STABLE CARBON AND NITROGEN ISOTOPE ANALYSES OF SUBFOSSIL RATS FROM LIANG BUA (FLORES, INDONESIA)

by

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B.S. Florida State University, 2009

A thesis submitted in partial fulfillment of the requirements
for the degree of Masters of Arts
in the Department of Anthropology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2011

ABSTRACT

This research study investigated the level of bone collagen preservation of rat femora from Liang Bua cave on the island of Flores, Indonesia, as well as conducted carbon and nitrogen stable isotopic analyses on well preserved samples. Although Flores is located in a hot intermediate tropical zone and the burial environment of the bone samples within the cave is considered less than optimal for collagen preservation, significant preservation of the bone collagen was found. Collagen yields, C:N ratio and carbon and nitrogen concentrations were investigated. However, this research study argues that carbon and nitrogen concentrations are an appropriate means to determine preservation on its own. According to the carbon and nitrogen concentration data 32 samples were considered well preserved. Carbon and nitrogen stable isotopic analyses were conducted on the 32 preserved samples. According to the carbon data a significant shift in the $\delta^{13}C$ values from a C_3 signature to a C_4 signature occurred prior to 2,750 years ago. This shift is indicative of the introduction of a non-endemic C_4 plant, which is believed to be either foxtail millet or sugarcane. Since this shift occurs abruptly it indicates that the introduction of agriculture to Flores occurred at one time and has continued to present day.

Dedicated to Mom and Dad

ACKNOWLEDGMENTS

I would like to thank my Mom and Dad for always supporting me and encouraging me to follow my dreams. Thank you for all of your endless love throughout the years and for being such amazing parents. I would also like to thank my siblings, Meghann, Josh and Ben. You guys are more than siblings to me, you are my close friends and I am very proud of all of your accomplishments. I would like to thank my fiancé and best friend, Anthony, for always being there for me, for putting up with the stresses that come with graduate work and reminding me that getting a master's degree is supposed to be difficult. I would like to thank my committee members, Dr. Tosha Dupras, Dr. Matthew Tocheri and Dr. John Schultz for supporting me during my Master's degree. I would like to especially thank my thesis committee chair, Dr. Durpas, for being so understanding and giving me so much guidance throughout the thesis writing process. I would like to thank the National Research and Development Centre for Archeology in Indonesia (ARKENAS), its director, Dr. Tony Djubiantono, and the Liang Bua Team (Thomas Suitkina, Wayhu Saptomo, Jatmiko, Rhokus Due Awe, Sri Wasisto, Kompyang, Dr. Matt Tocheri, Dr. Michael Morwood). I would like to thank the Smithsonian Isotope Lab Smithsonian Institution OUSS-MCI Stable Isotope Mass Spectrometry Facility and Dr. Christine France. This work was funded in part by a Smithsonian Small Grants Award to Dr. Matt Tocheri. Lastly, I would like to thank my two closest companions, Oliver and Sadie, for providing me with unconditional love and a comforting furry hug during the most difficult parts of my master's degree.

TABLE OF CONTENTS

LIST OF FIGURES viii

LIST OF TABLES ix

CHAPTER 1: INTRODUCTION 1

Research Objectives 5

Literature Cited 7

CHATPER 2: PRESERVATION OF BONE FROM LIANG BUA, FLORES 11

Introduction 11

Site Formation Background 12

Preservation within Liang Bua Cave 15

Diagenesis 17

Collagen Yields 18

Carbon and Nitrogen Concentrations 20

C:N Ratios 21

Materials and Methods 22

Results 24

% Collagen 25

C:N Ratio 28

Nitrogen Concentration 29

Carbon Concentration 30

Discussion 31

Conclusion 34
Literature Cited 38
CHAPTER 3: USING CARBON AND NITROGEN STABLE ISOTOPES TO INTERPRET DIETARY BEHAVIOR OF LIANG BUA RATS 42
Introduction 42
Rats of Flores 43
Stable Isotopes 44
Fractionation 45
Carbon 47
Nitrogen 48
Collagen 50
Early Agriculture in Island Southeast Asia 51
Materials and Methods 56
Results 58
Carbon 60
Nitrogen 62
Discussion 62
Conclusion 65
Literature Cited 66
CHAPTER 4: CONCLUSIONS 71
Literature Cited 73

APPENDIX: DATA FOR ALL OF THE SAMPLES USED IN THIS STUDY, INCLUDING: % COLLAGEN, WT % N, WT % C, AND C:N RATIOS 74

LIST OF FIGURES

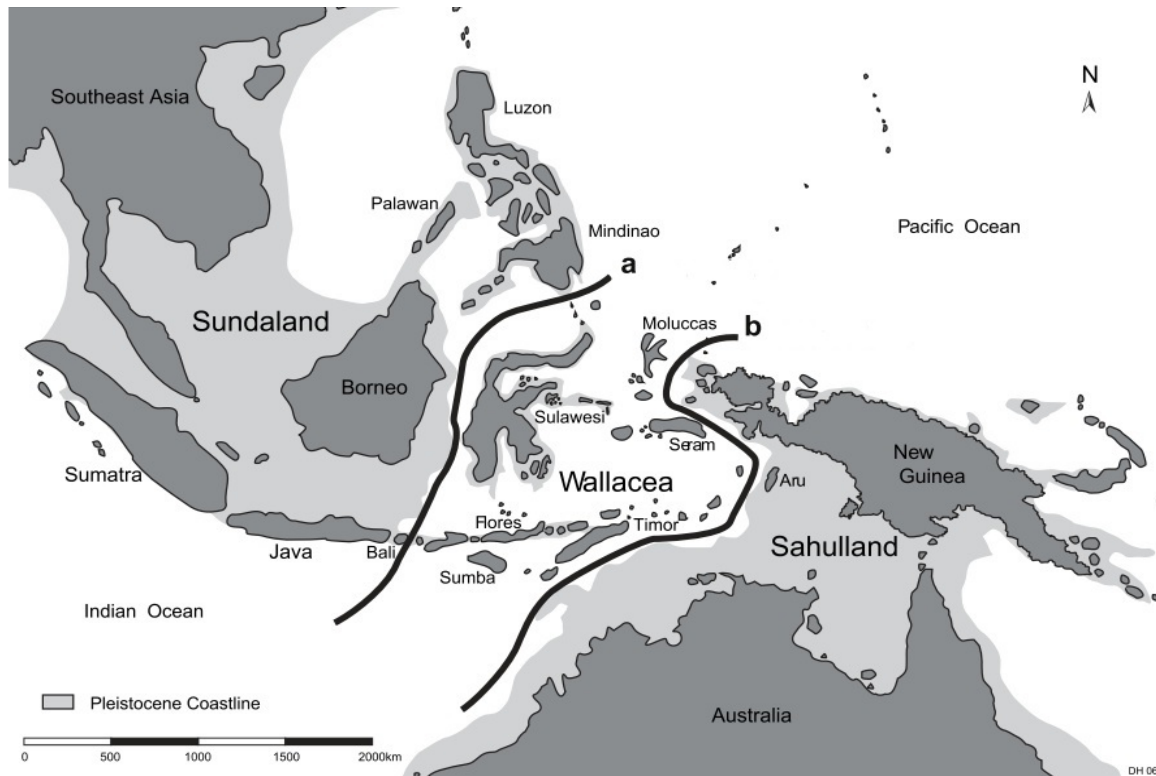
Figure 1. Location of Flores in Southeast Asia. (a) Wallace's Line and (b) Lydekker's Line are shown. 2
Figure 2. (a) Map showing the location ofwest Flores within the isalnds of Indonesia, located between mainland Southeast Asia and Austrailia. (b) Inset showing the location of the Liang Bua cave within the isalnd of Flroes. 13
Figure 3. Plan view of the Liang Bua cave site. Map depicts the larger front chamber and smaller rear chamber. 14
Figure 4. Graph showing % collagen vs. depth, including all collagen samples that yielded no measureable collagen. 24
Figure 5. Graph showing % collagen vs. depth. 26
Figure 6. Graph showing % collagen vs. depth including distinction between collagen yields of > 5%, 1-4% and < 1%. 27
Figure 7. Graph showing % collagen vs. depth including soil stratigraphy. Layers 7-9 are compoased of brownish silts and sandy silts. 28
Figure 8. Graph showing C:N ratio vs. depth. 29
Figure 9. Graph showing wt % N vs. depth. 30
Figure 10. Graph showing wt % C vs. depth. 31
Figure 11. Graph showing wt % N vs. collagen. Samples retaining appropriate wt % N values are outline by the solid red box. 32
Figure 12. Graph showing wt % C vs. % collagen. Samples that have retained appropriate wt % C values are outline by the solid red box. 33
Figure 13. Graph showing C:N ratio vs. % collagen. Samples retaining appropriate C:N ratios are outline by the solid red box. 34
Figure 14 (A) Carbon and nitrogen stable isotope analyses on Holocene dogs dated to 7,800 – 7,300 BC from North China indicate the presence of cultivated plants (Bettinger et al. 2010). (B) Pollen of the progenitor of foxtail millet, green bristlegrass found in North China dated to 12,000 years ago (Underhill 1997). (C) Single grain of foxtail millet from Timor dating to around 1,000 BC (Boomgard 2007). (D) Wad of sugarcane fiber found in an undated crevice (Horrocks et al. 2008). (E) Biomolecular evidence indicates the domestication of sugarcane in New Guinea (Lebot 1999). 52
Figure 15. Graph showing $\delta^{13}C$ values vs. depth. 60
Figure 16 Graph showing percentage of C4 plants in the diet using the average value for C4 plants of -12.5 61
Figure 17. Graph showing $\delta^{15}N$ values vs. depth. 62

LIST OF TABLES

Table 1 Data for 32 samples considered having well preserved collagen. 36
Table 2 Data for 32 samples considered well preserved including nitrogen and carbon isotopic values and the estimated percentage of C4 plants in the diet. 59

CHAPTER 1: INTRODUCTION

Flores is a small Indonesian island (14,000 km^2) located directly between mainland Southeast Asia and Australia (Figure 1). Throughout history, Flores has been continuously separated from the mainland by deep sea trenches known as Wallace's line and Lydekker's line (Morwood et al. 2009) (see Figure 1). Due to these deep sea trenches Flores has never been connected to either mainland even when water levels were low during glacial periods . Having always been surrounded by water, it would have been difficult for most Australian and Asian animals to successfully colonize Flores. This has resulted in Flores having an impoverished, although unique, island biogeography until the arrival of modern humans around 11,000 ya (Van den Bergh et al. 2009).



(Adapted from Morwood et al. 2009)
Figure 1. Location of Flores in Southeast Asia. (a) Wallace's Line and (b) Lydekker's Line are shown.

Archaeological excavations were first conducted on Flores in 1950 by Dutch priest Father Theodor Verhoeven (Morwood et al. 2009). Verhoeven excavated caves and limestone rock shelters throughout the island, including Liang Bua. In 1965, he returned to conduct more excavations at Liang Bua and found stone artifacts, faunal remains, and six Neolithic and Proto-Metallic age burials (Morwood et al. 2009). Beginning in 1978, R.P. Soejono followed Verhoeven's work at Liang Bua. Soejono excavated to a depth of 4.2 m, which has yielded a radiocarbon date of 10,000 BP (Morwood et al. 2009).

The most recent excavations at Liang Bua began in 2001 and are presently ongoing (Morwood et al. 2005; Morwood et al. 2004; Morwood et al. 2009). To interpret

the recent history of Southeast Asia, including studying the arrival of *Homo erectus* and modern *Homo sapiens* and the introduction of cultigens, domesticated animals, and new technology, excavations at Liang Bua took place as part of the "Astride the Wallace Line" project (Morwood et al. 2009). In September 2003, Liang Bua gained increased notoriety with the discovery of a partially complete hominin skeleton (LB1). Brown et al. (2004) proposed that the skeletal remains have taxonomically distinct characteristics and named a new hominin species, *Homo floresiensis*, using LB1 as the type specimen. Others, however, have argued that LB1 is a pathological anatomically modern *Homo sapiens* (Henneberg and Thorne 2004; Hershkovitz et al. 2007; Jacob et al. 2006; Kaifu et al. 2009; Martin et al. 2006; Obendorf et al. 2008; Oxnard et al. 2010; Richards 2006; Weber et al. 2005), although the majority of scientific evidence presented on the subject strongly supports *Homo floresiensis* as a valid taxon (Argue et al. 2006; Argue et al. 2009; Baab and McNulty 2009; Falk et al. 2009a; Falk et al. 2005; Falk et al. 2006; Falk et al. 2009b; Falk et al. 2007a; Falk et al. 2007b; Gordon et al. 2008; Jungers et al. 2009a; Jungers et al. 2009b; Larson et al. 2007b; Larson et al. 2009; Morwood et al. 2005; Tocheri et al. 2007).

Regardless of the debates over the taxonomy of the Late Pleistocene hominin remains from Liang Bua, there is no question that this cave preserves a unique faunal record which spans approximately the past 95,000 years (Van den Bergh et al. 2009). This extensive record includes, but is not limited to, the remains of Pleistocene fauna such as Stegodon (*Stegodon florensis*), Komodo dragon *(Varanus komodoensis*) and giant rats *(Papagomys armandvillei, Papagomys theodorverhoeveni and Spelaeomys florensis*)

(Morwood et al. 2009; Van den Bergh et al. 2009). Middle-sized rats from Flores include Komodomys and Floresomys (Musser 1981; Van den Bergh et al. 2009). The Polynesian rat (*Rattus exulans*) is found on Flores in the upper Holocene (Van den Bergh et al. 2009). The warty pig (*Sus celebensis*), Eurasian pig *(Sus scrofa*), and long-tailed macaque (*Macaca fascicularis*) were later introduced by modern humans during the Holocene between 7,000 and 4,000 years ago (Van den Bergh et al. 2009). The rats of Liang Bua are particularly interesting because they are found in large numbers and continuously throughout the stratigraphic sequence. The prevalence of the rat skeletal remains provides a prime opportunity to investigate levels of bone collagen preservation of the site, as well as examine changes to the local paleoecology through time. Reconstructing the diets of the Liang Bua rats may provide important new information about the ecology and environment surrounding Liang Bua during the past 95,000 years.

In this study, measures of diagenesis, and carbon and nitrogen stable isotope analyses of bone collagen from rat femora will be conducted. These analyses on samples recovered from throughout the stratigraphic sequence will make it possible to ascertain if any shifts occurred in available C_3 or C_4 plants, which would have made up a significant portion of the diet of the Liang Bua rats. In particular, the introduction of non-endemic C_4 cultigens such as foxtail millet will help discern when agriculturalists arrived. The archaeological record for early cultivation in Island Southeast Asia is very limited (Peregrine and Ember 2001). Only a small number of research projects on the introduction of agriculture to Island Southeast Asia have been conducted and a great deal of that research has yielded unreliable and unsecured dates (Meadow 1996).

The information on diet, such as possible plants consumed, can also help reconstruct the environment and habitat during the Pleistocene and Holocene.

The bone collagen stable isotope results will also make it possible to investigate the level of preservation of the collagen in the skeletal material. As evidenced from the stratigraphy of Liang Bua, the cave floor experienced intermittent pooling of water during seasonal monsoons throughout its prehistory (Westaway et al. 2009a). Today the Wae Racang River is located about 200 m away from and 30 m below the cave (Westaway et al. 2009b). However, until more recent times the Wae Racang River was located near the mouth of the cave and is thought to have periodically flooded the cave during the rainy season. Due to the cave's environment, its sedimentary soils are continually moist and damp. Preservation of skeletal material is greatly affected by the presence of groundwater in burial environments, especially when there is a recharging of fresh water (Hedges and Millard 1995). The rat bones could be affected by the varying amounts of water found present in the cave throughout its history. This type of analysis has not been previously conducted at Liang Bua and will indicate how well the rat bone collagen has survived.

Research Objectives

Using carbon and nitrogen stable isotope analyses, this research focuses on these rat bones in order to broadly 1) study the effects of stratigraphic position on collagen preservation in the site; and 2) reconstruct the diets of these rats and to use this information to make inferences about the surrounding ecology and environment. More specifically, this research aims to answer the following questions:

1. What are the effects of stratigraphic depth, unit, and geologic composition on the preservation of bone collagen within Liang Bua?
2. Is there evidence of dietary differences between rats of different body size?
3. Is there evidence of dietary change through time among the Liang Bua rats?
4. In what respects do the diets of the Liang Bua rats reflect human-induced changes to the surrounding ecosystem (e.g., the introduction of non-native plants and animals)?

This thesis will explore these research questions in the form of 4 chapters, including the introduction. Chapter 2 will specifically address issues of preservation in the Liang Bua cave. Chapter 3 will discuss the use of stable isotopes to interpret the diets of the Liang Bua rats. The possible introduction of new plants, shown by a shift in carbon, may help determine the timing of the arrival of agriculture onto the island of Flores. Lastly, chapter 4, the conclusion, will discuss and summarize the major findings of this research.

Literature Cited

Argue D, Donlon D, Groves C, and Wright R. 2006. *Homo floresiensis*: Microcephalic, pygmoid, Australopithecus, or Homo? J Hum Evol 51(4):360-374.

Argue D, Morwood MJ, Sutikna T, Jatmiko, and Saptomo EW. 2009. *Homo floresiensis*: a cladistic anlysis. J Hum Evol 57(5):623-639.

Baab KL, and McNulty KP. 2009. Size, shape, and asymmetry in fossil homins: the status of the LB1 cranium based on 3D morphometric analyses. J Hum Evol 57(5):608-622.

Falk D, Hildebolt C, Smith K, Jungers W, Larson S, Morwood MJ, Sutikna T, Jatmiko, Saptomo EW, and Prior F. 2009a. The Type Specimen (LB1) of *Homo floresiensis* Did Not Have Laron Syndrome. Am J Phys Anthropol 140(1):52-63.

Falk D, Hildebolt C, Smith K, Morwood MJ, Sutikna T, Brown P, Jatmiko, Wayhu Saptomo E, Brunsden B, and Prior F. 2005. The Brain of LB1, *Homo floresiensis*. Science 308:242-245.

Falk D, Hildebolt C, Smith K, Morwood MJ, Sutikna T, Brown P, Jatmiko, Wayhu Saptomo E, Brunsden B, and Prior F. 2006. Response to Weber et al.'s comment: "The brain of LB1, *Homo floresiensis*". Science:310, 246c.

Falk D, Hildebolt C, Smith K, Morwood MJ, Sutikna T, Jatmiko, Saptomo EW, and Prior F. 2009b. LB1's virtual endocast, microcephaly, and hominin brain evolution. J Hum Evol 57(5):597-607.

Falk D, Hildebolt C, Smith K, Morwood MJ, Sutikna T, Jatmiko, Wayhu Saptomo E, Imhof H, Seidler H, and Prior F. 2007a. Brain shape in human microcephalics and *Homo floresiensis*. Proceedings of the National Acadamy of Sciences 104:2513-2518.

Falk D, Hildebolt C, Smith K, and Prior F. 2007b. LB1's virtual endocast: Implications for hominin brain evolution. Faculty of Medicine Gadjah Mada University, Yogyakarta, Indonesia. 37-46 p.

Gordon A, Nevell L, and Wood B. 2008. The *Homo floresiensis* cranium (LB1): Size, scaling, and early *Homo* affinities. The National Academy of Sciences 105:4650-4655.

