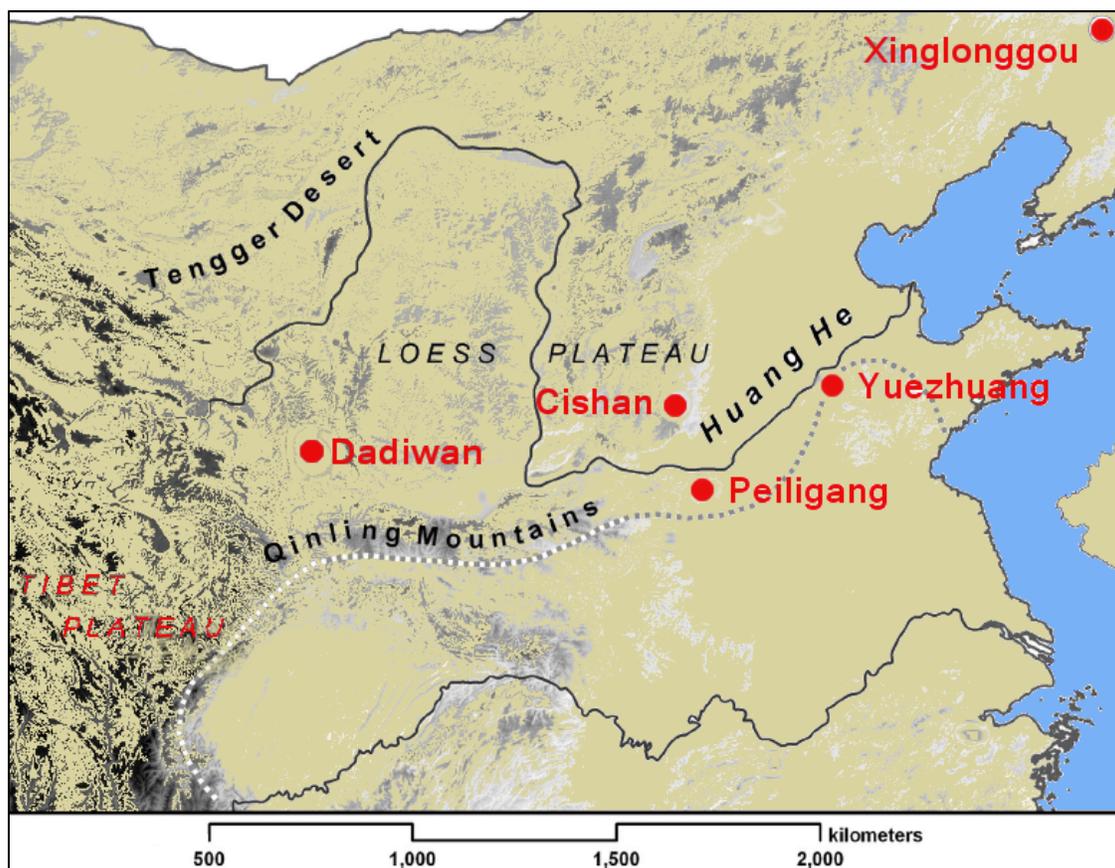


Figure 24. Locations of Chinese Middle Neolithic archaeological sites (adapted from Barton et al. 2009).

Whether the *scrofa-italica-sativa* domestication package that appeared in Southeast Asia and parts of Island Southeast Asia in the ensuing millennia was the result of population diffusion through Taiwan or Malaysia is contested. The “out of

Taiwan” model, asserts that people living in Taiwan during the Early Neolithic, often referred to as Proto-Austronesians, began moving southwards through the Philippines, and the Indonesian Islands with domesticated pigs, foxtail millet, rice, and Chinese sugarcane (*Saccharum sinense*), while replacing indigenous hunter-gatherer groups along the way (Glover 1976; 1977; Bellwood 1987, 2001, 2005; Chang 1996; Daniels and Daniels 1993) (Route 1, Figure 25). This model is supported by archaeological, linguistic, and genetic evidence (Bellwood and Hiscock 2005; Bellwood 2005; Xu et al. 2012). The alternative model, advocated by researchers such as Barton et al. (2009) and Solheim (2000; 2006) posits that agriculture arose in Island Southeast Asia independently. In this view, cultigens and domestic animals were circulated through Island Southeast Asia by means of the Nusantara maritime trading network that eventually expanded northwards, and domesticated pigs, as well as crops such as millet and rice may have been incorporated into this trade network from the north via the Malay Peninsula (Route 2, Figure 25). Though this model is a more recent development, there is some archaeological evidence to support it (Piper et al. 2009).

