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## When did the ancestors of Polynesia begin to migrate to Polynesia? The mtDNA evidence

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WHEN DID THE ANCESTORS OF POLYNESIA BEGIN TO MIGRATE TO  
POLYNESIA? THE mtDNA EVIDENCE

by

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A thesis submitted in partial fulfillment  
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**December 2009**

## ABSTRACT

### **When Did the Ancestors of Polynesia Begin to Migrate to Polynesia? The mtDNA Evidence**

by

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The timing and nature of the migration of the ancestors of the Polynesian people is debated by two competing theories. The “Express Train” and “Slow Boat” theories assert that the migration of the Proto-Polynesian people began around 6,000 years before present (BP) or around 10,000 years BP respectively. Through the use of haplogroups and specific genetic mutations a direct relationship between the Proto-Polynesians and modern Polynesians was attempted to test which of these theories was correct. The ancient skeletal remains from the island of Borneo currently housed at UNLV were used in this study as their dates fall within both theories’ geographic and temporal range and so held the potential to provide the genetic material required to test these theories.

The aim of this study was to genetically link these ancient skeletal remains to modern Polynesian people. However, the results obtained determined the samples were contaminated with DNA belonging to people outside of the Southeast Asian haplogroup and that any original DNA had become degraded. This meant that no further analysis could take place. These findings lead to the conclusion that collection practices need to be implemented by the excavators and curators of skeletal remains to reduce or eliminate accidental contamination.

## TABLE OF CONTENTS

ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	vi
CHAPTER 1 INTRODUCTION .....	1
Research Questions.....	3
CHAPTER 2 LITERATURE REVIEW .....	12
Express Train Model.....	15
Slow Boat Model .....	16
Material Cultural Research .....	17
Linguistic Research.....	20
Physical Research .....	22
Genetic Research .....	25
Women and Migration .....	27
Malaria .....	30
Malarial Counter Measures.....	31
Using Malarial Diseases .....	33
CHAPTER 3 MATERIALS AND METHODS .....	44
Cultural Material.....	45
Decontamination Guidelines.....	47
Decontamination Procedures .....	49
Property of Bone .....	49
Genetic Material Amplified .....	52
Bone Sample Extraction Procedure .....	53
Extraction and Amplification.....	56
CHAPTER 4 RESULTS .....	61
CHAPTER 5 DISCUSSION / CONCLUSION .....	64
Research Questions Discussion .....	67
Implications of Research.....	69
Archaeological Recovery Techniques .....	70
Sample Viability .....	71
APPENDIX 1 BURIAL AGES AND REMAINS.....	74
APPENDIX 2 BURIALS AND ARTIFACTS .....	76
APPENDIX 3 DESCRIPTION AND WEIGHTS .....	77
BIBLIOGRAPHY.....	79

VITA..... 88

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## CHAPTER 1

### INTRODUCTION

Great migrations of the human species have occurred multiple times over the course of prehistory. These migrations demanded the traversing through large tracts of land, open ocean, and the crossing of numerous habitats with varying environmental conditions. Through the use of multiple lines of anthropological evidence, migration theories have been developed to place a frame of reference for dealing with questions concerning where and when people began spreading out around the world. It is thought that the first major migration of members of the genus *Homo* began before the appearance of *Homo sapiens*. Paleontological evidence documents a great migration of *Homo erectus* out of Africa around 1 million years ago or more (Fleagle & Gilbert, 2008; Stoneking, Sherry, Redd, & Vililant, 1992; Wolpoff, Hawks, Frayer, & Hunley, 2001; Wolpoff, Tishkoff, Kidd, & Risch, 1996). This migration episode of *Homo erectus* spanned the continents of Asia and Europe.

Much more recently, *Homo sapiens* migrated out of Africa 55,000 to 85,000 years ago to populate the globe (Forster & Matsumura, 2005; Goebel, Waters, & O'Rourke, 2008; Stringer, 2003; Stringer, 2002). Archeological evidence indicates early humans migrated into the extreme North West corner of Asia 40,000 to 45,000 years ago (Goebel et al., 2008) Humans continued their easterly movement from Siberia over the land bridge created by lower sea levels during the last ice age. This migration of people occurred within the last 28,000 to 30,000 years (Goebel et al., 2008).

Human movement has been tracked through the recovery of archeological artifacts. These artifacts have been found on the eastern and southern edges of the North and South



American continents connecting humans to this region of the world at a time depth of at least 10,000 to 12,000 years ago (Goebel et al., 2008). Human remains have been unearthed around Lake Mungo on the southeastern end of the Australian continent (Forster & Matsumura, 2005). These have been dated to 50,000 years before present (BP). The discovery of human remains in the most distant regions of Australia suggests a much earlier initial migration to this continent (Barker, 2005; Bowler et al., 2003; Forster & Matsumura, 2005; Hudjashov et al., 2007; O'Connell & Allen, 2004; Roberts, Jones, & Smith 1990). The distances and environments covered by the human species 50,000 years ago were immense. Environments ranged from the Siberian arctic, to equatorial Indonesia, to a more temperate climate in Australia. The distances traversed and occupied by the human species from Siberia to Australia is in excess of 9,000 miles entailing large movements over vast stretches of land as well as across open ocean. The open ocean voyage of people to Australia was a great navigational feat that would not be surpassed by any human populations until 6,000 to 10,000 years ago by the ancestors of modern day Polynesian people who sailed out into the remote regions of the Pacific Ocean.

The last great human diaspora, prior to the 15<sup>th</sup> century's age of exploration, was the populating of the Polynesian islands. There are two competing models for the timing of the migration and original homeland of the Proto-Polynesians (these competing models will be discussed in more detail below). The widely accepted view of this 8,000 mile epic journey has the origin of the Proto-Polynesians, the ancestors of modern Polynesians, in Southeast Asia (Southeastern China and Taiwan) (Diamond 1988; Friedlaender et al., 2007; Melton et al., 1995; Oppenheimer & Richards, 2001). The migration is thought to have begun 6,000 years ago as a rapid spread through Taiwan and the islands of

Southeast Asia to their ultimate destination, 1,000 years ago at Easter Island the most remote of all the Polynesian islands. An alternative theory places the origins of the first Proto-Polynesians on the Island of Borneo (modern day Indonesia and Malaysia) 40,000 years ago, where they lived for tens of thousands of years prior to any further migration events (Friedlaender et al., 2008; Friedlaender et al., 2007; Matisoo-Smith & Robins, 2004; Oppenheimer & Richards, 2001). Within the last 10,000 years these Proto-Polynesians began to move out in all directions, but specifically into the eastern islands of the Pacific.

### Research Questions

The focus of this thesis addresses the timing of the migration as well as the place of origin for the Proto-Polynesians. When did the Proto-Polynesian people reach the region of Eastern Indonesia and, more specifically, the island of Borneo (Cox, 2005; Diamond, 1988; Friedlaender, Gentz, Green, & Merriwether, 2002; Hurles, Matisoo-Smith, Gray, & Penny, 2003; Oppenheimer, 2004; Oppenheimer & Martin, 2001; Redd et al., 1995; Richards, Oppenheimer, & Sykes, 1998; Whyte, Marshall, & Chambers, 2005)? A common factor in the two opposing migration theories is that this general region (Southeast Asian Islands) acted as the spring-board for the Proto-Polynesian peoples to make their way east into the islands of Polynesia. The question arises as to which of the specific Islands of Southeast Asia were populated (Borneo or Taiwan) by the Proto-Polynesian people this is a matter of debate between these competing models. In addition, there remains the issue of the timing of the migration. Between the two models there is a difference of some 6,400 years as to when the migration first occurred. Therefore, the

major questions to be answered are when the ancestors of the modern Polynesian people arrived at the islands of Southeast Asia and from what specific region did the migration occur.

One way to address these questions is to examine DNA from the human remains of these Proto-Polynesians. While skeletal material is available in the human remains collections at UNLV, there are supplementary questions that need to be addressed relating to the feasibility of obtaining such data. First, can genetic material be recovered from the skeletal remains (either bone or teeth) that have been buried for thousands of years in a tropical rainforest environment? Second, if genetic material can be obtained, which source provides the most complete genetic sequences, bone or teeth? Third, do loose teeth or teeth still embedded in the jaw yield more complete or least contaminated genetic sequences? Also, has the storage of the skeletal remains at the UNLV facility allowed for the recovery of ancient DNA without any major contamination of that genetic material?

The path of these maritime explorers is a difficult one to follow due to the antiquity of the event and the vastness of the Pacific Ocean. As a consequence, a multitude of anthropological techniques and disciplines are employed to unravel this impressive migration of people to the islands of Polynesia. Archaeological, linguistic, cultural, physical, and genetic evidence are being employed to unravel the origins and migration route of the Proto-Polynesians into the far reaches of Polynesia (Baker et al., 2000; Cox, 2005; Diamond, 1998; Hagelberg, 1994; Harrison & Medway, 1962; Hurles et al., 2003). The synthesis of anthropological data has lead to two distinct migration theories

involving the ancestors of the Polynesian people as mentioned above. They are known as the “Slow Boat” and the “Express Train” theories.

These two theories use much of the same anthropological data and techniques to validate their stance on when, and from what region of the world, the Proto-Polynesian people originated. The major differences between the two migration theories are the questions as to when and from where the ancestors of the Polynesian people began their migration. The “Slow Boat” theory has the Proto-Polynesian people living in and around modern day Indonesia and Malaysia 20,000 years BP and even earlier. Then at about 12,000 to 10,000 BP, the ancestors of modern day Polynesian people began migrating eastward toward the Polynesian Islands (Oppenheimer & Richards, 2001). They made their way out to the far reaches of the Polynesian islands, a distance of 8,000 miles, within the last thousand years. Due to the large time frame in which the Proto-Polynesians are thought to have spent in the Western South Pacific Islands, artifacts of culture, language, and genetics are all used to support the theory.

The second migration theory, known as the “Express Train,” has the Proto-Polynesian people migrating from China out toward Polynesia around 3,600 BP through Taiwan, then through the Philippines, and lastly out to the Polynesian Islands (Cox, 2005). The movement of the Proto-Polynesian people is thought to have occurred along the shorelines of the islands of Indonesia, Melanesia, and Micronesia until they finally spread out and settled on the islands of Polynesia. The relatively short time frame in which the “Express Train” migration is argued to have occurred is also supported by archeological, linguistic, physical, (dental and cranial metrics), and genetic evidence from the islands of Micronesia, Indonesia, Malaysia, and Melanesia.

Archeological evidence includes the presence of a specific pottery type known as Lapita pottery, which is associated with the Polynesian people and their ancestors. The tracking of Lapita pottery over time and geographic space allows for the formulation of answers as to when and where the creators originated. Both theories use the Lapita pottery to defend their positions; therefore, difficulties arise in determining which is the correct theoretical model. Similar problems arise with linguistics, wherein the evidence is used on both sides to defend either theory without completely discrediting the other model. Linguistics is used to associate modern Polynesian people with the Lapita pottery, thought to be brought by the Proto-Polynesian people. Conversely, others show that the indigenous linguistic families of Island Southeast Asia have the genetic mutations that are linked to modern Polynesians and subsequently their ancestors. Physical characteristics of modern Polynesians and those of island Southeast Asia are used on both sides of the theoretical debate as well. Specifically, cranial measurements are used to support the “Slow Boat” theory showing continuity between modern Polynesians and Southeast Asians with the ancient remains of those buried on the islands. Similar to the other lines of evidence, there are physical characteristics that support the “Express Train” theory. This line of evidence involves the examination and comparison of the dentition linking modern and ancient people to the region of Southern China and Taiwan. Archeological, linguistic, and physical data are important in the determination of the timing and origin of the Proto-Polynesian people; however, the focus of this study centers on the use of genetic data (modern and ancient).

Modern genetic evidence finds itself in the same quagmire as archeology and physical and linguistic anthropology. Analysis of modern individuals’ DNA has allowed for the

development of a “Molecular Clock”; a regression formula that predicts the amount of time necessary for mutations to occur in DNA strands. The short time frame in which the migration of the Proto-Polynesian migration took place impacts the range of dates that result from this formula (Cox, 2005). The “Molecular Clock” time frame has the specific genetic mutations of the modern Polynesian people occurring 0 to 50,000 years ago (Cox, 2005). This time frame is too imprecise to support, unequivocally, either migration theory. However, analysis of modern Polynesian DNA indicates these people have a mutation that is associated with malarial resistance (Kaneko et al., 1998). In other situations, one would assume that a genetic resistance to malaria would be developed evolutionarily over thousands of years. Yet, when looking at the Proto-Polynesian people and the geographic area, specifically island logistics, one must consider the genetic principles of bottlenecks and founder effect (Flint, Boyce, Martinson, & Clegg, 1989). Both of these genetic principles can accelerate the frequency of a genetic mutation down to only a few generations. Therefore, the use of malarial genetic mutations cannot be used specifically to determine the timing of the migratory movements by the Proto-Polynesian people.

This lack of clarity in the genetic data results in a stalemate between the two competing theories. Reaching an agreement as to when and from where the ancestors of modern day Polynesians came from is thus still a matter of debate. A way through this cloudy conglomeration of data is to shift from a focus on modern Polynesian DNA to that from ancient DNA from people in the region and from the times of contention. By looking at the specific remains of animals and individuals buried in the region, a better picture of the migration patterns of the Proto-Polynesian people can emerge.

DNA analyses performed on modern and ancient animals such as pigs (*Sus scrofa*) and rats (*Rattus exulans*) found on Polynesian Islands give another angle with which to explore the movements of the Proto-Polynesian people (Larson et al., 2007; Matisoo-Smith & Robins, 2004). Animals such as *R. exulans* are the largest populations of extant and ancient remains available for large scale genetic analysis (Matisoo-Smith & Robins, 2004). Genetic comparisons of modern and ancient animals are used to indicate a line of ancestry from the ancient burials to the modern living counterparts on the Polynesian Islands today (Hagelberg, Quevedo, Turbon, & Clegg, 1994). Subsequently, ancient DNA of rats and pigs is used to show the movement of these animals across the Islands of the Pacific and by association their human carriers (Matisoo-Smith, 2002). This analysis assumes that the Proto-Polynesian people brought every rat and pig to each of the Pacific islands rather than just trading for them with the indigenous populations of the islands. Rats can stowaway or be taken on board boats, specifically for food, without having any great ties to the people on any particular island (Matisoo-Smith & Robins, 2004; Murray-McIntosh, Scrimshaw, Hartfield, & Penny, 2007). Pigs, as well, can be traded or sold and do not have to be associated with one particular group of people. The use of ancient animal DNA may one day answer the question of the origin of the Proto-Polynesian people but currently the data is not definitive. Still, the use of ancient animal and human DNA has been employed in an attempt to develop a more precise time frame and location of the migration of the Proto-Polynesian people.