Hedges REM, and Millard AR. 1995. Bones and Groundwater: Towards the Modeling of Diagenetic Processes. J Archaeol Sci 22:155-164.

Henneberg M, and Thorne A. 2004. Flores human may be a pathological *Homo sapiens*. Before Farming 4:2-4.

Hershkovitz I, Kornreich L, and Laron Z. 2007. Comparative Skeletal Features Between *Homo floresiensis* and Patients with Primary Growth Hormone Insensitivity (Laron Syndrome). Am J Phys Anthropol 134:198-208.

Jacob T, Indriati E, Soejono RP, Frayer DW, Eckhardt RB, Kuperavage AJ, Thorne A, and Henneberg M. 2006. Pygmoid Australomelanesian *Homo sapiens* skeletal remains from Liang Bua, Flores: Population affinities and pathological abnormalities. Proceedings of the National Acadamy of Sciences 103:13421-13426.

Jungers WL, Falk D, Hildebolt C, Smith K, Prior F, Tocheri MW, Orr CM, Burnett SE, Larson SG, Djubiantono T and others. 2009a. The hobbits (*Homo floresiensis*) were not cretins. American Journal of Physical Anthropology, Supplement 48:124.

Jungers WL, Harcourt-Smith WEH, Wunderlich RE, Tocheri MW, Larson SG, Sutikna T, Due RA, and Morwood MJ. 2009b. The foot of *Homo floresiensis*. Nature 459(7243):81-84.

Kaifu Y, Baba H, Kurniawan I, Sutikna T, Saptomo EW, Jatmiko, Awe RD, Kaneko T, Aziz F, and Djubiantono T. 2009. Brief Communication: "Pathological" Deformation in the Skull of LB1, the Type Specimen of *Homo floresiensis*. Am J Phys Anthropol 140:177-185.

Larson SG, Jungers WL, Morwood MJ, Sutikna T, Jatmiko, Saptomo EW, Due RA, and Djubiantono T. 2007. *Homo floresiensis* and the evolution of the hominin shoulder. J Hum Evol 53(6):718-731.

Larson SG, Jungers WL, Tocheri MW, Orr CM, Morwood MJ, Sutikna T, Due Awe R, and Djubiantono T. 2009. Descriptions of the upper limb skeleton of *Homo floresiensis*. J Hum Evol 57:555-570.

Martin RD, MacLarnon AM, Phillips JL, and Dobyns WB. 2006. Flores hominid: New species or microcephalic dwarf? The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 288A(11):1123-1145.

Meadow RH. 1996. The Origin and Spread of Agriculture and Pastoralism in Northwestern South Asia. In: Harris DR, editor. The Origins and Spread of Agriculture and Pastoralism in Eurasia. Washington D.C.: Smithsonian Institution Press. p 390-412.

Morwood MJ, Brown P, Jatmiko, Sutikna T, Saptomo EW, Westaway KE, Due RA, Roberts RG, Maeda T, Wasisto S and others. 2005. Further evidence for small-bodied hominins from the Late Pleisotocene of Flores, Indonesia. Nature 437:1012-1016.

Morwood MJ, Soejono RP, Roberts RG, Sutikna T, Turney CSM, Westaway KE, Rink WJ, Zhao JX, Van den Bergh GD, Due RA and others. 2004. Archaeology and age of a new hominin from Flores in eastern Indonesia. Nature 431:1087-1091.

Morwood MJ, Sutikna T, Saptomo EW, Jatmiko, Hobbs DR, and Westaway KE. 2009. Preface: research at Liang Bua, Flores, Indonesia. J Hum Evol 57:407-449.

Musser GG. 1981. The giant rats of Flores and its relatives east of Borneo and Bali. American Museum of Natural History 169:67-176.

Obendorf PJ, Oxnard CE, and Kefford BJ. 2008. Are the small human-like fossil finds on Flores human endemic cretins? Proceedings of the Royal Society 275:1287-1296.

Oxnard CE, Obendorf PJ, and Kefford BJ. 2010. Post-cranial Skeletons of Hypothyroid Cretins Show Similar Anatomical Mosaics as *Homo floresiensis*. PLoS One 5(9):e13018.

Peregrine PN, and Ember M, editors. 2001. Encyclopedia of Prehistory: East Asia and Oceania. United States of America: Human Relations Area Files, Inc.

Richards GD. 2006. Genetic, physiological and ecogeographical factors contributing to variation in *Homo sapiens*: *Homo floresiensis* reconsidered. Journal of Evolutionary Biology 19:1744-1767.

Tocheri MW, Orr CM, Larson SG, Sutikna T, Jatmiko, Saptomo EW, Due Awe R, Djubiantono T, Morwood MJ, and Jungers WL. 2007. The primitive wrist of *Homo floresiensis* and its implications for hominin evolution. Science 317:1743-1745.

Van den Bergh GD, Meijer HJM, Awe RD, Morwood MJ, Szabo K, Ostende LWV, Sutikna T, Saptomo EW, Piper PJ, and Dobney KM. 2009. The Liang Bua faunal remains: a 95 k.yr. sequence from Flores, East Indonesia. J Hum Evol 57(5):527-537.

Weber J, Czarnetzki A, and Pusch CM. 2005. Comment on "The Brain of LB1, *Homo floresiensis*". Science 310(5746):236.

Westaway KE, Roberts RG, Sutikna T, Morwood MJ, Drysdale R, Zhao JX, and Chivas AR. 2009a. The evolving landscape and climate of western Flores: an

environmental context for the archaeological site of Liang Bua. J Hum Evol 57(5):450-464.

Westaway KE, Sutikna T, Saptomo WE, Jatmiko, Morwood MJ, Roberts RG, and Hobbs DR. 2009b. Reconstructing the geomorphic history of Liang Bua, Flores, Indonesia: a stratigraphic interpretation of the occupational environment. J Hum Evol 57(5):465-483.

CHATPER 2: PRESERVATION OF BONE FROM LIANG BUA, FLORES

Introduction

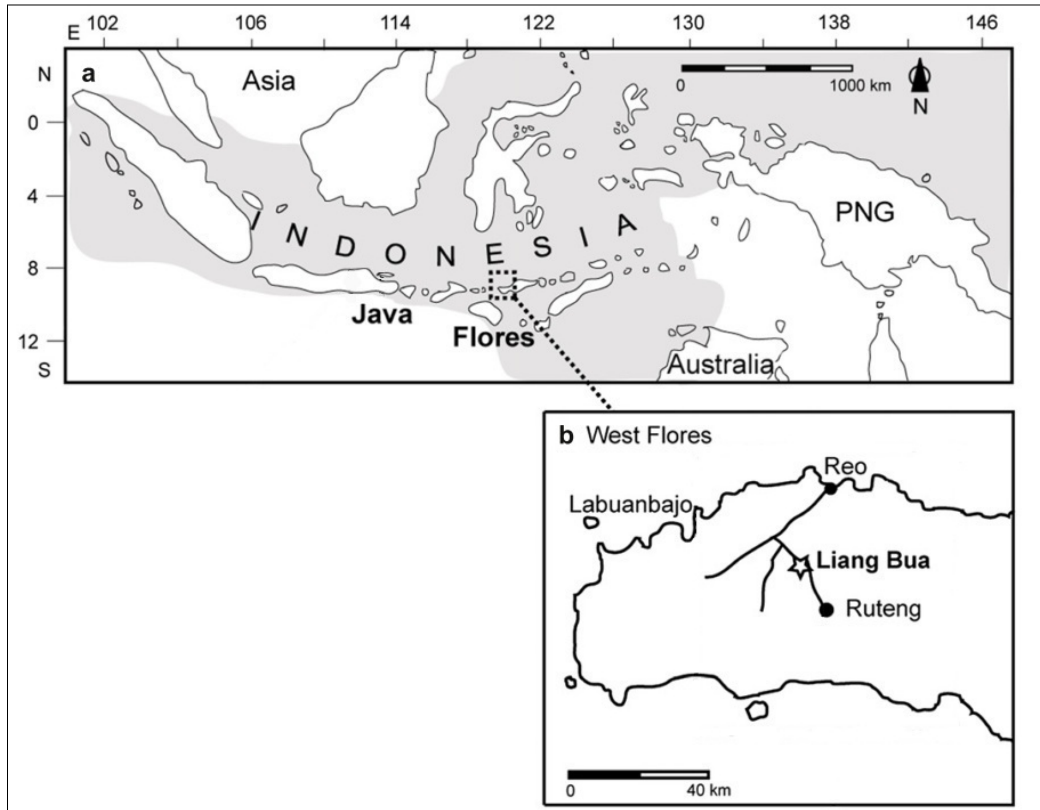
In archaeological contexts stable isotope analyses are typically used to reconstruct diet, weaning patterns and migration (Ambrose et al. 1997; Bocherens et al. 1994; DeNiro 1985; Dupras et al. 2001; Dupras and Tocheri 2007; Kellner and Schoeninger 2007; Larsen 1997; White et al. 2007). Many researchers have shown that in optimal environments, analyses of stable isotopes from bone collagen accurately reflect stable isotopic signatures (Lee-Thorp 2002; Lee-Thorp et al. 1989; Schoeninger 2005; Schwarcz and Schoeninger 1991). However, bone is known to undergo diagenetic processes which can alter the microscopic preservation of the bone and possibly corrupt stable isotopic data (Ambrose 1990; DeNiro 1985; Schoeninger et al. 1989; Van Klinken 1999). Some researchers have previously used diagenetic changes in bone as a reason for why stable isotopes are not an accurate means to reconstruct dietary behaviors (Ambrose 1993). More recently it has been shown that diagenetic changes to bone and collagen can be controlled for (Ambrose 1990; DeNiro 1985; Van Klinken 1999). Researchers using stable isotope analyses must first analyze their samples for possible degradation and contamination and then evaluate if samples must be discarded from the study (Ambrose 1990). It is important for researchers to understand how diagenesis can affect their samples, how to correctly prepare their samples, and how to accurately screen their data for contamination and diagenesis. Without these criteria stable isotopic study would be inaccurate and unreliable. This type of analysis has not been previously conducted on

skeletal remains from Liang Bua, and as such the state of bone collagen preservation within the cave is unknown. Sub-fossil rat femora were analyzed to determine if there are adequate levels of bone collagen preservation needed to conduct accurate stable isotope analyses.

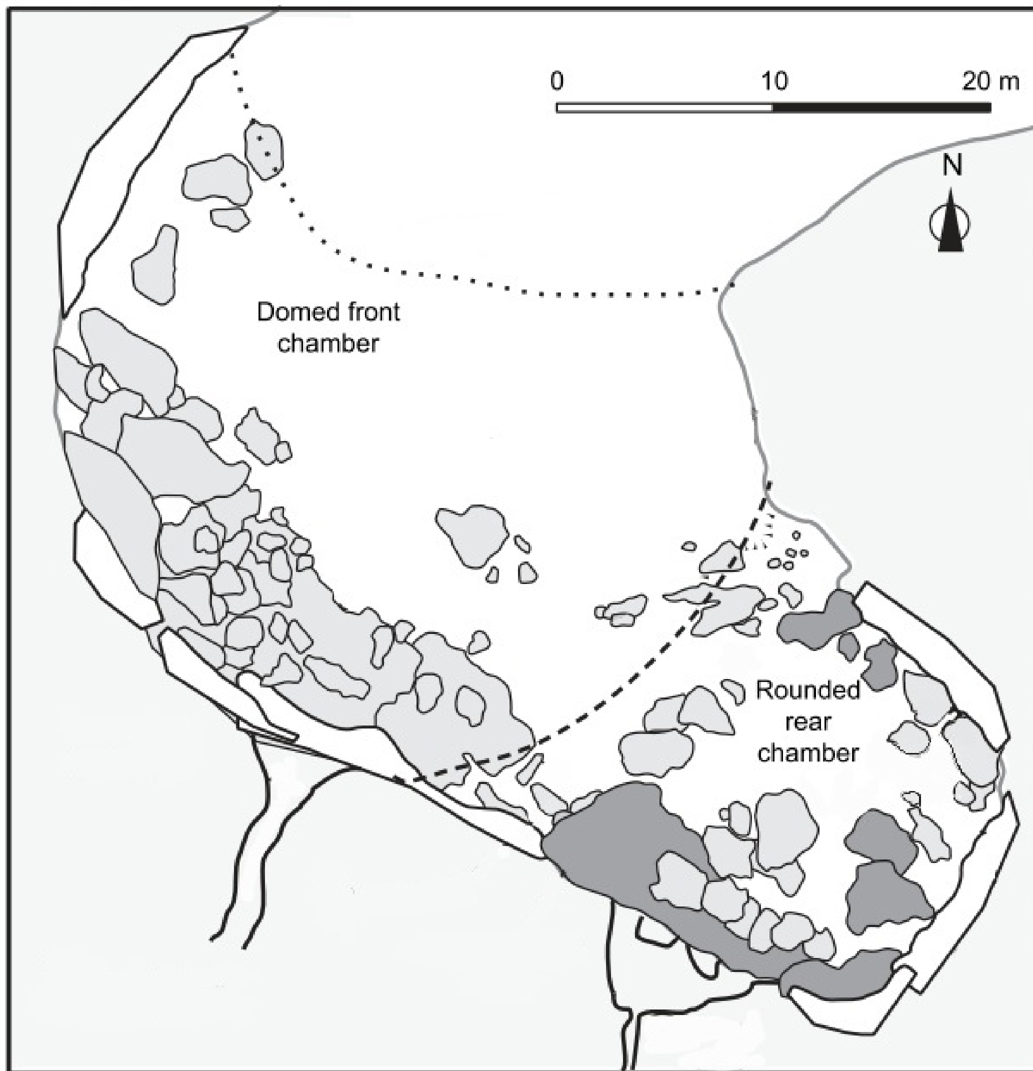
Site Formation Background

Liang Bua cave is located on the western side of Flores along the Wae Racang River Valley, 14 km north of the city of Ruteng (Morwood et al. 2004) (Figure 2). The cave opening is 30 m wide, 25 m in height and reaches a depth of 40 m (Aiello 2010; Morwood et al. 2004). It is composed of a larger front chamber and smaller rear chamber, which could have resulted from the merging of two separate caves (Figure 3).

Throughout most of the cave's history the floor of the front chamber was located about 10 m below the floor of the rear chamber. This resulted in the concentration of faunal and hominin activity in the front chamber (Westaway et al. 2009b). Between 10 and 2 kya the front chamber floor reached a similar elevation to that of the rear chamber (Westaway et al. 2009b).



(Adapted from Westaway et al. 2009a)
Figure 2. (a) Map showing the location ofwest Flores within the isalnds of Indonesia, located between mainland Southeast Asia and Austrailia. (b) Inset showing the location of the Liang Bua cave within the isalnd of Flroes.



(Adapted from Westaway et al. 2009a)
Figure 3. Plan view of the Liang Bua cave site. Map depicts the larger front chamber and smaller rear chamber.

The limestone cave was formed as a subterranean cavern 600 kya as a result of karst dissolution (Morwood et al. 2004; Westaway et al. 2009b). During the formation of the cave the subduction of tectonic plates caused the uplift of Flores' limestone beds. Uplift has been continuous throughout the island's history and is estimated to increase by

.02 to .05 mm per year (Westaway et al. 2009a). Due to this uplift over time more limestone has been exposed to aqueous solutions causing the dissolution of the limestone and the formation of the large chambered cave. Tectonic uplift combined with the continued down cutting of the Wae Racang River into the valley resulted in weakened cave chambers (Westaway et al. 2009a).

Around 190 kya the subterranean cave was uncovered when the Wae Racang River valley floor reached the depth of the cave (Westaway et al. 2009b). The initial exposure of the cave, caused by an influx of fast moving water from the river, is depicted by the alluvial conglomerate deposit in the cave's southern end (Westaway et al. 2009a). Sediments found in the floor of the cave, such as a coarse matrix of silty sand, also indicate strong movement of running water (Westaway et al. 2009b). Due to the presence of water in the cave, occupation is thought not to have been possible until ~100 kya. Longer term occupation has been argued to take place around 74-61 kya (Westaway et al. 2009b).

Preservation within Liang Bua Cave

Prior to this study the quality of bone collagen preservation of skeletal material from Liang Bua cave was mostly unknown. Although retrieval of aDNA from pig skeletal remains from Liang Bua was successfully carried out (Larson et al. 2007a), stable isotopic analyses has previously not been conducted. Bone collagen preservation in the cave may be poor due to the less than optimal burial conditions. The island of Flores experiences seasonal monsoons from the Asian monsoon system which cause the cave floor to undergo intermittent channeling and pooling of water (Westaway et al. 2009a).

The water channels form during rainy monsoon seasons, while the pooling of water occurs during dry seasons as result of decreased water flow (Westaway et al. 2009b). The monsoons are believed to have decreased between 36-17 kya resulting in dryer cave conditions (Westaway et al. 2009a). However, beginning around 17 kya there was a return of seasonal monsoons. This resulted in increased rainfall and thus wetter conditions in the cave during the Holocene (Westaway 2009). The cave likely also previously experienced seasonal flooding as a result of water overflow from the Wae Racang River. Although today the river is located about 200 m away and 30 m below the cave, it is known to have been previously located on a similar elevation (Westaway et al 2009b). At ~17 kya the river is noted to have been ~10 m below the cave (Westaway et al. 2009a).

Due to the cave's natural environment, its sedimentary soils are continually moist and damp, even during the dry season. Preservation of skeletal material is greatly affected by the presence of groundwater in burial environments, especially when there is a recharging of fresh water (Hedges and Millard 1995). Bone collagen of the rat skeletal remains could have been affected by the varying amounts of water found present in the cave throughout its history.

Other than the amount of water typically found present in the cave, Flores' natural climate may also affect the preservation of bone collagen. The island is located just south of the equator within the intermediate tropical zone. Bones located in tropical environments that experience significant precipitation and heat are likely to rapidly lose collagen (Lee-Thorp et al. 1989; Van Klinken 1999). According to Lee-Thorp and

colleagues (1989), bone samples in wet and warm environments, like that on Flores, experience loss of collagen preservation within 10,000 years.

Diagenesis

Diagenesis is defined as the "chemical, physical, and biological changes undergone by a bone through time," (White 2000:525). Diagenesis can occur in the form of both degradation and contamination. Degradation involves the breakdown of a bone sample such as the loss of bone apatite due to microbial attack or the leaching of collagen (Van Klinken 1999). Contamination occurs when exogenous contaminants are found present in a bone sample (Van Klinken 1999). For example, during burial bone undergoes ion exchange with its surrounding environment, such as the uptake of uranium from ground water (Hedges and Millard 1995). Bone can also absorb humic acids and polysaccharides which can cause contamination, although humic acids can also inhibit microbial attack on bone (Hedges and Law 1989). These microscopic changes cause bone to be altered from its original structure and makeup. However, without these diagenetic changes it is unlikely that bones and teeth would survive in the archaeological record. The chemical changes stabilize the bone and allow for its conservation (Lee-Thorp and Sealy 2008). Overall preservation of bone is known to vary greatly between different environments and even within the same site (Hedges et al. 1995). The degree of diagenesis in bone material is believed to result from the amount of time since death, the water in the burial environment, microorganisms in the area, and climate (Gutierrez 2001). Researchers in the stable isotopic community have established methods for

evaluating bone apatite and collagen for degradation and contamination (Ambrose 1990; DeNiro 1985; Schoeninger et al. 1989).