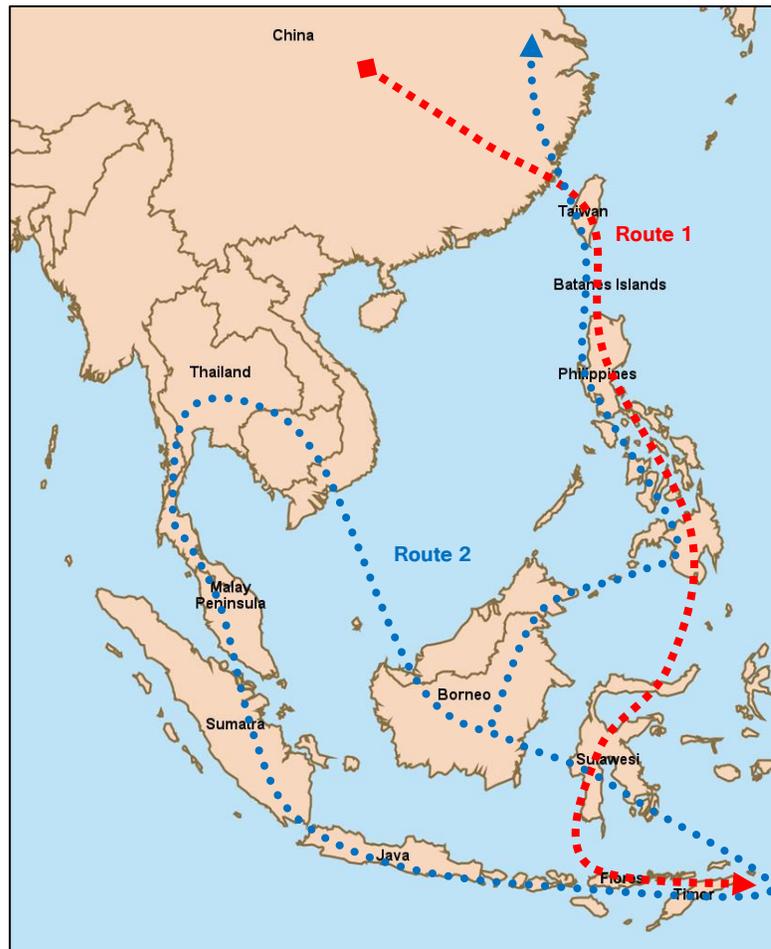


Figure 25. Proposed routes of domesticated plants and animals into Island Southeast Asia.

In any case, Eurasian pigs, foxtail millet, Asian rice, and Chinese sugarcane begin appearing at archaeological sites in progressively southern latitudes over the next three millennia. The earliest evidence for any of the four outside of China is between 5500 and 4800 yBP on the island of Taiwan, at sites associated with the Tapenkeng culture (Tsang 2005; Sagart 2008). Excavations have also established the development of a trade relationship between populations on Taiwan and the Batanes islands by approximately 4200 yBP (Bellwood and Dizon 2005; Bellwood 2008;

Bellwood and Oxenham 2008). This relationship involved the exchange of red-slipped and cord-marked pottery, slate knives, nephrite-jade adzes, and domesticated pigs and rice (Hung 2004; Bellwood 2011). There is also some indication that this trade relationship extended to the Philippine islands: by 4200 yBP, *S. scrofa* appears in multiple archaeological sites in the Philippines (Piper et al. 2009; Amano 2011). No evidence of millet has been found on either the Batanes or Philippine islands from this period, and whether this reflects the situation of domestication during this time, or is simply the result of an incomplete archaeological record is not currently known. There is also no evidence of Chinese sugarcane any farther south than Taiwan.

The “out of Taiwan” model becomes more complex after this point. Assuming that the Austronesian agricultural populations in the Philippines continued dispersing directly south, the next two islands that they would have encountered are Borneo and Sulawesi. Currently, there is no evidence of *S. scrofa* on Borneo, suggesting that the agricultural Austronesian population possibly circumvented Borneo in favor of Sulawesi, or the Moluccas. Barker and Richards (2012) instead suggest that the absence of *S. scrofa* and *S. italica* on Borneo indicates that both species reached their terminus in the Philippines. Additionally, the only other evidence for foxtail millet in Island Southeast Asia comes from Glover (1977: 43), who reports finding “one possible specimen” of foxtail millet in strata dating to less than 5000 yBP at the Uai Bobo 2 archaeological site on Timor. Timor is farther east along the Indonesian Island chain than is Flores, suggesting a possible route from the Philippines to the Moluccas to the Lesser Sunda Islands (Tryon 2006). During this period *S. scrofa* also appears in the archaeological

record on Flores, Timor, and the Northern Moluccas (Larson et al. 2007). According to the “Nusantao trade network” hypothesis, the semi-domesticated Eurasian pigs that appear on Flores at 4000 yBP are part of a “Pacific clade” that arrived via the Malay Peninsula instead of China. Large-scale haplotypic analyses also support this hypothesis (Larson et al. 2005; Larson et al. 2007; Dobney et al. 2008; Larson et al. 2009).