Mitochondrial DNA (mtDNA) can be used to determine the genealogical trees of people (Thalmann, Hebler, Poinar, Pääbo, & Vigilant, 2004). The use of genetic material has been made possible because of the development of the technique Polymerase Chain

Reaction (PCR) (Hagelberg et al., 1991). PCR allows for the amplification of small amounts of DNA strands. The ability to replicate millions of times a small amount of DNA has made genetic research possible when looking at ancient materials (Yang et al., 1997). The nature of the transference of mtDNA from the matrilineal line makes an excellent resource for migration studies (Friedlaender et al., 2008; Hagelberg et al., 1999; Hagelberg & Sykes, 1989; Hagelberg et al., 1994; Stone & Stoneking, 1993; Thalmann et al., 2004; Yang et al., 1997). The use of mtDNA as a tracking device of an ancestral lineage can be applied to both modern and ancient individuals (Cox, 2005; Hagelberg et al., 1994). A peoples' mtDNA lineage can be used by looking for distinct mutations that occur during the replication process of the mtDNA. By the identification of a mutation in mtDNA, an entire group of people can be traced back to a common ancestor (Hagelberg et al., 1999). As a result of the Anderson et al. (1981) research that completely sequenced the 16,569 base pairs that make up human mtDNA, the ability to recognize these mutations within the human mtDNA code is possible. The comparison of mtDNA of modern day people has yielded mutations that can be used to distinguish regional groups of people from all others around the world. Specifically, modern Polynesian people have a set of mtDNA mutations that distinguish them from all other humans. Modern Polynesians have a deleted non-coding section of DNA consisting of 9 base pairs between the genes cytochrome oxidase II and lysyl transfer RNA. In addition to this 9 base pair deletion, modern Polynesians have 4 base pair changes: 16,217 (T to C), 16,247 (A to G), 16,261 (C to T), and 16,189 (T to C) known as the "Polynesian Motif" (Hagelberg et al., 1999; Hagelberg et al., 1994). The combination of the 9 base pair deletion and the "Polynesian Motif" is found to occur in around 95% of all modern day



Polynesian people (Hagelberg et al., 1999). Through the development of a baseline for the genetic code of mtDNA, mutations/variations in the code are used to determine the migration of people from any geographic region (Pierson et al., 2006). Therefore, the sampling of ancient human remains in the region of Southeast Asia, in conjunction with using the specific genetic mutations of the Polynesian people, should aid in the answering of the questions: from where and when did the Proto-Polynesian people originate?

A previous line of ancient DNA research on Polynesian people focused on the ancient remains of people unearthed on Easter Island (Hagelberg et al. 1994). This study confirmed that the modern indigenous inhabitants of Easter Island expressed the same, “Polynesian Motif”, mtDNA mutation as those buried in an ancient cemetery on the island. Therefore, ancient DNA is a highly useful tool to determine an ancestral line for the people of Easter Island going back 1,000 years. However, the origin of these ancient people is still open for debate. Through the analysis of mtDNA of ancient remains from people in an area of migratory contention such as the island of Borneo (the Great Niah Cave in particular), a picture of mtDNA ancestry and geographic descent can be developed.

The location of the Niah Cave burial site is important in the determination of the validity of either migration theory. The Great Niah Cave is on the outer edge of the suggested migration route of the Proto-Polynesians following the “Express Train” model. In contrast, for the “Slow Boat” model, the Great Niah Cave is in the middle of the perceived settlement of the Proto-Polynesian people for tens of thousands of years. The Great Niah Cave has been used as a grave site for at least 40,000 years (Harrison, 1967, p140). The long history of use of the Great Niah Cave provides the time depth and

location to test for the genetic mutations unique to the modern day Polynesian people, thereby adding to the knowledge base developed anthropologically around the Proto-Polynesian people. The finding of the Polynesian Motif in a the human remains 3,600 years and older would support the “Slow Boat” theory that the ancestors of the modern Polynesians were in the islands of Southeast Asia for a much longer time than the “Express Train” theory. But, the discovery of the Polynesian Motif in just the youngest of the human remains supports the “Express Train” theory which expects the ancestral people of with the Polynesian Motif in the islands of Southeast Asia to be less than 3,600 to 1,000 years BP.

What this study will add to the discussion is the genetic make up of 24 ancient people found buried in the region of Southeast Asia, specifically from The Great Niah Cave on the island of Borneo, during the time frame of 11,700 to 1,870 BP. (Appendix 1) This time frame is significant in that it spans the “Slow Boat” and “Express Train” theories of when and where the migration of the Proto-Polynesians happened. The addition of specific genetic information of actual people from the region during the critical time of migration for both theories has the potential for ground-breaking discovery. This addition of ancient human DNA from the island of Borneo to the archeological, linguistic, physical, and modern genetic data will aid in narrowing regions in the search for the ancestors of the Polynesian people.

## CHAPTER 2

### LITERATURE REVIEW

The intrepid nature of the human species has enabled people to populate nearly every inch of the globe. The journeys of ancient peoples out of Africa, into the Americas, and Australia are the focus of ongoing research. In addition to the American and Australian migrations, the last great prehistoric journey began between 10,000 and 3,600 years BP and ended around 1,000 years BP with the populating of Easter Island in the remote Pacific Ocean. The most widely accepted view of this 8,000 mile odyssey has the Proto-Polynesian's origins in Southeast Asia and is known as the "Express Train" theory. This theory has the timing of the Proto-Polynesian people's migration beginning 6,000 years ago in Taiwan and ending about 1,000 years ago as they settled on the farthest islands of Polynesia (Cox, 2005; Diamond, 1988; Friedlaender et al., 2002; Hurles et al., 2003; Oppenheimer, 2004; Oppenheimer & Martin, 2001; Redd et al., 1995; Richards et al., 1998; Whyte et al., 2005).

Another view, known as the "Slow Boat" theory, also addresses both the time and location of the Proto-Polynesian migration. However, this theory places the ancestors of modern Polynesians on the Island of Borneo for the last 30,000 to 40,000 years. This theory states these Proto-Polynesians only began migrating in the direction of the islands of Melanesia, and ultimately Polynesia, about 10,000 years ago, reaching Easter Island 1,000 years ago (Cox, 2005; Diamond, 1988; Friedlaender et al., 2002; Hurles et al., 2003; Oppenheimer, 2004; Oppenheimer & Martin, 2001; Redd et al., 1995; Richards et al., 1998; Whyte et al., 2005). This debate about the migration of people into the islands of Polynesia uses many anthropological techniques and data from various sub-disciplines

to be put to use. Archaeological, linguistic, cultural, physical, and modern genetic evidence are being used to illuminate the timing and origin of the Proto-Polynesian's migration into the far reaches of Polynesia (Baker et al., 2000; Cox, 2005; Diamond, 1998; Hagelberg et al., 1994; Harrison & Medway, 1962; Hurles et al., 2003). However, the focus of this study will be on the extraction and detection of ancient mitochondrial DNA (mtDNA) markers from a selection of remains originating from Niah Cave on the island of Borneo.

The core of this research is twofold: 1. to isolate ancient DNA from a population of Proto-Polynesian people; and 2. to utilize genetic markers that aid in determining the ancient population's origins (Southeast Asia) and their biological heritage (Polynesian). The two predominant theories involving the migration of Proto-Polynesians into Polynesia, as outlined above, will be tested through the genetic analysis. The research will be accomplished through the use of skeletal remains of people from eastern Malaysia dating from 11,700 to 1,870 BP. Ancient DNA has not been tested in this region for the particular genetic marker of the Polynesian people; however, other forms of evidence have been collected and be outlined below.

The wide range of anthropological data collected from Southeast Asia and Polynesia has contributed to our understanding of a complex tapestry of human migration. The employment of various sub-disciplines of research are necessary to account for the broad impact that people can impose culturally, linguistically, and environmentally on isolated regions such as islands. Such a multidisciplinary approach can be viewed as a form of checks and balances working to assess the competing theories that have developed in an attempt to explain the migration pattern of the first Polynesians. Therefore, a

multidisciplinary approach is of paramount importance in determining the movements of humans through Southeast Asia and, ultimately, to Polynesia.

The history of the Polynesian people spans hundreds to thousands of years and different anthropological techniques are capable of aiding in the determination of the origin and timing of their migration. Cultural, linguistic, physical and genetic artifacts, for example, have been used to determine the origin of the Polynesian people. Through research conducted by researchers in these various sub-disciplines, theories of how the Proto-Polynesians moved and colonized the Polynesian islands were formulated. Based on the evidence collected, two main migration theories have been developed to explain the movement of people from Southeast Asia to the islands of Polynesia.

As stated above, the “Express Train” or “Out of Taiwan” model proposes a migration of the Proto-Polynesian people from Taiwan around 6,000 years ago (Cox, 2005; Diamond, 1988). During this migration the Proto-Polynesians stopped at various Southeast Asian islands as they made their way to the Polynesian islands. In contrast, the “Slow Boat” migration theory maintains that the Proto-Polynesians were in the region of eastern Malaysia/Indonesia and western Melanesia about 7,000 years earlier (Friedlaender et al., 2008; Oppenheimer & Richards, 2001) (Figure 1). Thus, the “Slow Boat” theory proposes a longer Proto-Polynesian occupation of in the islands of Southeast Asia than the “Express Train” theory.

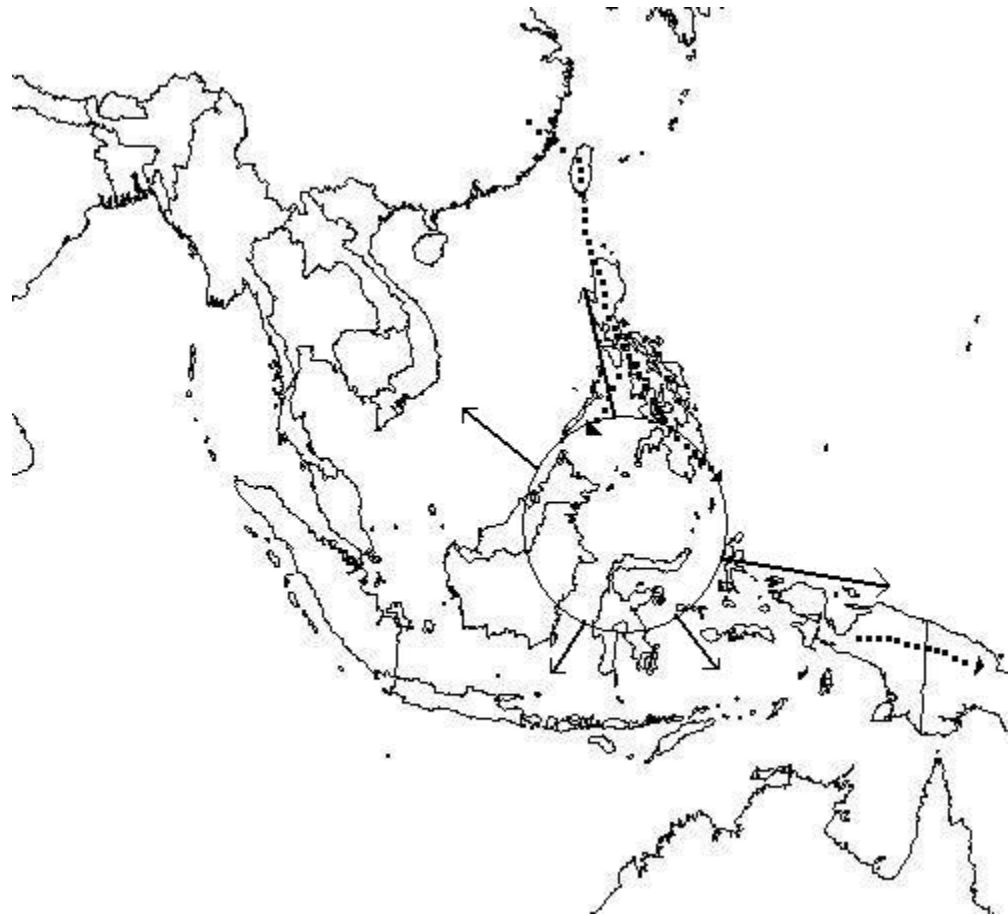


Figure 1. “Express Train” and “Slow Boat” Theories  
The small dotted lines represent route of the “Express Train” model for the Proto-Polynesian peoples’ movement as proposed by the “Express Train” model. The large dashed circle with lines projecting out represents the route of the Proto-Polynesian people according to the “Slow Boat” model.

### Express Train Model

The “Express Train” model proposes that the ancestors of the Polynesians were farmers that dispersed south from China/Taiwan, replacing the indigenous Australoid hunter/gatherer population, and who then voyaged east to Polynesia. The name given to the culture of the population migrating from Taiwan is Lapita (Diamond, 1988; Friedlaender et al., 2008; Matisoo-Smith & Robins, 2004). Archaeological artifacts collected in Southeast Asia are specifically associated with the Lapita culture. The Lapita

cultural complex is associated with notched pottery, domesticated animals, and highly refined navigational skills (Hagelberg & Clegg, 1993). With refined navigational skills came the ability to traverse large expanses of open ocean, which allowed for the settlement of the Polynesian islands.

The Lapita cultural materials reveal the migration route taken by the Proto-Polynesians from their origin in Southeast Asian. The migration of the Lapita people is thought to have begun within the last 6,000 years (Diamond 1988; Friedlaender et al., 2007; Melton et al., 1995; Oppenheimer & Richards, 2001). The people and their culture appear to have moved at a steady pace south through the Philippine islands over the course of 3,000 to 2,500 years. Subsequently, the Lapita people continued to migrate, moving to the islands of eastern Malaysia/Indonesia and western Melanesia. The Proto-Polynesians entered eastern Malaysia/Indonesia and western Melanesia after 3,500 years BP (Cox, 2005; Hurler et al., 2003) (Figure 1). The Lapita people are thought to have entered the Polynesian islands such as Fiji at around 1,000 years BP. This rapid expansion of people through that region left behind cultural, linguistic and genetic evidence that anthropologists use to substantiate the “Express Train” theory. Likewise, the “Slow Boat” model has evidence to bolster its explanation of the Proto-Polynesian migration.

