Spring 1-1-2011

Ancient DNA in Physical Anthropology: A Review

Jacqueline E. Broida
University of Colorado at Boulder, jacquelinebroida@gmail.com

Ancient DNA in Physical Anthropology:

A Review

by

Jacqueline E Broida

B.A., Miami University, 2006

A thesis submitted to the Graduate School of the

University of Colorado in partial fulfillment

Of the requirement for the degree of

Master of Arts

Department of Anthropology

2011

This thesis entitled:
Ancient DNA in Physical Anthropology: A Review
Written by Jacqueline E Broida
Has been approved for the Department of Anthropology

X

Dennis Van Gerven

X

Darna Dufour

X

Herbert Covert

Date _____

The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.

Broida, Jacqueline E (Masters, Biological Anthropology)

Ancient DNA in Physical Anthropology: A Review

Thesis directed by Full Professor Dennis VanGerven

The field of ancient DNA began in 1984 with the sequencing of quagga—an extinct member of the horse family—DNA and the development of PCR (Higuchi et al., 1984). Since then, ancient DNA has been used in physical anthropology. Ancient DNA has a variety of applications in anthropology including phylogentic relationships and human evolution, movement and migration, the study of hominin ancestors, sex determination, agriculture, animal domestication, nutrition, diseases, historical kinships, and primate conservation. In particular aDNA technology has given anthropologists the opportunity to study the history and pre-history of the agricultural expansion in the Pacific as well as the ability to learn more about the Neanderthals: what their mitochondrial genome was like, how much their genome differed from the modern human genome, their pigmentation, and their position in hominin phylogeny.

Table of Contents

Figures

Introduction

The field of Ancient DNA (aDNA) has been referred to as "black magic" (Hummel, 2003) and at least one researcher has suggested that only people with novel genomes should be able to work with ancient DNA (Stoneking, 1995). Particularly in the early days of the field, mystery pervaded certain aspects of PCR protocol and contamination from exogenous DNA. As the field has matured, the mystical overtones have given way to painstaking protocol and careful decontamination. As aDNA techniques have improved, the field has started contributing valuable information to other fields of inquiry, including physical anthropology.

The first and primary intent of this thesis is to examine ancient DNA's role in physical anthropology up to 2011 and to suggest future prospects for ancient DNA in this discipline. The second purpose of this thesis is to take this information and incorporate it into two case studies—the first one on Pacific migrations and the second one on the Neanderthals—to demonstrate how ancient DNA has actually been used in physical anthropology.

To facilitate these goals, this paper is organized into two sections. The first section includes a chapter on ancient DNA techniques, including environments necessary for DNA preservation, sources of ancient DNA, methods for extraction and preparation of ancient DNA, PCR protocol, and determining the authenticity of results. The second chapter is concerned with a history of the field and major questions and applications for ancient DNA in physical anthropology. The chapter begins with a brief history of the field of aDNA, looking specifically at a hallmark paper for aDNA within physical anthropology, *Ancient DNA Studies in Physical Anthropology* (O'Rourke et al., 2000). I suggest that the major applications for ancient DNA in physical anthropology are sex determination; primatology and primate conservation; studies on

hominin evolution, gene flow, and kinship; agriculture and disease; nutrition; social structure; human movement and migration; and human ancestors.

In part two I shall incorporate the methods, techniques, and history of part one into two case studies. The third chapter will be on ancient DNA research concerning Pacific migration during the agricultural expansion. Here I review what we know about Pacific migrations from the fields of linguistics and archaeology. I then examine what genetics, coupled with the other two fields of inquiry, have added to our understanding of Pacific prehistory. I examine the use of human, lizard, chicken, and rat ancient DNA and show how all of these lines of evidence have merged into a blurry picture of what was happening in the Pacific. The fourth chapter will look at what we have learned about Neanderthals from aDNA. First I briefly look at the history of Neanderthal research and then I review and analyze the methods, techniques, and results of Neanderthal ancient DNA studies to date.

I conclude by looking at problems and prospects for aDNA within physical anthropology. I shall suggest that some of the major prospect within physical anthropology are studying infectious disease and "diseases of affluence," multidisciplinary approaches to historical reconstruction, doing environmental reconstruction at archaeological sites, and better analyzing population interactions. Next I shall propose that some of the major challenges are contamination, sequencing technology, and small sample sizes.

Part One: Ancient DNA Research

Chapter One: Methods and Techniques

Working with and acquiring results from ancient DNA is like putting together a puzzle with missing pieces. Procuring the DNA is like trying to find an old puzzle in the closet. It helps to know the correct places to look and it can be under a lot of dust and debris. Choosing a tissue to work with is like trying to determine which puzzle to do—you only have so much time to devote to it. Sometimes there is only one puzzle to choose from. Sometimes there are many puzzles to choose from and you want to select the one that will be the most interesting. Extraction and preparation of the DNA is analogous to taking the puzzle out of a box, laying the available pieces on the table and flipping them all over to the correct side. When one performs PCR on DNA, one is trying to put the puzzle together: which piece goes where? Do these pieces belong together? Finally, one does an analysis: What is the most comprehensive picture? It is difficult to determine because many of the pieces are missing. Of course, there are aspects that help or hinder. If the picture is of something unique then it can be easier to decipher than if it is of something commonplace. It's a very puzzling field, ancient DNA.

DNA degrades over time. Thus, older DNA is susceptible to degeneration or complete disappearance. To date, only DNA 100,000 years old or younger can be extracted. Anything older is too degraded (Mitchell et al., 2005). To procure meaningful and significant results from an ancient DNA (aDNA) sample, many factors must be taken into account. One must consider the environment in which the sample was found, what tissue from which to take the sample, whether to use nuclear or mitochondrial DNA, the method used to extract the DNA, and how to avoid contamination in the process. It is equally important to consider the protocol and equipment one is using, as well as the set-up of the laboratory.

Procuring Ancient DNA

The first step in any aDNA analysis is finding intact DNA. This step in analogous to finding the puzzle, though one should not expect to find a complete puzzle by any stretch. There are two basic requirements for DNA preservation over time: rapid desiccation and the inhibition of bacterial activity (Pääbo, 1985; Höss et al., 1996; Wayne et al., 1999). High temperatures break down the hydrogen bonds between the bases, breaking the DNA apart. Wet environments foster oxidation that modifies the DNA. Oxidation is the process where the nitrous bases are altered and the sugars are broken down, destroying the DNA chain (Lindahl, 1993; Handt et al., 1994; Hofreiter et al., 2001). Therefore, the environments that support rapid desiccation are dry and cold[1] environments. Salty environments have also proven fruitful for aDNA researchers (Pääbo, 1985; Höss et al., 1996; Wayne et al., 1999). In special cases, however, "wet" samples may also contain aDNA. These "wet" environments are either water or humid environments. (One example of this is the Windover archaeological site.) In these special cases, it is the anaerobic nature of the area that protects the DNA from being damaged by bacterial or fungal activity (Doran et al., 1986; Hummel and Herrman, 1994; Lawlor, 1994). All these environments protect aDNA by preserving the bonds between the two bases as well as the sugars attached to bases. They also prevent the DNA from being mutated and degraded by foreign substances. Although it is not considered a foreign substance, UV rays may also damage DNA and these environments protect the DNA from ultraviolet damage as well (Höss et al., 1996). To see if DNA is still present in usable form within a specimen, amino acid racemization is used. This technique is minimally destructive and is a good estimate for depurination. If the results of an amino acid racemization show that DNA might remain, one may them test the specimen. If the

[1] The extreme example of a cold environmental preservation is the case of frozen remains. Frozen remains are protected from decay, humidity, and bacterial activity. In many cases, working with well preserved frozen samples is much like working with modern samples, if the preservation is complete enough (Nielsen et al., 1994 H&H; Grody, 1994 H&H).

results are negative, then one can save the specimen for morphological studies (Collins et al., 2009).

The next step in the process is to determine which tissue to use for the DNA extraction. The main tissues used for anthropological research are soft tissues, bones, and teeth. In cases where the remains are very well preserved, hair shafts are also used[2] (Wilson et al., 1995; Gilbert et al., 2007; Gilbert et al., 2008; Wolinsky, 2010). Coprolites may also be used to glean knowledge about disease and nutrition, though the technical aspects are not addressed here (see: Hofreiter et al., 2001). In many cases—such as archaeological sites—the choice is made simply by what tissues are available. Bones and teeth are generally the best preserved tissues and the only options. If there is a choice of tissues, there are other aspects to consider.

The first tissue used in ancient DNA studies was soft tissue (Higuchi et al., 1984; Paabo, 1985). Dried soft tissue is an excellent source of DNA because the desiccation protects it from hydrolytic damage (though not from oxidative damage). It is also easy to prepare. It does not require drilling or excessive handling or modification. Soft tissue has also been shown to produce a good DNA yield (Paabo, 1985; Paabo, 1989). The difficulty with soft tissue is that it is often handled while in the field. Historical and museum samples are particularly susceptible to a lot of handling. This can lead to a high probability of contamination. For this reason, soft tissue is ideally procured from below the surface of the specimen. Muscle is good because the skin above provides some protection from contamination. The brain and other organs can also yield DNA if they are preserved. These tissues also have the advantage of being particularly useful for studying infectious disease that often does not manifest itself on harder tissues (Salo et al., 1994).

[2] aDNA has also been extracted from seeds, egg shells, and feathers. While these are not often used in physical anthropology, they can be used to glean clues about agriculture and nutrition (Cooper, 1994; Ellegren, 1994; Rollo et al., 1994).

The difficulty with organ tissue, however, is that the organs' enzymatic activity sometimes degrades its own DNA (Cooper, 1994)

In many cases bone may be the only or best choice of tissue to use for extraction. The advantage of using bone is that DNA binds to the bone minerals and this may slow DNA degradation. In addition, the interior of bone is protected from contamination, provided the part one utilizes is not near a lesion or a break that would admit foreign DNA (Yang et al., 1998). If, however, one is searching for information on infectious disease, bone lesions are an excellent place to search for pathogen DNA (Baron et al., 1995). There is also the choice of using trabecular or compact bone. Trabecular bone has been shown to yield greater amounts of DNA, but compact bone has proven to yield DNA more reliably (Hermann & Hummel, 1994). There are also difficulties to working with bone. Breaking down the bone matrix is a laborious and time consuming process. Bone also contains PCR inhibitors, which can be a disadvantage when preparing the sample for analysis (Rohland & Hofreiter, 2007).

Teeth are another potentially good source of DNA. Teeth are very hard and resistant to decay. Also, utilizing the inside of the tooth helps prevent contamination. In addition, teeth have also been shown to have good DNA yield as the DNA is generally free of natural PCR inhibitors (Smith et al., 1993). In their 1994 study, Woodward et al. look at DNA extracted from both teeth and soft tissue. They found that eighteen of their twenty teeth samples produced DNA, while none of the soft tissues did (Woodward et al., 1994).

Extraction & Preparation

DNA extraction has been called "the most crucial step" in aDNA analysis (Hummel, 2003). Therefore, DNA extraction and preparation necessitates a very careful and

exacting approach. Despite the importance of being absolutely certain that the chosen procedure produced accurate and repeatable results, extraction and preparation protocol for aDNA is still far from uniform. Partially this is because of the nature of the material. Unlike modern DNA (which is always found in a state of wonderful completeness), ancient DNA is found in varying degrees of degradation. In addition, different tissues require different steps in preparation—and mitochondrial DNA preparation differs from nuclear DNA preparation. To make matters more complicated, not everyone agrees on the best method for extracting and preparing aDNA. Still, there are some basic steps that are true for all aDNA analyses. These steps include: sampling of the specimen, pretreatment, DNA extraction, DNA isolation, DNA concentration, and purification (Hummel & Herrmann, 1994; Handt et al., 1996;Yang et al., 1997).

In sampling, one attempts to find the best tissues for aDNA research in the specimen under study. The best tissue depends on two aspects: the first is which tissue is of interest for the study (i.e. if one is attempting to study a pathology, one may prefer the diseased lung to the healthy bone). The second aspect is to attempt to find the tissue that will have the least contamination. (Contamination is an extremely important issue in aDNA studies. It is mentioned in this section briefly and then further discussed below.) Taking samples from below the outer surface of the specimen guards against modern DNA contamination[3].

Pretreatment involves ridding the sample of previous contaminants. This involves treating the surface of the selected sample with something that will not harm the sample, but will remove other DNA. Ultraviolet light and bleach are common choices (Handt et al., 1994; Hummel & Herrmann, 1994; Zierdt et al., 1996; Austin et al., 1997; Yang & Watt, 2004; Kim et al., 2008). For hard tissues one may also use some harsher methods including acid treatment,

[3] This is particularly true in specimens that were used and handled before aDNA analysis, such as the Nubian mummy collection at The University of Colorado at Boulder.

blasting the specimen with high powered air, or rubbing the specimen with sterile sandpaper. All methods must be used with care. Treating the specimen too long will destroy endogenous DNA and not treating the specimen long enough may lead to residual exogenous DNA (Hagelberg, 1994; Hummel & Herrmann, 1994).

DNA extraction is the manual process of pulverizing the material being studied[4]. Manual extraction is relatively easy for soft tissue. One must cut it and crush it into smaller pieces. The process is much more difficult for hard tissues. Bone must be sawed in half longitudinally and then a small piece may be removed from the interior for sampling. The bone must then be left in a solution that breaks down the inorganic matter as well as PCR inhibitors (Hänni et al., 1995). This may take a couple of days. Then the bone needs to be crushed. Several methods have been favored for crushing bone. Researchers have used a sterilized mortar and pestle as well as coffee grinders. One of the best methods to date, however, is placing the sample in a sterile plastic bag, freezing it in liquid nitrogen, and crushing it with a hammer. This method is the best at preventing contamination (Hagelberg, 1994).