Isotopic Evidence for Subsistence Changes on Flores

Bone Collagen

The results of the analysis of the bone collagen samples suggest that there were significant changes in the diet of pigs living on Flores during the past 5160 yBP. Specifically, there is was an overall enrichment in ^{13}C with an associated decrease in ^{15}N that occurred relatively quickly at some point between 5160 and 2750 yBP. There are two possible scenarios that explain such a shift, both related to the introduction of domesticated pigs (*Sus scrofa*) onto the island by humans around 4000 yBP (Larson et al. 2005; Larson et al. 2007; Larson et al. 2009). In the first scenario, human populations reached Flores with domesticated pigs but did not bring any cultigens with them. Possibly as a result of their arrival, indigenous hunter-gatherer populations who also practiced small-scale cultivation of indigenous C_3 plants began clearing portions of the forest to accommodate increased population densities. Evidence for this scenario comes from Krigbaum (2003), who analyzed the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of tooth enamel in 36 adult humans at three different Neolithic archaeological sites in Borneo and like

this study, reported a slight increase in $\delta^{13}\text{C}$ values over time. Krigbaum (ibid.) suggests that because of the humid tropical climate of Borneo, humans would have had difficulty cultivating C_4 crops, such as foxtail millet, on any significant scale. Instead, he attributes enrichment in ^{13}C to the development of “open forest horticulture” (Krigbaum 2003: 301). This is known as the “canopy effect”, in which plants growing on the floor of a forest are depleted in ^{13}C relative to the plants that extend to the top of the canopy. This is due to decreased ventilation near the soil surface, as well as decreased photosynthetic activity due to decreased access to sunlight (Drucker 2008). When forests are cleared (presumably for the purpose of horticulture), the plants growing on the forest floor are able to take in more atmospheric carbon, and their levels of photosynthetic activity increase. If forest clearing occurred on Flores, as Krigbaum (2003) suggests it did on Borneo, then the enrichment in ^{13}C observed in the pig collagen samples would not necessarily be the result of C_4 cultivation, but of the elimination of the canopy effect. The forest horticulture hypothesis might also explain why depletion in ^{15}N is observed over time in both bone and dentin collagen. Land clearing increases the effects of erosion by removing natural barriers to processes such as slope wash that carry away nutrients and minerals in the topsoil. Continually growing a single crop in the same area can also lead to the depletion of soil nitrogen.

A major difference between Borneo and Flores, however, is that *S. scrofa* was never introduced onto Borneo during the Neolithic. Some studies have suggested that Borneo was something of an outlier, and may have been bypassed by migrating agricultural populations due to its location on the equator (Bellwood 2011). As

Krigbaum (2003) noted, it would have been difficult to grow crops here that were domesticated on the mainland because of the hot and humid climate. Additionally, the enrichment in ^{13}C on Borneo observed by Krigbaum (ibid.) is 1.2‰, and there is a ~4000-year hiatus between the two samples. Studies related to the canopy effect have demonstrated that there is depletion in ^{13}C of ~3‰ relative to the C_3 average (-27‰) for plants in the understory of forests (Jackson et al. 1993; Quade et al. 1995; Yakir and Israeli 1995; Schoeninger et al. 1998). However, enrichment in ^{13}C observed in the pig bone collagen samples from Liang Bua is much greater, around 7.36‰, suggesting that there may have been factors other than the removal of the canopy effect that attributed to the pattern of ^{13}C enrichment. As discussed earlier in this section, the dominant pattern throughout East Asia is one in which cultigens and domesticated pigs are always associated with one another. A second hypothesis about the shift in the isotopic values of pigs is that the human population that first brought *S. scrofa* onto Flores also brought domesticated crops from the mainland with it. Native Floresians, who practiced hunting and gathering as well as small-scale horticulture with indigenous C_3 plants, were quickly assimilated into the culture of the Austronesians, and began practicing agriculture on a more intensive basis (Bellwood 1996).

Unfortunately, no archaeobotanical material has been found yet on Flores that would reveal which, if any, cultigens were introduced during this colonization event. However, assuming for the moment that the isotopic signatures of the pigs reflect the integration of C_4 plants into their diet, the possibilities can be narrowed down by again consulting the archaeological evidence for plant domestication in mainland Asia.

Like many of the islands in Southeast Asia, the climate of Flores is classified as tropical rainforest, which is characterized by perpetually high temperature and precipitation. This means that only certain cultigens, specifically those that can tolerate such conditions, can be grown successfully on the island. China is the only one of the three agricultural centers identified in Asia that contains regions with subtropical and tropical climates, which means that cultigens introduced from this region would be somewhat preadapted to the environment of Flores. Further, only a few of the major crops associated with the Asian agricultural centers utilize the C₄ photosynthetic passageway. Among these are sorghum (*Sorghum bicolor*), barnyard millet (*Echinochloa utilis*), broomcorn millet (*Panicum miliaceum*), Chinese sugarcane (*Saccharum sinense*), and of course, foxtail millet (*Setaria italica*). Sorghum is ruled out because the available archaeobotanical specimens are generally associated with agricultural centers in Japan, and are not found in China or elsewhere until the 13th century AD (Crawford 1992). Similarly, barnyard millet is usually associated with Jomon cultural material, and is not found in mainland or Island Southeast Asia. As discussed previously, broomcorn millet appears to have been dropped in favor of rice in areas south of Japan. Some researchers, including Lebot (1999), Bird et al. (2004), and Anderson (2011), have suggested that sugarcane (*Saccharum sp.*) could have been independently domesticated within Wallacea, or introduced as part of a westward migration of domesticated crops out of Papua New Guinea around 3500 kyBP. However, there is no reliable archaeological or archaeobotanical indication of its presence within Wallacea during the Neolithic (Daniels and Daniels 1993). The isotopic

evidence from this study also suggests that though sugarcane could have played a role in the diet of pigs living on Flores, it did not contribute to their $\delta^{13}\text{C}$ signals. Relative to other C_4 plants, the protein content of sugarcane is very low, around 0.4g per 100g compared to estimates of around 12g per 100g in foxtail millet (Leach et al. 1996; Valentin et al. 2006). As the $\delta^{13}\text{C}$ values of bone collagen are derived from the protein component of diet, sugarcane would have made very little, if any, contribution to the carbon isotopic signals of the pig bone collagen (Ambrose and Norr 1993; Tieszen and Fagre 1993; Saunders et al. 1997; Valentin et al. 2006). This means that the enrichment in ^{13}C across geological time observed in the collagen samples must be from some other C_4 plant source.