Collagen Yields

Among the stable isotopic research community it is generally accepted that collagen concentrations (weight % of whole bone) can be used as an indicator of sample degradation (Hedges and Law 1989; Schoeninger et al. 1989; Van Klinken 1999). Beginning almost immediately post-mortem the amount of collagen in bone decreases, while the quality of the collagen also degrades (Hedges and Law 1989; Hedges et al. 1995). "The mechanisms of degradation (leading to the loss of collagen from bone) involve the gradual breakup of the collagen α-chains (breakage of peptide bonds between amino acids), followed by loss of larger or smaller peptides from the collagen triple-helical structure and loss from the bone through leaching" (Van Klinken 1999:688).

The rate of collagen degradation depends on the environment in which the bone is buried in and the climatic conditions (Van Klinken 1999). Loss of collagen occurs relatively slowly in temperate to subtropical environments and occurs relatively quickly in tropical environments due to heat (Nielsen-Marsh et al. 2000; Van Klinken 1999). Ambrose (1990) has demonstrated that the loss of collagen in African sites occurs in less than 3,000 years. Bone in wet and warm environments have significantly reduced or no collagen after 10,000 years (Lee-Thorp et al. 1989). The degradation of collagen is a cause for concern since it can compromise isotopic integrity and reduces the overall sample size, making the extraction of collagen more difficult (Van Klinken 1999).

Collagen in fresh bone accounts for about 22% of dry bone weight (Katzenberg 2000c; Schoeninger et al. 1989). It is generally accepted that collagen samples with < 5% collagen yields have "low" collagen yields and should be discarded from study (Schoeninger et al. 1989), because bones with < 5% collagen potentially have distorted carbon and nitrogen isotopic content (Schwarcz and Schoeninger 1991).

However, there is some disagreement on discarding all samples with less < 5% collagen. Van Klinken (1999) argues that collagen yields regarded as acceptable should depend on the geographic origin of the bone samples. Van Klinken (1999), who analyses bone samples from Europe, considers samples with as low as 0.5% collagen to be acceptable for analysis. However, Ambrose (1990), who conducts analyses on bones from Africa, considers bones with no lower than 3.5% collagen to be acceptable. Other authors (Ambrose 1993; Schwarcz and Schoeninger 1991) argue that samples with < 1% collagen should be discarded from study since these samples are likely to be enriched in nitrogen by 15‰.

It is also possible for collagen yields to be affected by loss of sample during preparation. When extracting collagen from a bone sample, the mineral portion of the bone is broken down by a 5% HydroChloric Acid solution. Once the bone apatite is dissolved, and only collagen remains, the collagen samples are run through a centrifuge and rinsed with water. This causes the collagen sample to break down into the water solution making it possible to lose sample while replacing the water during rinsing.

Carbon and Nitrogen Concentrations

Measurement of concentrations of carbon and nitrogen, expressed as wt % of collagen, are strongly argued to be excellent indicators of bone and collagen preservation (Ambrose 1990; Van Klinken 1999). Ambrose (1990) states that carbon and nitrogen concentrations in collagen are the most dependable tests of collagen preservation when a bone is transitioning from a well preserved bone to a poorly preserved bone. Carbon and nitrogen concentrations decrease as % collagen decreases. Once collagen yields reach 2%, carbon and nitrogen concentrations decrease rapidly (Ambrose 1990). According to Ambrose (1990) prehistoric bone collagen should have carbon concentrations > 13% and nitrogen concentrations > 4.8%. Van Klinken (1999) suggests an upper limit of nitrogen concentrations of 16%. Samples ranging outside of that limit are believed to have poor preservation and are typically removed from study.

Wt % C and wt % N are differentially affected by diagenetic factors. It is possible for a single sample to have acceptable wt % C values and not have acceptable wt % N values. Conversely, the wt % C could be well preserved while the wt % N is not. If either wt % N or wt % C are out of the accepted range then the C:N ratio will not be within the 2.9-3.6 range. However, it is also possible for both the wt % N and wt % C values to not be within the accepted range and inadvertently have a C:N ratio within the 2.9-3.6 range, thus giving a false reading of preservation within the sample when it is not actually well preserved.

C:N Ratios

Many researchers agree that carbon to nitrogen (C:N) atomic ratios in collagen are an acceptable indicator of collagen degradation or contamination (DeNiro 1985; DeNiro and Weiner 1988; Van Klinken and Hedges 1995). DeNiro (1985) suggests that collagen with C:N ratios outside of 2.9-3.6, the range of modern humans and animals, should be discarded from study. DeNiro (1985) argues that bone samples with C:N ratios outside the 2.9-3.6 range will most likely have altered $\delta^{13}C$ and $\delta^{15}N$ values. During diagenesis carbonate and phosphate can be exchanged or absorbed by bones, which can potentially alter the isotopic ratios of the bone. Samples that have significantly low or high carbon or nitrogen concentrations result in C:N ratios outside the expected range of 2.9-3.6. Lower carbon or nitrogen concentrations are believed to be the result of inorganic substances present in the sample, such as clays, minerals, or salts (Ambrose 1990; Van Klinken 1999). Samples with higher carbon or nitrogen concentrations are believed to be the result of organic carbon (Van Klinken 1999). Although collagen yields extracted from bone vary significantly between samples and/or studies, atomic C:N ratios are relatively constant (Ambrose 1990).

Some researchers argue that C:N ratios alone are not a reliable indicator of collagen preservation (Ambrose 1990; Schoeninger et al. 1989; Schwarcz and Schoeninger 1991). Schwarcz and Schoeninger (1991) state that it is possible for noncollagenous proteins to have C:N ratios within the generally accepted range of 2.9 to 3.6. Ambrose (1990) states that when collagen yields are exceedingly low, C:N ratios are

not a reliable source of determining collagen integrity. However, this method is still widely used to determine bone collagen preservation.

Materials and Methods

In this study, femora of large, medium, and small-sized rats from Liang Bua (Flores, Indonesia) were analyzed for carbon and nitrogen stable isotopes. Based on ^{14}C dates of charcoal samples from Liang Bua (Roberts et al. 2009) the oldest rat bone included in this study comes from stratigraphic deposits that are at least 19,200 years old. The specific species of rats used for the sample in this study are unknown; however, giant rats *Papagomys armandvillei*, *Papagomys theodorverhoeveni* and *Spelaeomys florensis*, middle sized rats *Komodomys rintjanus* and *Floresomys naso* and small sized rats *Rattus exulans* are possible candidates for the sample since they have all be identified as being present during the Pleistocene and Holocene. Prehistoric Floresian rats are differentiated into species based primarily on size, shape, and occlusal patterns of dentition (Musser 1981). Since the sample of this study includes only rat femora, species determinations of the sample could not be made. Although the small and medium sized femora cannot be convincingly attributed to species, they most likely belong to *Komodomys rintjanus*, *Floresomys naso* and *Rattus exulans.* Alternatively, the largest femora most likely belong to *Papagomys armandvillei*, *Papagomys theodorverhoeveni* and *Spelaeomys florensis.*

The rat bone samples were excavated from Sector XVI in 2008 as part of a collaboration between the Indonesian National Research and Development Centre for Archaeology and the University of Wollongong (Australia). Indonesian faunal expert, Rokus Due Awe, performed the taxonomic identifications and selected the best

macroscopically preserved specimens from each 10 cm spit (excavation unit) for this analysis.

Collagen was extracted from 136 individual rat femora to conduct carbon and nitrogen stable isotope analyses (Appendix 1). This extraction followed methods first described by Longin (1971) and Chisholm et al. (1989), and modified by the Laboratory for Stable Isotope Studies (LSIS) at the University of Western Ontario. The collagen extraction process began by washing all bones in distilled water in an ultrasonicator to rid them of humic soils. The samples were then dried in an oven at 70 degrees. Once dry, the bones were weighed. The bones were then soaked in a 5% HCL acid solution for as many days as was required until only collagen remained. Once this occurred the collagen was rinsed in a centrifuge with deionized water to remove all the HCL, and lastly rinsed with a sodium chloride solution to be sure that all humic soils were removed. After a final rinse in deionized water, 5 ml of deionized water was added to the sample and placed in an oven overnight at 90 degrees. This process was completed to break down the collagen bonds. The collagen-water solution was then transferred from the larger vial to a smaller 5 ml vial and placed again in the oven with no lid to evaporate the water and leave behind the collagen. The collagen was then weighed and the collagen yield was calculated as a measure of preservation. Between .5 and 1 umg of the sample was then weighed into silver tin capsules for the final analysis. Once the collagen was collected the samples were sent to the Smithsonian Institution OUSS-MCI Stable Isotope Mass Spectrometry Facility to be analyzed on a Thermo Scientific Delta V Advantage mass spectrometer.

Results

This research project began with 136 rat femora samples from Liang Bua (Appendix 1). After processing the samples, as described in the materials and methods section, 55 of the samples did not yield any measurable collagen. Samples with no measureable collagen are found throughout the stratigraphic sequence (Figure 4).

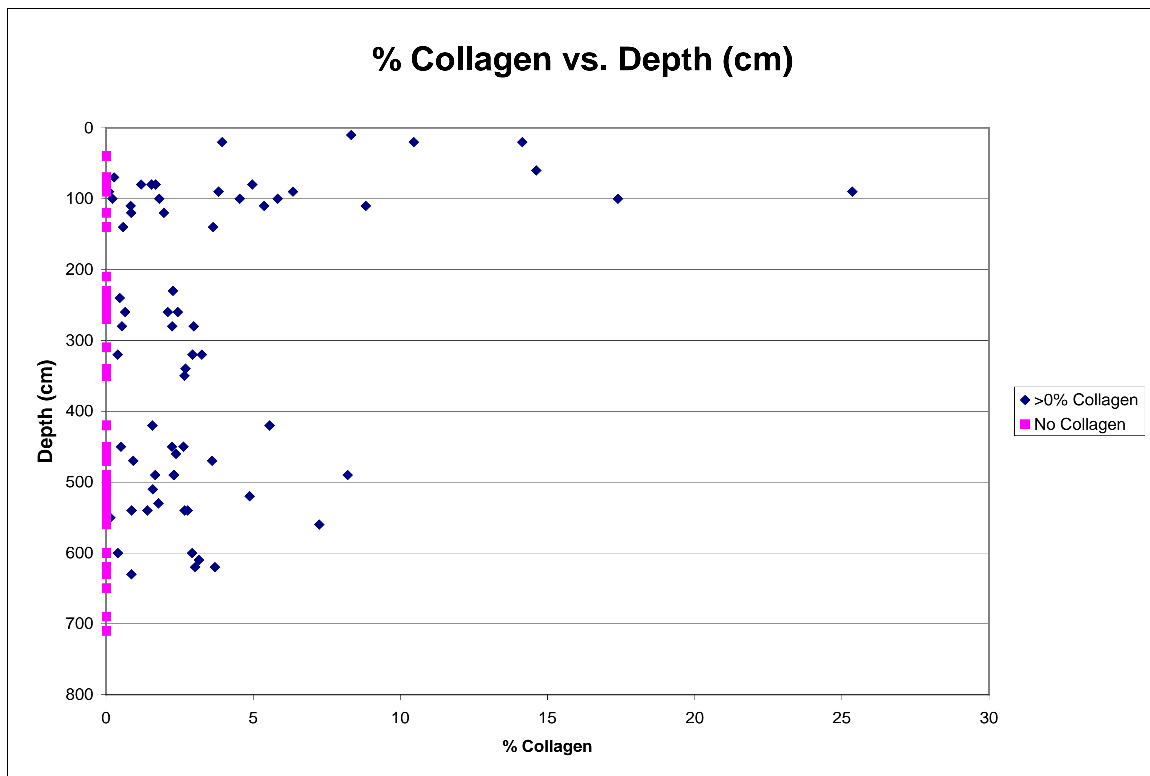


Figure 4. Graph showing % collagen vs. depth, including all collagen samples that yielded no measureable collagen.

The remaining 81 samples that did yield collagen were analyzed for wt % N, wt % C and the C:N atomic ratio in order to determine collagen preservation (Table 1). It should be noted that the following samples 42m.1, 42m.2, 43b.1 and 46s.1 did yield a

percentage of collagen, however, the original dry weight of the bone samples was undocumented so the percentage of collagen is unknown.

% Collagen

It is generally accepted that samples with < 5% collagen be discarded from isotopic study (Schoeninger et al. 1989). However, other researchers argue that samples with as low as 1% collagen should be considered preserved (Ambrose 1993; Schwarcz and Schoeninger 1991; Van Klinken 1999). Samples that have < 5% collagen have only the potential to have altered carbon and nitrogen values. Figure 5 shows % collagen plotted against depth and time. Eleven samples with > 5% collagen are less than 110 cm deep and less than 5,160 years old. The three samples that retain > 5% collagen deeper than 400 cm, 24m.2, 29m.2 and 37s.1, have low carbon and nitrogen concentrations and C:N ratios outside the 2.9 to 3.6 range. Thus, it is unlikely that these three samples have well preserved collagen.

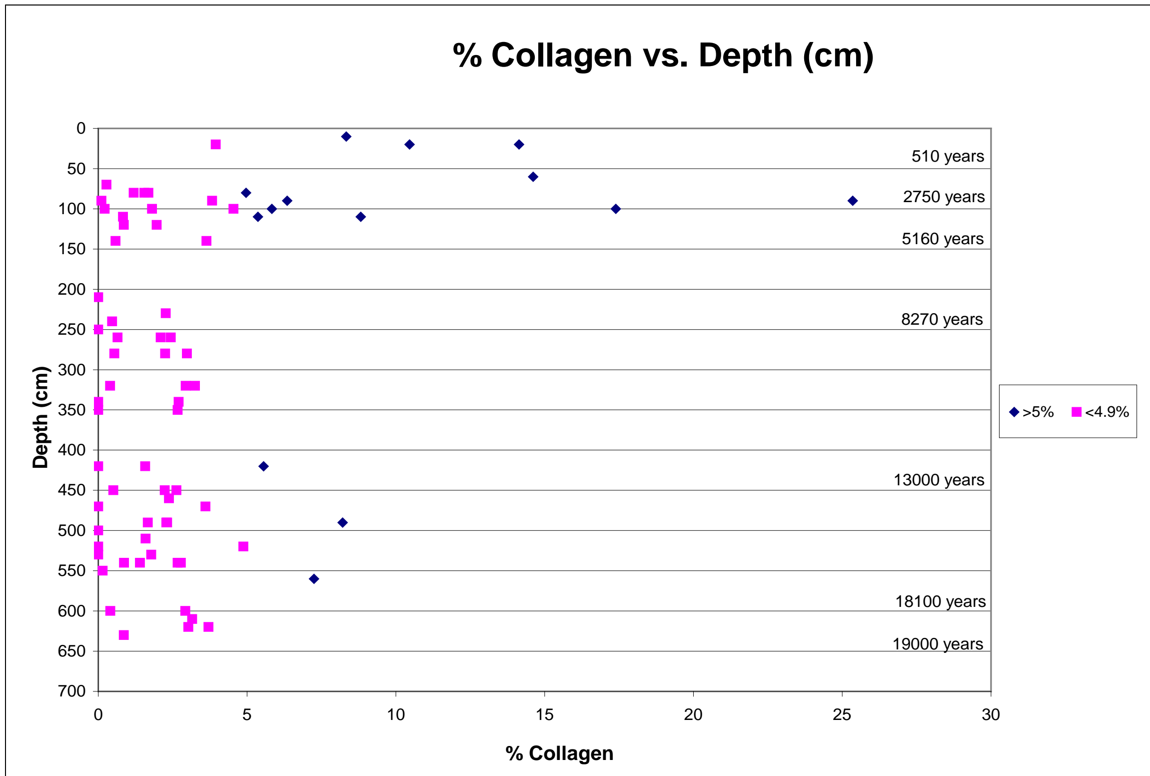


Figure 5. Graph showing % collagen vs. depth.

Figure 6 shows % collagen plotted against depth and time, including a distinction between collagen yields of > 5% collagen, 1-4.9%, and < 1%. Samples with between 1% and 4.9% collagen are consistently found from 20 cm to 620 cm. However, since samples with < 5% collagen are considered having "low" collagen yields it is still necessary to investigate other methods of determining preservation.

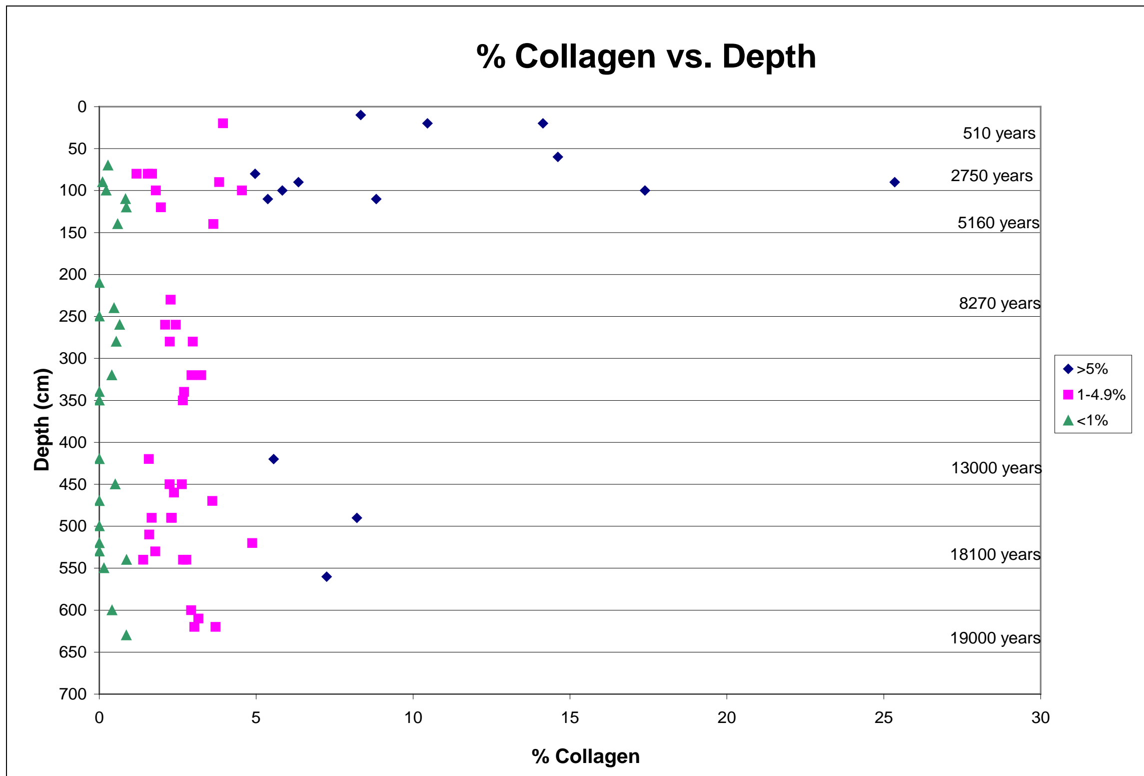


Figure 6. Graph showing % collagen vs. depth including distinction between collagen yields of > 5%, 1-4% and < 1%.

Figure 7 shows % collagen plotted against depth and time including soil stratigraphy of sector VII/XI which is adjacent to the sector this study's samples were excavated from. The soil types found in this sector include brownish clayey silts, sandy silts and white tuffaceous silts (Morwood et al. 2005). The specific texture, color and rock abundance vary by soil layer. This figure suggests that there is not a correlation between soil type and collagen yield.