Extracting DNA from teeth used to involve a similarly destructive process. First, one drilled into the outer part of the tooth and then sterilized the area. Then one drilled further into the tooth to collect the sample intended for analysis. There are two options for tooth material. One is to utilize the pulp and the other is to use both the pulp and the surrounding dentin (Woodward et al., 1994; Drancourt et al., 1998). This method worked well for extracting aDNA as the tooth cavity is well protected from contamination; however, teeth are important for morphological and isotopic studies, and drilling the tooth destroyed other valuable information. Another method, referred to as a "reverse root canal," offers a less invasive extraction technique.

[4] The process of DNA isolation is also referred to as "extraction" sometimes. Additionally, both extraction and isolation may be referred to together as "extraction."

Using this method, a small file accesses the opening of the canal. Then, selectively larger files open the root cavity and reach the dental pulp, which is extracted. This leaves the tooth mostly intact for other studies (Cobb, 2002).

DNA isolation involves leaving the tissue in a buffer to release the nucleic acids. The buffer consists of a Tris-HCL, EDTA, potassium salt, and proteinase K (Hummel & Herrmann, 1994). After the buffer, the protocol depends on which isolation method one is utilizing. While the protocols differ in details, there are five basic categories. These categories include: silica, chelex, phenol, chloroform, and boiling (Cano & Poinar, 1993; Hummel, 2003).

Boiling is almost never mentioned in aDNA literature. Chelex, phenol, and chloroform have all had success at yielding amplifiable DNA (Doran et al., 1986; Paabo 1989; Chou et al., 1992). Silica, however, is the preferred method. The advantages of silica are that it most reliably yields amplifiable DNA, it is quick and easy to use, and it helps remove PCR inhibitors that are present in bone (Boom et al., 1990; Hoss & Paabo, 1993; Cattaneo et al., 1997; Yang et al., 1998; Bouwman &Brown, 2002; Rohland & Hofreiter, 2007 a&b). In many cases, a combination of two or more of the isolation methods is used to procure the DNA. Sometimes they are used in sequence and sometimes concurrently. Utilizing multiple isolation substances can increase the likelihood of procuring pure DNA, particularly when working with difficult material, such as bone.

Once the DNA has been successfully isolated, it must be separated from the isolation substance, or concentrated, into pure DNA. This is done using pure sterilized water. After the DNA is concentrated, the researcher may choose to purify the DNA again using UV (Zierdt et al. 1996; Hummel, 2003). At this point, the DNA is ready to be amplified and analyzed.

PCR

There are several different assays used to analyze extracted DNA. Ancient DNA is unique in that it tends to be degraded. Degradation leads to short and small DNA yields. As polymerase chain reaction (PCR) is the usual and best technique to date for working with short and small DNA strands, it is the technique almost always used when working with aDNA[5] (Hummel et al., 1992; Hummel, 2003). PCR is a means of amplifying a piece of DNA so that it may be analyzed, and it consists of three basic steps with many different individualized accompanying methods. The basic steps are denaturation, annealing, and elongation (Palmirotta et al., 1997; Hummel, 2003). This process reproduces the DNA by being placed in a mixture of reagents and specifically targeted DNA primers. The DNA is pulled apart and denatured at high temperatures. Then the DNA is replicated in the latter two steps, doubling the yield of the DNA. The process is repeated as many times as necessary in cycles, each time doubling the DNA yield. PCR is a simple and easy technique for amplifying DNA; however, as with all things ancient DNA, there are several methods to improve the chances for aDNA yield from the PCR process (Weaver, 2008).

The first part of the PCR process is determining the primers and the reagents to be placed in the PCR mixture. This is a very individualized process and depends on what sequence one is hoping to amplify. The primers must match the sequence to be amplified[6]. After the solution is in place, there are several methods that have been employed to encourage DNA amplification for degraded DNA. (These methods are necessary because it is usually only safe to amplify aDNA for 30 cycles-- any more cycling risks analytical and contamination problems. However, only amplifying the DNA for 30 cycles leads to primer dimerization and small bands of inauthentic

[5] There are other methods for sequencing aDNA. A couple of these are addressed in chapter 4. However, these are still new techniques and are still prone to nucleotide substitutions and other errors (see: Prüfer et al.).

[6] In her 2003 book, Ancient DNA Typing, Hummel gives a detailed PCR explanation in chapter four and a laboratory protocol in chapter eight, section five. Because of this complete explanation, I shall not delve into it here.

DNA (Ruano et al., 1989; Don et al., 1991; Chou et al., 1992; Zierdt et al., 1996; Palmirotta et al., 1997; Hummel, 2003; Römpler et al., 2006).) The methods employed to overcome this hurdle are Hot Start, Touchdown, Booster, and Multiplex PCR. One or all of these may be used depending on the nature of the DNA.

Hot Start PCR involves keeping one or some of the reagents separate from the primer mixture until the temperature of the mixture is very high (80-90 degrees C). This prevents the mixture from partially amplifying the DNA early thus preventing misprimed sequences and primer dimerization. The end result is that the correct sequence is amplified without much interference from partial sequences. This is particularly true for fewer cycles (Chou et al., 1992; Zierdt et al., 1996; Rohland et al., 2004).

The Touchdown technique does not prevent mispriming like the Hot Start technique; however, Touchdown does prevent small bands of a misprimed or wrong product from dominating the target sequence. It does this by starting at a relatively high annealing temperature and slowly lowering the temperature each cycle for a few cycles. This gives the target sequence time to anneal and thus amplify as opposed to the smaller unwanted DNA sections (Don et al., 1991).

Booster PCR involves initially diluting the primers and then bringing the concentration back to normal levels. By using Booster PCR, the primers cannot use up extra enzyme in advance, allowing the DNA sample access to the enzyme it needs. This produces a better DNA product (Ruano et al., 1989).

Multiplex PCR analysis is performing multiple PCR analysis on the same sample at the same time. It is a two step process. In the first step, one uses multiple primers to simultaneously amplify different DNA segments. This step makes good use of a limited amount of DNA, but is

tricky. It is ideal to amplify segments that do not overlap and have distinctly different primers or one runs the risk that the sample will overlap and be misprimed (Römpler et al., 2006). The next step is to take the amplified product and run separate single PCRs on each strand that one is attempting to identify. The advantage of this technique is that one can test for multiple different sequences from one sample. For example, one can amplify segments from both the X and Y chromosomes simultaneously and thus determine sex (Palmirotta et al., 1997). One can also simultaneously look for mitochondrial DNA, nuclear DNA, and Pathogenic DNA (Hummel, 2003; Römpler et al., 2006).

Once the DNA has been amplified, there is enough DNA product to do an analysis on the sample. The samples are usually run on an agarose or polyacrylamide gel. The gel separates the DNA fragments by weight and the result appears like bands or blobs on the gel (Weaver, 2008). These bands and blobs may be analyzed by simple presence or absence or by more detailed means[7] (Hummel, 2003).

Contamination and Authenticity

There are two obstacles to overcome when verifying that one obtained authentic aDNA results. The obstacle that has received the most attention is contamination issues with the aDNA specimen. The less discussed and less prevalent concern is being able to distinguish the aDNA analysis as authentic DNA from an ancient degraded specimen. The first obstacle feeds into the second, but does not comprise it in its entirety.

Contamination and the knowledge of its risk pervade every step of the aDNA process. Specimens contain exogenous DNA from the soil surrounding then, bacterial and fungal

[7] Almost all molecular biology texts and manuals contain detailed descriptions on how to run a gel and interpret the results. It is a technical skill that one improves on with practice. The specifics of gel analysis are beyond the scope of this thesis.

invasions, and DNA from being handled; therefore, the first steps to preventing DNA contamination are ridding the specimen of present exogenous DNA, avoiding sampling areas that are particularly prone to foreign DNA (i.e. surfaces and lesions), and preventing additional contamination in the form of handling the specimen. In addition, modern DNA is preferentially amplified over ancient degraded DNA, so protocols must be in place to prevent contamination once the specimen is in the lab up through performing PCR and analyzing the product.

Some contamination controls are mentioned in *Extraction and Preparation* above. Contamination risk is such a natural piece of working with ancient DNA that it is impossible to discuss methodology without mentioning it. However, contamination is so important that it is often a topic of conversation on its own (Paabo 1989; Hagelberg, 1991; Hummel, 2003; Yang & Watt, 2004). As mentioned above, the first step when working with ancient DNA is to be prepared by handling the specimen as little as possible and wearing protective equipment, including gloves. When selecting the piece of the specimen for aDNA sampling, one should preferably select an area that is below the surface of the specimen. This will help avoid contamination from the soil and previous handling. Additionally, one should select an area away from any lesions or breaks that might admit foreign DNA (Cooper, 1994; Hagelberg, 1991; Hagelberg, 1994, Herrmann & Hummel, 1994; Lawlor, 1994; Zierdt et al., 1996).

It is ideal to wear all protective equipment at all times when working with specimens intended for aDNA sampling (Yang & Watt, 2004). However, this is not always possible. Specimens are often procured in the field without equipment designed to prevent contamination, particularly if the original intent of the study was not DNA analysis. Additionally, specimens gathered before the advent of the aDNA field were certainly not handled with the intent of analyzing their DNA, particularly in the case of museum specimens (Cooper, 1994). Yet,

because these specimens often yield valuable information, they are often used in aDNA studies. At the very least, once a specimen has been identified as a source of aDNA, precautions should be taken not to add to the contamination already present. These precautions include using disposable gloves (preferably two sets), face masks, hair covers, and sterile suits when working with ancient materials (Chou et al., 1992; Handt et al., 1994; Stoneking, 1995; Zierdt et al., 1996; Austin et al., 1997; Yang & Watt, 2004; Römpler et al., 2006). All equipment used needs to be sterilized and preferably disposable (Handt et al., 1994; Stoneking, 1995; Austin et al., 1997; Palmirotta et al., 1997;Yang & Watt, 2004; Römpler et al., 2006;). Another crucial aspect of contamination control is ridding the specimen of previous contaminants. This is already detailed above.

Ideally the laboratory used for an ancient DNA analysis should not be used for modern DNA samples. All the surfaces of the lab need to be sterilized with bleach and UV radiation (Handt et al., 1994; Stoneking, 1995; Austin et al., 1997; Palmirotta et al., 1997; Yang & Watt, 2004; Römpler et al., 2006). The equipment and chemicals should not be opened until they are ready to be used and should be sterile, if possible. (A concern that has been raised is the use of liquid nitrogen. It is common to use liquid nitrogen to freeze samples and keep them at an appropriately cold temperature. However, liquid nitrogen is not sterile and care should be taken to separate the sample from the actual liquid nitrogen (Fountain et al., 1997).)

As DNA research has become more routine, several companies provide kits and materials for DNA extraction. Kits are often used in modern DNA research and are being more frequently used in ancient DNA research. The advantage of using prepared kits and supplies is that contamination from the lab becomes less likely. However, sometimes there are trace amounts of contaminants on the ordered kits and supplies. Care needs to be taken to ensure that

the supplies are free of contaminants including the usual standard method of UV radiation (Hummel, 2003).

Once all precautions have been taken to assure the researcher that the samples are contamination free, one need still implement controls to verify that this is the case experimentally. These controls come in three forms: positive controls, negative controls, and reproducibility. Positive controls insure that the experiment was performed correctly and negative controls monitor for contamination (Hummel, 2003). In one negative control, a blank PCR is run alongside the aDNA sample. If anything is produced from the blank sample, then the aDNA sample is also suspect for contamination. Another good negative control is to include samples from soil or fauna that were taken from the same site as the sample under study. When these samples are run, one should verify that the sample under study and the negative controls are distinct (Hagelberg, 1994; Handt et al. 1994; Austin et al., 1997).

While positive controls verify that the PCR was done correctly (and are often used in modern DNA test), many aDNA researchers recommend against their use because they may contaminate the aDNA sample accidentally. The recourse for aDNA researchers is to reproduce the results in a separate laboratory (Handt et al., 1994; Cooper et al., 2000).

If all the above protocols are followed then it is likely that the results from the analysis are authentic. However, there are still a few reasons that the results may be suspect or unverifiable. The PCR procedure may produce nucleotide substitutions (Hansen et al., 2001; Pusch & Bachmann, 2004; Gilbert et al., 2006; Olivieri et al., 2010).To verify that some of the nucleotides are not PCR substation errors, one should to amplify several sequences from multiple clones (Bower et al., 2005; Spencer & Howe, 2010).

If the sequence produced from PCR is unduly long or if the resulting DNA does not fit expected parameters, then the results should be suspect (Handt et al., 1994; Austin et al., 1997; Cooper et al., 2000). Ancient DNA sequences are typically short. If a long sequence is produced, then the experiment should be redone. In addition, the researcher will want to carefully examine the preservation conditions. On occasion, some DNA is very well preserved and might justify a longer sequence. If the sequence does not fit expected parameters (i.e. if the quagga DNA had resembled an elephant more than a horse or if one found a novel gene that did not fit the population under study) then the results also need reexamined. There is not always a good recourse for these types of results. They should be sent to another lab to see if they can be reproduced. If they can be, this lends the results credibility, but not certainty.

Finally, there are three other difficulties that one can encounter when verifying authenticity. These difficulties are miscoding lesions, lack of appropriate statistical models, and indistinct results. PCR is a very sensitive process. Once one has run a successful PCR with no contaminants identified, there is still reason to doubt the PCR product. One reason for this is that the PCR process can occasionally cause single base substitutions (Hansen et al., 2001; Gilbert et al., 2007). Another reason to be wary of the result is that ancient DNA itself has been shown to be the cause of base substitutions in the PCR process (Pusch & Bachmann, 2004; see also: Binladen et al., 2006 for further discussion on base substitutions).