#### Slow Boat Model

The “Slow Boat” theory proposes a slower rate of migration for the Proto-Polynesian people. Rather than originating from China or Taiwan 6,000 years ago and moving rapidly to Polynesia, the Proto-Polynesian people have their origins in eastern

Malaysia/Indonesia and western Melanesia (Friedlaender et al., 2007; Friedlaender et al., 2008; Matisoo-Smith & Robins, 2004; Oppenheimer & Richards, 2001). The “Slow Boat” theory, like the “Express Train,” has the Proto-Polynesian’s origins in Southeast Asia; however, the “Slow Boat” theory places the Proto-Polynesian people in the islands of Southeast Asia (eastern Indonesia, island Melanesia) much earlier, at over 30,000 years BP. The “Slow Boat” theory assumes that the genetics of the modern day Polynesian people are a direct reflection of the prehistoric migrants into Polynesia (Friedlaender et al., 2008). The “Slow Boat” theory has the Proto-Polynesian people radiating out from eastern Indonesia and island Melanesia around 12,000 years BP (Figure 1). The Proto-Polynesian people are thought to have migrated in all directions. The construction of theoretical models through the use of a multidisciplinary approach, such as cultural artifacts, help to support and/or dispute both theoretical models.

### Material Cultural Research

Archeologists use artifacts associated with groups of people to track their movement and/or trade routes. As artifact types are associated with a particular culture they are dated using techniques like radiocarbon and bio-stratigraphy. The movement of people can be dated through the discovery of artifacts, such as pottery. Pottery is the preferred artifact rather than plant or animal domesticate remains due to the low survivability of these materials, especially in a tropical environment (Krigbaum, 2003). Through the analysis of pottery and stone tools, inferences are made about food production and sedentism. These inferences enable the researcher to develop a picture of how people



lived on the islands of Southeast Asia. Focus is placed on the pottery assemblages found throughout the South Pacific.

Red slipped pottery is one such artifact used to determine the presence of the Proto-Polynesian people (Oppenheimer & Richards, 2001). The archaeological evidence obtained through red slipped pottery supports a 4,000 years BP origin for the original Polynesian people. The existence of red slipped pottery in eastern Indonesia and western Melanesia at 4,000 years BP contradicts the case for the “Express Train” theory. The discrepancy lies in the specific location of the artifacts and a date nearly 1,000 years earlier than the “Express Train” theory predicts for the migration of the Proto-Polynesian people (Oppenheimer & Richards, 2001). This narrowing of the time interval decreases the likelihood of the “Express Train” theory and bolsters the “Slow Boat” theory (Oppenheimer & Richards, 2001). However, the red slipped pottery is not the only artifact assemblage used to indicate the movements of the Proto-Polynesian people.

The collection of artifacts most closely associated with the Proto-Polynesians is known as the Lapita cultural complex. This Lapita complex consists of a unique dentate-stamped pottery, hand tools, and plant and faunal remains (Larson et al., 2007; Smith, 1995). The poor survivability of faunal and plant artifacts severely reduce their numbers in the archaeological record but, if present, they provide rich sources of information about the lives of the ancient people being studied. For example, the existence of ovens and storage pits at Lapita sites indicate a level of plant domestication; faunal remains show a level of marine subsistence (Smith, 1995).

The overall similarity of a Lapita pottery style in conjunction with the vast distances over which the pottery is found suggests that there might have been a centralized location

for its production and subsequent distribution (Kennett, Anderson, Cruz, Clark, & Summerhayes, 2004; Smith, 1995). Commonalities of style are important in that they show the potential for shared ideas as well as movement of people. However, other forms of analysis such as plasma-mass spectrometry have provided conflicting conclusions in regards to the origins Lapita pottery. Analysis of style, technical aspects and chemical composition of the Lapita pottery has lead to the conclusion that the vast majority of the pottery found is of local origin (Kennett et al., 2004). However, exchanges of material such as obsidian, pottery, and shells indicate that long distance trade between Lapita communities occurred (Smith, 1995). The establishment of a marine-based economy of trade is a testament to the navigational proficiency that the Proto-Polynesian people would have needed to sail across the vast open ocean.

The argument that the Lapita complex moved into the islands of Southeast Asia and then to the Polynesian Islands is not without its' detractors. Smith (1995) argues that the Lapita complex known on Island Melanesia has no firm evidence of a predecessor in any other region. Without an alternative place of origin, the evidence pertaining to the Lapita cultural complex could be interpreted as support of the "Slow Boat" theory of migration in that there was not an outside group moving in, but rather that the complex development and the subsequent migration was done by those indigenous to the islands of Southeast Asia (Oppenheimer, 2004). Thus, specific sites, with artifacts connected to the Lapita complex, are used to support both migration theories.

Research using pottery has also been used to support the "Express Train" model. Archaeological research on the island of Mussau performed by Kirch (1989) show artifacts, dating back 1,600 years BP, to be a part of the Lapita culture. The artifacts

lacked a gradual progression in their development, indicating they had no precursor. This is substantiated by the oldest deposits that contain elaborate pottery (Diamond, 1988). The existence of a fully formed and sophisticated style of pottery is a powerful indicator of a culture moving into a region. The lack of developmental progression of the pottery on the island of Mussau indicates a rapid change in the culture of the people. This change in cultural artifacts can be explained by the “Express Train” theory where the material culture of the Mussau people was quickly replaced by those associated with the Laptia culture and language. The inability to confidently confirm either theory’s claim using pottery alone highlights the need for a multidisciplinary approach to get a consensus as to when the Polynesian islands were populated. Therefore, anthropologists use various disciplines to support either theory. One such discipline is the field of linguistics which studies language patterns and is used to connect groups of people across time and vast geographical distances.

### Linguistic Research

The accepted view for the Islands of Southeast Asia is that the Papuan language is the first linguistic group to the region and the Austronesian language groups were second (Friedlaender et al., 2008; Friedlaender et al., 2002; Pierson et al., 2006). The Papuan language group has a much greater degree of diversity and that is indicative of a much greater time depth in the region (Friedlaender et al., 2008; Pierson et al., 2006). The extent of this greater time depth places the Papuan language groups on the islands of Southeast Asia tens of thousands of years before the Austronesian language group (Friedlaender et al., 2002). The work of Whyte et al., (2005) allows for “Melanesian”

Papuan speakers and the Austronesian speaking people from Southeast Asia (“Express Train” theory) to have had a linguistic influence the ancestors of Polynesians. The acceptance of an influence makes distinction between the two theories more difficult to clearly define. Distinctions can be made by more closely examining the two models themselves and how they handle the linguistic evidence.

A rapid spread of people across the area of eastern Indonesia, island Melanesia, Tonga/Samoa as predicted by the “Express Train” would not produce a strong linguistic signal (Hurles et al., 2003). The result of this extended time for sharing means that many words of Papuan origin should be found in late Oceanic languages. As of yet, this word exchange has not been found, but the search for this connection has not been completely exhausted (Hurles et al., 2003). The relatively rapid expansion of the Lapita cultural complex into the islands of the Pacific Ocean is seen as the reason for only a small amount of word sharing between the long established Papuan speaking languages and the new Austronesian language group (Bellwood, 2001; Pierson et al., 2006).

Friedlaender et al. (2008) views the organization of linguistic diversity by island size and island topography. On average, the Papuan language groups are found in the more isolated and inner portion of the islands where along the shore-line of islands the Austronesian language group is more prevalent (Friedlaender et al., 2008). The prevalence of the Austronesian language along the coast lines is a strong indicator of the rapid movement of people along an island chain thereby bolstering the view of the “Express Train” proponents (Friedlaender et al., 2008). In addition to the focus of language groups along the coast, a lack of shared words between the Papuan and

Austronesian speakers is a strong indicator of rapid movement and strengthens the “Express Train” model.

The “Slow Boat” theory is not without its support from the field of linguistics. Friedlaender et al. (2008) sampled some 952 individuals from a variety of Polynesian islands. He suggested that the invading Austronesian languages were adopted by the Near Oceanic people 3,300 years BP with little or no genetic or marital exchange (Friedlaender et al., 2008). One can infer that the Austronesian speakers had a strong influence on the culture of the indigenous Papuan speaking people by the examination of the cultural remains.

These inferences can be deceiving if other anthropological disciplines are not employed. In a linguistic study coupled with a genetic study involving modern day people living in Polynesian, non-Austronesian speaking people possessed the specific genetic markers once only thought to be associated only with Austronesian people (Friedlaender et al., 2002). The connection of Non-Austronesian speaking people with a specific Polynesian genetic marker throws doubt to the validity of the “Express Train” theory of a quick advance by the Proto-Polynesian people with little or no contact with the indigenous island dwellers. Much of the cultural and linguistic findings are better used in conjunction with additional lines of evidence such as physical remains.

### Physical Research

Measuring the physical characteristics of Polynesian peoples, both living and long deceased, is yet another method used in the pursuit of unraveling their origins. First, the pre-Neolithic inhabitants of the Southeast islands are characterized by a protruding

glabellae, massive jaws, large teeth, and alveolar prognathism (Brothwell, 1960; Matsumura & Hudson, 2005). This collection of cranial metric traits is known as the “Australo-Melanesian” features. Craniometric analyses indicate a stronger affiliation of modern Polynesian people with those of Southeast Asia than to people in China and Taiwan (Oppenheimer, 2004; Pietrusewsky, 1997). This line of evidence therefore points to the “Slow Boat” model as the possible origin of the Polynesian people. The existence of the “Australo-Melanesian” features in modern Polynesian people indicates a longer time of habitation on the islands of Southeast Asia than the “Express Train” model predicts. On the contrary, Matsumura and Hudson’s (2005) craniometric data support the “Express Train” theory of migration. Matsumura and Hudson (2005) maintain their data points to a close affinity to modern Chinese and Taiwanese people.

Matsumura and Hudson (2005) focus on dental morphology in Southeast Asia (China and Taiwan), island Southeast Asia, and the Polynesian islands. The dental evidence centers on Sinodont and Sundadont characteristics which are used to show migration patterns. The morphological traits used to determine Sinodonty and Sundadonty site specific identifiable features of a tooth. In the study by Turner (1990), the terms Sinodont and Sundadonty were coined. Through the course of the study eight traits were found to show the most significant statistical differences between populations (Turner, 1990). Sinodont traits, found in high frequency in certain populations (see below) include the following seven traits: shovel shaped upper first incisor, double-shoveling upper first incisor, one rooted upper first premolar, enamel extension of upper first molars, peg/reduced/ congenitally absent upper third molar, lower first molars with deflecting wrinkles, and three rooted lower first molars (Turner, 1990). The eighth trait of four

cusped lower second molars is found in high frequency in Sundadont populations (Turner, 1990). The terms Sinodont and Sundadont are used to classify people from specific countries or regions of Northeast Asia, Southeast Asia, the islands of Southeast Asia, and Polynesia. Sinodont dentition is associated with “Northern Mongoloids” of China, Northeast Asia, and Native Americans. Sundadont dentitions are found in Southern Mongoloids of Indo-China, Island Southeast Asia, and Polynesia (Turner, 1990; Turner 2006). Using the regional characteristics of Southeast Asia it can be assumed that the people of eastern Indonesia, western Melanesia, and Polynesia would have the Sinodont characteristic and people of western Indonesia and Indo China would express the Sundadont trait (Turner, 1990; Turner, 2006). According to Matsumura and Hudson (2005), dental evidence indicates an increase in similarity between Mainland and the Islands of Southeast Asia starting around the Neolithic period. The connection between the Neolithic and the change in dental configuration strengthens the “Express Train” theory. In contrast, Turner (1990) finds that the morphological changes from Sundadont to Sinodont are most likely to have occurred *in situ* rather than from people migrating from the Asian mainland. Therefore, dental evidence can not alone give a definitive answer as to from where and when the Proto-Polynesian people originated.

Craniometric data provide conflicting results that can be used to support both models. Since physical characteristics can be used to bolster both models other data need to be used to determine the ancestry of these people. Genetic research is another related discipline that is used to illuminate lineages both modern and ancestral.

## Genetic Research

Genetic research using both animal and human subjects has been used to determine the movements of the Proto-Polynesian people. Animal genetic research mainly includes rats and pigs that are thought to have been brought to the islands of Polynesia purposely by, or as stowaways of, the ancestors of modern day Polynesian people. The genes of modern rats found on the islands of Polynesia are used to show a connection to the ancient rat DNA recovered from sites on the islands of Polynesia. Domestic animals such as pigs have a unique genetic profile which is different to that of feral pigs in the region of Southeast Asia. Thus, the genetic research performed on both modern and ancient animals, to show specific genetic similarities and differences between them.

*Rattus exulans* has a dispersal range from the mainland of Southeast Asia across the Islands of Southeast Asia and ending far out in the Pacific Ocean at Easter Island (Matisoo-Smith & Robins, 2004). Along with the wide dispersal of *R. exulans* the species itself is different from that of the ones brought to Southeast Asia by European explorers. In addition, *R. exulans* does not interbreed with the European rat and so allows for a diachronic approach to its' population studies (Matisoo-Smith, Allen, Ladefoged, Roberts, & Lambert, 1997; Matisoo-Smith & Robins, 2004). The remains of *R. exulans* are first seen in the Islands of Southeast Asia only in association with the Lapita cultural complex. Because of this association, it is an excellent animal to use to test the two migration models. The number of ancient rat skeletons at a site with ancient human remains outnumbers the people 10 to 1, if not 100 to 1 (Matisoo-Smith et al., 1997). Even though the numbers of ancient rats is much greater in comparison to the number of ancient people, the average femur weight of a rat is 0.1g. This causes difficulties in



ancient DNA extraction where the average amount of ground bone is 1.0g (Matisoo-Smith et al., 1997). The idea of *R. exulans* being a stowaway on the canoes of the Proto-Polynesians is possible but unlikely due to the behavior of the ancient rat's modern counterparts (Matisoo-Smith et al., 1997). The more common perception is that the rats were brought with the islands to be used as food (Matisoo-Smith et al., 1997; Murray-McIntosh et al., 1998).

Another animal associated with the Lapita cultural complex is the domestic pig or (*Sus scrofa*) (Larson et al., 2007). As with the rat, the ancestors of the modern feral pigs on the Polynesian islands are compared with their ancient counterparts. A connection has been established between the ancient *S. scrofa* and their indigenous counterparts (Larson et al., 2007). The genetic profile of the ancient and modern pigs does not match the modern or ancient pig specimens from China, Taiwan, and the Philippines. The appearance of the domestic pig in the Western islands of Southeast Asia (Moluccas and New Guinea) has been observed as far back a 3,500 years BP (Larson et al., 2007). The appearance of domestic pigs and their association with Lapita cultural complex suggests that the origins of the Proto-Polynesian people are not from mainland China or Taiwan (Larson et al., 2007).

The use of animals to definitively determine the origins of the Proto-Polynesian people is left up for debate as well with strong evidence to support either the "Express Train" or the "Slow Boat" theory. Therefore, when considering the association of animals and humans two points must be considered. Animals can be associated with specific human movement but they can also be used to establish a trade network in a region (Larson et al., 2007). Therefore, the use of animals in conjunction with human DNA has

the best potential to yield answers about the movements of people over the Pacific.