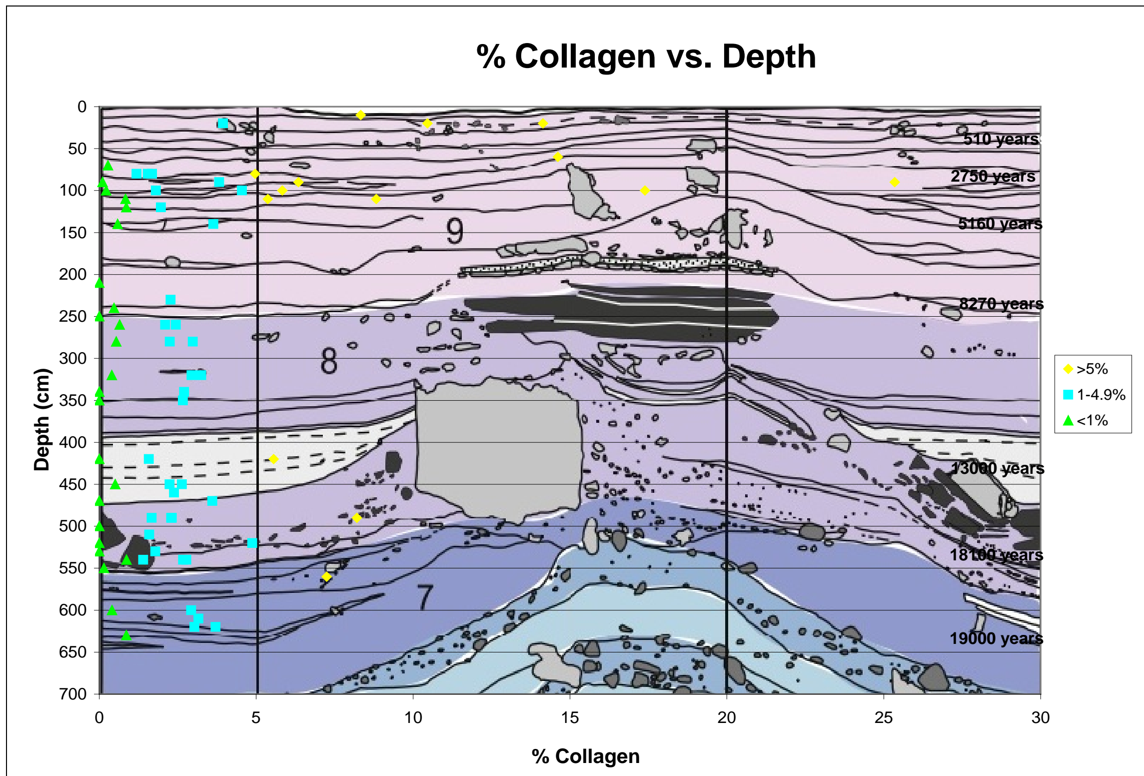


Figure 7. Graph showing % collagen vs. depth including soil stratigraphy. Layers 7-9 are composed of brownish silts and sandy silts.

C:N Ratio

It is generally accepted that samples with C:N ratios outside of the 2.9 to 3.6 range determined by DeNiro (1985) be discarded from study. However, samples with noncollagenous proteins and inappropriate carbon and nitrogen concentrations can inadvertently have C:N ratios within the 2.9 to 3.6 range (Schwarcz and Schoeninger 1991). Figure 8 shows C:N ratio plotted against depth and time. If a range of 2.9 to 3.6 C:N ratio was used to determine collagen preservation 31 samples would be considered well preserved. However, the samples below a depth of 500 cm that have C:N ratios within the 2.9 to 3.6 range have low carbon and nitrogen concentrations. Sample 32m.1 also has a low collagen yield of 1.5915%. Although sample 33m.1 has a collagen yield of

4.8780% its low carbon and nitrogen concentrations indicate that the collagen that is present is not well preserved. Another sample, 35m.4, has a C:N ratio just outside of the 2.9 to 3.6 range of 3.6398% also has very low carbon and nitrogen concentrations and low collagen yields. The C:N values for samples 32m.1, 33m.1 and 35m.4 are likely coincidences and not actually indicative of collagen preservation.

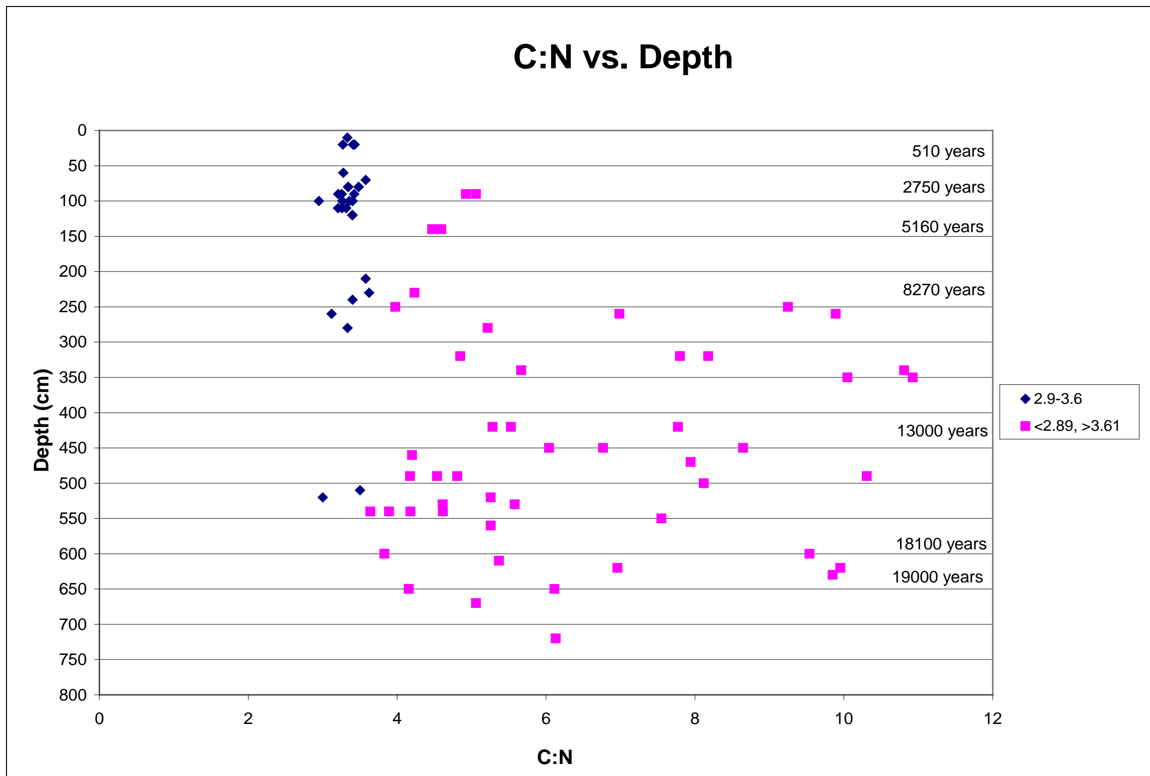


Figure 8. Graph showing C:N ratio vs. depth.

Nitrogen Concentration

Ambrose (1990) argues that carbon and nitrogen concentrations in collagen are the most reliable indicators of collagen integrity. Figure 9 shows wt % N plotted against depth and time. According to Ambrose (1990) samples with nitrogen concentrations greater than 4.8% are considered to have well preserved collagen. Van Klinken (1999)

argues for a slightly higher value of 11 wt % N and also argues for a 16 wt % N upper limit. Twenty-nine samples in Figure 9 fall within the range suitable for nitrogen concentrations in collagen. These samples range between 10 cm and 520 cm and have nitrogen concentrations ranging between 4.8% and 13.9%. However, after 260 cm nitrogen concentrations in most samples abruptly decrease below 4.8%.

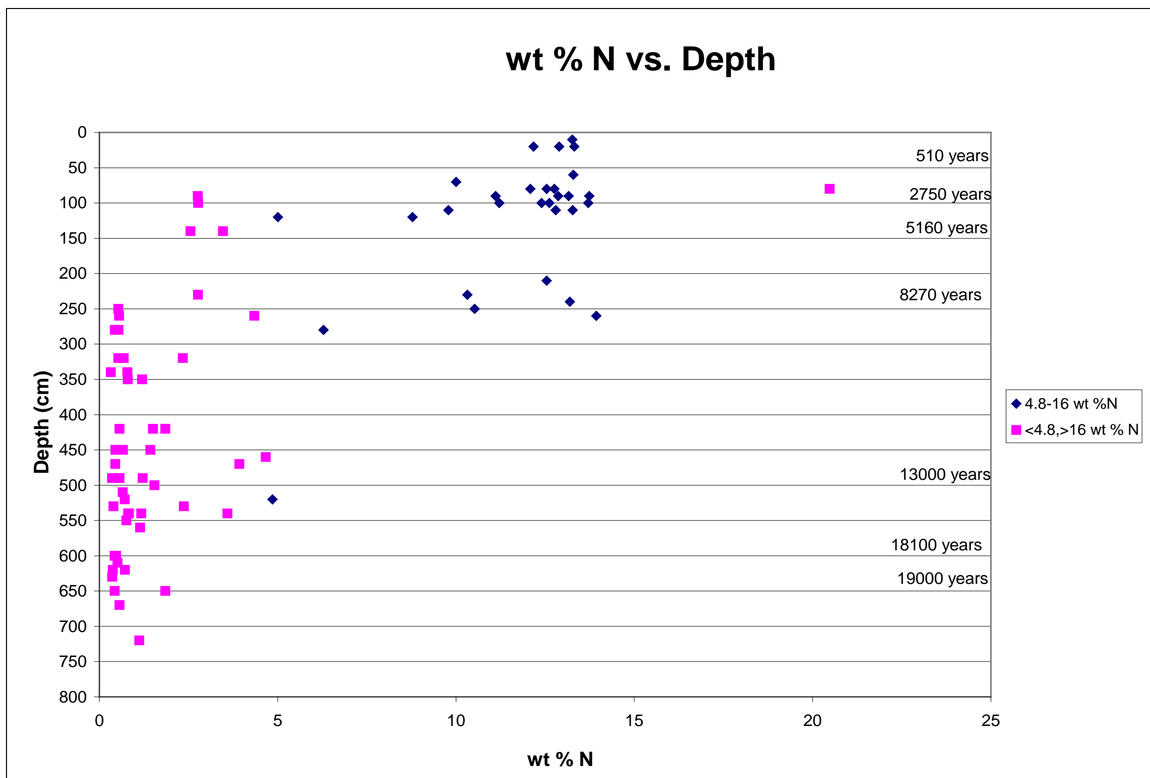


Figure 9. Graph showing wt % N vs. depth.

Carbon Concentration

According to Ambrose (1990) samples with carbon concentrations > 13% are considered to be well preserved. Figure 10 shows wt % C plotted against depth and time. Thirty-four samples fall within the range suitable for carbon concentrations. These samples range between 10 cm and 520 cm and have carbon concentrations ranging

between 13.28% and 58.7%. All samples below 520 cm have low carbon concentrations. The decrease in carbon concentration does not occur as abruptly as the decrease in nitrogen occurs.

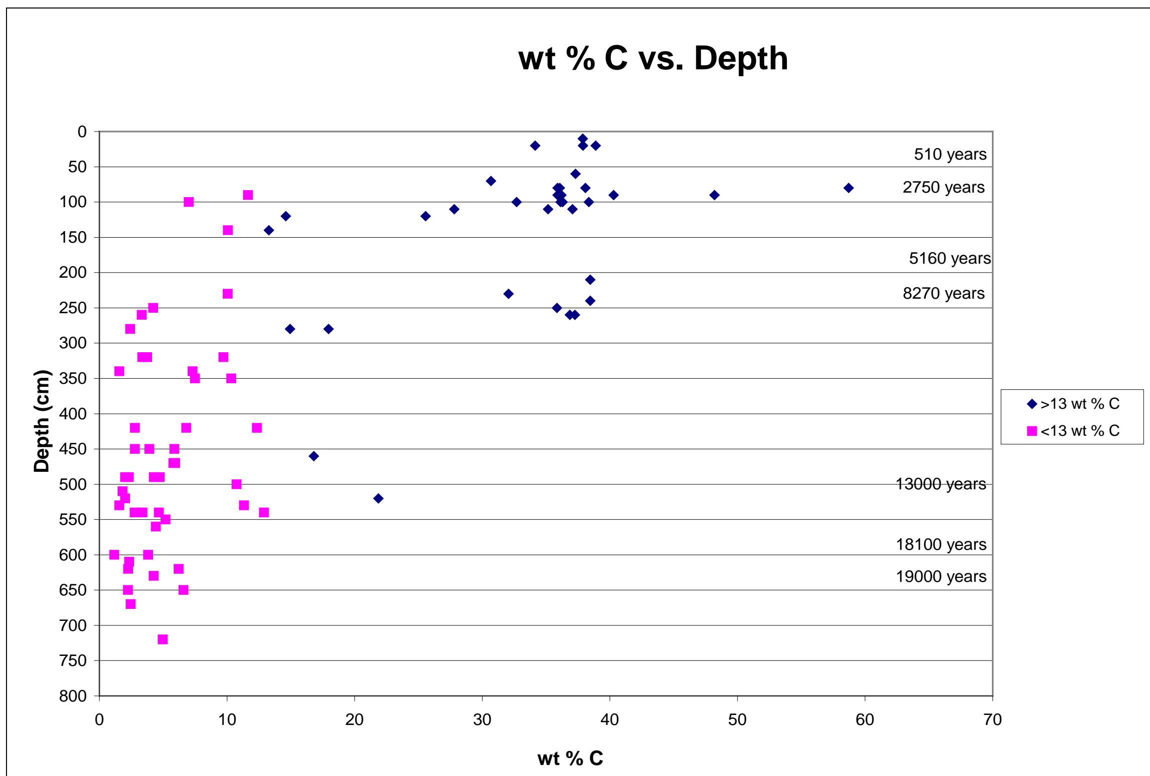


Figure 10. Graph showing wt % C vs. depth.

Discussion

The measure of sample preservation (Figures 4-9) is based on standards typically accepted in the study of bone collagen. Samples with < 5% collagen are usually discarded from stable isotopic study. However, this study specifically examined the wt % C and wt % N of each sample in order to determine if samples with less than 5% collagen retained appropriate values, regardless of % collagen.

Nitrogen concentrations are expected to range between 4.8 and 16 wt % N (Ambrose 1990; Van Klinken 1999). Samples outside of that range are most likely affected by diagenesis or contamination. Figure 10 shows wt % N plotted against % collagen. According to Figure 11, 18 samples with < 5% collagen have retained appropriate wt % N values.

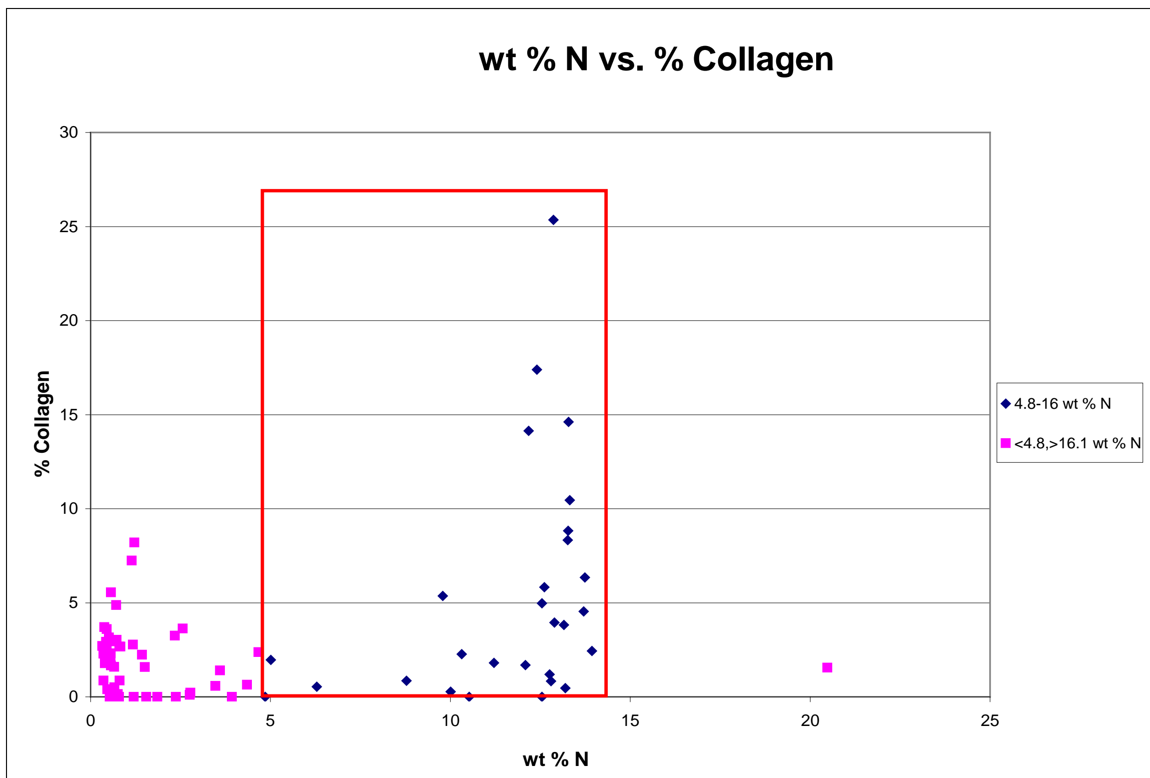


Figure 11. Graph showing wt % N vs. collagen. Samples retaining appropriate wt % N values are outline by the solid red box.

Carbon concentration are expected to be above 13 wt % C (Ambrose 1990). Samples with less than 13 wt % C are considered to be not well preserved. Figure 12 shows wt % C plotted against % collagen. According to Figure 12, 22 samples with < 5%

collagen have retained appropriate wt % C values.