Because many specimens are so old and the species may be extinct, there are often no good statistical parameters in place to determine if the results are statistically significant. This may not allow one to determine if the base substitution is to be expected or if it reveals something genetically distinct (or if it is a result of the above mentioned problem). Sometimes the significance must then be surmised by logic and the experience of the researcher.

The last difficulty is if one cannot distinguish authentic results from inauthentic ones. This is sometimes the case when the sequence processed is from a very stabile region or codes for a very common gene that has high frequency. One of the reasons that we know many of the Neanderthal mtDNA results are authentic is that they differ from the human pattern (Stoneking, 1995; Höss, 2000; Hummel, 2003). If the Neanderthal specimens were found to have the same mitochondrial genome as modern humans, one could not be sure that the results were not a matter of contamination.

Chapter Two: History & Applications

The ancient DNA world is not flat. When one reaches what appears to be the edge of the field of ancient DNA (1984), one does not fall off into a world of dragons and sea monsters, but rather revolves into the land of genetics and molecular biology. Even as the field of ancient DNA suffers its own trials and frustrations—problems with contamination, lack of DNA—it remains closely connected with the fields that preceded it. In addition, ancient DNA is increasingly not only a field of its own, but a tool that contributes to many other areas of research. As the field continues to technically improve, it increases its ability to answer a myriad of questions and addresses a plethora of problems. Ancient DNA is a field full of prospects dealing with some problems. This chapter details a brief history of aDNA before delving into the various ways that it has been employed in physical anthropology.

A Brief History & O'Rourke et al.

As almost every review on the uses of ancient DNA will note, the breakthrough for using ancient DNA was in 1984 when Higuchi et al. sequenced DNA from the quagga. The quagga went extinct in 1883. The individual being studied died 140 years before this study was performed. This paper provided an important discovery: it showed that procuring ancient DNA was possible. As this was an amazing discovery, a good deal of the paper was devoted to explaining how the feat was accomplished. After explaining the very difficult process of extracting the DNA, the authors discuss what they discovered from the quagga's mitochondrial DNA—the first application of ancient DNA. The authors discovered that the quagga's mitochondrial DNA was different than, but related to the zebra's. They also calculated that the common ancestor for the quagga and the zebra lived about three to four million years ago

(Higuchi et al., 1984). In 1985 Pääbo extracted DNA from an Egyptian mummy. While this paper was a discourse on ancient DNA preservation and extraction, it opened the window for the use of ancient DNA in anthropology (Pääbo, 1985a&b). Pääbo did not mention any applications for ancient DNA in 1985. Soon, however, ancient DNA technique had been somewhat mastered and a variety of applications beyond those first mentioned by Higuchi et al. were realized.

For the remainder of the 1980s, aDNA researchers were concerned with finding genetic relatedness and grouping haplotypes. By the early 1990s, researchers had grouped and studied mtDNA groupings from around the world as well as the Neanderthal mitochondrial genome (Horai et al., 1991; Torroni et al., 1993; Krings et al., 1997; Krings et al., 1999; Kolman & Tuross, 2000; Hofreiter et al., 2001). This research led to a wealth of information on global genetic diversity. It also allowed for DNA studies on ancient humans to be matched to a modern population. The 1990s saw many other developments in aDNA studies as well. In 1989 and 1991Horai et al. and Hagelberg et al. published their work concerning aDNA studies on hard tissues (Horai et al., 1989; Hagelberg et al., 1991). The ability to analyze hard tissues opened the door for a variety of studies on archaeological material that was not previously possible (Horai et al., 1991). Additionally, aDNA PCR techniques were improving rapidly. These new techniques ensured some DNA product that researchers could be reasonably sure was authentic.

In 1997 Audic and Béraurd –Columb announced that the current challenges for aDNA were validity and authenticity. They suggested that once authenticity was ascertained, aDNA could answer questions about ancient human populations, agriculture, and infectious disease. Wayne et al. added an additional list of possible applications in 1999. Their paper suggested that aDNA could also be used to answer questions on migration, admixture, and the tempo and mode of mitochondrial mutations.

In 2000, O'Rourke et al. wrote a review of ancient DNA in physical anthropology [8]. The O'Rourke et al. paper, *aDNA and Physical Anthropology*, is divided into two basic sections. The first section looks at techniques and technical aspects of ancient DNA studies and addresses the special concerns that are necessarily present when dealing with human DNA—contamination and authenticity. This section details how DNA degrades over time, different extraction methods, sources of DNA, and amplification methods.

The second section looks at applications for aDNA in physical anthropology. O'Rourke et al. looks at applications by region as opposed to topic, but the topics under the regions include Neanderthal studies, the Tyrolean Ice Man, Amerindian haplogroups, Polynesian origins, and Japanese lineage groupings. Additionally, O'Rourke et al. look at the use of aDNA in paleopathology and the use for nuclear DNA-- the most important aspect of which was sexing skeletal material. O'Rourke et al. concludes with future prospects. The authors believed that greater sample sizes as well as further utilization of museum specimens will yield more information on both humans and other primates.

aDNA and Physical Anthropology looked at many important aspects of ancient DNA within anthropology. Its applications in particular highlighted what aDNA had already contributed to anthropology with specific short case examples that allowed O'Rourke et al. to delve more deeply into the technical and theoretic aspects of aDNA analysis.

[8] Although this review is one of the best, there have been a few other reviews on ancient DNA—some within anthropology and some from other disciplines. For some other interesting reviews see: Rogan & Salvo, 1990; O'Rourke et al., 1996; Pääbo et al., 2004; Gilbert et al., 2005; Mulligan, 2006.

aDNA Applications

From 1984 to the present, aDNA studies have ranged from acquiring small amounts of mitochondrial DNA to determine phylogenies to sequencing the whole mitochondrial genome to a new field of paleogenomics that can study whole genomes and dynamics of extinct species (Hofreiter, 2008, Willerslev & Cooper, 2005). This expansion of research techniques and improved ways to acquire uncontaminated DNA has broadened the aDNA field and its applications. Many of these applications have fallen into the realm of physical anthropology and have broadened and complicated our understanding of the primate past.

There are a few different ways to group aDNA applications in physical anthropology. O'Rourke et al. 2000 grouped them by region of application (i.e. Neanderthals originated in Europe). This works well when one is addressing individual cases (Feldhofer). However, when one is addressing multiple studies from various regions, it is far simpler to cover them by application themes. There remain a couple difficulties with this approach. A thematic view does not differentiate the very real technical and genetic differences between working with mitochondrial and nuclear DNA. This paper seeks to resolve this by indicating, in each case, what DNA is used in the research. The other intricacy is that the research on many specimens falls into multiple thematic categories. This is unavoidable. This paper has attempted to divide the research into the best categories possible, realizing that the categories are not always a perfect fit. The applications for ancient DNA research include the following: studies on hominid evolution and microevolution, human movement and migration, human ancestors, sex determination, agriculture and domestication, disease, kinship and social structure, and primatology and primate conservation.

Phylogenetic Relationships & Human Evolution

The first studies done on human phylogenetic relationships and evolution were conducted using modern DNA. Allelic frequencies were collected from diverse population groups and then fit into population and demographic models. Statistical tests were done to determine human divergence, the rates of mutation, and how closely related different populations were to one another (Jorde, 1985; Cyran & Kimmel, 2010). Modern DNA was very informative about the past. It was and is a very useful tool to determine how closely related different populations were to one another and thus answered questions about population histories and migration. Combined with paleoanthropology, DNA has told us a lot about when and how we evolved. However, many of the conclusions about evolution and relatedness were based on assumptions that were favored by varying degrees by different researchers and statisticians. That is to say, that the results differed by which model was used (i.e. Wright-Fisher model) and which statistic accompanied the model (i.e. Chi squared, least squares regression).

Utilizing ancient DNA has helped us better understand both phylogenetics and evolution. While ancient nuclear DNA is still difficult to obtain in sufficient quantities, ancient mtDNA has helped us better understand mitochondrial rates of change, population ancestries, and—as a consequence of our adjusted timelines and an influx of ancient archaic DNA—our evolution (Lambert et al., 2002; Pennisi, 2002; Cyran & Kimmel, 2010). This data has been used for a variety of interesting studies including determining "mitochondrial Eve" (Seager, 1999; Cyran & Kimmel, 2010) and microevolution (Williams et al., 2002; Haak et al., 2005; Chan et al., 2006; Manica et al., 2007; Ermini et al., 2008; Melchior et al., 2008; Crubézy et al., 2010; Melchoir et al., 2010)[9].

[9] This paper has included studies of haplogroups, mtDNA mutations, and human bottlenecks as "microevolution."

Using ancient mtDNa as well as ancient Y-chromosome, X-chromosome, and nuclear DNA has provided a wealth of information. It has given us the ability to look at genetic diversity globally and by population, determining archaic human and modern human genetic admixture and looking at human origins (particularly interesting articles include: Pääbo et al., 1988; Handt et al., 1994; Merriwether et al., 1994; Kittles et al., 1999; Balter, 2008; Gibbons, 2009; and Kim et al., 2011) . Ancient DNA has helped fit discovered remains with their appropriate modern lineage which can help in political debates of ownership as well as help determine if finds are genuine (Melchior et al., 2008). Using aDNA has also opened up new areas of debate on many of these topics. Looking at Native American haplogroup studies is an excellent way to demonstrate the use of aDNA in phylogenetic studies.

It was not long after Higuchi discovered that aDNA could be procured than the technique was applied to Native American aDNA. The first study merely ascertained that one could retrieve DNA from archaeological Native Americans (Shearin et al., 1989). Once researchers were convinced that they could obtain viable aDNA, research focused on looking at DNA insertions, deletions, and substitutions to determine if there were (as previously thought) four founding haplogroups for the Native Americans, what that indicated about when the Native Americans arrived, and how closely they were related to each other.

Looking at the control region (D-loop) of mitochondrial DNA in archaeological samples indicates that there are five or more distinct haplogroups among the Amerindians. Considering it has been calculated that there is only one transition in the control region of the human mitochondrial genome, this is a substantial amount of mitochondrial diversity. Because genetic diversity is often lost when a small sub-set of a population splits off from a larger population,

this likely either demonstrates that the founding population was genetically diverse[10] or that there were separate waves of founding populations (Forster et al., 1996).

There are, however, difficulties when using aDNA to assign archaeological material to a population. It is relatively easy to assign a specimen that has all the diagnostic traits of any given haplogroup. There are currently five recognized Amerindean haplogroups: A,B,C,D, and X. Four of the haplogroups (A,C,D, and X) are identified by restriction sites and one haplogroup (B) is identified by a nine basepair deletion. However, each population is comprised of a mixture or a different frequency of the different haplogroups. Additionally, the results may be skewed by a PCR artifact or "jumping" PCR. This may result in a substitution that matches a haplogroup but is not genuine. European admixture should also be viewed with suspicion. While it is possible that the specimen under study has European mtDNA, these specimens must be discarded because the DNA is more likely contaminating DNA from the researcher than actual DNA from the specimen (Kolman & Tuross, 2000). Therefore, while DNA can certainly tell us a lot about Native American ancestry, it also opens questions about contamination and models or mtDNA haplogroup frequencies within a population. In the future as more contamination issues are resolved, aDNA is sure to tell us a lot more about Amerindian populations and their history.

Movement & Migration

Almost the next thought after determining genetic relationships between populations or over time, is determining what the relationship means for human movement or migration. Human movement and migration has been studied for a variety of times and areas including looking at Out-of-Africa modeling (see: Wolpoff et al., 2000; Adcock et al., 2001; Liu et al.,

[10] The founding population likely was fairly diverse. A Study by Horai et al. (1991) demonstrates that the founding population was Asian in origin. This put their mitochondrial diversity as less than that of African origin, but more than any other population group that split off after Asians did.

2006; Forster and Matsumura, 2005; Gunz et al., 2009; and Armitage et al., 2011 for various models) and the colonization of and movement around Europe (see: Balter, 2005; Larsen et al., 2007; Sampietro et al., 2007; Balter, 2009; Bramanti et al., 2009; Endicott et al., 2009; Helgason et al., 2009;and Haak et al., 2010, for newer studies), Asia (see: Ballinger, 1992; Lalueza-Fox et al., 2004; Xie et al., 2007; Zhang et al., 2007; Shi et al., 2008; Jin et al., 2009; Derenko et al., 2010), Australia (Adcock et al., 2001; Cooper et al., 2001), Polynesia (see chapter three), and the new world (Gibbons, 1993; Smith et al., 1999; Balter, 2008;).

The Numic expansion has been well studied in North America. Linguistic and archaeological evidence have suggested a Numic expansion out of the Great Basin area into the Northeastern part of the country. DNA evidence showed that there were indeed different frequencies of the different haplogroups among the different Amerindian populations. However, this did not demonstrate that there was a Numic expansion: only that these were separate populations separated by time and space. However, looking at the different haplogroups diachronically provided a clearer vignette.

The current Algonquian speakers have high frequencies of haplogroups A,C, and X, but low frequencies of haplogroups B and D. This frequency is not represented in the archaeological specimens. The ancient specimens actually exhibit not only different frequencies of the major haplogroups, but also more genetic variation within the major haplogroups. The pattern changes over time. The populations of older specimens show different frequencies of the haplogroups and plenty of mitochondrial diversity within the haplogroups. As one looks at younger archaeological specimens, the frequencies look more like those seen in current Algonquian speakers and there is less diversity within the haplogroups. Using these aDNA studies, it can be concluded that there

was a Numic expansion that resulted in incomplete replacement of the populations into which the Numics expanded (Kaestle & Smith, 2001; Shook & Smith, 2008).