Genetic research allows for the examination of humans in different levels of specificity. At one level the researcher can analyze an individual genetically down to what constitutes to a fingerprint. This level of genetic work focuses mainly on the nuclear DNA and provides a genetic fingerprint of the person. However, at another level there are genetic markers on nuclear DNA that allow researchers to track the movements of people over time and space. In the case of the Polynesian people, a blood disease Thalassemia, which has an evolutionary connection with the disease malaria, is being used. Other genetic research uses Mitochondrial DNA and Y-Chromosomal DNA. These sources of DNA in a cell allow for the tracking of a wider lineage of people. Nuclear DNA is passed down as a combination of both mother and father while Mitochondrial DNA is passed down from mother to offspring and Y-Chromosomal DNA is passed from father to son.

### Women and Migration

A rationale for the use of mtDNA considers the potential physical migration of the men vs. women and their subsequent genetic dispersal. If only the male Proto-Polynesian's colonized the Polynesian islands then just half the potential mtDNA would be spread out to the far reaches of the Pacific islands. Fortunately, inferences can be drawn indicating an active role for women in the migration of the Polynesian islands through the use of ethnographic accounts and mythological tales.

When using ethnographic and mythological tales, one must take into account that the Polynesians prior to European contact did not write down their history. Therefore, the earliest written information pertaining to the cultural habits of the Polynesians is based

upon the ethnographic observations by people of Western decent (Arredondo, 2000). In addition to the inherent cultural bias, these Europeans were only at any one particular island for a short period of time (Arredondo, 2000). The cultural bias and short contact time of these Europeans diminishes the accuracy and understanding of the cultural working formed over hundreds of years by the island societies.

Cultural changes and adaptations occur in every culture but in the case of the Polynesian people their culture was overtly changed by the Western missionaries (Harding, 1993). A Christian set of gender roles were imposed on the Polynesian people by the missionaries thereby influencing the modern view of women in Polynesia. This change makes it difficult to definitively determine the role of the Proto-Polynesian women. However, while cultural nuances of the Polynesian people may have been lost by the onset of colonization some practices lasted long enough to be recorded by Western observers.

Unlike in European cultures where title and prestige is passed down from father to son Polynesian title and prestige was passed through the matrilineal line (Gunson, 1987). Through time the cultural bias of European explorers and missionaries imposed a more Western cultural attitude diminishing the role of women in Polynesian society (Arredondo, 2000; Ralston, 1993). Understanding of the important role of women during pre-western contact suggests that this matrilineal system had a long prehistory. Assumptions that prestige would be built and maintained by women must be made. Thus women would have been a vital part of battles and voyages to new lands to increase their social standing. Therefore, the possibility of both men and women being active participants in the migration of the Proto-Polynesian people cannot be rejected. Another

way to see past the ethnocentric censorship of women's roles is to delve into the mythological stories of the Polynesian people.

Mythological stories spanning the whole of Polynesia from New Zealand to Hawaii depict women harnessing the energy of volcanoes, voyaging across great distances, displaying feats of great strength, and performing heroic deeds (Ralston, 1993). These mythological tales glorifying the exploits of women allow for the interpretation that women were regular participants and/or leaders in expeditions to the far reaches of the Polynesian islands. The surviving myths depicting the strength and power of women in the distant past may only scratch the surface of the importance of women. Nevertheless, the depiction of women as highly capable sailors would suggest that women were not left behind during the migration of the Proto-Polynesian people across the Pacific. The mythological descriptions of women would support the hypothesis that women were not just passive passengers but instead were leading expeditions to new islands of the Pacific. Unfortunately, the ability to know the entire impact women have had in the myths of the Polynesian people may never be known.

Prior to European contact some Polynesian names were gender specific, but most children were named after events, circumstances, or places (Gunson, 1987). Gender neutral names of ancestors make the tracking of deeds performed by a specific gender difficult if not impossible. However, the use of gender specific names in mythological tales along with knowledge that prestige passed along the female lineage strongly suggests that women were more than just passive observers during the migration of the Proto-Polynesian people across the Pacific Ocean.

This evidence increases the likelihood of both genders being represented during the initial migration doubles the chance to recover genetic material, specifically mtDNA. Thus the chance of recovering ancient DNA is bolstered for this study as is its subsequent use to track the timing of the Proto-Polynesian people's movements.

Looking first at nuclear DNA, difficulties arise as a result of DNA's inability to remain intact over time. The survivability of DNA ranges from 50 thousand to 1 million years depending on the environment in which the DNA has been interred (Hebsgaard, Phillips, & Willerslev, 2005; Hofreiter, Serre, Poinar, Kuch, & Pääbo, 2001; Lindahl, 1993; Pääbo & Wilson, 1991; Willerslev & Cooper, 2005). The low number of nuclear DNA strands (in comparison to mtDNA) and the low survivability of both mtDNA and nuclear DNA over time makes nuclear DNA less useful when looking for migration of people over vast amounts of time. However, this is not to say that nuclear DNA is not used; in fact, as discussed below, researchers (Harding & Clegg, 1996; Hill, Flint, Weatherall, & Clegg, 1987; Lie-Injo, Pawson, & Solai, 1985; Müller, Bockarie, Alpers, & Smith, 2003) are looking at a specific malarial mutation in the Polynesian people that may help determine their ancestors' movement in the distant past.

### Malaria

Malaria is a parasitic disease caused by a hematozoan from the genus *Plasmodium*. This infectious disease is concentrated in the tropical regions of the world. However, the tropics are not the only region of the globe that this disease can be found, the subtropics and temperate zones can be affected by the disease although to a lesser degree (Stinton, Bogin, Huss-Ashmore, & O'Rourke, 2000, p.280). The malarial disease

is not spread through direct contact between infected people. Malaria is spread through a mosquito vector. A mosquito bites an infected human and ingests the malarial zygotes in the blood of the human. The malarial zygotes mature into sporozoites in the digestive system of the mosquito and then migrate to its' salivary glands. The mosquito with the sporozoites then bites another human injecting the new host with the infectious material thereby perpetuating the cycle of infection (Stinton et al., 2000, p.235). The ability of malaria to be spread person to person through a highly mobile vector has allowed for this disease to become extremely prevalent in human populations.

#### Malarial Counter Measures

The human species has adapted genetically to the disease though the use of other genetic diseases, like sickle cell anemia, that are harmful in their homozygote expression but beneficial in the heterozygote expression. Blood pathogens are a powerful example of adaptation and the effects of natural selection on the human species (Hill et al., 1987). In areas with an endemic disease such as malaria, the continual exposure to the diseases can result in the development of immunities (Stirnadel, Beck, Alpers, & Smith, 1999). Humans have a highly polymorphic immune system that responds and adapts to hostile environments (malarial) using one of two methods: destroying the invading disease or developing a tolerance to it. In the case of most diseases that are endemic to an area in which humans live, the second approach is employed. This is seen with other deleterious diseases such as Thalassemia, where in its heterozygote form it curbs such deadly diseases as malaria (Müller et al., 2003). This reduces the affects of malaria but does not actively eliminate its presence from the infected host; therefore, it tolerates its presence

and allows the host to live in the endemic environment. This is not unlike other deleterious diseases found to exist in a precarious manner where the health of humans is concerned.

The most common of the deleterious diseases associated with hemoglobin and influenced by the malarial parasite is sickle cell anemia. However, in this study the focus will be on the hemoglobin disorder Thalassemia because it is more relevant as it has the potential to resolve the theoretical debates concerning Polynesian migrations.

Thalassemia is defined as a defective production of a of globin chain (alpha or beta). This improper synthesis of one globin chain means that the other chain is longer. As a result of this imbalance, the blood cell matures at an abnormal rate which in turn leads to the cell's premature death (Fortin, Stevenson, & Gros, 2002). This abnormality has generated several possible scenarios for the cell's tendency to resist malaria: thalassemic cells have higher levels of parasitic antigens on their surface, they are resistant to red blood cell clumping, and/or they are destroyed more easily by the phagocyte system (Allen et al., 1997; Fortin et al., 2002). The benefits of these various scenarios are still open to some debate in regards to their effectiveness in resisting malaria (Flint, Harding, Clegg, & Boyce, 1993; Hill et al., 1987; Stirnadel et al., 1999; Trent et al., 1985). However, population studies have made a strong correlation between the high frequency of the (alpha and beta) Thalassemia disease and malarial environments (Fortin et al., 2002; Kaneko et al., 1998; Stirnadel et al., 1999). This discussion illustrates that, through the exposure to endemic diseases, genetic changes can occur in local populations.

## Using Malarial Diseases

This genetic disease and the prevalence of its heterozygote expression are used to determine the origins and migration patterns of people around the world. The ability to track the different immune responses of the human species aids is pertinent to the origins of the Polynesian people debate. On a scale much smaller than global migration, the presence of such diseases as Thalassemia can be used to discover the settlement patterns of people on islands. One such example is on the island of New Guinea, a part of the island chain of Melanesia. On New Guinea, malaria is endemic and the frequency of alpha-Thalassemia is in the range of 81% (Lie-Injo et al., 1985). However, in the highlands of New Guinea malaria is absent. As one would expect in a malarial-free environment, alpha-Thalassemia is also absent in these highland populations (Lie-Injo et al., 1985; Müller et al., 2003; Hill et al., 1987).

Along with showing a strong correlation between malaria and alpha-Thalassemia other human patterns can be assumed. One such assumption is that the settlement patterns on New Guinea have been long and lacking in mixing between the highland and lowland peoples (Hill et al., 1987). If the upland and lowland peoples frequently interbreed then one would expect to see no difference in frequency of alpha-Thalassemia. This not being the case, other, alternative scenarios can be expounded upon and different settlement and migration patterns can be looked at or traced to explain the difference between the two populations in such close proximity. What can be taken from the New Guinea example is that close proximity is not always an indication of sameness in genetic adaptations of populations.



Despite the close proximity of Polynesia to Melanesia, malaria has never been an endemic problem. The lack of malaria in Polynesia is due to the absence of *anopheles*, the vector responsible for the dissemination of the disease between the human populations (Lie-Injo et al., 1985). Therefore, one would expect to see a lack of the diseases associated with a malarial environment in the Polynesian people.

In a study by Lie-Injo et al. (1985), 60 people of Samoa Polynesia descent were tested for alpha-Thalassemia. The entire sample lacked the abnormal alpha-Thalassemia fragment. This is what is to be expected in an area that lacks the malarial stressor. However, this study was only performed on a sample of 60 individuals from one particular Polynesian island. The lack of adaptive mechanisms in the Polynesian people to a malarial environment lends credence to a rapid migration of the Proto-Polynesian people from Southeast Asia and Melanesia into the islands of Polynesia. The “Express Train” model of migration benefits greatly from the lack of alpha-Thalassemia. It can be argued that the Proto-Polynesians were not in Melanesia long enough to develop the adaptation or to have interbred with the indigenous population for alpha-Thalassemia to show in their genetic makeup.

Further studies of the malarial marker alpha-Thalassemia brought to light a specific variation of that disease,  $-\alpha 3.7\text{III}$ , in Melanesia. The origin of the  $-\alpha 3.7\text{III}$  variation was found to be one of the oldest in Melanesia in regards to malarial selection (Harding & Clegg, 1996; Hill et al., 1986). The significance of this discovery is that samples from Polynesia were tested and found to have the  $-\alpha 3.7\text{III}$  variation. This cannot be explained by random mutation, because the frequency in which the  $-\alpha 3.7\text{III}$  variation was found to be around 23% (Flint et al., 1993; Harding & Clegg,

1996). Referring back to the models of migration, this would support the “Slow Boat” theory because the 23% frequency indicates a relatively strong influence of Melanesian genes in Polynesia. The Polynesian alleles can be seen as a subset of the Melanesian ones (Flint et al., 1989). This gene flow from Melanesia would not be possible at this frequency if the original colonizers of Polynesia moved rapidly through Melanesia as proposed by the “Express Train” model. However, there are those that see the presence of the -3.7III variation as support for the “Express Train” model.

Proponents of the “Express Train” model postulate that the higher-than-expected frequency of  $\alpha$ -3.7III is due to the Proto-Polynesian people stopping for a short time in these highly malarial regions and the frequency was amplified by this relatively brief contact with the malarial environment (Harding & Clegg, 1996). They see the malarial environment as being a strong enough factor in the survival of these new immigrants that they adapted quickly. Harding and Clegg (1996) do not say if this adaptation was due to direct genetic changes on the Proto-Polynesians or if the adaptations occurred through interbreeding between them and the indigenous people. Therefore, both scenarios are still viable possibilities. The use of disease markers confirms the general ancestry of the Polynesian people in that they come from an Asian and Melanesian ancestry (Harding & Clegg, 1996; Hill et al., 1987). Thus the genetic evidence reveals the origin of the Polynesian people and bottlenecks explains why the Thalassemia disease remains at such a high level in a non-malarial environment.

The island populations are those that are currently under investigation. Papua New Guinea is the largest of all the islands that make up Melanesia but these islands are spread

out over hundreds of miles in the Pacific Ocean. The process of bottlenecking and the founder affect therefore have a high potential of occurring in these isolated areas.

Founder affect can be characterized by an occurrence in which one inseminated female or a very small group of individuals relocate to a deserted location (such as an island) and establish a new colony. In this new environment, interbreeding would serve to decrease genetic diversity and the heterozygosity of the group should decrease (Nei, Maruyama, & Chakaborty, 1975).

Bottlenecking is a phenomenon where a once larger population is reduced dramatically to only a small number of individuals. These individuals are cut off from other populations, and are only able to reproduce with those in the group of survivors (easily seen in island situations) (Nei et al., 1975). This decrease in heterozygosity can lead to the survival of such deleterious genetic markers as Thalassemia in non-malarial environments. Increased homozygosity is understandable due to the low number people who could contribute to the genetic make up of the founding or bottlenecked population (Nei et al., 1975). The rate in which the heterozygosity and homozygosity changes in a population depends upon the number of individuals in the initial reduction event.

Nei et al. (1975) argued that the heterozygosity of the early Proto-Polynesians declined rapidly in a bottlenecking scenario. The actual rate at which the heterozygosity decreased is dependant on the intrinsic growth rate of the population. Depending on the intrinsic growth rate the decline in heterozygosity can take 20 to 300 generations to level out (Nei et al., 1975). The significance of this reduction in heterozygosity is that during this period low frequency alleles are the ones most likely lost from the population. Therefore, the presence of the Thalassemia disease and the Polynesian Motif in the

Polynesian population suggests that they occurred in high frequency prior to the Proto-Polynesian people's migration to the Polynesian islands. The frequency of 23% in the current Polynesian population lends credence to the "Slow Boat" model of migration showing a much longer occupation in Southeast Asia than suggested by the "Express Train" model. Also, when addressing the range of 20 to 300 generations, and assuming each generation lasts 30 years, the possibility for both theoretical models to be valid remains. The difficulty of determining an exact time of separation from the Proto-Polynesian people and the modern day Polynesians is therefore not clarified by the recognition that a bottleneck or founder effect had occurred.