Beyond the significant amount of evidence for it being cultivated as far south as the Philippines, and perhaps farther still in Timor, there are also a few practical reasons why foxtail millet could instead be responsible for the changes in the isotopic ratios of the pigs. Foxtail millet is hardy, and quite tolerant of different soil and climatic conditions. The direct progenitor of *S. italica* is the common summer weed *Setaria viridis*, which has simple soil requirements and is capable of yielding grain before having to grow very tall, a trait which it passed on to *S. italica* (Zohary and Hopf 2000; Brutnell et al. 2010). It can also be consumed by humans and livestock, as it was in many of the Neolithic sites in China. These traits would have made foxtail millet a good choice for humans introducing cultigens into new areas. Flores is also about as far south of the equator as the Philippines are north (see Figure 1), which suggests that it could have been successfully grown there, as it was on the Philippines.

Interestingly, the $\delta^{13}\text{C}$ values of the bone collagen sample in the stratigraphic layer closest to the ground surface at Liang Bua (30 cm) appears to shift back towards depletion in ^{13}C (Figure 7, Results section). While this layer is only represented by a single long bone fragment, the pattern is consistent with the $\delta^{13}\text{C}$ values of dentin collagen and enamel carbonate also present. This layer dates to 140 yBP, well after the time of contact with Portuguese traders and missionaries, and may indicate a shift towards the adoption of rice (*Oryza sativa*), which is a staple of the modern Floresian farming economy.

Dentin Collagen

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the dentin collagen samples reiterate the trend observed in the bone collagen samples towards ^{13}C enrichment and ^{15}N depletion over time. When compared to the isotopic values of bone collagen, these data also reveal potentially interesting, but currently uncertain information about differences in isotopic fractionation and rate of turnover between bone and dentin collagen.

Enamel Carbonate

$\delta^{13}\text{C}$ Values of Enamel Carbonate Samples

Analysis of the $\delta^{13}\text{C}$ values of tooth enamel carbonate, like the collagen samples, suggest that the diets of pigs living on Flores became more enriched in ^{13}C over geological time. However, relative to the $\delta^{13}\text{C}$ values of bone collagen from the same levels, the $\delta^{13}\text{C}$ values of tooth enamel are all enriched by approximately 7‰. This may be the result of the “trophic level effect” (Schoeninger and DeNiro 1984). When

consuming breast milk, the isotopic values of organisms are more enriched than those of their mothers because they are consuming their mothers' tissue, and are therefore one trophic level higher than they are. Most researchers report this pattern in $\delta^{15}\text{N}$ values exclusively (Dupras et al. 2001; Dupras and Tocheri 2007). However, Richards et al. (2002) also report the presence of the trophic level effect in the $\delta^{13}\text{C}$ values of infants relative to their mothers. Like many mammals, the tooth development of domesticated pigs (*S. scrofa*) is staggered. Adult M1s begin forming *in utero*, while M2s and M3s begin forming around 1 to 2 months, respectively (Magnell and Carter 2007). Crown formation of adult M1s is completed around 2 to 3 weeks after birth, while crown formation of the M2s and M3s is completed around 6 to 7 and 12 to 13 weeks after birth, respectively (Magnell and Carter, *ibid.*). Weaning begins around 4 to 5 weeks after birth as the piglet begins to incorporate solid food, and is completed between 8 and 17 weeks after birth (Newberry and Wood-Gush 1985; Jensen 1986; Jensen and Recen 1989; D'Eath and Turner 2009). Because of this, the $\delta^{13}\text{C}$ values of the tooth enamel carbonate samples likely reflect the fact that the pigs were still suckling during the formation of their adult dentition.

$\delta^{18}\text{O}$ Values of Enamel Carbonate Samples

Analysis of the $\delta^{18}\text{O}$ values in the enamel carbonate samples from Liang Bua reveals that, for the most part, $\delta^{18}\text{O}$ values did not vary significantly over geological time ($M = -4.77\text{‰}$, $SD = 1.12\text{‰}$). This supports the idea that Eurasian pigs quickly became an important part of the island ecosystem, and were bred and raised on Flores instead of being continuously imported from other islands as part of a trade route.