Human Ancestors

Most of the aDNA research that has been done on human ancestors has been done on Neanderthals (see chapter four). This is both because people find Neanderthals fascinating and because, after a few discredited studies, most researchers have determined that ancient DNA is only preserved for approximately 100,000 years (Mitchell et al., 2005) and many other hominin species fall outside that range. One of the difficulties researchers have had working on Cro-Magnon DNA is that it too closely resembles modern human DNA (Sampietro et al., 2007). This has made it very difficult to rule out contamination as the source of DNA in Cro-Magnon samples. However, in 2008, a study was performed on a Cro-Magnon individual, Paglicci 23, where the researchers were able to procure DNA from everyone who had ever handled the specimen. Paglicci's DNA did not match any of the handlers' DNA. While no one can be certain that the sample was not contaminated by an unknown source, these rigorous contamination controls lend credibility to the results. The results show that Paglicci 23's DNA is a common European mitochondrial group. This would indicate that there is a continual mitochondrial lineage from Cro-Magnon to modern Europeans (Caramelli et al., 2008).

An interesting study came out in 2010 that succeeded in complicating our known hominin family tree and possibly hominin dispersal theories as well. Some phalanges were found in Siberia that turned out to have mtDNA that differed substantially from both modern humans and Neanderthals. This would indicate that there was another hominin living in Siberia about 40,000 years ago. The unknown hominin's mtDNA differs from both modern human and

Neanderthal mtDNA; however, they lived in the same regions during the same time period (Krause et al., 2010). Interestingly, while Europeans do not share any DNA with this hominin, Melanesians share some of its DNA (Reich et al., 2010).

Sex Determination

One development that has been instrumental to answering anthropological questions on an individual and kinship level has been the ability to accurately assess the molecular sex of a skeleton. While osteologists can often determine the sex of an adult skeleton (Bass, 1971; White, 1991; Palmirotta et al., 1997), sub-adult skeletons and ambiguous adult skeletons cannot be sexed using traditional osteological methods[11] (Stone et al., 1996; Palmirotta et al., 1997). Molecular sex determination allows anthropologists to determine individual sub-adult sexes, revealing clues about demographics and social structure as well as social values and norms (Cherfas, 1991; Lassen et al., 2000).

Molecular sex determination using ancient DNA is not as simple as it would seem. Naturally, one needs to use nuclear DNA to determine sex. However, while mitochondrial DNA is present in many copies, nuclear DNA tends to provide only one copy. Additionally, unlike modern DNA where the Y chromosome is easy to identify, the degraded nature of aDNA means that the Y chromosome is not found whole. This problem was avoided by looking specifically at the amelogenin gene. Both the X and Y chromosomes contain the amelogenin, but the sequence differs in a 112bp fragment that can allow researchers to distinguish between individuals with both an X and Y chromosome and those with only the X chromosome. This technique is efficient and reliably determines sex in ancient samples with degraded DNA (Hummel, 1991; Stone et al., 1996; Cipollaro et al., 1998).

[11] It is also an excellent technique to use when the remains are too fragmentary to diagnose sex osteologically (Matherson & Loy, 2001).

Using ancient DNA researchers are able to determine the sex of infanticide victims. Knowing the sex of the victims and the ratio of the sexes can help explain the reasons behind the infanticide by looking at historical norms and analyzing sex ratios. It can also obfuscate them. In Roman times, female babies were often killed in preference to males. In a site in Ashkelon, Israel, a bathhouse was found that contained about one hundred infanticide victims. When they were sexed, however, more males were killed than females. This confronts the notion that males were preferentially saved. While this seemingly complicates the scenario, the researchers determined that the site may also be a brothel. If it was indeed a brothel, then perhaps although males are usually preferred, females were saved in this instance specifically for the brothel (Smith & Kahila, 1992; Faerman & Bar-Gal, 1998).

Agriculture and Domestication

Agriculture and animal and plant domestication are fascinating cases of aDNA studies in physical anthropology. While most of the other applications to anthropology involve human DNA, applications in the above area often use plant and animal DNA.

Archaeology was one of the main impetuses for aDNA plant studies. This is for a couple of reasons. First, archaeologists have long been interested in agriculture and plant domestication. Second, the genetic tools for domesticated plants were already at hand. Because the genomes of the domesticated plants are known, these sequences can be used as primers to retrieve aDNA sequences (Gugerli et al., 2005). Archaeologists have discerned quite a lot about agriculture by examining the remains of plants at archaeological sites. They have been able to look at morphological differences in domesticated plants to determine the what, when, and why of plant domestication (Balter, 2007). Ancient DNA studies of plant remains allow us to take these

studies further and learn more about plant domestication and the start of agriculture. Researchers have looked at plant DNA from archaeological sites and been able to determine the evolution of agricultural crops, their genetic diversity, how long the crops have been around, and what wild plants the domesticated crops came from (Goloubinoff et al., 1993; Brown et al., 1994; Doebley, 2006).

Ancient DNA can tell us many things about animal domestication. It provides information on when animals were domesticated, where they were first domesticated and where their wild lineages arose (Bar-Gal et al., 2002; Beja-Pereira et al., 2006). One of the major finds that aDNA has contributed to the study of animal domestication, is that it was a very complicated event that arose many more times than is evident from the archaeological record (Leonard et al., 2002; Larson et al., 2007; Larson et al., 2010; Speller et al., 2010).

Studies on pigs have shown that the Chinese Asian pig has a consistent mitochondrial link to ancient wild pigs in the region and that the rest of Asia shows a similar mitochondrial heritage with genetic admixture from local boars in the region (Larson et al., 2010). Studies on European pigs show a more complicated history. The modern European pigs show no ancestry to the Near Eastern pigs that were presumed to have migrated (with humans) to Europe. However, analysis of archaeological pig remains show that there were Near Eastern pigs in Europe. On closer examination it appears that Near Eastern pigs and European pigs were both domesticated (and that an Italian wild boar was domesticated separately from the other two pig lineages), but it is still undetermined whether the European pigs were domesticated before the Near Eastern pigs arrived or as consequence of their arrival. What is clear is that the European lineages were preferred and that those lineages replaced the Near Eastern lineages in Europe (Larson et al., 2007).

Nutrition

Although deciphering plant and animal domestication goes a long way to understanding what humans were eating at a particular site, one does not always find remains paired with domesticated flora and fauna. Sometimes a direct assessment of diet is more desirable. One can find food in the digestive tract if the remains are particularly well preserved (Rollo et al., 2002). Alternatively, one can utilize coprolites to analyze diet (Hofreiter et al., 2000; Poinar et al., 2001). Because one does not know what food was eaten, in order to determine diet from coprolites or pre-coprolites, one needs to use several different generalized primers. To determine plant matter, a primer used to capture all chloroplast DNA is used. After several amplifications with multiple clones, the sequences are matched up with the DNA of known plant species. If the sequences retrieved from the specimen differ by two or fewer nucleotides from a given plant species, that species can be assumed to have been eaten. The nucleotide substitutions can result from PCR problems or species variation, and can thus be assumed to be an authentic result (Hofreiter et al., 2000).

To determine what, if any, animal species was consumed, a general primer needs to be prepared that can cover a range of species that could have been eaten. While it would be fascinating to test for cannibalism, the DNA that is actually extracted from such an analysis would more likely be the individual from whom the coprolite originated. One can usually start by looking at genera that were present in the environment that the intestinal tract or coprolite was discovered. From there, one can determine the make-up of floral and faunal species eaten near the time of expulsion of the coprolite or shortly before death in the case of the intestinal tract (Poinar et al., 2001; Rollo et al., 2002).

This type of analysis was performed on Ötzi, the Tyrolean Iceman. Because Ötzi was naturally mummified and frozen, his intestinal tract was still intact. The contents of Ötzi's digestional system had already been morphologically and isotopically analyzed, but this led to confusing information. The isotopes suggested that Ötze was a vegetarian. aDNA researchers were able to look at Ötzi's last two meals by looking at the contents of the colon and the area of the intestines near the ilium. They sequenced DNA using primers to target mammal mitochondrial DNA as well as plant chloroplast DNA. They discovered that Ötzi's last meal was red deer and possibly cereal. The meal before that was ibex and cereal (Rollo et al., 2002).

Disease

Disease is prevalent today and has been prevalent in our evolutionary past. Ancient DNA studies of disease offer two rewards. The first lagniappe is the ability to look at how various diseases evolved which lends us clues on how to prevent and treat them currently (Blerkom, 2003; Minogue, 2010). The other interesting facet of aDNA studies on infectious diseases is to determine certain aspects of our past. Utilizing aDNA, researchers have been able to address questions about the emergence of certain diseases, how humans (and their ancestors) and diseases have co-evolved, how hominins have migrated with disease, and determine what diseases were represented by non-specific skeletal lesions (Salo et al., 1994; Spiegelman et al., 1994; Filon et al., 1995; Fricker et al., 1997; Nerlich et al., 1997; Braun et al., 1998; Drancourt et al., 1998; Yngvadottir, 2007; Haensch et al., 2010; Suzuki et al., 2010). Through an association with other lines of evidence, looking at ancient diseases can also tell us about a past population's nutritional status, hygiene, and population density by looking at which diseases spread and what mechanisms those pathogens used (Baron et al., 1996).

One of the infectious diseases that has been studied intently has been tuberculosis. Tuberculosis can leave skeletal and soft tissue evidence, but the markers are non-specific and can be caused by other diseases as well. However, since the discovery that pathogenic aDNA can be detected in soft tissue, bone, and calcified remains, researchers have been able to determine if an individual with suspect lesions or other indicators, did indeed have tuberculosis. Researchers have also been able to ascertain that TB can be detected even in remains that do not exhibit physical manifestations of the disease (Salo et al., 1994; Baron et al., 1996; Braun et al., 1998; Donaghue et al., 1998). In addition to finding ancient individual cases of tuberculosis, researchers have determined that tuberculosis was present in the New World before the arrival of Columbus and other Europeans (Salo et al., 1994; Braun et al., 1998).

Kinship & Social Structure

Kinship and social structure analysis fall in between the level of individual and population analyses. Studies looking at kinship and social structure may make use of both nuclear and mitochondrial DNA. Using aDNA can broaden our understanding of a particular archaeological site. Specifically, it can help researchers determine if the site contains a single genetically linked population or if the site has been inhabited multiple times by different populations (Hauswirth et al., 1994; Ricout et al., 2006). It can also show if an archaeological population is genetically related to people still living in the region or where that population originated from (Oster et al., 2010).

On a more specific level, aDNA can illuminate family relationships at a particular site. Such is the case at two Yakut burial sites[12]. Here researchers found five skeletons at two different sites. The first site contained three male skeletons. The researchers were able to

[12] See also: Keysert-Tracqui et al., 2003, for another interesting case or paleo-family relationships.

determine that two of the skeletons were father and son. They rated it at sixty-eight percent likely that the third skeleton was a first cousin to one of the other two skeletons. The other site contained two adult female skeletons. Analysis at this site—both autosomal and mitochondrial—could find no relationship between the two female skeletons. They were able to use this evidence and other archaeological evidence to deduce that the Yakut buried at the two sites practiced a patrilineal burial system and that females of a certain clan were likely not related to one another (Ricaut et al., 2006).

Primatology & Primate Conservation

Much like with disease, ancient DNA applications for primates have both informational and functional applications. aDNA is useful for identifying unknown specimens both in the museum and from specific sites (Bailey et al., 1999; Xing et al., 2007). This information can tell us not only what species the specimen is, but can also reveal information about past association or proximity to people, as in the case of a Barbary Macaque found in Pompeii (Bailey et al., 1999).

The other main intellectual concern with looking at primate aDNA is reconstructing phylogenies (Caccone & Powell, 1989; Miyamoto & Goodman, 1990; Clarke & Whyte, 2003; Xing et al., 2007). While DNA from extant and living primates can tell us a lot about the primate phylogenetic tree, it still leaves some relationships between different species nebulous.

Ancient DNA can also be used in primate conservation. aDNA can indicate evolutionary diversity within a species, which can help conservationists assess its current variability. It can also determine past habitats. Knowing the past habitat of a species can yield clues as to if it is

suited to its current home (or just pushed there from necessity) and tell those in charge of repatriation where the species might thrive (Cooper et al., 1996; Hofkin et al., 2003).

Summation

Physical anthropology has been able to use ancient DNA for a host of applications, as evinced above. However, the above vignettes provide only a limited understanding of how ancient DNA is being used in each of those themed applications. In order to give a more nuanced portrait of ancient DNA in physical anthropology, chapters three and four will look at more specific topics in which ancient DNA has been employed. Specifically, chapter three will look at the Neanderthal studies and all the information that has been gleaned from the Neanderthals that apply to the various topics in this chapter. Chapter four continues in the same vein to look at the different theories on Polynesian history and prehistory.

Part Two: Ancient DNA Case Studies

Chapter Three: Pacific Migrations

Migrations among the Pacific Ocean islands have been a topic of interest for linguists, archaeologists, and geneticists for over two centuries. All these approaches suggest that the peopling of the Pacific was a complicated process, particularly where Polynesia was concerned. Ancient DNA has only recently entered the discussion. It provides new insights and complicates the picture of what was happening in the Pacific, particularly in Polynesia. It also provides a good vignette of how ancient DNA may be used within the framework of an intricate and layered debate, as well as how it can be used in combination with other lines of reasoning to understand the Pacific migrations. This chapter is divided into four sections. The first section reviews what we know about Pacific migrations and the theories on which they are based. The second section reviews what we know from linguistics and the third section looks at the archaeological evidence for migration. Section four looks at what genetic studies, especially ancient DNA, have contributed to the ongoing discussion of the peopling of the Pacific.