The Polynesian populations are genetically homogeneous over all the islands (Trent et al., 1985) indicating they went through some kind of dramatic decrease in population size. The two genetic markers the Polynesian Motif and Thalassemia are both present in the modern Polynesian population. The presence of the Polynesian Motif in a high frequency of around 80-90% is understandable due to the benign nature of this genetic marker. According to current research this arrangement of nucleic acids does not cause any deleterious effects on its carrier; in contrast, the deleterious potential of Thalassemia has affected its frequency in the Polynesian population. The Thalassemia's 23% frequency is not nearly as high as that of the Polynesian Motif but still is significant in a non-malarial environment. Both markers show potential in determining the migration patterns of the ancestors of the Polynesian people. Genetic researchers use levels of homozygosity in order to determine the origins and movements of people in regions like the South Pacific, but there are limitations to the degree of precision with which these methods can determine the timing of the migration.

The potentially wide time range of the migration of the Proto-Polynesians is disheartening in its lack of precision. Also, the use of cultural artifacts and linguistic data can be misleading in that their presence does not necessarily indicate the actual people's movement into an area. The only solid conclusion one can reach is that the language and artifacts such as pottery were in the region by a certain time. Studies using genetic characteristics of modern subjects for the determination of the origins of the Polynesian people give a more definitive answer as to the connection between the Polynesian people and their ancestors.

These genetic techniques are not without their problems either. The major problem stems from the large, imprecise time interval that is currently projected by the "Molecular Clock" because of the nature of genetic data collected. The molecular clock method uses modern nuclear DNA to determine the timing of origin of certain genetic traits. However, ancient DNA has the potential to narrow the wide time interval. The inability of nuclear DNA to remain as an intact strand for long periods of time and the low number of nuclear DNA strands have caused other scientists to look to mtDNA to address their research questions.

Within a single cell there is one set of nuclear DNA; but, in that same single cell there are a thousand to ten thousand times more mtDNA that can be used to determine ancestry (Hagelberg et al., 1991). Along with the greater number of mtDNA strands present per cell, mtDNA is found in every human cell. Y-chromosomal DNA is only found in males, which would reduce any sample size by half, furthermore, regions on mtDNA have unique characteristics pertaining to Polynesian people and so mtDNA will be used in this research project.

As stated above, genetic material may be limited to only one specific gender (Y-Chromosomal DNA) leaving out an entire section of the population limiting its usefulness. Another potential pitfall of genetic analysis is the potential for samples to be contaminated through a multitude of sources. The extraction of a DNA sample, whether by a technician in the laboratory or by an archaeologist in the field, has the potential to become contaminated. However, the use of proper extraction procedures by both scientists reduces the chance of contamination.

Mitochondrial DNA has its own methodological difficulties to consider when interpreting the significance of results. Mitochondrial DNA has potential to be completely lost within one generation. If a mother only has sons then in the next generation her mtDNA will not be passed on to the next generation. Therefore, with mtDNA research there is potential for huge spikes and drops in frequency of mtDNA markers. To counter this problem, when using mtDNA to determine ancestry two separate regions of the mtDNA are sequenced.

The use of genetic affiliation in determining Polynesian origin is a two step process. The first step is to test for a Hypervariable section I (HVS I) of mtDNA between the cytochrome oxidase II (COII) and the lysyl transfer RNA (tRNA<sup>Lys</sup>) genes. This is an intergenic region that usually contains two tandemly repeated copies of a 9-base pair sequence (CCCCCTCTA) (Hagelberg et al., 1994). The absence of one of these two repeats characterizes people from Polynesia, Asia, and people native to America. This 9-base pair deletion is found in other populations over the globe but has its highest prevalence within the three groups mentioned. The existence of the 9-base pair deletion is a good initial indicator that the individual has ancestry from Asia but is not definitive;

therefore, another test is needed to prove this. In conjunction with the 9-base pair deletion, a more specific target region is examined focusing on four specific base pair substitutions on the mtDNA and is the second step of the research process.

This second, more specific, step of the genetic test differentiates people from the rest of the world from those from Polynesia. Between mtDNA bases 16,215 and 16,410 there are four base pair substitutions 16,217 (T to C), 16,247 (A to G), 16,261 (C to T), and 16,189 (T to C) that are only found in people from Polynesia (Cox 2005; Hagelberg et al., 1994; Melton et al., 1995; Oppenheimer, 2004; Redd et al., 1995; Richards et al., 1998; Whyte et al., 2005). This sequence of substitutions is known as the “Polynesian Motif” and, as of yet, has only been seen within that particular population.

There are distinct advantages to using mtDNA as the target source of genetic material especially in ancient remains. The large number of mtDNA within one cell increases the chances of the recovery of intact genetic material. The mtDNA is seen as a way of illuminating a genealogical tree to show relationships between ancestral and modern people (Thalmann et al., 2004). Therefore, by extracting mtDNA and testing for these base pair sequences, genetic affiliations can be assigned.

The presence of the Polynesian Motif in modern populations of Oceania is used to develop a molecular clock. Using the molecular clock method, the Polynesian Motif’s age is estimated to be 17,000 years. However, the confidence intervals are so high that the range of possible development of the Polynesian Motif allows for dates of 34,500 to 5,500 years before present to be possible (Oppenheimer, 2004; Richards et al., 1998). Regardless of the wide range of dates during which the mutation is thought to have developed, the calculation places the origin of the Polynesian Motif to be greater than

predicted by the “Express Train” model; therefore, the use of the genes from modern people supports the “Slow Boat” model of migration.

There are anthropologists that see these data as inconclusive and have performed their own analysis of modern nuclear DNA from the Pacific region. Cox (2005) used a new set of modern genetic samples and previously studied samples in order to determine a more precise time frame for the development of the Polynesian Motif. Cox (2005) used DNA samples of modern people from eastern Indonesia, Samoa, Papua New Guinea, Taiwan, and the Cook Islands collected by Richards et al. (1998). These samples, along with new data from Santa Cruz, Indonesia, and Taiwan, were used to develop a different range of dates for the molecular clock. According to Cox (2005), the molecular clock estimates of the Polynesian Motif range from 0 to 52,500 years BP. Therefore, the findings of this study could not exclude the origins of the Proto-Polynesian people from Taiwan (Cox, 2005). Thus, the “Express Train” model can not be discounted. The wider time range, which allows for the possible validity of the “Express Train” model, is more accurate because of the number of samples used in the study. This large sample size gives a better determination of the range of time during which the Polynesian Motif could have developed. However, the wide interval produced by modern DNA studies calls for a narrowing of this time frame in order to better illuminate the migration of the Proto-Polynesian people.

Hagelberg et al. (1994) extracted ancient DNA from a skeletal sample from Easter Island. The DNA from this ancient sample ranged in age from 1,100 to 1,680 AD and 1,680 to 1,868 AD. The samples were examined for the presence of the 9-base pair sequence and the Polynesian Motif (Hagelberg et al., 1994). This research demonstrated



that the modern indigenous people of Easter Island are the direct descendants of that prehistoric population. The genetic marker on the HVS I of the mtDNA, in conjunction with the presence of the Polynesian Motif, was used to verify the relationship. This finding that ancient indigenous people had the genetic markers indicative of modern Polynesians as early as 1,100 AD is a powerful tool in determining timing of the peopling of Easter Island. These results are of interest to this study because of the extraction techniques used and viability of DNA in skeletons stored in collection rooms for decades.

The importance of Hagelberg et al.'s (1994) research for this current study is in that DNA that is around 1,000 years old can be extracted and amplified. This study is extracting and amplifying DNA that is over 1,700 years old and the positive results of the Easter Island experiment is encouraging for the successful recovery of ancient genetic material from other specimens. In conjunction with the explanation of migration patterns of the people of Polynesia, this research showcases ancient DNA's ability to take a leading role in determining past populations' origins as opposed to cultural, linguistic, physical, and modern genetic studies (Hagelberg & Sykes, 1989).

This ancient DNA research entails taking samples from 28 individual skeletons that based on radiocarbon dates between 11,700 and 1,870 years BP and performing genetic tests to determine the haplogroups, the presence of the 9-base pair deletion, and the Polynesian Motif. These individuals originated from Niah Cave in eastern Indonesia; a region pertinent to both migration theories. DNA from individuals as old as 11,700 years BP will allow for the testing of the two competing migration theories. Figure 2 indicates with a dot where Niah Cave is located on the island of Borneo and how its' location relates to the migration routes of both theoretical models.

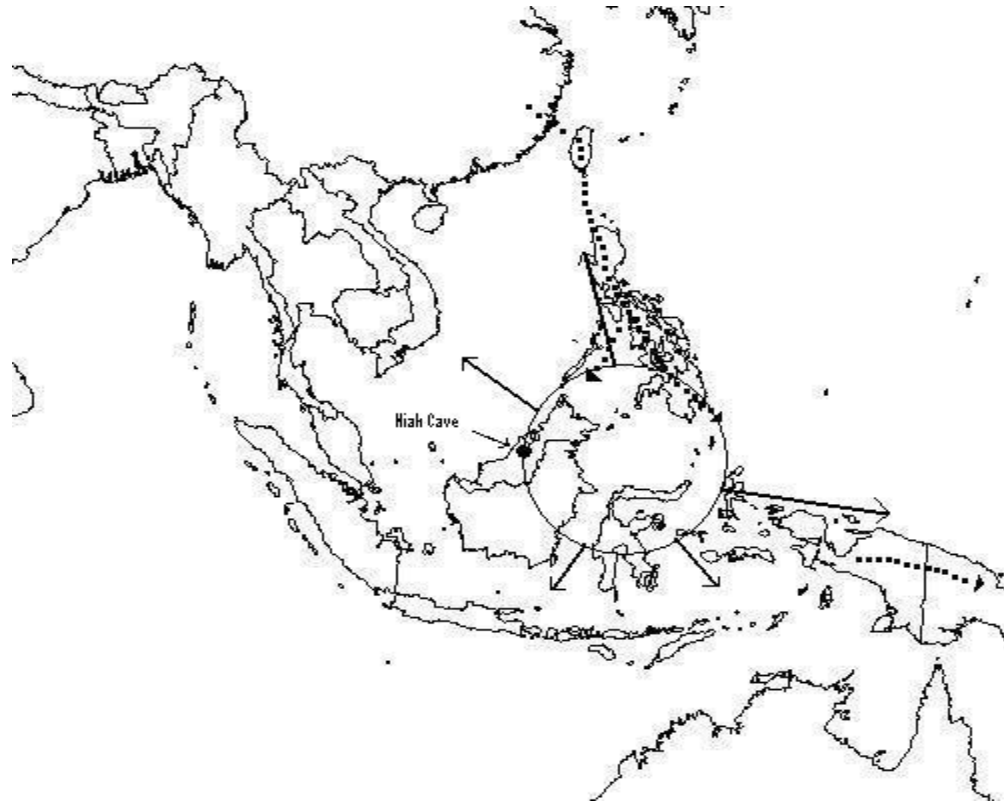


Figure 2. The dot on Borneo is the approximate location of the Great Niah Cave in relation to the migration routes proposed by “Express Train” and “Slow Boat” models.

The ultimate goal of this research is to add to the body of anthropological evidence in regards to the origin and the migration patterns of the Polynesian people. The null hypothesis of this research is that the genetic evidence will substantiate the “Express Train” theory of Polynesian origin. Therefore, no sign of the Polynesian Motif is to be expected in any of the samples dating older than 3,500 years BP. The alternative hypothesis is that the Polynesian Motif is found in individuals older than 3,500 years BP, this would support the “Slow Boat” model of migration.

## CHAPTER 3

### MATERIALS AND METHODS

The aim of this study is to extract mtDNA from samples of bone and teeth that are pertinent to the debate over the origin of the modern Polynesian ancestors. This study will attempt to determine the haplogroup of the individuals as well as to isolate the hypervariable section I of the mtDNA which will identify the presence, or not, of the Polynesian Motif. The presence/absence of these regions will add to the data available to assess the veracity of the “Slow Boat” vs. “Express Train” models of Polynesian origins.

The samples used in the study originate from burials excavated in the Great Niah Cave located in the Sarawak National Park in Northeastern Borneo in the country of Malaysia. The Great Niah Cave is a massive complex of underground caverns, the cave floor measures around ten hectares and the cave height reaches as high as 75 meters. There is one single main entrance to the western side of the cave and from there the cavern divides into smaller caves and channels (Barker et al., 2000).

The archaeological excavations of the Great Niah Cave taken since 1962 and have unearthed skeletal remains dating as far back as 39,600 BP (Harrison, 1967). The long term use of the Great Niah Cave as a burial site indicates a significant time of human habitation in Northern Borneo. What can be extrapolated from this occupational time is a long human habitation of the Southeast Asian islands both south and east of the island of Borneo. The evidence of people living in and around the Great Niah Cave for forty millennia increases the likelihood that cultural remains and genetic material deposited in the burials would be preserved. The burials on the islands of Malaysia, Indonesia and Melanesia have been used to indicate the timing and origin of the Proto-Polynesian

peoples' movements (Brooks, Heglar, & Brooks, 1977; Harrison, 1967; Harrison & Medway, 1962). Skeletal and dental evidence from the Great Niah Cave have the potential to add clarity to this issue because of the age of the site and the long cultural sequences present there.

### Cultural Material

The cultural material extracted from the cave consisted of rings, bone carvings, tooth pendants, turtle tools, points, and spatulas (Harrison & Medway, 1962), as well as soil samples and human and faunal remains that span thousands of years of use (Harrison, 1967) (Appendix 2). The continuous use of the site meant that archaeologists could recover artifacts that over a long cultural sequence. Archeologist analyzing the artifacts found that over the occupation period there was a change in material culture (Harrison, 1967). This change in material culture indicates either an influx of a new group of people or a point in time where the indigenous people underwent a period of innovation.

Analysis of material remains found in conjunction with skeletal remains from the Great Niah Cave indicates that these people were not associated with the Lapita cultural complex and therefore are not associated with the Proto-Polynesian people (Harrison & Medway, 1962).

However, as discussed earlier, the cultural remains can be interpreted in many ways and multiple lines of evidence should be considered before determining the ancestry of any group of people. Further, materials recovered from Niah Cave burials are limited in number with some burials devoid of cultural artifacts entirely. Thus the lack of

association with the Lapita culture does not take away from the potential of the physical; remains giving some insight into the Proto-Polynesian origin debate.