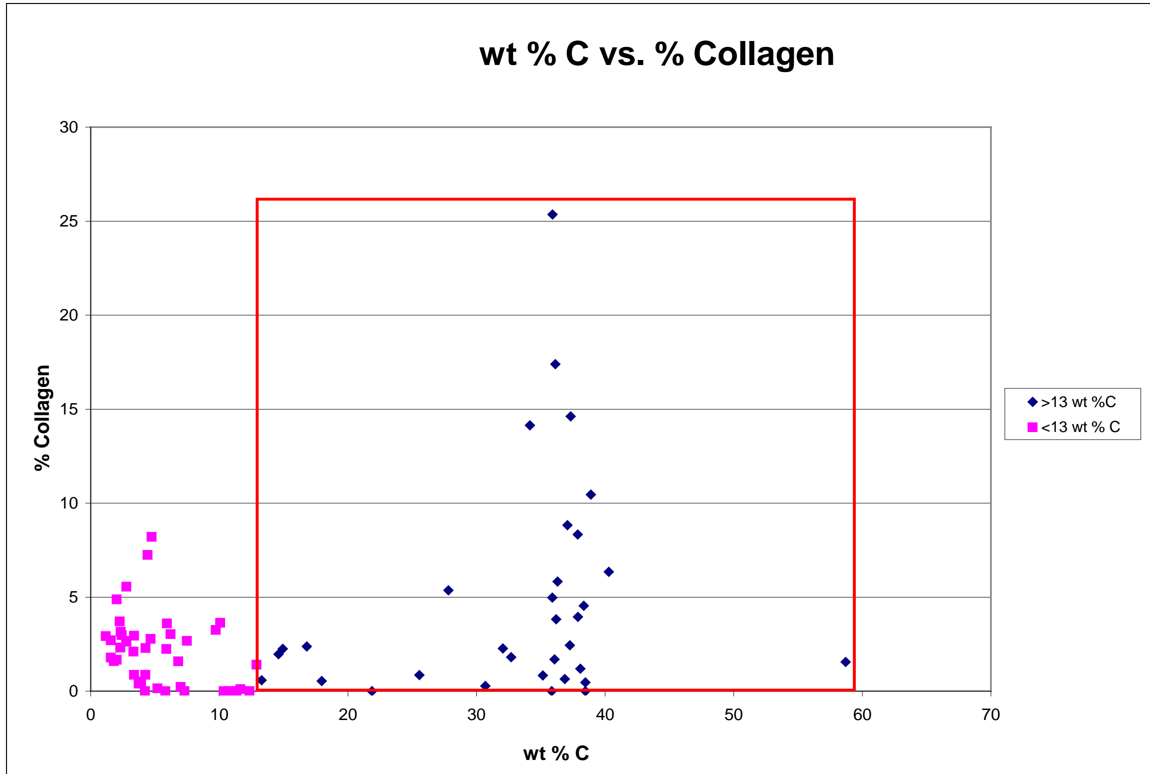


Figure 12. Graph showing wt % C vs. % collagen. Samples that have retained appropriate wt % C values are outline by the solid red box.

Many researchers follow DeNiro's (1985) suggestion that the C:N ratio of collagen samples be between 2.9 to 3.6, the ratio of modern day humans and animals. Samples outside of this range are typically considered not well preserved. Figure 13 shows the C:N ratio plotted against % collagen. According to Figure 13, 17 samples with < 5% collagen have values within the accepted range.

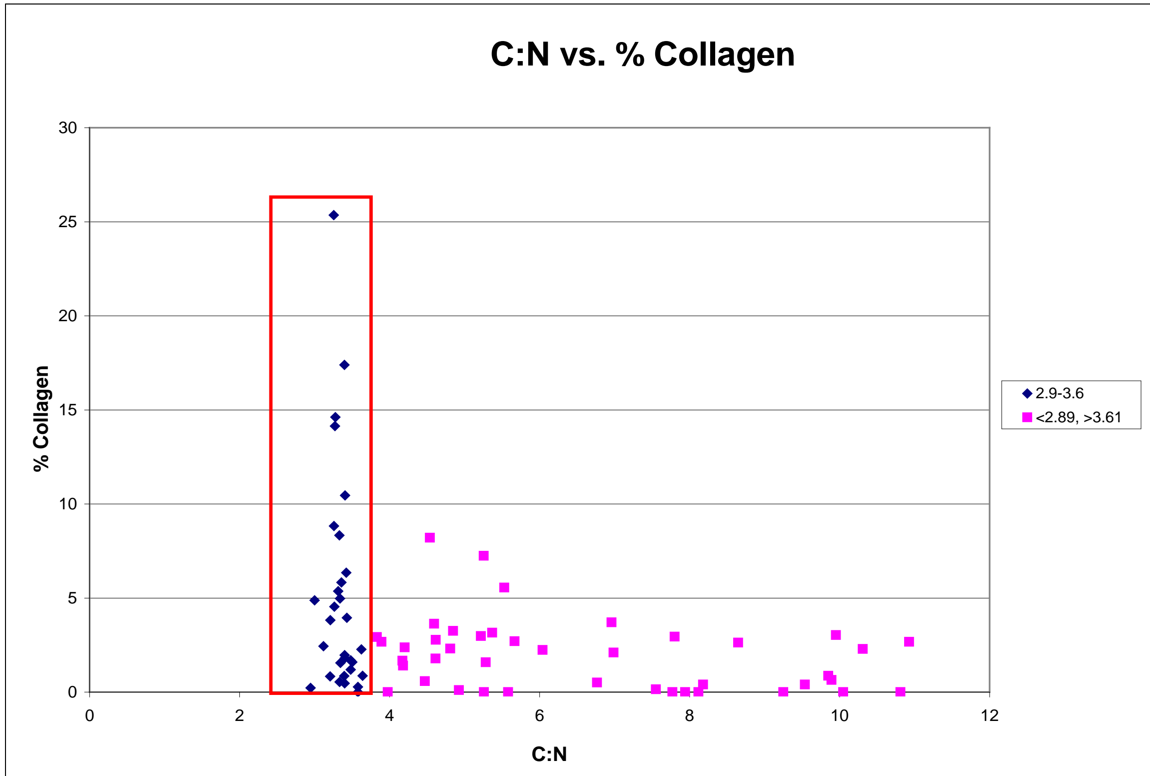


Figure 13. Graph showing C:N ratio vs. % collagen. Samples retaining appropriate C:N ratios are outline by the solid red box.

Conclusion

Many researchers consider samples with < 5% collagen to be not well preserved. However, according to the data from this study it is possible for carbon and nitrogen concentrations to be within the accepted ranges and considered well preserved even when the sample has yielded < 5% collagen.

According to Ambrose (1990) bone collagen samples with nitrogen concentrations > 4.8% and carbon concentrations > 13% are considered to have well preserved collagen. In this study 29 samples have nitrogen concentrations > 4.8% and 32 samples have carbon concentrations > 13%. These samples are within the carbon and nitrogen concentration ranges proposed by Ambrose (1990) and are considered well

preserved. Two additional samples (16b.1 and 18m.1) have carbon concentrations within the accepted range, however, the $\delta^{13}C$ values are not within the expected range and are not indicative of collagen preservation. Although 18 of the samples with appropriate nitrogen concentrations and 22 of the samples with appropriate carbon concentrations have < 5% collagen, it has been argued (Ambrose 1990) that carbon and nitrogen concentrations are more reliable in indicating collagen preservation than collagen yields. Thus the carbon and nitrogen concentrations are used as the determining factor to indicate collagen preservation of a sample.

There are 10 samples that have appropriate values for all of the methods used to evaluate preservation including % collagen, C:N ratio, wt % C and wt % N. However, I argue that concentrations of carbon and nitrogen are an appropriate means to determine preservation on their own and an additional 22 samples are considered well preserved. In total 32 samples are thought to have well preserved collagen and are considered to be appropriate for stable isotopic study (Table 1).

Table 1 Data for 32 samples considered having well preserved collagen.

Sample ID	Depth (cm)	% Collagen	wt %N	wt %C	C:N (atomic)
1m.1	10	8.33	13.26	37.87	3.33
2m.1	20	10.46	13.32	38.89	3.41
2m.2	20	14.14	12.17	34.15	3.27
2s.1	20	3.95	12.89	37.89	3.43
4m.1	60	14.61	13.29	37.31	3.28
5b.1	70	0.28	10.00	30.69	3.58
6b.1	80	1.68	12.08	36.07	3.48
6b.2	80	1.55	20.48	58.70	3.34
6m.2	80	4.97	12.54	35.90	3.34
6s.1	80	1.19	12.76	38.08	3.48
7m.2	90	3.83	13.16	36.19	3.21
7m.3	90	25.35	12.87	35.90	3.26
7s.2	90	-6.12	11.11	48.20	5.06
7s.3	90	6.35	13.74	40.29	3.42
8m.2	100	1.81	11.21	32.69	3.40
8m.3	100	5.83	12.61	36.30	3.36
8m.4	100	4.55	13.71	38.35	3.26
8s.1	100	17.39	12.40	36.14	3.40
9m.1	110	0.83	12.79	35.16	3.21
9m.2	110	5.37	9.79	27.81	3.31
9m.3	110	8.82	13.27	37.08	3.26
10b.1	120	0.86	8.78	25.56	3.40
10b.2	120	1.96	5.01	14.60	3.40
11b.1	140	0.58	3.47	13.28	4.47
12s.1	210	0	12.54	38.47	3.58
13s.2	230	2.27	10.32	32.05	3.62
14m.1	240	0.47	13.20	38.47	3.40
15s.1	250	0	10.52	35.85	3.97
16s.1	260	2.44	13.93	37.25	3.12
18b.2	280	0.54	6.29	17.96	3.33
26m.1	460	2.38	4.67	16.81	4.20
33s.1	520	0	4.85	21.86	5.26

Other than the presence of water in the cave and the warm tropical environment additional factors might have affected bone collagen preservation of the rat femora

sample. A portion of the sample was from that of juveniles with missing epiphyses or the epiphyses were not completely fused. Some of the samples were also fragmentary. These two factors expose the cortical bone and could have left the bone increasingly vulnerable to degradation. Although this research study did find significant results and was able to report preserved collagen, future stable isotopic research would benefit from the use of femora from that of larger mammals, as thicker cortical bone may provide increased collagen preservation.

Literature Cited

Aiello LC. 2010. Five Years of *Homo floresiensis*. Am J Phys Anthropol 142:167-179.

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J Archaeol Sci 17(4):431-451.

Ambrose SH. 1993. Isotopic Analysis of Paleodiets: Methodological and Interpretive Considerations. In: Sandford MK, editor. Ivestigations of Ancient Human Tissue. Langhorne, Pennsylvania: Gordon and Breach Science Publishers.

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, and Krueger HW. 1997. Stable Isotopic Analysis of Human Diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol 104:343-361.

Bocherens H, Fizet M, and Mariotti A. 1994. Diet, physiology and ecology of fossil mammals as inferred from stable carbon and nitrogen isotope biogeochemistry: implications for Pleistocene bears. Paleogeogr Paleoclimatol Paleoecol 107:213-225.

Chisholm BS, Nelson DE, and Schwarcz HP. 1982. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216(1131-1132).

DeNiro MJ. 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to paleodietary reconstruction. Nature 317:806-809.

DeNiro MJ, and Weiner S. 1988. Organic-Matter Within Crystalline Aggregates of Hydroxyapatite - A New Susbtrate for Stable Isotopic and Possibly Other Biogeochemical Analyses of Bone. Geochim Cosmochim Acta 52(10):2415-2423.

Dupras TL, Schwarcz HP, and Fairgrieve SI. 2001. Infant feeding and weaning practices in Roman Egypt. Am J Phys Anthropol 115:204-212.

Dupras TL, and Tocheri MW. 2007. Reconstructing Infant Weaning Histories at Roman Period Kellis, Egypt Using Stable Isotope Analysis of Dentition. Am J Phys Anthropol 184:63-74.

Gutierrez MA. 2001. Bone diagenesis and taphonomic history of the Paso Otero 1 bone bed, pampas of Argentina. J Archaeol Sci 28(12):1277-1290.

Hedges REM, and Law JM. 1989. The radiocarbon dating of bone. Applied Geochemistry 4:249-253.

Hedges REM, and Millard AR. 1995. Bones and Groundwater: Towards the Modeling of Diagenetic Processes. J Archaeol Sci 22:155-164.

Hedges REM, Millard AR, and Pike AWG. 1995. Measurements and Relationships of Diagenetic Alteration of Bone from Three Archaeological Sites. J Archaeol Sci 22:201-209.

Katzenberg MA. 2000. Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. In: Katzenberg MA, and Saunders SR, editors. Biological Anthropology of the Human Skeleton. New York: Wiley-Liss, Inc. p 305-327.

Kellner CM, and Schoeninger MJ. 2007. A simple carbon isotope model for reconstructing prehistoric human diet. Am J Phys Anthropol 133(4):1112-1127.

Larsen CS. 1997. Bioarchaeology: Interpreting behavior from the human skeleton. Lasker GW, Mascie-Taylor CGN, Roberts DF, and Foley RA, editors. New York, New York: Cambridge University Press.

Larson G, Cucchi T, Fujita M, Matisoo-Smith E, Robins J, Anderson A, Rolett B, Spriggs M, Dolman G, Kim TH and others. 2007. Phylogeny and ancient DNA of *Sus* provides insights into neolithic expansion in island southeast Asia and Oceania. Proc Natl Acad Sci U S A 104(12):4834-4839.

Lee-Thorp J. 2002. Two decades of progress towards understanding fossilization processes and isotopic signals in calcified tissue minerals. Archaeometry 44:435-446.

Lee-Thorp J, and Sealy J. 2008. Beyond documenting diagenesis: The fifth international bone diagenesis workshop. Palaeogeography, Palaeoclimatology, Palaeoecology 266(3-4):129-133.

Lee-Thorp JA, Sealy JC, and van der Merwe NJ. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. J Archaeol Sci 16(6):585-599.

Longin R. 1971. New Method of Collagen Extraction for Radiocarbon Dating. Nature 230(5291):241-242.

Morwood MJ, Brown P, Jatmiko, Sutikna T, Saptomo EW, Westaway KE, Due RA, Roberts RG, Maeda T, Wasisto S and others. 2005. Further evidence for small-bodied hominins from the Late Pleisotocene of Flores, Indonesia. Nature 437:1012-1016.

Morwood MJ, Soejono RP, Roberts RG, Sutikna T, Turney CSM, Westaway KE, Rink WJ, Zhao JX, Van den Bergh GD, Due RA and others. 2004. Archaeology and age of a new hominin from Flores in eastern Indonesia. Nature 431:1087-1091.

Musser GG. 1981. The giant rats of Flores and its relatives east of Borneo and Bali. American Museum of Natural History 169:67-176.

Nielsen-Marsh C, Gernaey A, Turner-Walker G, Hedges REM, Pike AWG, and Collins M. 2000. The Chemical Degradation of Bone. In: Cox M, and Mays S, editors. Human Osteology: in Archaeology and Forensic Science. London: Greenwich Medical Media.

Roberts RG, Westaway KE, Zhao JX, Turney CSM, Bird MI, Rink WJ, and Fifield LK. 2009. Geochronology of cave deposits at Liang Bua and adjacent river terraces in the Wae Racang valley, western Flores, Indonesia: a synthesis of age estimates for the type locality of *Homo floresiensis*. J Hum Evol 57(5):484-502.

Schoeninger MJ. 2005. Stable isotope studies in human evolution. Evolutionary Anthropology 4(3):83-98.

Schoeninger MJ, Moore KM, Murray ML, and Kingston JD. 1989. Detection of bone preservation in archaeological fossil samples. Applied Geochemistry 4:281-292.

Schwarcz HP, and Schoeninger MJ. 1991. Stable Isotope Analyses in Human Nutritional Ecology. Yearbook of Physical Anthropology 34:283-321.

Van Klinken GJ. 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J Archaeol Sci 26(6):687-695.

Van Klinken GJ, and Hedges REM. 1995. Experiments on Collagen-Humic Interactions: Speed of Humic Uptake, and Effects of Diverse Chemical Treatments. J Archaeol Sci 22:263-270.

Westaway KE, Roberts RG, Sutikna T, Morwood MJ, Drysdale R, Zhao JX, and Chivas AR. 2009a. The evolving landscape and climate of western Flores: an environmental context for the archaeological site of Liang Bua. J Hum Evol 57(5):450-464.

Westaway KE, Sutikna T, Saptomo WE, Jatmiko, Morwood MJ, Roberts RG, and Hobbs DR. 2009b. Reconstructing the geomorphic history of Liang Bua, Flores, Indonesia: a stratigraphic interpretation of the occupational environment. J Hum Evol 57(5):465-483.

White CD, Price TD, and Longstaffe FJ. 2007. Residential histories of the human sacrifices at the moon pyramid, Teotihuacan. Ancient Mesoamerica 18:159-172.

White TD. 2000. Human Osteology. San Diego, California: Academic Press.

CHAPTER 3: USING CARBON AND NITROGEN STABLE ISOTOPES TO INTERPRET DIETARY BEHAVIOR OF LIANG BUA RATS

Introduction

Carbon and nitrogen stable isotope analyses of bone collagen have been extensively used to determine dietary behaviors of prehistoric humans and animals (DeNiro and Epstein 1978a; DeNiro and Epstein 1981). However, stable isotopic study of skeletal remains from Liang Bua cave on Flores has not previously been conducted. To determine the dietary behaviors of animals living on Flores during the Pleistocene and Holocene, carbon and nitrogen stable isotope analyses were conducted on collagen from 32 rat femora samples. These samples were selected from the stratigraphic sequence to determine if any shifts in the consumption of C_3 or C_4 plants occurred through time. The introduction of non-endemic C_4 plants, such as foxtail millet, may help determine when agriculturalists first arrived on Flores. Currently the archaeological record for early cultivation in Island Southeast Asia is very limited. Researchers have interpreted the movement of agriculturalists in this region based on limited data, such as a single grain of cereal found in pottery sherds, as well as skeletal remains of domesticated dogs and pigs (Boomgaard 2007; Noerwidi 2009). This research study will help provide information on when agriculturalists arrived on Flores as well as help reconstruct the environment and paleoecology during the Pleistocene and Holocene.

Rats of Flores

On Flores, the family Muridae is composed of twelve different extinct and extant species of rats and mice, four of which are considered endemic species of giant rat (Musser 1981). The four species of giant rat include *Papagomys armandvillei, Papagomys theodorverhoeveni, Spelaeomys florensis*, and *Hooijeromys nusatenggara*. *P. armandvillei* is an extant species of rat and is the largest giant rat known from Flores, found through the Pleistocene and Holocene.(Van den Bergh et al. 2009). *P. theodorverhoeveni* has also been found present in both the Pleistocene and Holocene. This species is similar in morphology to *P. armandvillei*, but has smaller teeth and different occlusal patterns (Van den Bergh et al. 2009). *Spelaeomys florensis* is found in the archaeological record during the Pleistocene and Holocene and is thought to have gone extinct around 4-3 kya (Van den Bergh et al. 2009). Lastly, *Hooijeromys nusatenggara*, which is morphologically similar to *P. armandvillei* and *P. theodorverhoeveni*, is found in the middle Pleistocene. Middle-sized rats from Flores include Komodomys and Floresomys (Musser 1981; Van den Bergh et al. 2009). The Polynesian rat (*Rattus exulans*) is not found on Flores until the upper Holocene (Van den Bergh et al. 2009).

Possible dietary items for the Liang Bua rats include grasses, buds, flowers, leaves, fruit, snails, insects, earthworms, and fungi (Musser 1981; Pisanu et al. 2011). Specific diets for each species of rat would vary depending on their particular niche in the habitat and specific adaptations (e.g., molar morphology). Rats are known to be omnivorous opportunistic eaters and thrive due to their ability to consume a wide ranging

diet and live closely with humans. Upon the arrival of agriculturalists to Flores, the rats of Liang Bua would have easily adapted to consume plants introduced by humans. In this study, rat femora were sorted into approximate size categories (small, medium, and large) such that the stable isotope results may suggest some evidence of differential diets between rats of differing size, used as a proxy for likely taxonomic differences.

Stable Isotopes

Isotopes are atoms of an element that have the same number of protons, but differ in the number of neutrons (Mays 2000a; Schoeninger 1995b). Unlike unstable isotopes used for radiometric dating, such as ^{14}C, stable isotopes are not radioactive and do not decay, or transmute, over time (Larsen 1997). Thus, stable isotope analyses provide an accurate representation of the stable isotopes in an animal at the time of death. Controlled feeding studies have shown that stable isotopes in animal tissue and bone accurately reflect its diet (DeNiro and Epstein 1978b). In archaeological contexts, stable isotope analyses, particularly carbon and nitrogen, have been conducted on a myriad of tissues including hair, nails, skin, muscle tissue, bone apatite, tooth enamel, and bone collagen. These tissues have been analyzed to reconstruct diet, weaning patterns and migration (Ambrose et al. 1997; Bocherens et al. 1994; DeNiro 1985; Dupras et al. 2001; Kellner and Schoeninger 2007; Larsen 1997; White et al. 2007).