Background

Map from Hagelberg,Electrophoresis 18: 1529-1533

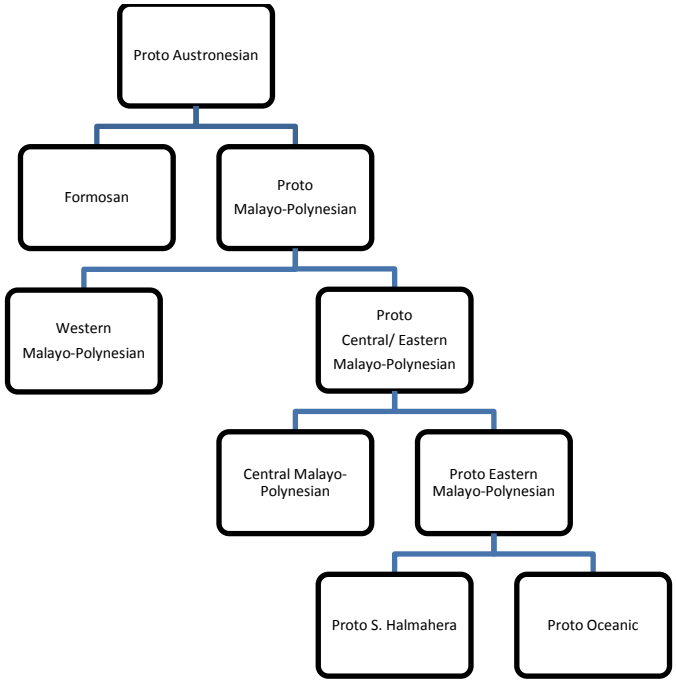
The Pacific Islands are divided into three geographic regions. Melanesia is north of Australia, south of Taiwan, and east of Indonesia. It is comprised of the Bismarck Archipelago in its northwest corner, the Solomon Islands, Vanuatu, the Fiji Islands, and New Caledonia on its southern extremity. The islands north of Melanesia are part of Micronesia. Micronesia's main Island complex is the Marshall Islands. To the east of both Melanesia and Micronesia is Polynesia. The Hawaiian Islands form the northern border of Polynesia, New Zealand is the southern border and the far eastern edge is Easter Island, or Rapa Nui. Polynesia is subdivided into Western Polynesia and Eastern Polynesia. Western Polynesia's main islands are Samoa and Tonga. Eastern Polynesia is the larger section of Polynesia and includes the Hawaiian Islands, New Zealand, Rapa Nui and the smaller islands in-between.

The different geographic regions nominally reflect differences in language and culture. The history and prehistory of the regions, however, is intertwined. Researchers have been trying to answer questions the peopling of the region. They want to know who was migrating in the region, where and in what direction they were migrating, where they came from, and if they made it all the way to South America (Kirch & Green, 1992; Lawler, 2010).

There are two basic theories on migration and expansion in the Pacific—Express Train and Slow Boat. Both are oversimplifications, but they are a good launching point. The Express Train model was popularized by Jared Diamond in 1988. This model suggests a rapid expansion into Polynesia. It entails a Taiwanese origin for Polynesians, links them directly to the Lapita culture, and suggests little or no admixture from Melanesia before the expansion into Polynesia (Diamond, 1988; see also: Bellwood & Dizon, 2008). The Slow Boat model (also called Entangled Bank or Pulse and Pause), suggests a much more complex migration. It still places the origin of dispersal in Southeast Asia, but points out that some evidence shows that Indonesia, not

Taiwan, is the origin of the Polynesians. This model not only allows for genetic admixture with Melanesians, but believes that it is the likely possibility. This is because in the Slow Boat model, the Polynesians move throughout Southeast Asia and Melanesia before gradually extending themselves into Polynesia. Along the way they came into contact with and shared genes with the local people (Oppenheimer & Richards, 2001; Gray et al., 2009).

Linguistics



Produced from from Ross, 2008, Past Human Migrations

Linguistics was the first field to take an interest in the Pacific Islands and their past (Pawley & Ross, 1993). Linguistics was actually interested in the history and spread of the Austronesian language family. Yet, as linguists delved into the history of Austronesian, they were able to uncover clues about the language family's origin and, by extension, they discovered quite a bit about the people who spoke it, their culture, and their migrations. When linguists

discuss Pacific migrations, what they are referencing is the spread of the Austronesian language family, and presumably its speakers, throughout the Pacific.

Austronesian is one of the world's largest language families (Hagelberg et al., 2008). Austronesian languages are currently spoken in Taiwan, Micronesia, Melanesia, and Polynesia. While these far flung areas all speak languages from the same language family, linguists were not initially clear on how the languages were related and where Proto-Austronesian—as the presumed ancestral language it termed—originated (Ross, 2008).

Linguistics calculates distance to a presumed most recent common ancestor—a proto language-- in much the same way as genetics accomplishes the task. Genetics looks at mutations over time and calibrates a 'molecular clock' using an out-group. Linguists look at phonemes, units of speech sound, and how they change over time (Atkinson & Gray, 2005; Blench et al., 2008). When linguists look at multiple modern languages and historic languages through texts, they are able to determine the directional change in phonemes over time. This process both helps reconstruct proto languages, and determines a time scale for those changes. The first step to reconstructing past languages is to establish what modern languages are related. Once it was ascertained that many of the Oceanic languages were related to each other, the next step was to figure out how the languages were related. Generally, languages that share many cognates and have similar sound constructions are closely related. The more similarities two languages contain, the more closely related they are (Dunn et al., 2005).

Formosan is the grouping of languages spoken by the Taiwanese aborigines and it is the group of languages most closely related to the ancestral Proto- Austronesian. There is still some discussion as to if Proto-Malayo-Polynesian stemmed directly from Proto-Austroneasian or if the Formosan languages gave rise to the languages of Melanesia and Polynesia (Ross, 2008). Most

lineages show the former. After Proto-Malayo-Polynesian splits off from its ancestral language, it subdivides many more times until one reaches the Proto-Oceanic language group. There are several Oceanic languages and they differ, but not all that much. While there is quite a bit of diversity in the Austronesian language family, the Oceanic languages are fairly homogenous (Pawley & Ross, 1993).

Linguistics, like genetics, tends to determine origin by diversity. That is, genetics looks to the place of the most genetic diversity of a species as its origin. One of the key reasons many anthropologists believe that Africa is the cradle of humanity is the diversity of Africans' DNA.Taiwan and the islands in its immediate vicinity is the area that contains the most diversity within the Austronesian language family. Hence, many linguists place Taiwan as the origin of Austronesian (Ross, 2008), though some believe Indonesia is a more likely origin (see: Capell, 1962). The Oceanic languages, by contrast, are not diverse. This shows a direction for the dispersal of the Austronesian languages—and the people who spoke them. Because Taiwan is the most diverse, linguists believe that the family originated there, then spread to the Melanesian region before heading into Western Polynesia. There seems to be a linguistic pause in Western Polynesia before a rapid language expansion into Eastern Polynesia (Pawley & Ross, 1993;Gray et al., 2009).

There is more linguistic evidence to suggest a sea-faring migration along this general route. This evidence comes in the form of common cognates between the various languages in the Pacific. Many of the words that are shared across this expanse are words concerning agriculture and canoe parts (Bellwood, 1991). Wet rice is commonly grown in Taiwan and was the first type of rice to be grown there. Most of the Austronesian languages have similar terms for wet rice, with the most archaic form of the term originating in Taiwan. This as well as

common plant terms, words for pig and dog, and fishing terms suggest a Taiwanese origin. Another group of terms that share similarities across many Austronesian languages are canoe parts and outrigger terms. These terms not only suggest a common language origin, but also a shared sea-faring culture. Interestingly, there are no outrigger terms in Formosan. Linguists do not know if the terms were lost or if they never existed. This could have bearing on the origins of the sea-faring culture as well as the language family (Pawley & Ross, 1993).

Archaeology

Linguistic reconstructions are an excellent tool for looking at migrations in the Pacific. However, they only go so far. As the proto-Austronesian languages were oral traditions, they left no written texts of their existence. Archaeology is a good next step in understanding Pacific migrations, as archaeologists are able to find material evidence of a past culture.

One of the first discoveries that archaeologists made was a distinctive type of red pottery with intricate designs. This pottery became associated with a prehistoric culture called the Lapita, and was known as their 'calling card.'The Lapita are also associated with pigs, chickens, and rats (Vilar et al., 2008). Archeologists first found evidence of the Lapita on the Bismarck Archipelago which they inhabited 32,000 BP (Pawley & Ross, 1993). Because Lapita pottery and occasional remains are found all the way into Samoa and in significant quantities in Vanuatu, archaeologists have assumed them to be the first people to colonize more remote Oceania (Pawley & Ross, 1993). Evidence for Lapita migrations also comes from radiocarbon dating of obsidian that shows it travelled long distances within and between archipelagos (Finney, 2007). Few human remains are found at Lapita archaeological sites. Most that have been found have been in Vanuatu (Manila, 2006). However, those that have been found have

been dated. Results of radiocarbon dating on Vanuatu show people associated with the Lapita culture there just before 2000 BP, thus representing the Late Lapita Period (Petchey et al., 2011).

Geographically, there is no trace of the Lapita east of Samoa. Their pottery disappears. There is also a substantial time gap between when the last Lapita sites are found and when Polynesian cultural sites appear. Up until recently, many archaeologists believed that the Polynesians are the direct descendants of the Lapita people with no genetic admixture (Lawler, 2010). They believed that the Polynesians continued east and migrated to Rapanui and possibly to South America (Lawler, 2010)[13]. A study using high-precision radiocarbon dating shows that the Society Islands were colonized by 1025-1120 CE. After about seventy to one hundred twenty-five more years, the remainder of the islands was colonized. This study shows a rapid migration and supports the Express Train model of Pacific colonization (Wilmshurt et al., 2011).

Despite the earlier belief that Polynesians descended directly from the Lapita, there is growing evidence among the archaeological community that the picture may be more complicated. First there was the growing recognition that the Lapita may not be a single culture, but a cultural complex containing multiple cultures (Szabo, 2008; Terrell, 2009). This has vast implications for migrations, including trade and different cultures migrating along different routes. The second reason that the picture seemed more complicated is that archaeologists working in the region began to suspect that some of the carbon dates were incorrect. They believed that part of the carbon deposits were not results of human activity, but of natural phenomenon (Lawler, 2010). This would indicate that there may not have been people there at the time the carbon dating shows. Additionally, carbon dates on bone have been shown to be unreliable until recently. Bone is a very difficult material to date because the dating can be

[13] Much of the evidence that Polynesians (or the Lapita) made it to South America is archaeological. Archaeologists dug up skulls that have morphological characteristics of both the Lapita and Polynesians (Lawler, 2010). Additionally, there is evidence from a chicken bone that seemed to come from a Polynesian chicken (Storey et al., 2010).

affected by the diet of the person before he or she died. Marine foods, in particular, can skew dating results. While dating bones is still challenging, isotope analysis can help determine diet and that can be used to help calibrate the radiocarbon dates. Currently, archaeologists are re-examining some of the old material and establishing more credible dates (Petchey et al., 2011; Wilmshurst et al., 2011).

Genetics & Ancient DNA

Earlier archaeological research tended to agree with linguistic evidence for a Austronesian-Lapita Express Train migration from Southeast Asia into Western Polynesia and then into Eastern Polynesia. However, as more archaeology is done, redone, and reanalyzed throughout the Pacific, it is becoming more likely that there was not a simple quick migration across the Pacific Ocean, but a complex set of migrations. Genetic and Ancient DNA studies have helped add to the complexity.

Mitochondrial DNA is usually used in migration studies. It is an excellent source of information because it is presumed to be selectively neutral and mutates rapidly compared to nuclear DNA. In Polynesia there is a common mitochondrial haplotype termed the 'Polynesian motif.' This haplotype is characterized by a nine base pair deletion in the control region (CoII/tRNALYS) compared with the human reference sequence (Anderson et al., 1981; Hagelberg & Clegg, 1993; see also: Andrews et al., 1999). In addition to the deletion, a true Polynesian motif mtDNA contains three nucleotide substitutions. There is a T to C substitution at position 16, 217, an A to G substitution at position 16,247, and a C to G substitution at position 16,261 (Hagelberg, 1994). The Polynesian motif is found in high frequencies in Polynesia, particularly as one moves east. In Easter Island, the ancient population samples almost hit fixation with this particular haplotype (Hagelberg et al., 2008; Vilar et al., 2008). Thus, while

DNA studies have not tended to lump prehistoric people into a single population—they started researching too late for that—they look at the Polynesian motif much in the same way that linguists view the spread of Austronesian and archaeologists look at the movement of the Lapita Cultural Complex: the Polynesian motif is a way for DNA researchers to track migration.

Modern genetic work has contributed substancially to our understanding migrations in the Pacific. Modern Polynesians show the most mitochondrial diversity closest to the western edge of Polynesia. As one looks at mtDNA moving east, there is less diversity and the frequency of the Polynesian motif increases. The 9bp deletion is present in all Austronesians today, but is not present in non-Austronesian populations (Merriwether et al., 1999). Running counter to this, Y chromosome analyses show clear Melanesian admixture throughout Polynesia (Ohashi et al., 2006)[14]. The mitochondrial DNA suggests the Express Train migration throughout the Pacific Ocean Islands. The Y-chromosome data, however, is consistent with the Slow Boat model. In both cases, the results suggest an East Asian origin for Pacific migrations (Lum & Cann, 1998; Loo et al., 2008).