The skeletal remains from the Great Niah Cave can be used to indicate cultural change or population movements. The ability to test the genetic profile of skeletal remains that span several thousand years still allows the assessment of population migration in the region and allows tests of how these occupants compare to the indigenous people currently occupying the islands of Southeast Asia and Polynesia (Table 2). In particular, the human remains extracted from Niah Cave dated between 11,700 and 1,870 years BP are of particular interest for the study because they have the potential to add additional information to test the “Slow Boat” and “Express Train” theories.

A portion of the skeletal remains extracted from Northeastern Borneo is currently housed at the University of Nevada, Las Vegas (UNLV). Brooks et al. (1977) performed radiocarbon dating on thirty individuals from the UNLV Niah Cave samples housed at UNLV. Of the 30 individuals tested, 28 were dated between 11,700 and 1,870 years BP (Brooks et al., 1977) (Appendix 1).

Historically, radiocarbon dating has been a reliable and quite useful tool to date recent skeletal remains. This does not mean, however, that radiocarbon dating is not without its shortcomings. The ability to date objects is contingent on three independent quantities: average cosmic ray intensity; magnitude of the earth’s magnetic field; and the degree of oceans mixing. All three of these quantities are averaged over an 8,000 year period (Libby, 1963). These three factors taken into account and averaged into the radiocarbon estimate allow for a more accurate formulation of dates.

## Decontamination Guidelines

The issue of contamination is of concern when working with ancient DNA. Measures must be taken in order to eliminate foreign modern DNA. The ability to avoid contamination of the samples can be done through strict adherence to procedures regarding isolation of area, controls, reproducibility, and independent replication (Caramelli et al. 2008; Cooper & Poinar, 2000; Kwok & Higuchi, 1989; Linderholm, Malmstrom, Linden, Holmlund, & Götherström, 2008; Yang & Watt, 2005). While the ideal laboratory is one dedicated to work on ancient DNA exclusively, this is not always a financial possibility. Therefore, in laboratories that handle both modern and ancient DNA, the practice is to work with the oldest material first and move on to the next oldest and so on to reduce the probability of contamination due to sample sizes (Cooper & Poinar, 2000). The strict adherence to this procedure assures the researcher the best possible results that will allow the replication of the work by others.

Using the guidelines for a successful ancient DNA extraction set forth, by Cooper and Poinar (2000), the extraction process was carried out. Two different samples were extracted from different sections of the individual (i.e. femur, humerus, or teeth), and by obtaining the same results from the selected samples would strengthen the results of the research. Reproducibility of data is important, so too is the independent replication of the samples tested. An independent laboratory should be given their own set of extracted samples (i.e. femur, humerus, or teeth) to confirm the results of the other laboratory. The confirmation of test results from a different, independent laboratory would demonstrate the reproducibility of the ancient DNA extraction process for both laboratories (Cooper &

Poinar, 2000). This powerful declaration of reproducible test results allows for more definitive conclusions to be drawn in the discussion.

Originally, the plan for this research was to carry out DNA extraction at the Shadow Lane Campus DNA facility and also to send samples to a second laboratory to confirm the results. Due to the unforeseen closing of facilities at UNLV, there was no second laboratory for the independent testing. In spite of this, the testing proceeded at the University of Adelaide, Australian Centre for Ancient DNA; a facility dedicated to the recovery of ancient DNA. While this was not an ideal circumstance, the DNA tests proceeded because of the world class facilities at the DNA facility in Australia. The Australian Centre for Ancient DNA is overseen by Dr. Alan Cooper, whose article, *Ancient DNA: Do It Right or Not At All*, contains the procedure followed by researchers when considering decontamination and reproducibility issues related to ancient DNA recovery. Samples for DNA analysis were obtained following the decontamination procedures of ancient material found in Cooper and Poinar (2000). There was no reason to doubt that the recovery of the genetic material would be handled properly and with the utmost care in Dr. Cooper's laboratory. In addition, the Australian Centre for Ancient DNA has performed ancient DNA test on bones from the region of Malaysia in the past and are fully aware of the difficulties of amplification of DNA from a tropical region. With these factors above considered the research proceeded. However, because the results could no longer be checked by a second lab, the elimination of contamination was of increased importance.

## Decontamination Procedures

Decontamination procedures for ancient human remains center on the use of two methods. The two decontamination procedures utilize ultraviolet light (UV) and bleach to destroy modern DNA that may have been transmitted onto the ancient material during its extraction from the ground. UV light exposure consists of the bone being placed in a UV hood for 15 minutes on every side (Handt, Krings, Ward, & Pääbo, 1996; Montiel, Malgosa, & Francalacci, 2001). Bleach is placed on the surface of teeth to destroy contaminate DNA. Equipment used during the extraction process is submerged for 30 minutes in bleach to destroy any DNA contaminates. By taking precautions to reduce the introduction of foreign contaminates from the surface of the bones, teeth, and equipment, the only DNA to be extracted is assumed to be the ancient DNA (Table 1). The decontamination process does destroy the ancient DNA on the surface of the bone, but the bone matrix houses many more cells inside. After the initial surface decontamination the bone matrix itself, which houses the genetic material, must be opened with as little damage to the fragile genetic material inside.

## Property of Bone

Extractable template quantity, PCR inhibitors, and template quality are the major concerns to consider when dealing with ancient DNA extraction (Yang et al., 1997). Extractable template quantities and PCR inhibitors are the two factors that can be influenced and improved by laboratory techniques and procedures. Through the use of a chemical called EDTA (ethylenediaminetetraacetic acid) and the manipulation of pH during the decalcification phase of the extraction process, an increase in quantity and



decrease in inhibitors is achieved to optimize the quantity of DNA to be amplified. The maximizing of DNA to be amplified is critical due to the nature of ancient DNA. Ancient DNA must be considered by the researcher to be of poor quality and thereby difficult to extract. From the time the organism's life ends the process of DNA degradation begins and, while environmental conditions can speed up the degradation process, the cells locked deep inside the boney matrix or tooth are more likely to be intact for a longer time. In order to get to the genetic materials such as calcium and hydroxyapatite must be removed. Calcium and hydroxyapatite are the factors addressed to increase the quantity and decrease the inhibitors in order to optimize the amplification of ancient DNA.

Calcium, which is a major component of bone, subsequently plays an equally important role in the successful amplification of DNA. The presence of free calcium in a sample to be amplified can significantly inhibit the amplification process. The free calcium present in a mixture is due to the grinding of the bone. Calcium is positive and DNA is negative and in nature these two materials will attract, inhibiting the DNA amplification process later. This reduction in amplification can cause a sample to yield lower amounts of DNA than actually present. To reduce the amount of free calcium during the amplification process, EDTA is utilized because it binds to the free calcium and then is removed before amplification. EDTA is more attracted to calcium than DNA and so can then be more easily removed before the amplification process. Hydroxyapatite is the other main factor that can reduce the amplification of DNA.

Hydroxyapatite can inhibit the amplification of DNA in two different ways. The negative charge of the DNA molecule shows an affinity for the phosphate group on the Hydroxyapatite (Okazaki, Yoshida, Yamaguchi, Kaneno, & Elliot, 2001), causing the

DNA in solution to be drawn to the Hydroxyapatite instead of staying in the solution. Subsequently, this can reduce DNA available for amplification. This strong affinity of DNA for the positive charged phosphate group will reduce the number of free DNA strands available during the lysis stage of the process. The way around this problem is to reduce the amount of Hydroxyapatite in the sample being used. This leads to the second characteristic of Hydroxyapatite which inhibits DNA amplification.

Hydroxyapatite is the inorganic component of bone which gives the bone its rigid characteristics. This structure surrounds the osteocytes and cartilage of the bone both of which contain genetic material. The manner of inhibition is due to the structure of the Hydroxyapatite. During the lysis and amplification stage, the genetic material is unable to be utilized because Hydroxyapatite reduces the ability to get at the genetic material. By the dissolution of the Hydroxyapatite in solution more genetic material is made available for amplification. However, through the dissolution of Hydroxyapatite, more calcium is placed in solution which, as discussed earlier can inhibit amplification. The amount of inhibitors in solution can cause the dissolution process to slow or even stop (Christoffersen, 1981). The way to keep the dissolution process continuing forward is to use EDTA at a pH that is conducive to the dissolution of Hydroxyapatite (Elliott et al., 2005; Okazaki et al., 2001) and still is available to bind with the free calcium thereby reducing inhibition. The pH ranges suggested for the dissolution of Hydroxyapatite start as high as 8 and go as low as 4.0 (Christoffersen, 1981; Christoffersen & Christoffersen, 1984; Christoffersen, Dohrup, & Christoffersen, 1998; Dorozhkin, 2002; Elliot et al., 2005; Mavropoulos et al., 2003; Okazaki et al., 2001; Purdy, Embley, Takii, & Nedwell, 1996; Schaad, Poumier, Voegel, & Gramain, 1997). The most commonly used pH of

EDTA during the dissolution of Hydroxyapatite has been found to be 8.0. Therefore, a pH of 8.0 was used in this study as well. The protocol to be followed was one of a total demineralization of the bone matrix to maximize the recovery of DNA from the samples. Once freed, the region of DNA of interest is determined and set primers are used to amplify the specific section for study.

### Genetic Material Amplified

The genetic code of the people of Polynesia of interest in this study is restricted to a 180 base pair segment in a control region of mtDNA. This location in mtDNA is in the Hypervariable Section I (HVS I) which includes the nucleotide positions from 16,189-16,360. The HVS I haplotype is found in all Polynesian populations that have been studied (Gongora et al., 2008; Hagelberg et al., 1991; Redd et al., 1995; Whyte et al., 2005;). This HVS I is located on the mtDNA and subject to variation in length between the COII gene and the tRNALys gene (Friedlaender et al., 2002). This 9-base pair deletion is not uncommon in other populations across the globe. Therefore, an additional, more specific, identification and genetic marker is used at the nucleotide positions 16,217 (T to C), 16,247 (A to G), and 16,261 (C to T) in the HVS I this set of transition mutations is known as the Polynesian Motif (Redd et al., 1995). The nucleotide position 16,189 (T to C) can be included within this motif to further the identification of the Polynesian Motif (Whyte et al., 2005). With the target region of the mtDNA set, the decontamination procedures and the collecting procedures for the necessary amount of skeletal material could begin.

## Bone Sample Extraction Procedure

The DNA extraction was performed in three distinct and separate phases. The first phase entailed the separation of the sample material from the main portion of the bone. The second phase was to proceed with the extraction of the DNA from its bone matrix. The third phase was to have been a two step process involving the amplification and analysis of the DNA. Phase one was performed at UNLV where the bone samples were extracted from the larger bone matrix. The Australian Centre for Ancient DNA indicated a preference to have the teeth still imbedded in the mandible or maxilla. This preference was met when possible, but there were eight cases where individual teeth not within bone were sent to maximize the possible set of results. The sample material was then packaged and shipped to the Australian Centre for Ancient DNA for the second and third phase of DNA extraction and amplification.

The first phase of the extraction process entailed the elimination of the surface contaminants so as to decrease the likelihood of amplifying modern DNA. In addition to surface contaminants, care was taken to extract enough of each sample from the original bone to allow the amplification process the best opportunity for success. At the same time, conserving as much of the bone as possible is important for future researchers and techniques. The initial step used to decrease the chance of contamination was to extract bone samples first from the oldest samples to the youngest. By proceeding with the extraction in this manner, there was a reduced chance of younger genetic material contaminating the older ones since they likely contained a smaller quantity of mtDNA to begin with (Cooper & Poinar, 2000; Kwok & Higuchi, 1989). Along with reducing the

chance of samples contaminating one another, decontamination methods involving the equipment in the procedure were implemented as described above.

The materials involved in the extraction process were disposable and used only once. All gloves, masks, face shields, hair nets, lab coats, sandpaper, weigh boats, and saw blades were used specifically for one specific bone sample (Montiel et al., 2001). For example, burial 4B bone samples were taken from two separate long bones belonging to that individual. All the above listed materials were replaced with new ones before the second long bone from the same burial labeled 4D had its sample extracted. In addition to the use of disposable equipment, the materials in direct contact with the bones were soaked in a bleach solution for 30 minutes prior to their use (Montiel et al., 2001; Montiel, Malgosa, & Subira, 1997; Sarkar & Sommer, 1990). There were two pieces of equipment reused during the extraction of all the physical remains: a scale and a positive flow PCR hood were used every time. Prior to, and after, each extraction, both scale and hood were sprayed with a 10% bleach solution (made fresh each day) and “DNA Away” was applied to the equipment. “DNA Away” is a commercial product designed to break apart DNA strands thereby being an effective cleaner during ancient DNA extractions. After the application of bleach and “DNA Away” the surfaces of both the scale and PCR hood were allowed to air dry. The PCR hood is equipped with an ultraviolet light bulb and after each cleaning with bleach the UV light was left on for 15 minutes as an additional precautionary measure. Just prior to extraction of a smaller fraction of bone from the parent long bone the saw blade, weigh boat, sandpaper, and the parent bone were placed in the PCR hood and the UV light was turned on for 15 minutes (Gongora et al., 2008; Montiel et al., 2001; Montiel et al., 1997; Sarkar & Sommer, 1990). The bone

and all the materials were turned over and the UV light applied for a further 15 minutes. After the surface decontamination procedures were completed, extraction commenced.

The long bone was examined for the appearance of cracks or fissures in the extraction region. The extraction site was determined by looking along the diaphysis for a suitable area devoid of bone markings. This was done so that these morphologically significant sites can still be studied in the future. Once a section of the long bone was selected, the area was lightly sanded to remove any extraneous surface debris (Montiel et al., 2001). After the completion of a light sanding the saw blade was used to extract a rectangular section of bone that runs longitudinally perpendicular to the long axis across the diaphysis of the bone. The amount of bone extracted was restricted to approximately 2 grams when possible (Appendix 3).

For the extraction of ancient mtDNA samples such as the ones from the Great Niah Cave, a sample size of 2 grams of dry bone has been found to be necessary to achieve positive results (Hagelberg & Sykes, 1989). The large amount of dry bone to be used in the extraction and amplification process is due to the low survivability of intact mtDNA. When looking at specimens older than 1,500 years BP, the mtDNA is most often found in fragments ranging in sizes of 100 base pairs to 500 base pairs (Hagelberg & Sykes, 1989; Willerslev & Cooper, 2005). Thus, there is a need to obtain a relatively large sample in order to recover enough genetic material as to make it possible to amplify the small fragments of DNA. Once the smaller section of long bone was extracted and weighed the piece of bone was placed in a labeled zip-lock bag to be shipped to the Australian Centre for Ancient DNA for phases two and three of the process. Forty-eight samples were sent to the Australian Centre for Ancient DNA, 23 of these were teeth (Appendix 3).