Relation of Bone Collagen Results to Previous Research on Flores

Previous research by Anderson (2011) examined the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen in several species of rat (*Papagomys sp.*, *Spelaeomys florensis*, *Komodomys rintjanus*, *Floresomys naso*, *Rattus exulans*) from sector XVI at Liang Bua. Anderson (ibid.) also identifies a significant shift in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the bone collagen samples starting at a depth of 110 cm, which also dates to between 5160 and 2750 yBP. Throughout the stratigraphic series, the $\delta^{13}\text{C}$ values of the rat bone collagen range from -11.14‰ to -21.19‰. These values, down to a depth of 280 cm, are plotted alongside the $\delta^{13}\text{C}$ values of the pig collagen in Figure 26. The most noticeable aspect of Figure 26 is the observed range of variation in the rat bone collagen samples. This is probably a function of the large sample size, but it may also have to do with dietary differences between the different species of rats included in Anderson's (ibid.) analysis. However, in both the rat and pig bone collagen samples, the general trend is towards enrichment in ^{13}C over time. Additionally, it appears that rats began experiencing enrichment in ^{13}C somewhat earlier than earlier than pigs did, at 120 cm. This could be due to the fact that no pig bones from spit 12 were analyzed, but it might also suggest that rats were able to access C_4 plant material sooner than the pigs did. This is expected given the fact that rats are opportunistic omnivores. If humans began cultivating foxtail millet or other C_4 plants on Flores, it would likely take some time to build up enough of a surplus to begin using it as feed for pig herds. However, rats could have gained immediate access to any food crops being grown or stored, and their isotopic signals would have reflected the incorporation of these materials.

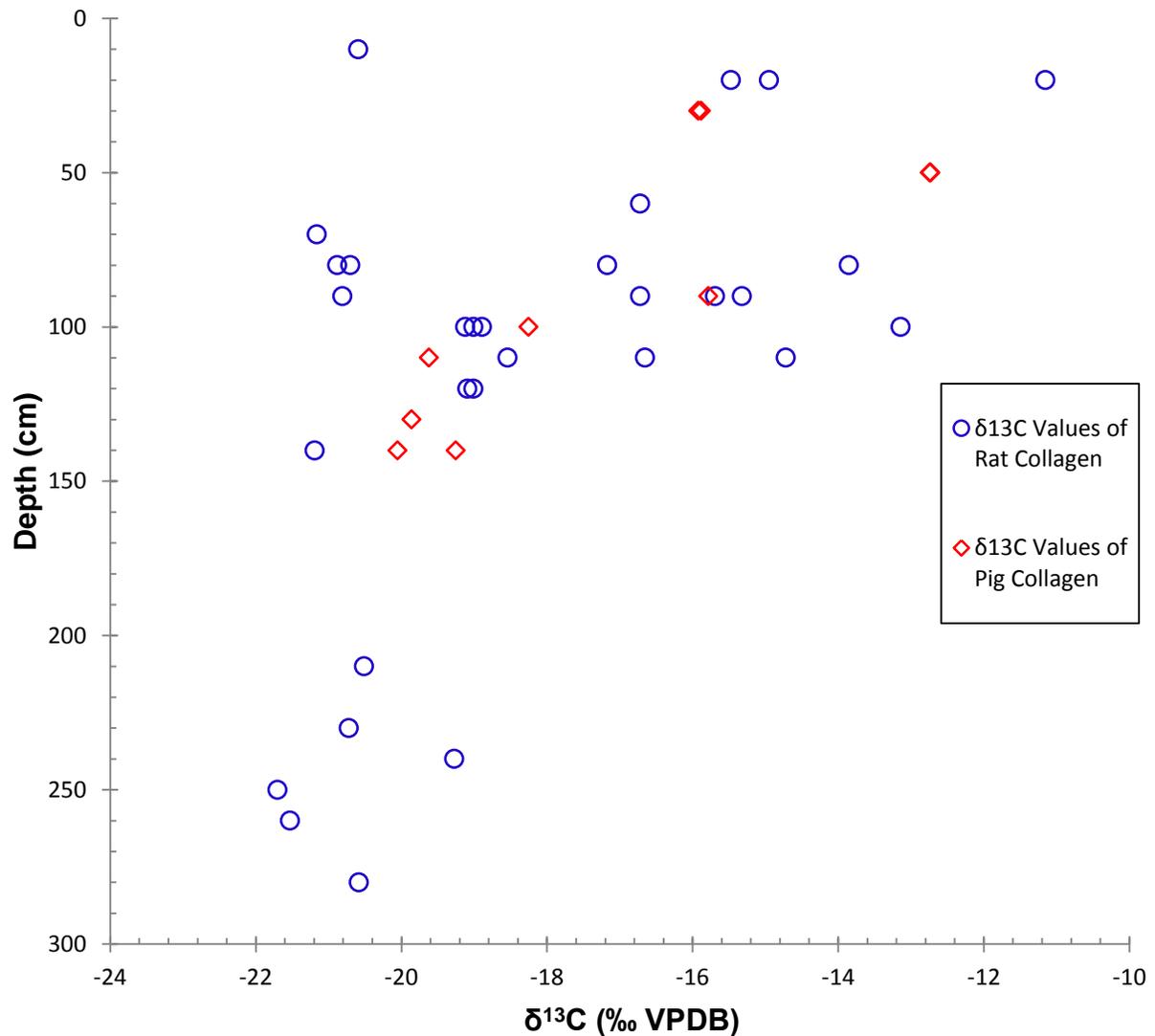


Figure 26. $\delta^{13}\text{C}$ values of rat and pig bone collagen samples at Liang Bua versus depth.