As looking at Polynesian and Melanesia mitochondrial DNA and Y-chromosome segments was providing mixed results, geneticists tried using other means to discover the migration route eastward into the Pacific. Oppenheimer & Richards 2001looked at the Polynesian motif and tried to determine its origins. The nine base pair deletion is a fairly common phenomenon in Asia and occurs with some frequency throughout the region. However, it is the deletion as well as the three base substitutions that really constitute the Polynesian motif. Interestingly, the full motif is not seen in Mainland Asia or in Taiwan. It is not seen in its full form until Indonesia, which again, suggests a slower migration and a stop in Indonesia

[14] With a complicated migration history, it is almost expected that the results from different studies will differ, based on island sampled and the number of people in the study (Addison & Matisoo-Smith, 2010). However, in this case, the results could also reflect sex biased admixture, which is known to happen frequently in Polynesia (Cann, 2001; Deguilloux et al., 2011).

(Oppenheimer & Richards, 2001; see also: Hagelberg et al., 2008). Researchers looking at a thalassaemia mutation (alpha 3.7 III) noted that Polynesians have high frequencies of the mutation, which is odd for people living outside of malaria zones. The researchers discovered the same mutation in New Guinea, also suggesting a slow migration east (Addison & Matisoo-Smith, 2010).

While genetic studies have contributed greatly to our understanding of Pacific human migrations, they have limitations. In some cases, the genetic information may have been lost through genetic drift, and modern people are not truly representing the genetic variability of the past (Addison & Matisoo-Smith, 2010). A more specifically Pacific problem is that, in many cases, people of European descent have replaced or reduced the Polynesian population on some islands (Hagelberg et al., 1994).

In 1993 Hagelberg and Clegg first sequenced bone from a Polynesian site and discovered that it had the nine base pair deletion. As this was relatively early in aDNA studies, much of the focus was on the possibility of extracting DNA and the paper did not comment on implications for Polynesian origins. Hagelberg returned to do further analysis on Polynesian ancient mtDNA. Her initial results demonstrated that there was not much mitochondrial diversity in the ancient Polynesians and that they all carried the nine base pair deletion as well as the three nucleotide substitutions, which seemed to support the fast train hypothesis (Hagelberg et al., 1994). Later, Hagelberg was able to test both Polynesian and Lapita archaeological sites. She found that the Lapita bones lacked the Polynesian motif and did not contain the nine base pair deletion. This was an important find because it showed that the Polynesians were probably not direct descendents of the Lapita without admixture. It also demonstrated through looking at the

frequency and pattern of mitochondrial mutations at various sites, that there was probably a back migration west after the initial colonization of East Polynesia (Hagelberg, 1995, 2008).

Hagelberg's aDNA analysis added complexity to the history of Pacific migrations, but it did help clarify that there was a lot more going on than researchers initially realized. It also helped fill a void in the understanding of the DNA of the people who had lived there in the past. However, aDNA studies on human remains in the Pacific are difficult because the tropical environment is not conducive to DNA preservation, there are not many human remains, and because for many cultures residing on the islands, it is taboo to test the dead (Matisoo-Smith & Allen, 2001; Holden, 2004; Addison & Matisoo-Smith, 2010; Storey et al., 2010). This led aDNA researchers to look at other ways to find ancient DNA.

What the researchers found were rats, chickens, and lizards. Rats and chickens are considered commensal animals. That is, they reside with humans and are associated with their remains. Chickens are part of the Lapita Cultural Complex and rats (*Rattus exulans*) have been found to reside with people in Polynesia, possibly as food. Lizards are presumed to have also accompanied people on their migrations across the Pacific (Austin, 1999; Matisoo-Smith & Allen, 2001; Storey et al., 2010). The lizards DNA emphatically supported the Express Train hypothesis. There lizards are all closely genetically related and their DNA analysis relates them to Asian lizards with differentiating mitochondrial mutations increasing as they move east. Since there is not a lot of variation between the lizards and because their mitochondrial lineage arose first in Asia, it appears that the lizards, at least, migrated across the pacific very quickly (Austin, 1999).

Studies on rat aDNA have proven useful because there are many more rat remains than human remains. This is particularly true now that their remains are being more carefully

salvaged from archaeological sites and used for aDNA research (Matisoo-Smith & Allen, 2001). Researchers have discovered three haplogroups (I, II, and III) for *Rattus exulans*. The haplogroups are geographically divided. Haplogroup I is only found in Southeast Asia. Haplogroup II is found in both Southeast Asia and Near Oceana (which encompasses West Polynesia) and Halpogroup III is only found in East Polynesian. This demonstrates that there were probably two or more waves of migration that brought rats to the Pacific. It also shows that haplogroup III is not closely related to the Southeast Asian rats (Matisoo-Smith & Robins, 2004).

Chicken aDNA is a relatively new pursuit. What researchers have discovered is that the main chicken haplogroup associated with the Lapita Cultural Complex is haplogroup E. This haplogroup is not only associated with the Lapita, but also with their descendents. Modern chickens in India, China, and the Middle East are also haplogroup E. This haplogroup was found in archaeological sites on Hawaii, Samoa, Nive, Tongo, Easter Island, and Chile. This lends weight the theory that Polynesian culture did descend from the Lapita Cultural Complex as well as to the theory that the agricultural migration across the Pacific made it all the way to South America. However, it is still too early in chicken aDNA studies to truly understand the migratory route of the people who carried them (Storey et al., 2010).

Ancient DNA has added to our understanding of the complexity of the event and demonstrated that there is room—and a necessity—for new theories on Pacific migrations. It has shown at the very least that there have been multiple waves of migration and that the last uninhabited places on Earth were not colonized in one fast simple fell swoop, but that the people and their culture were complex and diverse, and that we do not yet know the full picture of what was happening in the Pacific.

Chapter Four: The Neanderthals

Neanderthals are an excellent case study for ancient DNA studies. To begin with, Neanderthals have a long history outside of the field of aDNA. This history includes archaeology and paleoanthropology that help us understand Neanderthals from multiple view points. Neanderthals also make an excellent case study because we already have a fair amount of knowledge about them and new knowledge is continuously forthcoming.

Neanderthal aDNA provides excellent examples for different techniques and technologies. They do a wonderful job of elucidating problems with contamination and authenticity. These issues affect our understanding of the results and theories that are generated from ancient DNA. Ancient DNA studies on Neanderthals also illustrate many of the applications detailed in Chapter Two. Naturally, any study on Neanderthals is also a study on human phylogeny. Neanderthals have told us a lot about our phylogenetic relationships and our evolution, as well as their own. As aDNA technology becomes more sophisticated and as we accrue more information about them, Neanderthals are helping researchers delve into areas such as sex determination, migration and movement, and kinship structure. There is also potential for Neanderthals to tell us about diseases that evolved in our hominin relatives.

This chapter looka at all the ancient DNA work utilizing Neanderthals to date. It reviews the evolution of the field, the hazards it has faced, and what new knowledge has been gleaned from Neanderthal DNA. To this end, this chapter is divided into three sections. The first section looks at a general background to Neanderthal studies by looking at the history of contact with Neanderthal fossils and archaeological sites—and what we have learned via those lines of evidence. The second section will follow a chronological course through the major Neanderthal

DNA finds, focusing on what we have learned and what the difficulties with the research were. The last section will look at what other information Neanderthal DNA can explain.

Background

The first Neanderthal fossil was discovered in 1856. The fossil was found in the Neander Valley and thus was given the name Neanderthal (Tattersall, 1990; Trinkaus & Shipman, 1993; Tattersall, 1995; Gamble, 1999; Klein, 2003). The original debate around the specimen centered on determining whether it was a pathological human or an ancient human. As more people looked at the specimen and more specimens were found, the debate slowly shifted and more questions began to arise. Eventually, there was a general acceptance that Neanderthals represented a separate hominid lineage, presumably a dead end that *Homo sapiens sapiens* out competed. In 1927, a *Science* supplement was published in which Hrdlička posited the notion that Neanderthals were not a failed side lineage to our own, but our direct ancestors and a "phase" in our development (Science supplement, 1927).

Using archaeological sites, fossils, and radiocarbon dating, archaeologists determined that the Neanderthals lived from 300,000 to 60,000 years ago (Tattersall, 1995). In some pockets of Europe, Neanderthals survived even later to as recently as 28,000 years ago (Finlayson et al., 2006; Delson & Havarti, 2006). This indicates that Neanderthals and Cro-Magnon's (who are presumed to be ancestral to modern humans) overlapped in time and space for 10,000 years or more. This means that Neanderthals may not have been only ancestral to modern humans, but contemporaneous for some time. That changed the framework of the phylogenetic question. The new questions concerned the time period in which modern humans and Neanderthals became separate species and if they interbred. This debate has continued until the present time; however,

it continually increases in complexity and nuance and has been a central issue of Neanderthal DNA studies.

Archaeology and paleoanthropology not only provided the first clues about Neanderthals—what they looked liked, how they are related taxonomically to modern humans, and how they lived—but they still continue to not only provide further insights, but also shape the research that is done in Neanderthal studies. Some of the questions that these fields have brought up along the way (in addition to those listed above) are: what kind of culture or cultures did they have, how did they adapt to their environments, did they have spoken language, and why did they eventually die off (Gamble, 1999)? We have only just started to answer these questions, but it is helpful to the understanding of Neanderthal DNA studies to review what is known from other lines of evidence.

Skeletal morphology based on several specimens indicates that Neanderthals were similar to modern humans, but clearly distinct in several ways. The most distinctive aspects of the Neanderthal are found in the skull. Neanderthals had long and low cranial vaults. They had occipital buns in the back of their skull and pronounced mid-facial prognathism in the front of their skulls. The lateral sides of their skulls tended to bow out. Additionally, Neanderthals possessed a brow ridge, lacked a chin, had a distinctive gap between their third molars and the vertical ramus, and they had a different configuration of the bony inner ear (Tattersall, 1999; Klein, 2003).

Neanderthals also had different body builds than modern humans. They are sometimes described as looking like modern people who live in cold climates. They are slightly shorter than modern humans and more robust. They had proportionately short limbs and large joint surfaces (Tattersall, 1999).

Neanderthals are known from over three hundred archaeological sites, indicated by either artifacts or processed animal bones (Klein, 2003). Sites and fossils have been found all over Western Europe and as far east as Uzbekistan (Krause et al., 2007). They ranged as far north as Wales and as far south as Gibraltar. Neanderthals are associated with Mousterian tools and technology at many of the sites. This indicates a certain intelligence for perceiving and shaping tools for a specific purpose. Archaeological sites are also sometimes found with temporary structures in place that appear to be hearths and, in one case, a hole where a tent stake would have been inserted in the ground. All these findings indicate that Neanderthals had culture. Fewer and more controversial findings have hinted at more complex cultural habits. These include evidence for burnt ochre, burial, and defleshing of bone[15] (Tattersall, 1999).

Neanderthal DNA

One of the first pieces of archaic *Homo* DNA was found in 1995. However, the DNA itself was not ancient. What was found is termed a nuclear 'fossil' of DNA. A nuclear DNA 'fossil' is a piece of mitochondrial DNA that has inserted itself in the nuclear genome. In this particular case, a part of the mitochondrial control region was found inserted into Chromosome Eleven of the modern human genome. While mitochondrial DNA evolves more rapidly than nuclear DNA, once it is inserted into the nuclear genome, it turns into a pseudo-gene. The pseudo-gene is expected to evolve at a much slower rate, if at all. This is because the pseudo gene loses its ability to replicate itself as a gene. The researchers looked at one hundred thirty-four people from populations around the world (including both African and non-African populations). The pseudo-gene was found in all the people tested. From that it may be deduced

[15] The defleshed bone has been argued as both evidence for ritualistic second burial or cannibalism (Tattersall, 1999). Either way, this is evidence for ritual behavior among the Neanderthals.

that the pseudo-gene was inserted into the human nuclear genome before the human species differentiated itself into its modern form. As such is the case, what this study has observed is in a very true sense a fossil—and a way to determine *Homo* mitochondrial history as well as a way to refine the *Homo* taxonomic tree (Zischler et al., 1995).

A couple years later, ancient DNA studies had progressed to the point that researchers were willing to take on the mitochondrial DNA of a real Neanderthal. By this point, popular and scientific opinion was largely in favor of the Out-of-Africa model of human evolution and migration and against the idea that modern human ancestors and Neanderthals mated. They believed that Neanderthals were an evolutionary dead-end (Kahn & Gibbons, 1997).

In 1997 Krings et al. managed to sequence the first piece of Neanderthal DNA—from the Neanderthal type specimen. By this time, concerns with contamination and authenticity had been voiced and heeded. The authors were very careful in their study and followed the contamination controls that have been detailed in Chapter One. Still, there were problems. The authors sequenced part of the hypervariable region 1 (HVR1) of the Feldhofer mtDNA out of the right humerus cortical bone. They found that twenty-two of their thirty clones contained seven nucleotide substitutions when compared to the human mitochondrial reference sequence (Anderson et al., 1981). Four more of the clones contained sequences that were close to the reference sequence and presumed to be contamination from the researchers. The remainder of the clones contained some variations that were closer to the Neanderthal sequences and were presumed to be PCR errors. The Neanderthal mtDNA proved to be very different from modern human DNA. When the researchers looked at the whole mitochondrial control region, they found that the Feldhofer individual had a total of twenty-seven differences when compared to the reference sequence; whereas, humans differ from each other at only a few points, if at al.

The Feldhofer sequence did not match any of the researchers' mitochondrial DNA. Just to be sure that it was authentic, the researchers also tested to be sure that it was not a nuclear insertion from a modern human. When it was determined that the DNA was not an insertion, the researchers could be reasonably sure that the sequence was authentic. For many reasons, it is fortuitous that the Feldhofer sequence differed so greatly from modern human DNA. This lends much credence to its authenticity. Had the Neanderthal sequence matched that of modern humans, it would be impossible to rule out contamination as the cause (Krings et al., 1997).