The 23 samples containing completely intact teeth (not having the pulp chamber exposed to the environment) were shipped to the Australian Centre for Ancient DNA. Procedures for isolated teeth and/or teeth in jaws were the same as those used in extracting samples from long bones described above. The surface decontamination of a tooth or mandible was performed by placing the samples individually in the PCR hood for 15 minutes of UV light exposure on each side. The tooth or mandible was then weighed and placed in its own labeled plastic zip-lock bag, just as in the case of the long bone samples.

#### Extraction and Amplification

The sample material was then packaged and shipped to the Australian Centre for Ancient DNA for phases two and three, entailing DNA extraction and amplification. These two phases of DNA extraction and amplification were performed at a facility dedicated to ancient DNA research. Dr. Wolfgang Haak, the Senior Research Associate at the Australian Centre for Ancient DNA and his staff, began the preparation process for the samples. The following section regarding the methods used were reported to me by Dr. Haak.

The preparation process first began by looking over the samples sent and determining the three best samples that could possibly yield DNA. The purpose of selecting three samples was to assess the potential of DNA recovery from the sample lot as a whole. Dr. Haak determined that samples 4D, 19A, and 22B (Table. 1) had the most promise for DNA recovery. The first step at this phase of the procedure was to prepare the bone and teeth samples.

The long bone samples 4D and 22B were placed under UV light for 30 minutes on each side. The surface of the long bone was removed using disposable cutting blades. Afterwards each of the sample long bones were cut into small sections with new disposable blades and placed into a Sartorius Mikrodismembrator which uses ball bearings to crush the bone into a fine powder. Sample 19A, a molar, required a different preparation method. When sample 19A, a molar, was prepared it was first wiped with commercial bleach. The surface of the tooth was removed with a disposable blade. The removal of the tooth surface material was done so to reduce the possibility of external contaminants being introduced to the dentine of the tooth during the cutting process. A new disposable cutting blade was used to section the tooth at the cement enamel junction. After having cut open the tooth, the dentine was drilled out of the root and collected to be analyzed. Once the sample's preparations were all complete the extraction of DNA began.

Each of the three samples were placed in 3ml of 0.5M EDTA pH 8.0, 300 $\mu$ l 5% N-Lauroylsarcosine and 30ul Proteinase K (20mg/ml) (Gongora et al., 2008; Hagelberg et al., 1991; Hagelberg et al., 1994; Hagelberg & Sykes, 1989). This process simultaneously opens up the free osteocytes in the ground up material and gently dissolves the bone freeing more cells to be lysed. The mixture of bone and reagent was continuously mixed at 37°C for eight hours. The freed DNA was isolated through two washes containing phenol/chloroform/isoamylalcohol (25:24:1) at a pH of 8.0. The third and final wash was comprised entirely of chloroform. After this final wash the DNA was concentrated in an Amicon Ultra-4 filter (50 kDA) (Gongora et al., 2008; Hagelberg et al., 1991; Hagelberg et al., 1994; Hagelberg and Sykes, 1989; Montiel et al., 2001; Montiel et al., 1997).



Sterile water was used with the filter to retrieve a final volume between 40 and 60µl for samples 4D, 19A, and 22B. The newly concentrated samples of DNA were then taken to be amplified.

After the genetic material has been freed from its bone or enamel matrix, the process of amplification occurred. The target region for amplification is the HVS I. This region was amplified using singleplex PCR which uses overlapping primers so as to completely cover the HVS I. By using this system, the base pairs amplified span the HVS I. The mixture of reagents consist of 1 X Buffer, 2.5 mM MgCl<sub>2</sub>, 0.25 mM of each dNTP, 400µM of primer, 1mg/ml RSA (Sigma), 2 U of Amplitaq Gold Polymerase and 2µl of the DNA that was extracted (Gongora et al., 2008; Hagelberg et al., 1991; Hagelberg et al., 1994; Redd et al., 1995; Whyte et al., 2005). The thermocycling of the materials consisted of activating the enzyme at 95 °C for six minutes. After the initial denaturing of the DNA at 95°C for 30 seconds, a 30 second period DNA annealing at 60°C was allowed to occur. Immediately following the 30 second period of annealing, the elongation process occurs at 65°C for 30 seconds and finally the period of extension of the DNA at 65°C for 10 minutes was allowed to take place. The denaturing, annealing, and elongation processes was conducted 40-45 times in order to ensure the amplification of the specific sections of DNA determined by the primers introduced to the reaction mixture. Along with the three samples of extracted DNA to be tested two blanks and two PCR negatives were prepared to be run concurrently through all the PCR cycles. The blanks and negatives are run simultaneously with the samples to check the researcher's sterilization techniques and the condition of the PCR reagents. If the sample blanks or PCR negatives yielded any DNA results then the contaminated product or products would have to be

eliminated before running any more of the test samples. No such contamination was found in any of the blanks run for samples 4D, 19A, or 22B. With the conclusion of the amplification process the genetic material was sent to be analyzed.

All of the PCR products were placed on an agarose gel and checked by electrophoresis. The successfully amplified products of the PCR magnification were sequenced using the Big Dye Terminator 3.1 Kit. The specific manufacture instructions were followed during the sequencing as to yield the optimal results from the kit. The samples sequenced were placed in the 3130xl Genetic Analyzer and the software Sequencer the DNA was aligned and read.

A SNaPshot reaction was conducted on the 22 Single Nucleotide Polymorphism (SNP) multiplex using 5 $\mu$ l of the PCR products from above. This test utilized the SNaPshot reaction kit from Applied Biosystems. For optimization of the process the manufacturer's instructions were followed. The addition of 10% ammonium sulphate was necessary to reduce the presence of artifacts during the SNaPshot reaction. The denaturing, annealing, and elongation cycles are similar to that found in the multiplex PCR approach. There are differences in times and temperatures. Denaturing of the three samples occurs at 96°C for 10 seconds, annealing occurs at 55°C for five seconds and the elongation period takes place at 60°C for only 30 seconds. This process was repeated 35 times to amplify the DNA properly. After the 35 cycles have been completed the single base extension reaction was refined through a series of reactions involving 1 U SAP incubated at 37°C for 40 minutes then the mixture was heated to 80°C for 10 minutes. After this reaction process 2 $\mu$ l of each of the three samples single base extension reactions were mixed with 11.5 $\mu$ l of HiDi Formamide and 0.5 LIZ-120 size standards.

This final mixture was then run through the 3130xl Genetic Analyzer. The data collected was then processed by Genemapper v2.5 software. The results of this analysis are reported in the following chapter.

## CHAPTER 4

### RESULTS

The decontamination extraction and amplification of the mtDNA was performed in accordance with the guidelines discussed in the previous chapter. Both UNLV and the Australian Centre for Ancient DNA facilities performed separate decontaminations of the sample material in order to reduce potential contamination by extraneous modern DNA. During the extraction and amplification phases at the Australian Centre for Ancient DNA, the protocols for each phase were followed in accordance with the laboratory standards and/or commercial kits. This chapter will present the results of the decontamination, extraction, and amplification process. The tables below are provided to better illustrate the results of the project.

There were a total number of five singleplex PCR's performed on each extracted sample. In sum, 15 reactions concerning samples 4D, 19A, and 22B were tested (Table 1). A single multiplex PCR was performed involving three reactions pertaining to the three samples. As seen in Table 5, for sample 4D the primers 15996 to 16142, 16209 to 16348, and 16287 to 16410 were not able to be amplified. Amplification for sample 4D primers 16117 to 16233 and the 22 SNPs were successful. For sample 19A, primers 15996 to 16142 and 16287 to 16410 were not successfully amplified. Successful amplification of sample 19A occurred with primers 16117 to 16233, 16209 to 16348, and the 22 SNPs. The primers 15996 to 16142, 16117 to 16233, and 16209 to 16348 were unsuccessfully amplified for sample 22B. Successful amplification occurred with the 16287 to 16410 and 22 SNPs primers in regards to sample 22B. The following table illustrates the test reactions.

Table 1 Amplification results of mtDNA

(+) representing a successful amplification of mtDNA and an (\*) represents an unsuccessful attempt at amplification of the mtDNA with the primers.

Sample Number	Primer Starting Points on Low and High End of mtDNA				22 SNPs mtDNA
	L15996 H16142	L16117 H16233	L16209 H16348	L16287 H16410	
4D	*	+	*	*	+
19A	*	+	+	*	+
22B	*	*	*	+	+

The Genetic Analyzer and Sequencer Software was used to scrutinized samples 4D, 19A, and 22B and displayed the corresponding haplogroup for the sequences analyzed (Table 2).

Table 2 Sequencing results of successful amplification regions

Sample Number	Sequence Range of Successful Amplification	Haplogroup
4D	16118-16233	H
19A	16118-16346	H
22B	16288-16409	H

The 22 SNP multiplex analyzed 22 specific base pair sites and the Genemapper v2.5 software displayed the specific base pairs and associated haplogroup (Table 3).

Table 3 22 SNPs multiplex typing

		Sequence Range of Successful Amplification																					
Sample Number		13928_R9	3594_L2	10550_K	11467_U	4248_A	8994_W	13263_C	13368_T	11719_preHV_R0	8280delB	4580_V,M3	6371_X	10238_N1	7028_H	10034_I	5178_D	14766_HV	12705_R	10873_N	12612_J	2758_L0'1	10400_M
4	D	C	G	T	A		G	T	G	C	A	C	G	T	G	T	G	G	G	A	A	G	
19	A	C		T	A	T	A	T		C	A	C	G	T	G	T	G	G	G			G	G
22	B	C		T	A				G		A		G	T	G	T	G	G			A		

A preliminary analysis was performed on samples 4D, 19A, and 22B to determine if any mtDNA could be extracted. The results indicate that the H haplogroup was present in all three of the samples tested. Therefore, further testing was suspended. The finding of the H haplogroup in all of the samples tested indicated the samples were from people not indigenous to Southeast Asia. These results and implications will be discussed in more detail in the final chapter.

## CHAPTER 5

### DISCUSSION / CONCLUSION

The amplification process for 4D, 19A, and 22B was less than 50% successful. In all, only seven out of the 15 primer segments were successfully amplified. This low success rate is not out of the ordinary when attempting to amplify ancient DNA. Researchers working with ancient DNA anticipate a low amplification success rate due to the degraded nature of the samples. Short overlapping DNA primers are used to overcome the natural degradation of DNA over time and the negative effects the environment inflicts on the DNA molecule. The rationale for using overlapping DNA primers is to reconstruct a complete DNA sequence from a once fragmented strand of DNA. The primers used in this study were 146 to 116 base pairs in length so as to amplify mtDNA segments that have degraded from the much larger original strand. The isolation of small primer segments is used to counter the natural degradation of the DNA molecule over time. As stated above the breakdown of the DNA molecule was considerable even with the use of primers with so few base pairs. Therefore, the low rate of successful amplification is attributed to the highly degraded nature of the genetic material. This being said, any genetic material successfully amplified can be used to determine the sample's ancestry.

Looking at the data, the primer segments 15996 to 16142 and 16287 to 16410 found in Table 1 do not contain any of the base pairs that comprise the "Polynesian Motif". Only sample 22B had success in the amplification process for section 16287 to 16410. Samples 4D and 19A failed to amplify both primer segments. Successful amplification of

the mtDNA region containing a portion of the “Polynesian Motif” was produced by only one of the two long bone samples 4D and 22B.

The primer regions that contain the “Polynesian Motif” are 16117 to 16233 and 16209 and 16348. In the amplification process of sample 22B the primers were unable to successfully amplify DNA from the 16117 to 16233 and 16209 and 16348 base pair sections. Therefore, sample 22B was unsuccessfully amplified in both primer sections that contain portions of the “Polynesian Motif”. However, the primers for sample 4D were able to amplify mtDNA within the region of 16117 to 16233 base pairs. This section of amplification covers two of the base pairs associated with the “Polynesian Motif” 16189 and 16217.

The tooth sample 19A had successful amplification of the HVS I between 16117 to 16233 and 16209 to 16348. These primer regions cover the base pairs that encompass the “Polynesian Motif” 16189, 16217, 16247, and 16261.

The significance of the long bone and tooth results is that a possible determination of the specific HVS I region for 19A and a partial profile for 4D could be made. Therefore, a determination of the presence or absence of the “Polynesian Motif” could be visualized. This step was not attempted due to the results presented in the fifth and final column in Table 1.

The primers run under the heading of 22 SNPs mtDNA in the last column of Table 1 were found to be successful in all three samples. This primer set is used to determine the haplogroups for each of the individual samples tested.

Haplogroups are genetic mutations associated with the mtDNA or the Y-chromosome. These haplogroups, have been well researched in human populations from



a wide variety of regions around the globe. Haplogroups represent people who are found in a large general region of the world like Europe or Asia. As a result of extensive research projects by various scientists, these haplogroups can now be narrowed to identify people from specific regions like Eastern Europe or Oceania. These haplogroups are assigned letters to represent a specific set of DNA sequences that occur in high frequency in certain populations and so correspond to a particular group of people (i.e. European or Asian). Haplogroups associated with Asian and Oceanic ancestry are labeled as B and P respectively (Merriwether et al., 2005).

For this research a 22 SNP multiplex typing kit was used to determine the ancestry of the amplified genetic material. The 22 SNPs multiplex typing yielded data that associates base pairs with the haplogroups H, HV, I, J, K, RO, T, U, V, W and X (Table 3). These haplogroups are associated with people living in Europe (Alvarez-Ingesias et al., 2008; Mederios, Sucena, Ribeiro, Espinheira, & Geada, 2008; Parsons & Coble, 2001).

The reported haplogroup H is found in 40% of modern European people. The data from Table 3 indicates 11 of the 22 SNPs to be associated with people of European descent. Moreover, the SNP position 7028G and 14766G found on Table 3 are the primary indicators of the H haplogroup (Butler, 2005, p. 286). Therefore, presence of base pairs 7028G and 14766G signify the remains to be of European descent.

Haplogroups of Asian and/or Oceanic descent are B and P (Merriwether et al., 2005). Individuals of Asian or Oceanic ancestry are found to have the nucleotide Thymine at positions 7028 and 14766 (Butler, 2005, p. 286). Since Thymine was not detected at either base pair position it can not be concluded that the samples have ancestry of Asian or Oceanic origin. Therefore, one can only conclude that the samples tested were

contaminated with genetic material of European origin. The recognition that the apparently best preserved samples amplified at a less than 50% success rate and were themselves contaminated caused the discontinuation of any further amplification of the Niah Cave remains.

The results of the haplogroup test negated the need to test for the nine base pair deletion found in people of Asian and Polynesian descent. The haplogroup results did not warrant the cost or time needed to test for a genetic marker not specific to the Polynesian people.