The $\delta^{15}\text{N}$ values of the rat bone collagen range from -1.56‰ to 9.73‰ (Anderson 2011). These values, down to a depth of 280 cm, are plotted alongside the $\delta^{15}\text{N}$ values of the pig collagen in Figure 27. Again, there is some degree of scattering in the $\delta^{15}\text{N}$ values of the rat bone collagen samples, probably due to the large size of the sample,

or because of differences in the subsistence activity of different rat species. However, the general trend of both data sets is towards depletion in ^{15}N over geological time.

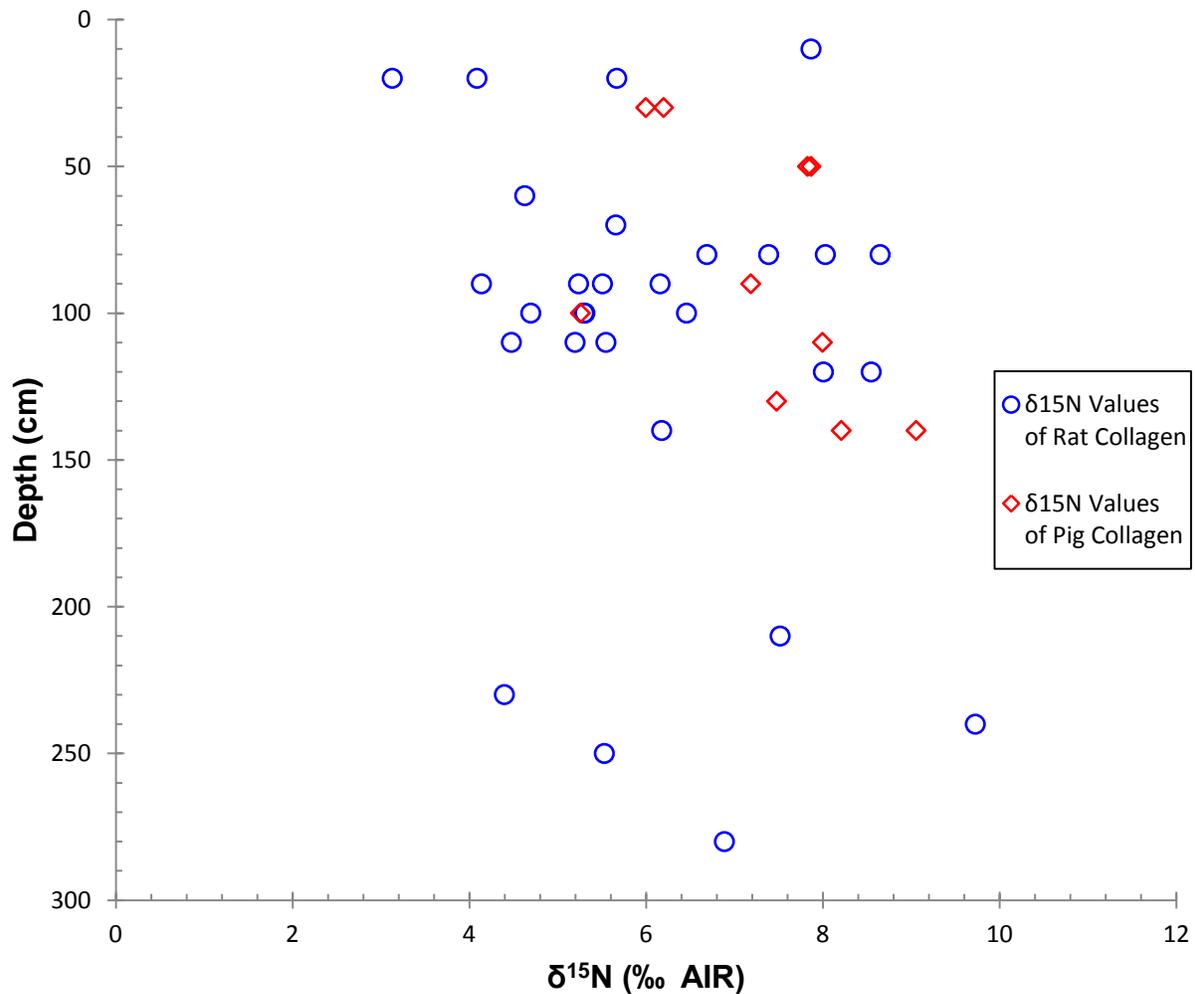


Figure 27. $\delta^{15}\text{N}$ values of rat and pig bone collagen samples at Liang Bua versus depth.

The concurrence of the rat and pig bone collagen isotope values supports Anderson's (2011) results, and suggests that the changes on Flores during this time were widespread, affecting the diets of species living in multiple ecological niches.