Sequencing the first Neanderthal mitochondrial DNA was a great success—and it opened the door for a great deal of debate. After analyzing the sequence, the authors determined that the most recent common ancestor (MRCA) between modern day humans and Neanderthals occurred four times as far back as the MRCA for all modern humans. This put the divergence date between modern humans and Neanderthals at around 550,000 years ago. Additionally, the authors saw no evidence that humans and Neanderthals shared mitochondrial DNA and that Neanderthals differed from modern humans about three times as much as modern humans inter-vary. The authors carefully stated (and many agreed) that the evidence seemed to support the Out-of-Africa model for human evolution (Khan & Gibbons, 1997; Krings et al., 1997; Ward & Stringer, 1997).

Not everyone agreed with Krings et al.'s analysis. While no one doubted the authenticity of the DNA, there was disagreement on the analysis, particularly where it concerned Out-of-Africa and human-Neanderthal genetic admixture. Nordborg answered with a cautionary article on the ability to incorrectly reject inbreeding. Nordberg determined that there was likely no "random" mating between humans and Neanderthals, but that the study was a small sample size, thus increasing the possibility of inaccurate assumptions on admixture (Nordborg, 1998). Others

argued that the mitochondrial DNA did not completely reject admixture. It was also argued that DNA could have been transferred through the male line (Y chromosome)[16]. The main argument was that Neanderthals could have interbred with the ancestors of modern humans and their mtDNA could have been lost through genetic drift (Manserscheid & Rogers, 1996; see also: Pääbo, 1999).

In 1999 Krings et al. sequenced the Hypervariable Region II (HVRII) of the type specimen. This gave more data on the Feldhofer Neanderthal and Neanderthal DNA in general, but Krings et al. came to the same basic conclusions and the Multiregionalists did as well (Krings et al., 1999). Then in 2000, Ovchinnikov et al. sequenced DNA from another Neanderthal specimen. This specimen came from the Mezmaiskaya Cave in the northern Caucus. The specimen was dated to approximately 29,000 years before present and thus, was one of the later surviving Neanderthals. Ovichinnikov et al. sequenced a 345 base pair (bp) segment of HVRI. Again, careful contamination control protocol was followed and the segment was deemed authentic.

When the Mezmaiskaya specimen was compared to the Feldhofer individual, it was found that the two Neanderthals shared nineteen differences in the mtDNA when compared with the reference sequence and that their mtDNA clustered together distinctly separately from modern humans, who formed a cohesive cluster of their own (Ovchinnikov et al., 2000). The Ochinnikov study produced more debate. Several people hailed it as an excellent study that has contributed to our understanding of human evolution (see: Krings et al., 2000; Höss, 2000) while others believed that nothing could be proven by the two Neanderthal's DNA (See Relethford, 2000, 2008; Wolpoff et al., 2004).

[16] Interestingly, while the idea that there would be only a one way transfer of mitochondrial DNA has been largely un-argued and ignored, it has been shown to happen in other species. In cases of wolf and coyote mating, the coyote mitochondrial lines show up in the wolf population, but wolf mitochondrial DNA does not appear in coyote populations. It is speculated that this is because of different breeding practices. The hybrids tend to mate with wolves, not coyotes (Pilgrim et al., 1998).

While the Multiregion/Out-of Africa debate continued, it was clear that new lines of evidence were needed to determine if Neanderthals had contributed to the modern human gene pool. The evidence came in two forms. Adcock et al. showed that an anatomically modern specimen (as determined morphology) showed evidence of a mitochondrial trait that is found only in modern Australians. Supporters of the multiregional theory argued that this showed a more complex system of archaic admixture in human ancestors (Adcock et al., 2001; Relethford, 2001). However, the Adcock study has been questioned for authenticity both because of contamination and because remains that old found in warm environments are not expected to still contain DNA (Cooper et al., 2001; Ovchinnikov et al., 2001).

Other approaches lent support to the Out-of-Africa model of human evolution. Two studies were done on early anatomically modern humans. One study claimed to be able to distinguish between Cro-Magnons and Neanderthals through DNA testing. The other study looked at two Cro-Magnons and confirmed that they fell within the range of moderns humans (as did the Lake Mungo specimen) while Neanderthals still maintained their own separate mtDNA cluster (Sholz et al., 2000; Caramelli et al., 2003). The first study came under scrutiny for soil contamination which would have certainly tainted all the samples (Geigl, 2001). The second study, however, did not come under scrutiny.

By 2005 eight different Neanderthal individuals had had at least part of their mitochondrial genomes sequenced (Lalueza-Fox et al., 2005). The results demonstrated that Neanderthals constantly clustered together away from modern humans. One study looked at two hominins that were argued to be “transitional” form between Neanderthals and modern humans based on their morphology-- Vindija and Mladeč. The results of the study failed to show any

admixture. The individual from Vindija clearly clustered with Neanderthals while the individual from Mladeč clustered with modern humans (Serre et al., 2004).

The influx of several different Neanderthal genomes showed that Neanderthals tended to have low mtDNA diversity (much like modern humans do), but there is no indication in their mtDNA that there was a population bottleneck prior to their extinction. From that, researchers were able to surmise that Neanderthals tended to live in small population groups that would not encourage much mtDNA diversity[17] (Serre et al., 2004; Lalueza-Fox et al., 2005). Because not much more could be determined with the Neanderthal samples at that time, many scientists involved with looking at Neanderthal population genetics turned to computer models and statistical simulations to learn more about Neanderthal population genetics and the possibility of Neanderthal admixture (Currat & Excoffier, 2004; Weaver & Roseman, 2005; Wall & Hammer, 2006; Zilhão, 2006; see also: Hodgson & Disotell, 2008; Herrera et al., 2009; Dodge, 2010; Endicott et al., 2010 for other models).

Then, in 2006, Svante Pääbo announced that he was going to sequence the Neanderthal nuclear genome (Holden, 2006). There were and are several problems with sequencing the genome. The first difficulty is that nuclear DNA is found in low-copy numbers and is therefore more susceptible to degradation and more difficult to retrieve viable sequences. For Neanderthals, this is especially problematic because many of the specimens are very old. The second difficulty is that the nuclear genome is very long and is very difficult to sequence using PCR. Therefore, both to be able to retrieve the very small amounts of degraded nuclear DNA left in the Neanderthal specimens and to tackle the shear length of the Neanderthal nuclear genome, new techniques and new technologies had to be created.

[17] Archeological evidence supports this (Tattersall, 1999).

The first technology to be created was the GS20 454 sequencing system. This system utilizes a clone library and thus circumvents bacterial cloning. (Bacterial cloning is a labor and DNA intensive process because the bacteria do not always correctly incorporate the DNA and there is a lot of waste in the process of producing viable DNA clones.) The double-stranded nuclear DNA is ligated to two different synthetic oglionucleotides. Then each successful DNA strand is put on a sepharose bead and subjected to emulsion PCR. This process can potentially make millions of copies of a DNA molecule. The sequences are then determined by pyrosequencing. This system has some drawbacks. It has a tendency for purines to be overrepresented at 5' end, cytosine residues, and difficulty averaging overhanging ends (Briggs et al., 2007).

The third (and final) difficulty is determining authenticity. As the Neanderthal genome is 99.5% the same as the modern human genome, it can be difficult to parse out which sequences are authentic and which are sources of contamination (Noonan et al., 2006).

For all the difficulties associated with the process, sequencing the Neanderthal genome has been able to tell us a lot about Neanderthals and the history of human evolution. In November of 2006, two studies of the Neanderthal nuclear genome were published. An analysis led by Green looked at one million base pairs of nuclear DNA from the 38,000 year old Vindija 80 specimen (Vi-80). Vi-80 was chosen because it was shown to have low levels of contamination and its HVRI had already been successfully sequenced. The GS20 454 sequencing system 'hit' about 3.61 bases per every ten thousand in the autosomal genome. It also 'hit' parts of the X and Y chromosomes. Green et al. 2006 determined that the ancestors of modern humans and Neanderthals diverged around 500,000 years ago. Green et al. 2006 also calculated that the ancestral population before the human/Neanderthal split was between 0 and 12,000 breeding

females, with 3,000 being the most likely. While this was a huge success, Green et al. 2006 cautioned that more sequencing would need to be done to determine a consensus sequence and sort out errors from real Neanderthal base differences (Green et al., 2006).

Noonan et al. 2006 also sequenced part of Vi-80's genome. They used a different technique called high-throughput sequencing that utilizes a metagenomic library. Despite using the same specimen as the Green et al. 2006 study, the Noonan et al.2006 study came up with different results. Again, they found that they 'hit' part of the Y-chromosome, determining that Vi-80 was male. They also identified thirty-four human-specific substitutions in the human genome and one hundred seventy-one Neanderthal-specific substitutions (when comparing modern humans, Neanderthals, and chimpanzees). This would mean that the Neanderthals had many times the substitutions that modern humans had. Because this seemed unlikely, the researchers attributed at least some of the substitutions to sequencing errors. In particular, C to T and A to G base substitution errors were common. Noonan et al. 2006 also calculated that the MRCA between modern humans and Neanderthals lived around 706,000 years ago and that the two species diverged about 370,000 years ago (Hurtley & Szuromi, 2006; Noonan et al., 2006; Pennisi, 2007).

The Green et al. 2006 and Noonan et al. 2006 studies produced a great deal of debate. Much of the general concern was generated because two different new techniques determined different results when working on the same specimen. Several pundits were disturbed that the technology yielded different data. It was suggested that careful attention should be paid to the tendency of the systems to change bases and to look at the ratios of the changes to help determine which bases were correct and which were sequencing artifacts. When the data was reanalyzed, the dates for divergence were still inconsistent and most researchers determined that

much of the results were generated by sequencing errors (Briggs et al., 2007; Wall & Kim, 2007; Hofreiter, 2008; see also: Vives et al., 2008; Green et al., 2009; Gigli et al., 2010; for other discussions on technology and aDNA errors). The suggestion was made that a more hypothesis-driven approach to looking at Neanderthal nuclear DNA may be beneficial (Erren et al., 2007).

The first two nuclear genes that were sequenced from a Neanderthal were FOXP2 and MC1R. Geneticists have determined that the FOXP2 gene has something to do with speech. They are not sure what, but experiments demonstrate that people who have their FOXP2 gene impaired cannot speak. Researchers determined that the Neanderthals do have the modern form of FOXP2 in their genome. While this does not prove that Neanderthals could speak, it also does not demonstrate that they could not (Culotta, 2007).

MC1R is a gene that controls pigmentation. There are certain variants of the gene that cause it to lose part of its functioning. This loss of function results in a light skin pigmentation and blond to red hair in modern humans. The MC1R gene was discovered in Neanderthals from Monti Lessini in Italy and El Sidrón in Spain. In both individuals, a variant of the gene was discovered that has not been found in modern human populations. This shows that the results were not a product of contamination, but since the variant had never been observed before, researchers were not sure if the gene would actually express itself in the same manner as it does in red-headed modern humans. In order to test this and see if the variant reacts similarly, researchers expressed it in a cell culture. The variant did demonstrate that it functioned the same way as the variant for red-heads does in modern humans. However, because some of the DNA processed revealed other variants of MC1R, the authors were not sure if the Neanderthal individuals were homozygote or heterozygote for this gene (Lalueza-Fox et al., 2007; Ledford, 2007).

In the ABO blood group, the O allele is the youngest allele and yet it predates the speciation event in modern humans. The O allele is defined by the Δ261 deletion. Therefore, it was an interesting experiment to see if Neanderthals also contained the Δ261 deletion. Researchers looked at two Neanderthal specimens from El Sidrón. These specimens were chosen for the analysis because they were excavated in 2006 under controlled conditions specifically designed for use in aDNA research. When the specimens' mtDNA was analyzed, it was shown to have low levels of contamination. This made these two specimens ideal for the study. Since the Δ261 deletion is still present in modern human populations, the researchers took the added precaution of analyzing the specimens' Y chromosomes. Both specimens yielded Y chromosomes and the chromosomes were distinct from those of modern humans. With these controls in place, the Neanderthal specimens were sequenced using multiplex PCR to determine if they contained the deletion. Out of ever eleven samples taken from the specimens, nine yielded the deletion. As the deletion is the 'consensus' sequence, the researchers believe that the Neanderthal individuals were homozygous for the O allele and that the other two samples are evidence of slight modern human DNA contamination or PRC errors (Lalueza-Fox etal, 2008).

The next gene to be sequenced was the gene for bitter taste receptors, TAS2R38. Variations in this gene code for different amino acids that can detect bitter tastes. People that are homozygous or heterozygous for the proline-alanine-valine (PAV) amino acid sequence can taste PTC. People who are homozygous for the alanine-valine-isoleucine (AVI) variation of the gene cannot taste PTC. It has been suggested that the ability to taste this bitterness would have been evolutionarily advantageous because it would have prevented people from eating too many bitter vegetables that could affect the thyroid. In modern populations, seventy-five percent can taste PCT whereas twenty-five percent cannot. When the El Sidrón 1253 individual was tested for this

gene, slightly over fifty-five percent of the sequences showed the sequence for a PTC taster, while the other slightly under forty-five percent of the sequences showed the sequence for a non-PTC taster. Therefore, it is likely that El Sidrón 1253 was a heterozygote taster (Lalueza-Fox et al., 2009).