#### Research Questions Discussion

The first research question posed in chapter I inquired is the recovery of genetic material possible from the Niah cave skeletal remains. The answer to that question regarding ancient DNA recovery points to the genetic material not being able to be recovered from the skeletal remains (either bone or teeth) buried for thousands of years in a tropical rainforest environment. The genetic material's true origin was found to be European, not Asian, nor from the Great Niah Cave, from which they were thought to originate. Of all the bones and teeth source materials, the tooth 19A housed the most complete mtDNA strand that could be retrieved. However, this information does not provide compelling evidence in regards to the population migration models outlined previously. Instead it indicated that this particular sample was contaminated with European DNA and that somehow that DNA entered the tooth pulp chamber causing contamination. Therefore, the conclusion reached by this analysis is that ancient DNA cannot be recovered from the current Niah cave samples housed at UNLV.

The second research question inquiring as to which source material provided the most complete genetic sequence yielded results. The answer is that the tooth sample 19A was the most successful in amplifying DNA but as stated above the genetic material obtained was contaminated. The conclusion to be drawn from the contaminated results is that if DNA contaminants reach a pulp chamber of a tooth the material is better protected and subsequently can be amplified better than contaminated bone samples. As for this studies question regarding the source material no significant conclusions can be deduced from these results.

Research question three regarding the loose teeth or teeth still embedded in the jaw yielding a more complete or least contaminated genetic sequences was not answered during this research. During the selection process by the Australian laboratory no embedded teeth were selected for amplification. Visual inspection of the collection of teeth by the scientists at the Australian Lab resulted in their decision that the loose molar 19A possessed the best opportunity to yield ancient DNA. The findings of the research that the DNA was of European decent halted any further extraction of DNA from any of the other samples available. Therefore, the comparison between loose and embedded teeth could not be made.

The final research question considered whether the storage of the skeletal remains at the UNLV facility allowed for the recovery of ancient DNA without any major contamination. The answer is not clear since the initial excavations were conducted by scientists of European descent. Thus contamination could have occurred in the field or at any time during the curation of the material at UNLV. The Great Niah Cave skeletal remains at UNLV are not suitable for genetic testing using today's technical facilities. As

genetic testing techniques improve ways to extract the ancient DNA from foreign contaminants maybe developed. Therefore, until such time as contaminate removal techniques improve the Niah Cave collection should not be considered for ancient DNA research.

In regards to the overall goal of the project, the data retrieved does not contribute evidence to support or refute either the “Slow Boat” or “Express Train” theories. As stated above, DNA from people indigenous to Island Southeast Asia or continental Asia was not retrieved from the samples tested. Therefore, the broad goal of this research to add to the understanding of the migration of the Proto-Polynesian people was unsuccessful.

#### Implications of Research

The “Slow Boat” and “Express Train” models represent the two most supported hypotheses proposed to explain the migration of humans from Asia to the farthest reaches of the Polynesian Islands. The replacement theory (“Express Train”) and a multi-regional theory (“Slow Boat”) both have their proponents and detractors. The use of multiple lines of evidence from anthropological sub-disciplines such as linguistics, archaeology, and physical anthropology challenge hypotheses and help prevent researchers from assuming that models can be solved through the use of one discipline alone (Hurles et al., 2003). Thus, the addition of ancient DNA research is of great value to the multidisciplinary approach to the understanding of the migration of the human species.

The use of ancient DNA as evidence to determine migration patterns is important in the field of anthropology; but technique itself has value both inside and outside

anthropology. The ability to extract DNA from samples that have resided in a tropical rainforest setting for thousands of years will strengthen the viability of conducting ancient DNA experiments in regions that are thought to be too difficult to obtain viable information. Even though this research was unable to extract uncontaminated DNA, the potential data that can be gained from similar research still warrants this type of research. The success of this type of research is influenced by the practices that occur far from the laboratory. The extraction and handling of the skeletal remains at the archeological site need to be amended/improved to allow for the best possible results in the laboratory.

#### Archaeological Recovery Techniques

Although the original goals of this research could not be completed, what can be learned from this research is that the extraction of skeletal remains demands a great level of care to reduce the exchange of genetic material between the excavator and the sample. The genetic material recovered from this research was highly degraded, which meant that the foreign DNA was old or had been nearly destroyed during the decontamination procedures. The decontamination procedures performed are meant to destroy any surface contaminants on the remains prior to the cutting of the bone or opening of the tooth. Although one cannot completely rule out the UNLV or Australian facility for the source of contamination, the more likely source of contamination would be at the site of extraction where the bones and teeth were washed and handled without gloves and masks.

New procedures must be implemented so as to reduce the exchange of DNA into the remains. By stopping the practice of washing the bones there is a lesser chance of foreign

DNA penetrating the deeper regions of the bone that the genetic researcher targets. The need to wash bones in order to see critical features is understood but doing so with gloved hands and with clean water (deionized if possible). The practice of wearing gloves when handling skeletal material and changing them when new remains are encountered would greatly reduce the contamination from the excavator and the cross contamination of remains of different people. The need for excavators to wear hair-nets, body suits, and face shields at the dig is not what is being asked only the need to attempt to reduce the risk of surface and deep contamination of the skeletal material. Being aware of the potential transfer of genetic material to the bone by the excavator and taking simple measures (wear gloves) to reduce the chance of DNA transfer would aid the genetic research in isolating the ancient DNA trapped within the bone.

### Sample Viability

Along with viability, the issue of artifact storage can be addressed with this research. The current practices and procedures of artifact storage and collection are addressed insofar as obtaining ancient DNA from samples is concerned. The current practices of storage in museums and university collection rooms is a question as to if the bone is being properly preserved.

The presence of European DNA in the three samples indicates that somewhere during the handling of the skeletal remains a significant amount of foreign DNA was introduced so as to penetrate into the deeper lamella of the bones. The likeliest introduction of this foreign DNA would be at the archaeological site over 40 years ago. The washing of the bones at the site to remove the excess dirt likely transferred the foreign DNA into the

deeper layers of the skeletal material. This deep penetration is assumed due to the procedures followed by the researchers to remove the potentially contaminated surface of the bones and teeth. The finding of European haplogroup H in the tooth sample also implies the contamination took place 40 years ago at the time of the initial collection. A longer time frame for the contamination of the samples is inferred due to the less than 50% success rate for the amplification of the DNA present. If the current research teams had contaminated the skeletal material a more complete and higher yield of DNA would have been resulted. However, while one cannot rule out the possibility that the DNA was from either research team extracting and amplifying the sample, and that somehow, through the decontamination process, it became degraded, this possibility is very unlikely.

The predominant argument against the current researchers contaminating the samples is the tooth. During the two separate decontamination phases prior to the extraction of dentine, the only liquid placed on the tooth surface was bleach to destroy any outside contaminants. Regardless of who might have contaminated the samples, the fact remains that the only DNA to be amplified points to people of European decent. While it cannot be definitively determined whose DNA was amplified, the issue of storage can be addressed.

The UNLV collection room is an area solely dedicated to the storage of skeletal remains. Currently UNLV is in the process of re-boxing their collection of skeletal remains. The new set of boxes for the skeletal remains will aid in the preservation of the DNA present in the samples, but in the past some remains shared a single storage box which could have compromised the integrity of the remains DNA. The practice of

keeping all the skeletal remains separated will aid future genetic research in reducing contamination.

The controlled environment of the collection room and the restricted access to the room are also important and necessary factors in reducing the potential contamination of the skeletal remains in regards to genetic research. Any future remains stored at the UNLV collections room would be preserved adequately in such an environment. However, if genetic research is to continue at UNLV, additional procedures would need to be implemented immediately. Procedures of wearing gloves, lab coats, and masks before entering the room would need to be executed. In addition, the collection of the genetic profile of all individuals entering the collection room would need to be collected and put on file for future reference. Therefore, this research regarding the storage of skeletal remains at UNLV sees the current practices to be sufficient when performing morphological measurements on skeletal remains but insufficiently rigorous for ancient DNA research in its current state.



APPENDIX 1

BURIAL AGES AND REMAINS

Niah cave Radiocarbon Dates and the description of the skeletal remains

Samples for the Express Train Model		
Burial Number	Age of Skeletal Remains B.P.	Comments Description
3	1930 to 1870	2 teeth in mandible, 3 teeth loose RI, LM, and LPM
10	3420 to 3320	4 shafts radius and ulnas, Mandible with molars and premolars in sockets
36	3420 to 3320	Skull fragments, 3 loose teeth LM2, RM2, and LP3 and right maxilla
50	2270 to 2210	6 loose teeth LM2, RI2, RC1, LM2, RP3, and RM2, 6 Diaphysis portions of long bones
57	2590 to 2520	5 loose teeth LC1, LM1, RI1, RP3, LM1, LM2, and RP4, 5 diaphysis long bones
60A	3040 to 2960	2 loose teeth LM3 and LM2 Mandible, Canines and premolars, 5 diaphysis long bones
67	2710 to 2630	No teeth, 7 diaphysis long bones
69	3260 to 3170	half of a mandible, 2 intact R molars
75	2700 to 2630	No long bones, 4 loose teeth, and (7 total) intact 2 L and 2 R mandible, 3 in maxilla
102	2740 to 2660	6 diaphysis long bones, 17 teeth intact with mandible and maxilla mainly with maxilla
125	2810 to 2730	4 intact not well preserved teeth, 2 diaphysis long bones covered in some type of foreign substance
133	3060 to 2980	No teeth, Only long bone diaphysis
135	2970 to 2880	One molar loose, fragmented diaphysis covered with red material as well as the molar
Samples for the Melanesian Migration (Slow Boat Model)		
Burial Number	Age of Skeletal Remains B.P.	Comments Description
54	10900 to 10600	1 tooth, 6 fragmented diaphysis long bones
66	7050 to 6850	4 long bones from infant
76	4290 to 4160	No teeth, 4 diaphysis long bones

83	8230 to 8000	4 teeth in the L and R maxilla, Only bone fragments not necessarily long bones
92	7350 to 7140	small amount of material, No teeth, 3 shafts of long bones
110	5130 to 4990	3 teeth LM1, LM3, and RM1, 2 diaphysis (only 3 total shafts)
115	4780 to 4650	4 loose teeth RP3, RI2, RP4, and LM1, 5 partial diaphysis long bones
146	11700 to 11400	2 intact teeth LP2 and LM1, 4 pieces of diaphysis long bones
147	7220 to 7020	No teeth usable, 3 diaphysis with pieces glued on
155	8080 to 7850	One intact molar, 4 loose teeth RM1, RM2, I, RC1, and LI2, 5 diaphysis long bones
	Samples in Transitional Time Period	
Burial Number	Age of Skeletal Remains B.P.	Comments Description
30	3820 + 485	No teeth, 2 Diaphysis long bones
68	3660 + 100	No teeth, 4 diaphysis long bone shafts 3 of which are glued
77	3580 + 70	An entire maxilla all but 1 canine, 5 diaphysis long bones
123	3590 + 160	7 intact teeth on left side of mandible, 3 loose teeth, 5 diaphysis long bones

APPENDIX 2

BURIALS AND ARTIFACTS

Niah cave burials and artifacts associated with skeletal remains

Burial Number	Burial Artifacts
146	Hematite and blackening of bones
54	None
83	Quartz crystal Fire striker, Chert, Rhinoceros teeth, Hematite staining of bones
155	None
92	None
147	Blackened bones
66	Wood Coffin
110	Bamboo Wrapper
115	Pandon Leaf Mat, Bamboo Wrapper
76	Wood Coffin
30	Hard Stone Chip, Potstones, Bone Needles, Toy Jar
68	Pandon Mat Pillow, Pandon Leaf Mat, Bamboo Wrapper
123	Pandon Leaf Mat, Bamboo Wrapper
77	None
10	Wood Coffin, Pandon Leaf Mat
36	Wood Coffin, Possible Bronze Knife
69	Earthenware, Jar
133	Two Bone Pendants*, Pandon Leaf Mat, Phallic Pebbles rubbed with Hematite
60A	Pillow of textile, Glass Bead, Bamboo Wrapper
135	None
125	None
102	Bamboo Wrapper
75	Wood Coffin
3	Bamboo Casket, Wooden pillow

\* Native people of Borneo do not wear this style of pendant (Harrisson, p173: 1967)

APPENDIX 3

DESCRIPTION AND WEIGHTS

This list consists of the renumbered samples and their weight at the time of shipping to the Australia Centre for Ancient DNA

Burial Numbers	Modified Burial Numbers	Brief description of samples	weights
146	1A	Maxilla plus 2 teeth attached	24.72g
146	1B	Long bone	1.78g
54	2A	Tooth plus surrounding bone (in tube)	4.74g
54	2B	Long Bone	1.94g
83	3A	Right Maxilla with 3 teeth attached	31.54g
83	3C	Left Maxilla with 3 teeth attached	30.37g
155	4B	Long Bone	1.58g
155	4D	Long Bone	3.17g
92	5B	Long Bone	2.21g
92	5D	Long Bone	1.92g
147	6B	Long Bone	2.43g
147	6D	Long Bone	1.96g
66	7B	Long Bone	1.58g
66	7D	Long Bone	1.13g
110	8A	One tooth (molar in tube)	2.29g
110	8B	Long Bone	1.97g
115	9A	One Tooth (incisor in tube)	1.83g
115	9B	Long Bone	1.51g
76	10B	Long Bone	2.21g
76	10D	Long Bone	1.75g
30	11B	Long Bone	1.60g
30	11D	Long Bone	1.55g
68	12B	Long Bone	1.82g
68	12D	Long Bone	2.18g
123	13A	Left half of Mandible with the first four on the right side	63.61g
123	13C	Left half of Mandible with the first four on the right side	63.61g
77	14A	The entire maxilla plate, all the teeth but the right canine broken off	54.38g
77	14C	The entire maxilla plate, all the teeth but the right canine broken off	54.38g
10	15A	Right half of mandible with teeth (two good molars)	43.55g
10	15B	Long Bone	1.70g
36	16A	Portion of Right Maxilla with 4 teeth attached	20.19g

36	16C	One tooth (molar in tube)	2.19g
69	17A	Left half of mandible with two good molars	57.20g
69	17C	Left half of mandible with two good molars	57.20g
133	18B	Long Bone	2.47g
133	18D	Long Bone	1.97g
60A	19A	One Tooth (premolar in tube)	1.97g
60A	19B	Long Bone	2.70g
135	20A	One Tooth (premolar in tube)	1.49g
135	20B	Long Bone	13.80g
125	21A	Right partial mandible with 3 teeth only one good molar	46.61g
125	21B	Long Bone	2.23g
102	22A	One tooth (molar in tube)	2.41g
102	22B	Long Bone	3.07g
75	23A	Right Maxilla with 3 teeth attached	24.15g
75	23C	One Tooth with piece of bone attached (molar in tube)	3.42g
3	24A	One Tooth (premolar in tube)	2.07g
3	24C	One Tooth (incisor in tube)	1.24g

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