Summary

As the data demonstrate, there was a distinct shift in the isotopic values of pigs living on the island of Flores during the past 5160 yBP. In all likelihood, this is attributable to the introduction of cultigens onto the island at some point between 5160 and 2750 years ago that utilize the C₄ photosynthetic pathway. Based on its presence in other archaeological sites in Island Southeast Asia, the most likely candidate is foxtail millet (Piper 2009; Bellwood 2011; Barker and Richards 2012). It is also possible that the shift had less to do with the introduction of an agricultural package from the Asian mainland, and more to do with changes in indigenous farming techniques and the elimination of the “canopy effect”, as Krigbaum (2003) suggests occurred on Borneo. However, given the small degree to which the canopy effect affects $\delta^{13}\text{C}$ values, as well as the close association between domesticated pigs and cultigens in Asia, this was probably not the main factor behind ¹³C enrichment in pigs on Flores. Interestingly, the isotopic values from the collagen samples used in this study suggest that, at all stratigraphic levels, there is at least some amount of C₄ plant matter being consumed by pigs (Results section, Figure 8). This could be attributed to the pigs ingesting small quantities of tropical C₄ weeds or grass that may grow on Flores while grazing, though there is no direct evidence to support this.

CHAPTER 6: CONCLUSION

The reconstruction of past human activity in Southeast Asia and Island Southeast Asia remains a challenging puzzle for archaeologists to solve. In the past 70,000 years, this region has witnessed the arrival and departure of multiple groups of humans, flora, and fauna. This study presents the results of an analysis of the isotopic values of bone and teeth belonging to individuals of genus *Sus* that lived on the island of Flores throughout the past 5160 years. The isotopic data from this study suggest that at some point between 5160 and 2750 yBP, there was an abrupt shift towards enrichment in ^{13}C and depletion in ^{15}N , while $\delta^{18}\text{O}$ values remain stable, a finding supported in part by the results of Anderson (2011). There are two possible explanations for this pattern, both of which involve the arrival of semi-domesticated Eurasian pig (*Sus scrofa*) onto Flores, which the genetic evidence suggests took place around 4000 yBP, as well as a shift in subsistence patterns (Larson et al. 2005; Larson et al. 2007; Larson et al. 2009). In both cases, humans would have been responsible for the arrival of pigs on Flores, since it would have been nearly impossible for these pigs to arrive on the island unassisted. The $\delta^{18}\text{O}$ values suggest that once *S. scrofa* appeared on Flores, they became an important part of the island ecosystem, and were continually bred and raised on Flores.

In one scenario, humans intentionally introduced *S. scrofa* onto the island, and at the same time began clearing swaths of forest in order to intensify the cultivation of indigenous C_3 crops. Because of the “canopy effect”, C_3 crops grown in the forest understory before the forests were cleared would be slightly depleted in ^{13}C , and this depletion would be reflected in the $\delta^{13}\text{C}$ values of pigs from deeper strata (van Klinken

1999; Drucker et al. 2008). After *S. scrofa* arrived and the clearing of forests occurred, crops, and consequently pigs, may have become enriched in ^{13}C . The concomitant depletion in ^{15}N could be the result of exhaustion in soil nitrogen associated with land clearing and erosion. There is some evidence for the forest-clearing scenario occurring on the nearby island of Borneo (Krigbaum 2003).

However, a major difference between Borneo and Flores is that *S. scrofa* was likely not introduced onto Borneo during the Neolithic (Krigbaum 2003; Barker et al. 2007; Barker and Richards 2012). Archaeological evidence from Neolithic sites in other areas of mainland and Southeast Asia supports the idea that there was a close association between domesticated pigs and domesticated crops, such that the arrival of one is usually indicative of the arrival of the other. Additionally, the enrichment in ^{13}C observed in the pig bone collagen samples is greater than that typically observed with the removal of the canopy effect. A more likely scenario is that humans introduced *S. scrofa* onto Flores along with C_4 cultigens brought via the “out of Taiwan” route. If this was the case, the shift towards enrichment in ^{13}C suggests that the cultigen introduced onto the island was a C_4 plant, which in all likelihood, was foxtail millet (*Setaria italica*). Though direct evidence of prehistoric foxtail millet has not yet been found on Flores, there is some suggestion that it was present on Timor, which is directly east of Flores, in stratigraphic layers less than 5000 years old (Glover 1977). The shift towards ^{15}N depletion in the pigs could again be caused by erosion and land use associated with agriculture, or it could reflect a decrease in dietary ^{15}N values associated with increasing

reliance on foxtail millet, which is depleted in ^{15}N relative to C_3 plants (DeNiro and Epstein 1981).

In any case, further resolution of subsistence changes on Flores, as well as Island Southeast Asia as a whole, will require continued research in both the field and the lab. Moving forward, there are two lines of potentially important evidence that are undeveloped. The first is the creation of an island-wide food web that incorporates the data from this study, as well as from Anderson (2011) with isotopic data from other mammals living on Flores during the Holocene. The second is the creation of an oxygen isotope index derived from meteoric waters on Flores and other locations in Island Southeast Asia. Both have the potential to reveal more data about the complex and historical relationships between geography, humans, plants, and animals.