Differences in the abundance of stable isotopes are very small and as a result are measured in the form of ratios between lighter and heavier stable isotopes (Ambrose 1993). These measurements are reported in delta values or "δ" units as parts per thousand (‰) relative to an international standard (Larsen 1997). The internationally recognized

standard for carbon is V-PDB (Vienna Pee Dee Belenmite) marine limestone (Ambrose 1993). The equation used to determine carbon isotopic ratios is:

$$\delta^{13}C_{(PDB)} = \left[\frac{^{13}C/^{12}C_{Sample}}{^{13}C/^{12}C_{PDB}} - 1 \right] X\ 1000‰ \tag{3.1}$$

The standard for nitrogen is the atmospheric N_2 [AIR]. The equation used to determine nitrogen isotopic ratios is

$$\delta^{15}N_{(AIR)} = \left[\frac{^{15}N/^{14}N_{Sample}}{^{15}N/^{14}N_{AIR}} - 1 \right] X\ 1000‰ \tag{3.2}$$

Dietary resources typically contain less ^{13}C than the standard; as a result carbon isotope ratios are most often represented as negative values. Conversely, most often sources contain higher amounts of ^{15}N than the standard and are represented by positive values (Ambrose 1993).

It is possible to calculate the percentage of C_4 plants (PC4) in the diet by using the following equation:

$$PC_4 = \left(\frac{\delta_c - \delta_3 + \Delta_{dc}}{\delta_4 - \delta_3} \right) X\ 100 \tag{3.3}$$

where δ_c is the measured isotopic value of the collagen, δ_3 = -26.5, the average isotopic value for C3 plants, δ_4 = -12.5‰, the average isotopic value for C_4 plants, and Δ_{dc} = -5 (Schwarcz 1989; Schwarcz et al. 1985).

Fractionation

As mentioned previously, stable isotopes have the same number of protons and electrons, but differ in the number of neutrons (Larsen 1997). This varying number of

neutrons causes isotopes of the same element to differ in their atomic mass. Isotopes with additional neutrons have more mass and as a result are "heavier" (Ambrose 1993). The difference in mass causes isotopes to undergo chemical reactions at different rates. Heavier isotopes undergo chemical reactions at a slower rate than lighter isotopes since their chemical bonds form and break more slowly (Schoeninger 1995). The difference between the rates of reaction results in discrimination or fractionation against the heavier isotopes of an element (Ambrose 1993). Stable isotopes with lighter atomic masses, such as carbon and nitrogen, have an increased degree of fractionation. This is due to lighter isotopes having a proportionally larger difference in mass than heavier isotopes (Schoeninger 1995a).

Isotope fractionation is the amount of change in stable isotope ratios when a stable isotope substrate is converted from one or more substances into a new product (Schoeninger 1995a). Isotope exchange reactions (also called equilibrium isotope distribution) and kinetic processes are two of the main mechanisms that cause isotope fractionation (Ambrose 1993). Isotope exchange reactions are reactions in which a physical property, such as evaporation or condensation, causes a reaction (Schoeninger 1995a). For example, when water evaporates, isotopically heavier water (${}^{1}H_2{}^{18}O$) will evaporate more slowly than isotopically lighter water (${}^{1}H_2{}^{16}O$). This results in an enrichment of the isotopically heavier water remaining in the liquid phase, while the isotopically lighter water is enriched in the vapor phase (Ambrose 1993). Kinetic processes are unidirectional reactions caused by the addition of biochemical properties such as photosynthesis in plants and collagen synthesis in bone (Schoeninger 1995a).

Carbon

There are two main stable isotopes of carbon, ^{12}C and ^{13}C, used in stable isotope analyses. ^{13}C uses C_3 (Calvin), C_4 (Hatch-slack), and Crassulacean Acid Metabolism (CAM) photosynthetic pathways. Browsing animals are more likely to consume C_3 plants, while grazing animals are more likely to consume C_4 plants. C_3 plants include trees, shrubs, tubers, wheat, and rice, whereas C_4 plants include savannah grasses, amaranths, sedges, millet, sugar cane, maize and sorghum (Larsen 1997; Lee-Thorp 2003). The CAM pathway is utilized by succulents and cacti which have values similar to both C_4 and C_3 plants and is less commonly studied (Larsen 1997). The amount of C_4 plants in the diet is especially important when determining consumption of certain cultivated plants such as millet, sugarcane and maize.

The carbon source for all terrestrial plants is CO_2. When CO_2 is used from the atmosphere the heavier ^{13}C isotope is less discriminated against by C_4 plants. This results in less negative $\delta^{13}C$ values in C_4 plants than in C_3 plants (Larsen 1997). $\delta^{13}C$ values in C_4 plants range from -9‰ to -14‰ and average around -12.5‰, while C_3 plants range from -20‰ to -35‰ and average around -26‰ (Katzenberg 2000a; Larsen 1997). Due to the difference in $\delta^{13}C$ values it is possible to discern whether an animal's diet was composed mostly of C_3 plants, C_4 plants or was a mixed consumer lying somewhere in the middle. In comparison, to diet, human bone collagen is enriched by 5‰ and bone apatite is enriched by 9.5‰ (Ambrose and Krigbaum 2003b; Katzenberg 2000a).

Carbon can be used to determine the consumption of marine versus terrestrial plants and animals. This is possible due to marine and terrestrial organisms acquiring

their carbon from sources that have different $\delta^{13}C$ values. Marine organisms derive carbon from dissolved bicarbonate which has a $\delta^{13}C$ value of 0‰. In comparison terrestrial organisms derive carbon from atmospheric carbon dioxide which has a $\delta^{13}C$ value of -7‰ (Katzenberg 2000c). Marine organisms typically have $\delta^{13}C$ values in-between C_3 and C_4 values. For instance, marine fish have a $\delta^{13}C$ value of -17‰ and marine mammals have a $\delta^{13}C$ value of -17.5‰ (Mays 2000b). Research that studies both nitrogen and carbon are believed to garner the most accurate results when determining consumption of marine versus terrestrial plants and animals (Schoeninger et al. 1983).

Nitrogen

Nitrogen is another commonly used stable isotope and has two stable isotopes, ^{14}N and ^{15}N, that are used in stable isotope analysis. Ninety-nine percent of nitrogen is held in the atmosphere or ocean as N_2, with the majority in the atmosphere (Larsen 1997). Atmospheric nitrogen has a value of 0‰. Animals and plants need nitrogen to form essential amino and nucleic acids; however, N_2 must be converted in order to be utilized. There are two ways in which this occurs. One way involves direct nitrogen fixation from the air by leguminous plants, such as beans or peas, by means of symbiotic bacteria from the genus *Rhizobium* (Katzenberg 2000c). This results in $\delta^{15}N$ values being similar to that of the atmosphere. Alternatively, non-nitrogen fixing plants absorb forms of nitrogen that are decomposed from organic matter in the soil such as ammonia (NH_2) and nitrate (NO_3) (Katzenberg 2000b). This results in $\delta^{15}N$ values being more positive than that of the atmosphere with $\delta^{15}N$ values averaging around +6.5‰.

Nitrogen is used to help determine diet by means of animal and plant trophic levels. Animals are enriched in $\delta^{15}N$ based on their trophic level with each successive level in the food chain enriched by 3‰ (Katzenberg 2000b). For instance, grazing animals have $\delta^{15}N$ values averaging around +8.5‰ (Mays 2000b). Since $\delta^{15}N$ values vary by trophic level it is possible to use nitrogen to determine weaning patterns. When infants are born they have $\delta^{15}N$ values similar to their mothers. However, once they begin to feed on their mother's milk they are at a higher level in the food chain causing their $\delta^{15}N$ values to become enriched. Infants that have not been weaned off of their mother's milk have $\delta^{15}N$ values enriched by 2-3% compared to older individuals (Fogel et al. 1989).

$\delta^{15}N$ values are believed to be altered by climate and habitat (Ambrose 1991). Thus it is not possible to directly compare animals from different environments. Animals and plants living in a hot and arid habitat have higher concentrations of $\delta^{15}N$ values than do animals living in a cool and wet habitat. The higher values of $\delta^{15}N$ are due to water stress. In an attempt to conserve water in hot and dry environments animals excrete more urea in relationship to water. This results in more ^{14}N being excreted by the body and more ^{15}N being held by the body (Katzenberg 2000c). Nitrogen is also useful in determining marine versus terrestrial diets. In comparison to terrestrial organisms marine organisms have about 6 to 8‰ more positive $\delta^{15}N$ values. For instance, marine fish have a $\delta^{15}N$ value of +13‰ and marine mammals have $\delta^{15}N$ value of +15‰ (Mays 2000b). This more positive $\delta^{15}N$ value is due to the fact that most nitrogen results from terrestrial bacterial activity and because nitrogen is more limited in marine ecosystems (Schoeninger 2005).

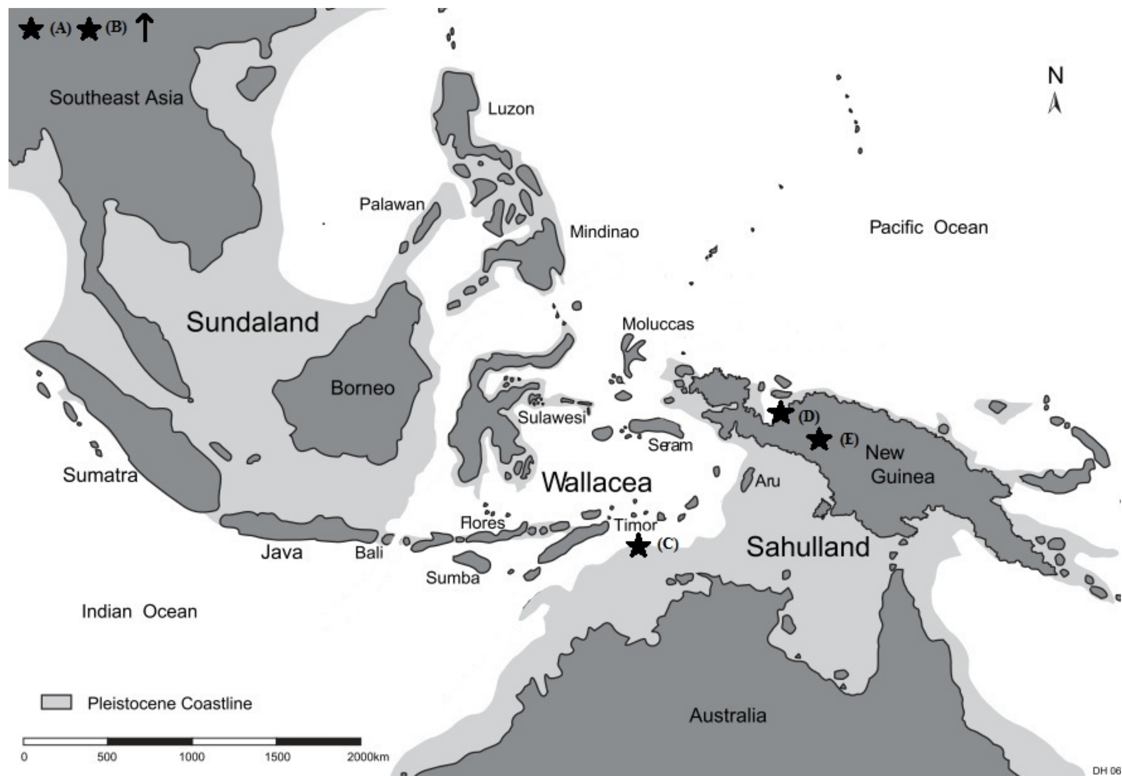
Collagen

Living bone is composed of water, mineral and organics (Nielsen-Marsh et al. 2000). The mineral portion of bone accounts for 70% of dry bone weight with the organic portion accounting for the remaining 30% (Wood 1990). Ninety percent of the organic component of bone consists of collagen and non-collagenous proteins, which account for about 22% of total dry bone weight (Katzenberg 2000c; Schoeninger et al. 1989). Type I collagen is found in bone, tendons and skin and is composed of three amino acid chains, two a_1 chains and one a_2 chain, formed into a triple helix (Nielsen-Marsh et al. 2000; Schwarcz and Schoeninger 1991). Extensive cross-linking between each of the chains allows the collagen to be relatively insoluble and less likely to degrade (Nielsen-Marsh et al. 2000). This allows collagen to preserve better and not be as readily subjected to diagenetic effects as bone apatite (Larsen 1997). The sample of rat skeletal remains used in this study spans the past 20,000 year history of Liang Bua. A pilot study has demonstrated that bone collagen is still present and, in some occasions, well preserved. It is believed to take 10 to 30 years for collagen to be completely replaced allowing for a long-term view into isotopic values of the animal (Mays 2000b). Although some researchers argue collagen represents the whole diet, other researchers suggest collagen may only represent the protein portion of the diet since it is composed of both essential and non-essential amino acids (Ambrose et al. 1997; Ambrose and Krigbaum 2003a; Larsen 1997).

Early Agriculture in Island Southeast Asia

The introduction of agriculture and the migration of people through Island Southeast Asia have been of particular interest to the scientific community (Bellwood 2005; Bellwood 1997; Donahue and Denham 2010; Zohary and Hopf 2000). The exact origin and time frame of which the spread of cultivation took place has remained uncertain due to limited archaeological and botanical evidence. However, it is currently believed that C_4 cultigens, foxtail millet, *Setaria italica*, and sugarcane, *Saccharum robustum*, as well as the C_3 cultigen, rice, *Orzya sativa*, were introduced to the region during the initial introduction of agriculture. The introduction of C_4 cultigens into the region should be distinguished by a shift in $\delta^{13}C$ values from a C_3 signature to a C_4 signature.

Cultivation in Island Southeast Asia stems from China, one of the major centers of agricultural origins. The cultivation of rice and foxtail millet first began in North China between 6400 and 5000 BC (Zohary and Hopf 2000). Carbon and nitrogen stable isotope analyses conducted on Holocene dogs (*Canis familiaris*) from the Dadiwan of the Laoguantai tradition in North China indicate the presence of cultivated plants in the dog's diet (Bettinger et al. 2010) (Figure 14). The Laoguantai tradition has been radiocarbon dated to 7,800 – 7,300 calBP (Bettinger et al. 2010). Based on evidence from Dadiwan, millet-based agriculture in the area became intensive within 1,000 years of initial cultivation (Bettinger et al. 2010).



(Adapted from Morwood et al. 2009)

Figure 14 (A) Carbon and nitrogen stable isotope analyses on Holocene dogs dated to 7,800 – 7,300 BC from North China indicate the presence of cultivated plants (Bettinger et al. 2010). (B) Pollen of the progenitor of foxtail millet, green bristlegrass found in North China dated to 12,000 years ago (Underhill 1997). (C) Single grain of foxtail millet from Timor dating to around 1,000 BC (Boomgard 2007). (D) Wad of sugarcane fiber found in an undated crevice (Horrocks et al. 2008). (E) Biomolecular evidence indicates the domestication of sugarcane in New Guinea (Lebot 1999).

The movement of agriculture out of China did not occur quickly. Although agriculture was taking place in China by 6500 BC it was not until 3500 BC that agriculture appears in the archaeological record in Taiwan (Bird et al. 2004). The cultivation of rice and foxtail millet was continued there by the Neolithic Dapengkeng culture. The cultivation of rice is known through the discovery of impressions of rice in cord marked and incised pottery sherds from the Suogang site on the Penghu Islands

(Bellwood 2005). Through time the type of pottery produced by the Dapengkeng culture transitioned into the red slipped style pottery that is eventually seen in Indonesia. Although not all researchers agree (Donahue and Denham 2010), some researchers propose an 'Out of Taiwan' model in which agricultural populations spread to the coasts of Taiwan, traveled south through the Philippines and eventually reached the islands of Indonesia around 2500 to 2000 BC (Bellwood 2005; Bellwood 1997). Due to the lack of evidence in the archaeological record for the cultivation of plants during this time period, it is difficult to know exactly how the spread of agriculture occurred in this region.

Although there is still some debate within the scientific community biomolecular evidence indicates that wild sugarcane, *Saccharum robustum*, a C_4 plant, was originally domesticated in New Guinea (Lebot 1999) (Figure 14). New Guinea is thought to have had an independent emergence of agriculture either before or coinciding with the emergence of agriculture in mainland Southeast Asia (Bird et al. 2004). Exact dates of the domestication and spread of sugarcane are uncertain, however, it is thought to have spread eastward out of New Guinea and into Island Southeast Asia prior to the expansion of Astronesians into the region (Donahue and Denham 2010). Botanical evidence for the cultivation of sugarcane is especially limited, however, a wad of sugarcane fiber has been discovered in an undated crevice at the Yuku rock shelter in New Guinea (Horrocks et al. 2008). There is currently no evidence for early cultivation of sugarcane on Flores.

Prior to the arrival of agriculture, the Australo-Melanesians living in Island Southeast Asia were egalitarian hunter-gatherers (Boomgaard 2007). They subsisted off of small mammals such as bats and rodents, marine resources, as well as exploited

naturally occurring, aroids, yams, bananas, and breadfruit (Bellwood 1987). Due to food availability and environment it is most likely that these people lived in small groups (Boomgaard 2007). They are also believed to have lived in caves and had a stone tool industry of scrapers and blades. Plant remains discovered in Timor include gourd, bamboo, and chestnut, as well as Job's tears, betel vine, and acreca nut dating to 3000 BC (Bellwood 1997; Boomgaard 2007).

Archaeological evidence indicating agriculture in the Sunda Islands is particularly limited; however, there are some agricultural indications. Remains of domesticated dogs and pigs found in Timor are believed to have been transported from Java or Sulawesi (Noerwidi 2009). Excavated caves in Eastern Timor have revealed pottery similar to that found in Northern Borneo dating between 2,500 and 2,000 BC (Noerwidi 2009). The Neolithic Kendeng Limbu site in East Java revealed stone adze and red slipped pottery. The Kendeng Lembu site has fertile volcanic soils and is also located next to water sources making it an ideal place for agriculture. This site, however, has not been dated reliably (Noerwidi 2009). Although exact places of origins are unknown and debated, cultigens such as taro, sago, yams, bananas, and coconuts are believed to have been first domesticated in the Island Southeast Asian region (Bellwood 1997). Palynological evidence provides further support for the cultivation of plants in Taiwan, Sumatra, and Java. The pollen during this time period shows that a significant amount of forest clearing was taking place. This most likely indicates that forests were being cleared to create land for agriculture (Bellwood et al. 2006). The type of pottery that is associated with agriculture in Taiwan, red slipped pottery, appears in Eastern Indonesia around 2000 BC

and continues until the Early Metal Phase around 500 BC. Red slipped pottery is not found present in Western Indonesia. However, evidence of rice production is found with the cord marked or paddle-impressed pottery that was produced (Bellwood 2005).