APPENDIX A: BONE SAMPLE DATA

Sample ID	Date excavated	Sector	Spit	Depth (cm)	Dry bone weight (g)	Bone
LB-1B	8/5/2008	xvi (16)	3	30	3.942	Fragmentary long bone
LB-2B	8/5/2008	xvi (16)	5	50	1.300	Phalanx
LB-4B	8/8/2008	xvi (16)	9	90	6.588	Fragmentary radius
LB-5B	8/9/2008	xvi (16)	10	100	1.086	Intermediate phalanx
LB-6B	8/9/2008	xvi (16)	11	110	1.211	Fragmentary metatarsal
LB-7A	8/12/2008	xvi (16)	13	130	4.127	Fragmentary calcaneus
LB-8C	8/13/2008	xvi (16)	14	140	7.092	Fragmentary distal humerus
LB-8D	8/13/2008	xvi (16)	14	140	6.062	Fragmentary radius

APPENDIX B: BONE COLLAGEN DATA

Sample ID	Empty vial weight (g)	Vial weight with collagen (g)	Collagen yield (g)	C:N ratio (Atomic)	wt% C	wt% N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
LB-1B-1	9.978	10.404	0.426	3.25	45.50	16.31	-15.88	6.20
LB-1B-2	4.725	4.861	0.136					
LB-2B-1	9.974	10.083	0.109	3.26	43.60	15.63	-12.73	7.87
LB-2B-2	4.668	4.711	0.043					
LB-4B	11.514	11.694	0.180	3.28	33.21	11.79	-15.78	7.19
LB-5B	6.152	6.203	0.051	3.21	42.37	15.40	-18.25	5.26
LB-6B	6.174	6.184	0.010	3.32	31.07	10.93	-19.62	8.00
LB-7A-1A	6.058	6.120	0.062					
LB-7A-1B	6.101	6.109	0.008	3.30	35.90	12.71	-19.86	7.48
LB-7A-2	11.459	11.482	0.023					
LB-8C	11.654	11.675	0.021	3.38	36.42	12.59	-19.25	9.06
LB-8D	11.522	11.641	0.119	3.31	30.59	10.77	-20.05	8.21

APPENDIX C: TOOTH SAMPLE DATA

Sample ID	Date excavated	Sector	Spit	Depth (cm)	Dry whole tooth weight (g)	Tooth
LB-1A	8/5/2008	xvi (16)	3	30	1.826	Lower I2
LB-2A	8/5/2008	xvi (16)	5	50	5.370	Lower M3
LB-3A	8/6/2008	xvi (16)	8	80	3.474	Lower M3
LB-3B	8/6/2008	xvi (16)	8	80	1.998	Lower I2
LB-4A	8/8/2008	xvi (16)	9	90	1.750	Lower I2
LB-5A	8/9/2008	xvi (16)	10	100	0.560	Lower I2
LB-6A	8/9/2008	xvi (16)	11	110	5.139	Lower M3
LB-8A	8/13/2008	xvi (16)	14	140	5.035	Molar
LB-8B	8/13/2008	xvi (16)	14	140	1.720	Premolar

APPENDIX D: DENTIN COLLAGEN DATA

Sample ID	Dry dentin weight (g)	Empty Vial Weight (g)	Vial weight with collagen (g)	Collagen yield (g)	C:N ratio	wt% C	wt% N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
LB-1A-1	1.496	4.597	4.650	0.053	3.26	44.93	16.06	-20.04	6.78
LB-1A-2		4.635	4.724	0.089					
LB-2A-1	1.470	9.945	9.991	0.046	3.29	42.49	15.10	-15.73	7.93
LB-2A-2		9.937	9.997	0.06					
LB-3A	0.878	11.495	11.541	0.046	3.28	36.55	12.99	-19.42	7.76
LB-3B-1		11.422	11.43	0.008					
LB-3B-2	1.428	11.424	11.582	0.158	3.30	38.47	13.34	-18.92	7.62
LB-3B-3		9.904	9.918	0.014					
LB-3B-4		9.895	9.935	0.04					
LB-4A-1	1.153	11.48	11.537	0.057	3.60	32.89	10.57	-19.69	7.68
LB-4A-2		11.478	11.588	0.11					
LB-5A	0.296	11.492	11.574	0.082	3.30	43.75	15.45	-16.83	8.24
LB-6A-1	1.204	11.478	11.513	0.035	4.75	18.41	3.98	-20.89	8.06
LB-6A-2		11.514	11.523	0.009					
LB-8A	0.546	11.465	11.491	0.026	3.33	36.49	12.80	-20.29	9.38
LB-8B	0.890	11.519	11.522	0.003	3.30	35.84	12.67	-20.34	8.99

APPENDIX E: ENAMEL CARBONATE DATA

Sample ID	Dry enamel weight (g)	Empty vial weight (g)	Vial weight with carbonate (g)	Carbonate yield (g)	$\delta^{18}\text{O}$ (‰) [VPDB]	$\delta^{18}\text{O}$ (‰) [VSMOW]	$\delta^{13}\text{C}$ (‰)
LB-1A	1.748	4.747	4.765	0.018	-5.83	24.92	-12.79
LB-2A	1.615	4.677	4.686	0.009	-4.26	26.53	-9.27
LB-3A	1.694	4.675	4.688	0.013	-5.18	25.59	-11.90
LB-3B	1.994	4.659	4.675	0.016	-2.93	27.89	-11.81
LB-4A	1.800	4.636	4.646	0.010	-4.17	26.62	-11.66
LB-5A	1.976	4.584	4.601	0.017	-6.87	23.86	-11.05
LB-6A	1.774	4.693	4.707	0.014	-4.27	26.52	-13.75
LB-8A	1.949	4.595	4.607	0.012	-4.96	25.81	-13.11
LB-8B	1.846	4.716	4.730	0.014	-4.53	26.26	-13.00

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