Although rice was a major part of the agricultural repertoire in mainland Southeast Asia, as agricultural populations moved into Island Southeast Asia, the cultivation of rice diminished (Bellwood 1997). While today rice is grown in many different environments, early rice forbears were most likely sensitive to changes in environmental conditions. Early rice is believed to have had photoperiod sensitivity. This means that when agriculturalists brought rice into new latitudinal zones the change in daylight lengths would have caused the plant to not flower properly (Bellwood 1997). In addition to these changes, islands located within five degrees north and south of the equator, such as Borneo and Sulawesi, have equatorial climates that are especially not conducive to the cultivation of cereals. Islands in equatorial climates have reduced seasonality with a longer rainy season and consist of thick rain forests with vegetation that grows quickly in the wet climate (Bellwood 2005; Bellwood 1997). Slash and burn techniques to clear the dense trees would have depleted nutrients in the clayey soils making cultivation increasingly difficult (Boomgaard 2007). This would have forced Austronesian speaking colonists to depend on cultigens that do grow well in the area such as tubers and fruits.

It is believed that once agricultural populations traveled south, away from the equator, and into a more seasonal tropical climate that the production of rice resumed (Glover and Higham 1996). Indonesian islands located south of the equator in the

intermediate tropical zones, which include Java, Bali, Sumbawa, Lombok, Flores, and Timor, have seasons regulated by monsoons. The winter rainy season has low rainfall followed by an extended dry season. In addition these intermediate tropical islands have rich volcanic soils suitable for agriculture (Bellwood 1987).

It is unclear whether or not millet would have diminished as severely in equatorial climates as rice. However, millet is a warm season crop and grows well in dry conditions (Zohary and Hopf 2000). This indicates that millet would not have grown well in equatorial climates that have an extended rainy season. Contrastingly the cultivation of millet would have been very successful in the long summer dry season in intermediate tropical environments south of the equator. Although archaeological data for this time period is limited there is some evidence of millet being produced in the Lesser Sundas by the discovery of a grain of foxtail millet from Timor dating to around 1000 BC (Boomgaard 2007) (Figure 14).

Materials and Methods

In this study, femora of large, medium, and small-sized rats from Liang Bua (Flores, Indonesia) are analyzed for their carbon and nitrogen stable isotopes. Based on ^{14}C dates (Roberts et al. 2009) the oldest rat bone included in this study comes from a stratigraphic level that is at least 19,200 years old. The specific species of rats used for the sample in this study are unknown; however, giant rats *Papagomys armandvillei*, *Papagomys theodorverhoeveni* and *Spelaeomys florensis*, middle sized rats *Komodomys rintjanus* and *Floresomys naso* and small sized rats *Rattus exulans* are possible candidates for the sample since they have all be identified as being present during the

Pleistocene and Holocene. Prehistoric Floresian rats are differentiated into species based primarily on size, shape, and occlusal patterns of dentition (Musser 1981). Since the sample of this study includes only rat femora, species determinations of the sample could not be made. Although the small and medium sized femora cannot be convincingly attributed to species, they most likely belong to *Komodomys rintjanus*, *Floresomys naso* and *Rattus exulans.* Alternatively, the largest femora most likely belong to *Papagomys armandvillei*, *Papagomys theodorverhoeveni* and *Spelaeomys florensis.*

The rat bone samples were excavated from Sector XVI in 2008 as part of a collaboration between the Indonesian National Research and Development Centre for Archaeology and the University of Wollongong (Australia). Indonesian faunal expert, Rokus Due Awe, performed the taxonomic identifications and selected the best preserved specimens from each 10 cm spit (excavation unit) for this analysis.

Collagen was extracted from 136 individual rat femora to conduct carbon and nitrogen stable isotope analyses. This extraction followed methods first described by Longin (1971) and Chisholm et al. (1989), and modified by Laboratory for Stable Isotope Studies (LSIS) at the University of Western Ontario. The collagen extraction process began by washing all bones in distilled water in an ultrasonicator to rid them of humic soils. The samples were then dried in an oven at 70 degrees. Once dry, the bones were soaked in a 5% HCL acid solution until only collagen remained. The bones were subsequently washed again in a centrifuge with deionized water and lastly rinsed with a sodium chloride solution to be sure that all humic soils were removed. The collagen was then placed in an oven at 90 degrees to break down the collagen in 5 ml of deionized

water. The water solution was then transferred from the larger vial to a smaller vial and placed again in the oven with no lid to evaporate the water and leave behind the collagen. The collagen was then weighed and the collagen yield was calculated to help determine preservation. Between .5 and 1 umg of the sample was then weighed into silver capsules for the final analysis. Once the collagen was collected the samples were sent to the Smithsonian Institution OUSS-MCI Stable Isotope Mass Spectrometry Facility to be analyzed on a Thermo Scientific Delta V Advantage mass spectrometer.

Results

This research project began with a sample of 136 rat femora. After the preservation of the samples was determined (see Chapter 2), 32 samples were considered well preserved and were analyzed for carbon and nitrogen stable isotopic data, as well as the percentage of C_4 plants in the diet (Table 2).

Table 2 Data for 32 samples considered well preserved including nitrogen and carbon isotopic values and the estimated percentage of C4 plants in the diet.

Sample ID	**Depth (cm)**	**^{15}N values (‰)**	**^{13}C values (‰)**	**PC4 (%)**
1m.1	10	7.87	-20.59	6.48
2s.1	20	5.67	-14.95	46.77
2m.1	20	4.09	-11.15	73.96
2m.2	20	3.13	-15.47	43.09
4m.1	60	4.63	-16.72	34.16
5b.1	70	5.66	-21.16	2.43
6s.1	80	6.69	-17.17	30.94
6m.2	80	8.65	-20.88	4.40
6b.1	80	7.39	-20.70	5.70
6b.2	80	8.03	-13.85	54.64
7s.2	90	4.14	-20.81	4.93
7s.3	90	5.24	-16.72	34.12
7m.2	90	5.51	-15.32	44.13
7m.3	90	6.16	-15.69	41.48
8s.1	100	6.46	-19.12	17.02
8m.2	100	5.31	-18.89	18.65
8m.3	100	5.30	-19.01	17.76
8m.4	100	4.70	-13.14	59.70
9m.1	110	5.55	-14.72	48.43
9m.2	110	5.20	-16.65	34.61
9m.3	110	4.48	-18.54	21.15
10b.1	120	8.01	-19.09	17.20
10b.2	120	8.55	-19.01	17.79
11b.1	140	6.18	-21.19	2.21
12s.1	210	7.52	-20.51	7.07
13s.2	230	4.40	-20.72	5.60
14m.1	240	9.73	-19.27	-1.40
15s.1	250	5.53	-21.70	-0.23
16s.1	260	-1.56	-21.53	15.93
18b.2	280	6.89	-20.58	6.60
26m.1	460	7.23	-21.31	1.39
33s.1	520	3.89	-22.16	-4.69

Carbon

Figure 15 shows $\delta^{13}C$ values plotted against depth. According to this figure six samples (12s.1, 13s.1, 14m.1, 15s.1, 16s.1, and 18b.2) located between 210 and 280 cm and two samples (26m.2 and 33s.1) located between 460 and 520 cm have $\delta^{13}C$ values indicative of a C_3 plant diet. The first six samples are approximately 5,160 to 8,270 years old with values ranging from -19.27‰ to -21.69‰, while the other two samples are at least 13,000 years old and have values of -21.3‰ and -22.15‰. A significant change in $\delta^{13}C$ values of the samples is seen beginning at 110 cm, which is dated to between 2750 and 5,160 years ago (Roberts et al. 2009). These $\delta^{13}C$ values range from -11.14‰ to -21.19‰ and indicate a clear shift in the rat's diet to include C_4 plants.

Figure 15. Graph showing $\delta^{13}C$ values vs. depth.

Figure 16 shows the percentage of C_4 plants in the diet. The figure supports the carbon isotopic data in that prior to 13,000 years the rats of Liang Bua were consuming a diet that did not include C_4 plants. Approximately 5,160 to 8,270 years there is also limited consumption of C_4 plants. Between 2,750 and 5,160 years ago, around 110 cm, there is a significant increase in the percentage of C_4 plants in the diet. Twenty cm deep sample 2m.2 has the most percentage of C_4 plants in the diet at 73.9%.

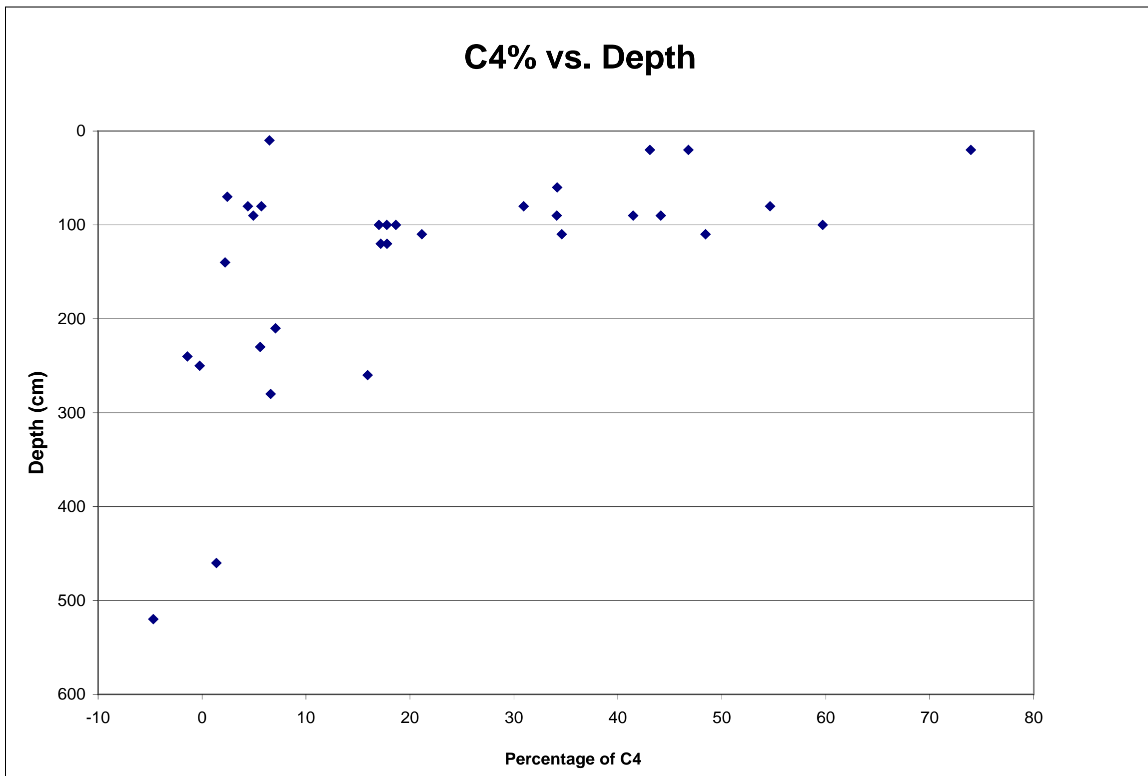


Figure 16 Graph showing percentage of C4 plants in the diet using the average value for C4 plants of -12.5

Nitrogen

Figure 17 shows $\delta^{15}N$ values plotted against depth. The $\delta^{15}N$ values for the sample range widely from -1.56‰ to 9.73‰. Samples above 110 cm have $\delta^{15}N$ values that range between 3.13‰ to 8.65‰.

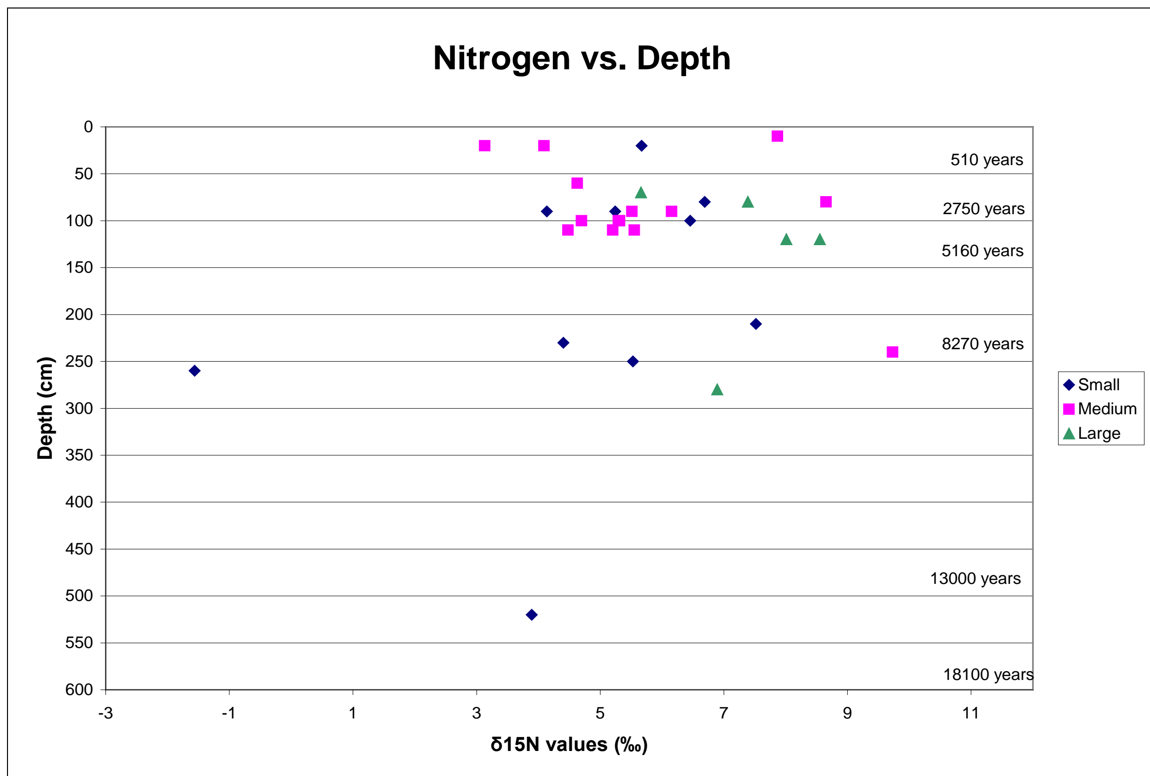


Figure 17. Graph showing $\delta^{15}N$ values vs. depth.

Discussion

The Out of Taiwan model is one of the main theories proposed for the spread of agriculture into Island Southeast Asia. According to this model agriculture reached Taiwan around 3500 BC, spread south through the Philippines and into Island Southeast Asia around 2500 to 2000 BC (Bellwood 2005; Bellwood 1997). However, as some researchers have discussed, botanical and archaeological evidence for the cultivation of

plants in this region is limited (Donahue and Denham 2010). This research study provides new data for the introduction of agriculture into Island Southeast Asia.

Carbon and nitrogen stable isotopic data indicate that a non-endemic C_4 plant, most likely foxtail millet or sugarcane, was introduced onto Flores prior to 2,750 years ago but after 5,160 years ago. Eight samples located between 210 cm and 520 cm have $\delta^{13}C$ values ranging from -19.27‰ to -22.15‰. C_3 plants have $\delta^{13}C$ values ranging from -20‰ to -35‰ and average around -26‰ (Katzenberg 2000a; Larsen 1997). According to the carbon data, the rats of Liang Bua were consuming a diet consisting of C_3 plants around 5,160 to 8,270 years ago and prior to 13,000 years ago. Sometime between 2,750 and 5,160 years ago there was a significant shift in the rats' diet to include C_4 plants. $\delta^{13}C$ values of 23 samples located between 10 and 140 cm range from -11.14‰ to -21.19‰. These values are between what is typically expected for C_3 plants (-20‰ to -35‰) and C_4 plants (-9‰ to -14‰) with an emphasis on the consumption of C_4 plants. This indicates that there was an introduction of C_4 plants prior to 2,750 years ago and the rats were consuming both C_3 and C_4 plants.

The shift in the carbon data from a C_3 signature to a C_4 signature between 2,750 and 5,160 years ago corresponds with archaeological evidence for the introduction of Neolithic cultural material. Around 4,000 years ago the Long-tailed macaque, Eurasian pig (*Sus scrofa*), Javanese or short-tailed porcupine (*Hystrix javanaica*) and Masked palm civet (*Paradoxurus hermaphrodites*) were introduced onto Flores and are seen in the archaeological record of Liang Bua (Van den Bergh et al. 2009). The introduction of the

fauna coincides with the appearance of pottery and stone ground adzes (Van den Bergh et al. 2009).

Denham and Donahue (2010) argue against the Out of Taiwan model and instead suggest that the introduction of agriculture and material culture into the region occurred from multiple sources and networks of people. This argument is largely based on the lack of archaeobotanical evidence indicating agricultural movement into the region. However, data from this research brings new information to the study of how and when agriculture spread into Island Southeast Asia. According to the carbon stable isotopic data, agriculture, as indicated by the introduction of non-endemic C_4 plants, began in the area surrounding Liang Bua sometime between 2,750 and 5,160 years ago. The shift in $\delta^{13}C$ values from a C_3 signature to a C_4 signature occurs abruptly and indicates that there was a rapid change in the plants growing in this area. The data mirrors the Out of Taiwan theory in that at one time the cultivation of plants and the start of agriculture occurred.

The interpretation of nitrogen data is more complex, as diet and physiological factors can introduce significant individual variation. The variation among the data for $\delta^{15}N$ values may result from differences between individual rat's diets, especially the amount of animal protein intake, and/or metabolic variation, including illness and pregnancy. Vegetation comprises the majority of a rat's diet, however, rats are opportunistic eaters and will eat animal protein when it is available. For example, rats have been reported to scavenge dead seabirds, as well as acquire juvenile turtles and eggs for consumption in island habitats (Pisanu et al. 2011). The variation in the nitrogen data could also be the result of illness or pregnancy (Williams 2008) or breastfeeding

juveniles. At this stage, the nitrogen data does not contribute to our understanding of agriculture or plant intake. There does not appear to be a strong difference in the $\delta^{13}C$ values or the $\delta^{15}N$ values between the small, medium and large sized rats.

Conclusion

The introduction of agriculture into Island Southeast Asia is currently based off of limited archaeological and botanical data. Although some evidence has been found for the cultivation of rice and foxtail millet these sites have yielded unreliable and unsecured dates. Similarly there is little archaeobotanical evidence for the initial cultivation and dispersal of sugarcane into Island Southeast Asia. The carbon and nitrogen stable isotopic data from this study provide new evidence for the introduction of agriculture into the Island Southeast Asia region. According to the carbon isotope data, a significant shift in the $\delta^{13}C$ values from a C_3 signature to a C_4 signature occurred between 2,750 and 5,160 years ago. This shift is indicative of the introduction of a non-endemic plant, which is most likely either foxtail millet or sugarcane. Since this shift occurs abruptly it indicates that the introduction of agriculture to Flores occurred relatively quickly and has continued to present day. Due to Flores' geographic location in Eastern Indonesia it is likely that non-endemic cultigens were introduced into the island Southeast Asia region through the spread of agriculture sometime prior to 5,160 years ago.

Literature Cited

Ambrose SH. 1991. Effects of Diet, Climate and Physiology on Nitrogen Isotope Abundances in Terrestrial Foodwebs. J Archaeol Sci 18:293-317.

Ambrose SH. 1993. Isotopic Analysis of Paleodiets: Methodological and Interpretive Considerations. In: Sandford MK, editor. Ivestigations of Ancient Human Tissue. Langhorne, Pennsylvania: Gordon and Breach Science Publishers.

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, and Krueger HW. 1997. Stable Isotopic Analysis of Human Diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol 104:343-361.

Ambrose SH, and Krigbaum J. 2003a. Bone chemistry and bioarchaeology. J Anthropol Archaeol 22(3):193-199.

Ambrose SH, and Krigbaum J. 2003b. Bone chemistry and bioarchaeology. Journal of Anthropological Archaeology 22:193-199.

Bellwood P. 1987. The Prehistory of Island Southeast Asia - A Multidisciplinary Review of Recent Research. Journal of World Prehistory 1(2):171-224.

Bellwood P. 2005. First Farmers: The Origins of Agricultural Societies. Madden, MA: Blackwell Publishing.

Bellwood PS. 1997. Prehistory of the Indo-Malaysian Archipelago. Honolulu, Hawaii: University of Hawaii Press.

Bellwood PS, Fox JJ, and Tryon DT. 2006. The Austronesians: historical and comparative perspectives Canberra, Australia: ANU E Press.

Bettinger RL, Barton L, and Morgan C. 2010. The Origins of Food Production in North China: A Different Kind of Agricultural Revolution. Evolutionary Anthropology 19:9-21.

Bird MI, Hope G, and Taylor D. 2004. Populating PEP II: the dispersal of humans and agriculture through Austral-Asia and Oceania. Quat Int 118:145-163.

Bocherens H, Fizet M, and Mariotti A. 1994. Diet, physiology and ecology of fossil mammals as inferred from stable carbon and nitrogen isotope biogeochemistry: implications for Pleistocene bears. Paleogeogr Paleoclimatol Paleoecol 107:213-225.

Boomgaard P. 2007. Southeast Asia: An Environmental History. Santa Barbara, California: ABC-CLIO, Inc.

Chisholm BS, Nelson DE, and Schwarcz HP. 1982. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216(1131-1132).

DeNiro MJ. 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to paleodietary reconstruction. Nature 317:806-809.

DeNiro MJ, and Epstein S. 1978a. Influence of Diet on Distribution of Carbon Isotopes in Animals. Geochim Cosmochim Acta 42(5):495-506.

DeNiro MJ, and Epstein S. 1978b. Influence of diet on the distribution of carbon istopes in animals. Geochimica et Cosmochimica Acta 42(295-406).

DeNiro MJ, and Epstein S. 1981. Influence on Diet on the Distribution of Nitrogren Isotopes in Animals. Geochim Cosmochim Acta 45(3):341-351.

Donahue M, and Denham T. 2010. Farming and Language in Island Southeast Asia: Reframing Austronesian History. Curr Anthropol 51(2):223-256.

Dupras TL, Schwarcz HP, and Fairgrieve SI. 2001. Infant feeding and weaning practices in Roman Egypt. Am J Phys Anthropol 115:204-212.

Fogel M, Tuross N, and Owsley D. 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations. Carnegie Institution of Washington. 111-116 p.

Glover CI, and Higham CFW. 1996. New evidence for early rice cultivation in South, Southeast and East Asia. In: Harris DR, editor. The origins and spread of agriculture and pastoralism in Eurasia. Washington, D.C.: Smithsonian Institution Press. p 413-441.

Horrocks M, Bulmer S, and Gardner RO. 2008. Plant microfossils in prehistoric archaeological deposits from Yuku rock shelter, Western Highlands, Papua New Guinea. J Archaeol Sci 35:290-301.

Katzenberg M. 2000a. Stable Isotope Analysis: A Tool For Studying Past Diet, Demography, and Life History. In: Katzenberg M, and Saunders S, editors. Biologial Anthropology of the Human Skeleton. New York: John Wiley and Sons. p 305-327.

Katzenberg MA. 2000b. Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. In: Katzenberg MA, and Saunders SR, editors. Biological Anthropology of the Human Skeleton. New York: Wiley-Liss, Inc.

Katzenberg MA. 2000c. Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. In: Katzenberg MA, and Saunders SR, editors. Biological Anthropology of the Human Skeleton. New York: Wiley-Liss, Inc. p 305-327.

Kellner CM, and Schoeninger MJ. 2007. A simple carbon isotope model for reconstructing prehistoric human diet. Am J Phys Anthropol 133(4):1112-1127.

Larsen CS. 1997. Bioarchaeology: Interpreting behavior from the human skeleton. Lasker GW, Mascie-Taylor CGN, Roberts DF, and Foley RA, editors. New York, New York: Cambridge University Press.

Lebot V. 1999. Biomolecular evidence for plant domestication in Sahul. Genet Resour Crop Evol 46(6):619-628.

Lee-Thorp J. 2003. What do Stable Isotopes tell us about Hominid Dietary and Ecological Niches in the Pliocene? International Journal of Osteoarchaeology 13:104-113.

Longin R. 1971. New Method of Collagen Extraction for Radiocarbon Dating. Nature 230(5291):241-242.

Mays S. 2000a. New Directions in the Analysis of Stable Isotopes in Excavated Bones and Teeth. In: Cox M, and Mays S, editors. Human Osteology In Archaeology and Forensic Science. London, England: Greenwich Medical Media Ltd. p 425-438.

Mays S. 2000b. New Directions in the Analysis of Stable Isotopes in Excavated Bones and Teeth. In: Cox M, and Mays S, editors. Human Osteology: In Archaeology and Forensic Science. London: Greenwich Medical Media Ltd. p 425-438.

Musser GG. 1981. The giant rats of Flores and its relatives east of Borneo and Bali. American Museum of Natural History 169:67-176.

Nielsen-Marsh C, Gernaey A, Turner-Walker G, Hedges REM, Pike AWG, and Collins M. 2000. The Chemical Degradation of Bone. In: Cox M, and Mays S, editors. Human Osteology: in Archaeology and Forensic Science. London: Greenwich Medical Media.

Noerwidi. 2009. Archaeological Research at Kendeng Lembu, East Java, Indonesia. Bulletin of the Indo-Pacific Prehistory Association:26-32.

Pisanu B, Caut S, Gutjahr S, Vernon P, and Chapuis JL. 2011. Introduced black rats Rattus rattus on Ile de la Possession (Iles Crozet, Subantarctic): diet and trophic position in food webs. Polar Biol 34(2):169-180.

Roberts RG, Westaway KE, Zhao JX, Turney CSM, Bird MI, Rink WJ, and Fifield LK. 2009. Geochronology of cave deposits at Liang Bua and adjacent river terraces in the Wae Racang valley, western Flores, Indonesia: a synthesis of age estimates for the type locality of *Homo floresiensis*. J Hum Evol 57(5):484-502.

Schoeninger MJ. 1995a. Stable isotope studies in human evolution. Evolutionary Anthropology 4(3):83-98.

Schoeninger MJ. 1995b. Stable Isotopes Studies in Human Evolution. Evolutionary Anthropology 4(3):83-98.

Schoeninger MJ. 2005. Stable isotope studies in human evolution. Evolutionary Anthropology 4(3):83-98.

Schoeninger MJ, Deniro MJ, and Tauber H. 1983. Stable Nitrogen Isotope Ratios of Bone-Collagen Reflect Marine and Terrestrial Components of Prehistoric Human Diet. Science 220(4604):1381-1383.

Schoeninger MJ, Moore KM, Murray ML, and Kingston JD. 1989. Detection of bone preservation in archaeological fossil samples. Applied Geochemistry 4:281-292.

Schwarcz HP. 1989. Ancient Maya Diet - As Inferred From Isotopic and Elemental Analysis. Jounral of Anthropological Sciences 16(5):451-474.

Schwarcz HP, Melbye J, Katzenberg MA, and Knyf M. 1985. Stable Isotopes in Human Skeletons of Southern Ontario - Reconstructing Paleodiet. J Archaeol Sci 12(3):187-206.

Schwarcz HP, and Schoeninger MJ. 1991. Stable Isotope Analyses in Human Nutritional Ecology. Yearbook of Physical Anthropology 34:283-321.

Underhill AP. 1997. Current issues in Chinese neolithic archaeology. Journal of World Prehistory 11(2):103-160.

Van den Bergh GD, Meijer HJM, Awe RD, Morwood MJ, Szabo K, Ostende LWV, Sutikna T, Saptomo EW, Piper PJ, and Dobney KM. 2009. The Liang Bua faunal remains: a 95 k.yr. sequence from Flores, East Indonesia. J Hum Evol 57(5):527-537.

White CD, Price TD, and Longstaffe FJ. 2007. Residential histories of the human sacrifices at the moon pyramid, Teotihuacan. Ancient Mesoamerica 18:159-172.

Williams LJ. 2008. Investigating Seasonality of Death at Kellis 2 Cemetery Using Solar Alignment and Isotoptic Analyses of Mummified Tissues. London, Ontario, Canada: University of Western Ontario. 271 p.

Zohary D, and Hopf M. 2000. Domestication of Plants in the Old World. New York, New York: Oxford University Press.

CHAPTER 4: CONCLUSIONS

The island of Flores is located in a historically rich geographic location. Human migration throughout Island Southeast Asia has sparked research interest in the region in regards to the spread of agriculture. Flores is also well known to have a unique island biogeography, which includes stegodon, komodo dragons and giant rats (Morwood et al. 2009; van den Bergh et al. 2009). The island of Flores would have been difficult to colonize since it has never been connected to a mainland continent, even during periods of glacially low sea levels. The Liang Bua rats are found throughout the stratigraphic sequence and their stable isotopic levels provide insights into the ecology and environment of Flores over the past 13,000 years.

Prior to this study, bone collagen preservation of skeletal remains from Liang Bua was unknown. Flores is located south of the equator within the intermediate tropical zone. Tropical environments are well known to have poor bone collagen preservation that results from significant amounts of precipitation and heat (Lee-Thorp et al. 1989, Van Klinken 1999). Due to this, some researchers have indicated that bone collagen preservation in Liang Bua is unlikely (Roberts et al. 2009). This research study conducted carbon and nitrogen stable isotopic analyses on 136 rat sub-fossil femora samples in order to determine the level of preservation within the Liang Bua cave. Collagen yields, C:N ratio, wt % C and wt % N were investigated in order to determine the preservation of the samples. According to Ambrose (1990) prehistoric samples with carbon concentrations > 13% and nitrogen concentrations > 4.8 % are considered to be

well preserved. Although samples with < 5% collagen are typically considered not well preserved, this study yielded 18 samples with appropriate nitrogen concentrations and 22 samples with appropriate carbon concentrations that have collagen yields of < 5%. I argue that concentrations of carbon and nitrogen are an appropriate means to determine preservation and that a total of 29 samples have appropriate nitrogen concentrations and 33 samples have appropriate carbon concentrations.

Carbon and nitrogen stable isotopic analyses were conducted on the samples considered well preserved. According to the carbon isotope data, samples located between 210 and 520 cm have $\delta^{13}C$ values ranging between -19.27‰ and -22.15‰. These values are indicative of a diet consisting of C_3 plants. Samples located between 110 and 10 cm have $\delta^{13}C$ values ranging between -11.14‰ and -21.19‰. These values indicate a shift in the rat diet to include C_4 plants. This shift occurred abruptly at sometime between 5,160 and 2,750 years ago and most likely indicates the introduction of agriculture onto the island of Flores, particularly the cultivation of the C_4 plants foxtail millet and sugarcane. Since Flores is located in Eastern Indonesia the introduction of non-endemic cultigens into the island Southeast Asia region would have taken place sometime prior to 5,160 years ago.

The $\delta^{15}N$ values for the rat samples range widely between -1.56‰ to 9.73‰. The variation among the $\delta^{15}N$ values is most likely the result of differences in protein intake between individual rats diet, as well as illness, pregnancy or breastfeeding adult females and breastfeeding juveniles.

Literature Cited

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J Archaeol Sci 17(4):431-451.

Lee-Thorp JA, Sealy JC, and Van der Merwe NJ. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. J Archaeol Sci 16(6):585-599.

Morwood MJ, Sutikna T, Saptomo EW, Jatmiko, Hobbs DR, and Westaway KE. 2009. Preface: research at Liang Bua, Flores, Indonesia. J Hum Evol 57:407-449.

Roberts RG, Westaway KE, Zhao JX, Turney CSM, Bird MI, Rink WJ, and Fifield LK. 2009. Geochronology of cave deposits at Liang Bua and adjacent river terraces in the Wae Racang valley, western Flores, Indonesia: a synthesis of age estimates for the type locality of *Homo floresiensis*. J Hum Evol 57(5):484-502.

Van den Bergh GD, Meijer HJM, Awe RD, Morwood MJ, Szabo K, Ostende LWV, Sutikna T, Saptomo EW, Piper PJ, and Dobney KM. 2009. The Liang Bua faunal remains: a 95 k.yr. sequence from Flores, East Indonesia. J Hum Evol 57(5):527-537.

Van Klinken GJ. 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J Archaeol Sci 26(6):687-695.

APPENDIX: DATA FOR ALL OF THE SAMPLES USED IN THIS STUDY, INCLUDING: % COLLAGEN, WT % N, WT % C, AND C:N RATIOS

Sample ID	Depth (cm)	% Collagen	wt %N	wt %C	C:N (atomic)
1m.1	10	8.33	13.26	37.87	3.33
2m.1	20	10.46	13.32	38.89	3.41
2m.2	20	14.14	12.17	34.15	3.27
2s.1	20	3.95	12.89	37.89	3.43
3s.1	40	-31.25			
3s.2	40	14.04			
3m.1	40	-6.25			
4m.1	60	14.62	13.29	37.31	3.28
5b.1	70	0.28	10.00	30.69	3.58
5b.2	70	0.78			
5s.1	70	10.64			
6b.1	80	1.685	12.08	36.07	3.48
6b.2	80	1.55	20.48	58.70	3.34
6m.1	80	-11.82			
6m.2	80	4.97	12.54	35.90	3.34
6s.1	80	1.19	12.76	38.08	3.48
7b.1	90	0.11	2.76	11.65	4.92
7m.1	90	42.42			
7m.2	90	3.83	13.16	36.19	3.21
7m.3	90	25.35	12.87	35.90	3.26
7s.1	90	-1.41			
7s.2	90	-6.12	11.11	48.20	5.06
7s.3	90	6.35	13.74	40.29	3.42
8m.1	100	0.22	2.77	7.00	2.95
8m.2	100	1.81	11.21	32.69	3.40
8m.3	100	5.83	12.61	36.30	3.36
8m.4	100	4.55	13.71	38.35	3.26
8s.1	100	17.39	12.40	36.14	3.40
9m.1	110	0.83	12.79	35.16	3.21
9m.2	110	5.37	9.79	27.81	3.31
9m.3	110	8.82	13.27	37.08	3.26
10b.1	120	0.86	8.78	25.56	3.40
10b.2	120	1.96	5.01	14.60	3.40
10s.1	120	0			

Sample ID	Depth (cm)	% Collagen	wt %N	wt %C	C:N (atomic)
11b.1	140	0.58	3.47	13.28	4.47
11m.1	140	3.63	2.56	10.07	4.59
11s.1	140	-1.47			
12s.1	210	0	12.54	38.47	3.58
13b.1	230	-1.70	2.77	10.05	4.23
13s.1	230	0			
13s.2	230	2.27	10.32	32.05	3.62
14m.1	240	0.47	13.20	38.47	3.40
14m.2	240	0			
14s.1	240	0			
15s.1	250		10.52	35.85	3.97
15b.1	250	0.62			
15bs.1	250	0	0.53	4.23	9.25
15s.2	250	1.28			
16b.1	260	0.65	4.35	36.87	9.89
16s.1	260	2.44	13.94	37.25	3.12
16s.2	260	2.10	0.55	3.32	6.99
16s.3	260	0			
17s.1	270	0			
17s.2	270	-2.08			
18b.1	280	2.98	0.54	2.413	5.22
18b.2	280	0.54	6.29	17.96	3.33
18m.1	280	2.25	0.44	14.94	39.92
19s.1	310	0			
19s.2	310	2.13			
20m.1	320	0.40	0.53	3.74	8.18
20m.2	320	3.25	2.34	9.73	4.85
20m.3	320	2.94	0.69	3.38	7.80
21b.1	340	2.70	0.32	1.57	5.67
21m.1	340	0			
21m.2	340	0	0.79	7.30	10.81
22m.1	350	2.67	0.80	7.49	10.92
22m.2	350	0	1.20	10.33	10.05
23m.1	350	0			
23s.1	350	0			
23s.2	350	0			

Sample ID	Depth (cm)	% Collagen	wt %N	wt %C	C:N (atomic)
23s.3	350	0			
24m.1	420	1.58	1.50	6.81	5.28
24m.2	420	5.56	0.57	2.78	5.53
24s.1	420	40.91			
24s.2	420	0	1.85	12.34	7.77
24s.3	420	-2.60			
25b.1	450	0.51	0.66	3.92	6.77
25m.1	450	2.24	1.43	5.88	6.03
25m.2	450	2.63	0.45	2.78	8.65
25s.1	450	3.57			
25s.2	450	1.33			
26m.1	460	2.38	4.67	16.81	4.20
26s.1	460	0			
26s.2	460	0			
27m.1	470	0.93			
27m.2	470	0	3.93	5.80	7.94
27m.3	470	1.64			
28m.1	470	3.60	0.45	5.94	17.19
28m.2	470	0			
28s.1	470	-3486.05			
29b.1	490	0.40			
29m.1	490	2.31	0.56	2.31	4.81
29m.2	490	8.21	1.22	4.73	4.54
30b.1	490	1.67	0.57	2.03	4.17
30b.2	490	1.34			
30m.1	490	2.29	0.36	4.27	10.31
31b.1	500	0	1.55	10.75	8.12
31s.1	500	0			
31s.2	500	0			
32m.1	510	1.59	0.66	1.81	3.50
32m.2	510	0			
32s.1	510	0			
33m.1	520	4.88	0.71	2.03	3.00
33s.1	520	0	4.85	21.86	5.26
34b.1	530	0	2.37	11.34	5.58
34b.2	530	1.78	0.40	1.56	4.61

Sample ID	Depth (cm)	% Collagen	wt %N	wt %C	C:N (atomic)
34s.1	530	0			4.18
35b.1	540	1.40	3.59	12.89	4.18
35m.1	540	2.77	1.18	4.67	4.62
35m.2	540	0			
35m.3	540	2.67	0.83	2.77	3.89
35m.4	540	0.87	0.81	3.38	3.64
36b.1	550	0.15	0.76	5.19	7.55
36m.1	550	1.69			
36s.1	550	1.23			
36s.2	550	0			
37s.1	560	7.25	1.14	4.43	5.26
37s.2	560	0			
37s.3	560	22.22			
38b.1	600	0.41	0.47	3.82	9.54
38m.1	600	2.93	0.43	1.17	3.83
38m.2	600	0.38			
39s.1	610	3.16	0.51	2.34	5.37
40m.1	620	3.70	0.38	2.26	6.96
40s.1	620	5.88			
40s.2	620	3.03	0.72	6.21	9.95
41m.1	630	0.86	0.36	4.25	9.85
41m.2	630	3.00			
41s.1	630	10			
42m.1	650		1.85	6.59	4.16
42m.2	650		0.43	2.24	6.14
42s.1	650				
43b.1	670		0.56	2.45	5.06
44b.1	690				
45s.1	710				
46s.1	720		1.12	4.97	6.13