The microcephalin (MCPH1) gene controls brain size. Haplogroup D is a derived haplogroup that originated between sixteen thousand and sixty-two thousand years ago. Because of its recent origin and because it is found in seventy percent of the world's population (with higher frequencies found in Eurasia and lower frequencies found in Africa), it was thought that haplogroup D may have been a result of archaic admixture. If a Neanderthal had the D haplogroup, then that would lend support to the admixture theory. To that end, the Monti Lessini individual was tested for the MCPH1 gene. Monti Lessini, however, proved homozygous for a non D haplogroup (Lari et al., 2010).

While a sample of one does not disprove admixture, this study was largely received as another piece of evidence that Neanderthals did not contribute to the modern human genome. However this stance was shortly re-examined as researchers who were still looking at the whole Neanderthal genome came out with new studies. Burbano et al. 2010 came out with a study that looks at a new technique, Array-Based Sequence Capture, that can target Neanderthal DNA even when an aDNA sample is over ninety-nine percent microbial (Burbano et al., 2010). In the same issue of *Science*, Green et al. 2010 came out with a draft genome using the high-throughput sequencing system. While there were some microbial issues, because the human and chimpanzee genomes have already been sequenced, the researchers were able to extract the Neanderthal DNA without too many microbial issues. They discovered that some genes, particularly some regulatory genes, that are 'fixed' in modern humans are variable in Neanderthals. This indicates

that the fixed genes are of relatively recent fixation and there was more variation in our more immediate ancestors. The researchers also discovered that Neanderthals share significantly more genes in common with non-Africans than with African populations. Specifically, one to four percent of the non-African genome may be from archaic admixture. This suggests that European Cro-Magnon populations were breeding with Neanderthals (or some other archaic hominin). Because Neanderthals share more substitutions with non-African populations, Out-of-Africa is still the dominant evolutionary model, but with archaic admixture mixed in (Dalton, 2010a &b; Green et al., 2010; Hurtley, 2010).

The complete Neanderthal mitochondrial genome has also been sequenced (Morgan, 2008). The excitement of being able to sequence the nuclear genome aside, there is still a great deal of information that the mitochondrial genome can provide. Once the Neanderthal mitochondrial genome was sequenced, it was able to decipher more about Neanderthal population history and about modern humans (Briggs et al., 2009). One of the most interesting findings of the Green et al. 2008 study was that there seemed to be some positive mitochondrial selection. That is to say, that the changes seen in mitochondrial DNA may not always be neutral. Specific evidence for this was determined from the COX2 gene. In modern humans the COX2 gene has an excess of amino acid divergence that is consistent with positive selection. When the modern human gene is compared to the Neanderthal COX2 gene, it can be noted that the COX2 gene has experienced four amino acid substitutions since the modern human and Neanderthal lineages diverged. The study also looked at the overall mtDNA substitution rate for Neanderthals and found that it was high. This is typically seen in small populations, which indicates that Neanderthals had small population groups (Green et al., 2008).

The Neanderthal mitochondrial genome suggests that Neanderthals lived in small population groups that had low diversity—about one-third the amount of mitochondrial diversity of modern humans (and half the diversity of non-Africans) (Morgan, 2008; Briggs et al., 2009; Laursen, 2009; Pennisi, 2009). The mtDNA also shows that there were three different regional groups of Neanderthals (Fabre et al., 2009).

One of the most interesting studies that has been performed on Neanderthal mitochondrial DNA looks at twelve individuals from El Sidrón. The remains were presumed to be deposited in close chronological order[18] and are comprised of six adults, three adolescents, two juveniles, and one infant. Of the adults, three were determined to be male and three female based on a morphological analysis. One of the adolescents was determined to be male based on molecular sexing and the second is believed to also be male as determined by the size of the canine teeth. When the researchers looked at the twelve individuals' mitochondrial lineages, they found three different lineages (comprised of seven, four, and one individual, respectively). All three of the adult males are from the same mitochondrial lineage and all three of the adult females are from separate mitochondrial lineages. This possibly indicates patrilocal mating behavior among Neanderthals. Another interesting find from this study is that one of the juveniles and the infant are of the same mitochondrial lineage as one of the adult females. This indicates, at least in this instance, that there was about a three year birth interval, the same as is seen in modern hunter gatherers (Lalueza-Fox et al., 2011).

Looking at human mitochondrial DNA, researchers were able to discover a 'fossil' in our DNA that must be attributed to a hominin ancestor. Recently, Ochinnikov and Kholina discovered two numts in the human genome that match Neanderthal mitochondrial DNA. This indicates that those segments of mitochondrial DNA are of ancient origin (as they are found in

[18] It is speculated that the remains were from cannibalistic behavior (Lalueza-Fox et al., 2011).

both modern humans and Neanderthals). This means that Neanderthal DNA not only gives us the opportunity to look at Neanderthals and modern humans, but may also provide the opportunity through 'fossils' to look at other hominins. Because the numt were found in modern humans and Neanderthals, it was also present in the ancestor that gave rise to both species (Ochinnikov & Kholina, 2010).

Further Neanderthal Research

Since we have been able to look at Neanderthal DNA, new finds and new theories have been proposed that give shape to ancient DNA studies and that can be tested utilizing ancient DNA. One of the great mysteries that remain is why the Neanderthals went extinct. In 2008, a study was published that suggested the possibility that Neanderthals went extinct because of Transmissible Spongiform Encephalopathies (TSEs). The hypothesis was generated based on Kuru, a disease present in the Fore of Papua New Guinea. The disease spread through a funerary ritual of eating dead relatives. Because there is some evidence of cannibalism in Neanderthal populations, it was suggested that Neanderthals could transmit TSEs much like the Fore (Underdown, 2008). While researchers may not be able to find evidence of TSEs in Neanderthals, because it tends to manifest itself in the brain, there is potential to look for other infectios diseases in the Neanderthals as well as hereditary diseases.

Ancient DNA can also help identify to which species a specimen belongs. In many cases, the morphology of a specimen may be difficult to determine. Many earlier hominins have been determined as "hybrids" between Cro-Magnon and Neanderthals. The Vindija specimen that has already been used for aDNA studies was one of these. In that case, it was determined that it was indeed a Neanderthal, just with finer features than some other Neanderthal specimens. Other undistinguishable specimens could also be tested. Not only would this research tell a lot about

the individual specimen, but it could also provide information on gene flow and, possibly, Cro-Magnon/Neanderthal admixture. New work on Neanderthals and a better understanding of modern human genetics may also lend more clues on Neanderthal and modern human ancestors, through the use of ‘fossils’ common to both species.

Ancient DNA: Problems & Prospects

The field of ancient DNA was born in 1984 and is now twenty-seven years old. Since its inception aDNA was challenged by the ability to avoid contamination and prove that it was a legitimate field with authentic results. It was also an enticing and promising field laden with hope that this would allow us to delve further into our past and more fully understand human history and prehistory. To date, aDNA has outgrown many of its problems and has lived up to much of its potential.

PCR technology is what made ancient DNA studies possible. The first attempts at utilizing PCR for ancient DNA were inefficient and fraught with contamination problems. New techniques such as Hot Start, Touchdown, Booster and Multiplex PCR allowed for better DNA acquisition. In addition to these techniques for improved DNA yield, advances in the understanding of how susceptible ancient DNA is to contamination, prompted a call for better protocol. The protocol guidelines—difficult, expensive, and complex as they are—allowed aDNA researchers confidence in the authenticity of their results.

Once DNA could be procured and the results could be authenticated, ancient DNA opened the doors for a plethora of research opportunities in Physical Anthropology. Even before the year 2000, ancient DNA was being used in phylogenetic, movement and migration, sex determination, disease, Neanderthal, and kinship and culture studies. Since 2000 ancient DNA has told us increasingly more about these studies. It has started to do so with greater efficiency and less cost, thus allowing more work to do be done. Since 2000, aDNA has also become increasingly important in primate conservation, agriculture and domestication, nutrition, and telling us more about our hominin ancestors.

Problems

Despite its many great successes, the field of ancient DNA still must wrestle with a few problems. Contamination remains a big issue with all studies. Because studies still cannot entirely rid their samples of contamination, there are still concerns over the authenticity of samples that resemble the reference sequence or that cannot be distinguished from contamination. The Neanderthals are a good example of this. Researchers believe that the Neanderthal samples are authentic because they are so different from modern human samples. The Neanderthals mitochondrial DNA clusters together and away from all human DNA. This lends it credibility. With the nuclear DNA, one of the reasons that we know that Neanderthal pigmentation is not a result of exogenous modern DNA is that the Neanderthal mutation of the COX2 gene is not seen in modern humans. However, we still cannot be sure whether an individual Neanderthal is heterozygous or homozygous for a particular allele because modern human alleles are still present in the resulting DNA extraction, despite careful controls.

The difficulty with contamination and authenticity is seen from a different angle when we look at Cro-Magnon DNA. The difficulty with the Cro-Magnons is that, as far as anyone can determine, they fall within modern human range for DNA variation. Because the differences between Cro-Magnons and modern humans cannot be seen, the authenticity is always suspect, despite the utmost care.

Technology is another difficulty with which ancient DNA is still struggling. PCR and its variations have made ancient DNA analysis possible. However, PCR is inefficient and it is difficult and expensive to process long segments of DNA using PCR. Some new technologies have been developed, such as the GS20 454 sequencing system and the Array-Based Sequence Capture used on Vi-80. These new technologies helped protect against contamination and

allowed for the sequencing of the whole Neanderthal genome, but the produce many known errors (and possibly some unknown errors as well). This is in part why Neanderthals seem to have several times the mutations that modern humans do since the hominin-chimpanzee split.

Sample size is another problem that persists in aDNA studies. There are not that many specimens that have persisted in an environment conducive to DNA preservation. This precludes too large a sample size and that makes the data pre-statistical. In many cases that is not a problem. For instance, in the Neanderthal studies, knowing that one Neanderthal had red hair and light pigmentation greatly increases our knowledge about the Neanderthals. In such a situation, it is also possible to assume that this was not the only Neanderthal individual with this pigmentation. While we cannot conclude that *all* Neanderthals were red-heads, we can conclude that *some* Neanderthals were red-heads. The study of Pacific migrations, on the other hand, would greatly benefit from more samples. Because most of the DNA researchers are looking at frequencies, larger sample sizes affect the results. Fortunately, in many case, there is the possibility for larger sample sizes. First, there remain plenty more specimens that have not yet been found and each one will increase our sample sizes and knowledge. Second, now that ancient DNA studies are becoming more common and more cost effective, more samples are being specially treated with DNA and contamination in mind and more aDNA studies are being done.

Prospects

Ancient DNA was a source of evidence in the discussion on whether *Mycobacterium tuberculosis* was present in the New World before the arrival of the Europeans. It has also offered insights into other infectious diseases and promises to keep doing so. As mentioned

above, it may also be possible to learn more about infectious diseases present in Neanderthal and other archaic hominin species. Additionally, in the future it should be able to offer insights on hereditary diseases as well. Presumably, mutations that today cause 'diseases of affluence' were once advantageous to human survival. Genetics is currently learning more about these diseases and the molecular biology behind them. It is possible that ancient DNA studies can spot these mutations in ancient samples and this should lend us more clues on the evolution of 'diseases of affluence.'

Ancient DNA has proven a useful tool in understanding kinship structures, migrations, and human ancestors. This is already partially informative about past population interactions. With continued research, it should be possible to use ancient DNA to put many of these divergent fields of study together and with the combined whole, learn much more about how past populations interacted with each other. This will give us a much clearer picture of our past. Additionally, we should be able to combine ancient DNA studies with other disciplines to increase our knowledge even more. To some extent, this is already the case.

The Pacific Migrations case study illustrated how ancient DNA studies—of humans, rats, chickens, and lizards-- combined with linguistics and archaeology has increased our understanding of the peopling of the Pacific. True, at the moment, ancient DNA has only complicated an already complex understanding of our history, but this is to be expected. Most reconstructions of the past are not simple and straight-forward, but fraught with subtleties. Ancient DNA has excellent prospects for contributing to these complexities and thus increasing our knowledge of what actually happened in the past.

On a slightly smaller scale, using ancient plant and animal DNA, aDNA has the potential to help reconstruct past flora and fauna at archaeological sites. This becomes increasingly

plausible as aDNA technology improves, long DNA segments can be sequenced, and multiple organisms can be sequenced simultaneously without mispriming. This can increase our knowledge of the environments that archaeological populations lived in and thus our knowledge of the people at those sites and what their lives were like.

Anthropology is a holistic field of study. It searches out understanding of people not just in one time or place, but in many. It seeks to look at people from all over the world from a cultural and a biological viewpoint. As such, time depth is also an important part of the discipline. Archaeology and paleontology have greatly increased our knowledge and understanding of past populations and extinct hominins. Ancient DNA studies have contributed even more to our understanding of the past-- and our understanding of humanity. Ancient DNA is a useful tool in physical anthropology. It has already provided new insights in many different anthropological areas of interest and will contribute many more.

References Cited

Adcock GJ, Dennis ES, Easteal S, Huttley GA, Jermiin LS, Peacock WJ, Thorne A. 2001. Mitochondrial DNA sequences in ancient Australians: implications for modern human origins. *Proc. Natl. Acad. Sci. USA* 98: 537-542.

Addison DJ & Matisoo-Smith E. 2010. Rethinking Polynesian origins: a West-Polynesia Triple-I model. *Archaeol. Oceania* 45: 1-12.

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics* 23: 147.

Armitage SJ, Jasim SA, Marks AE, Parker AG, Usik VI, Uerpmann H-P. 2011. The southern route "Out of Africa": evidence for an early expansion of modern humans into Arabia. *Science* 28: 453-456.

Atkinson QD & Russell GD. 2005. Curious parallels and curious connections: phylogenetic thinking in biology and historical linguistics. *Systematic Biology* 4: 513-526.

Audic S & Béraud-Colomb E. 1997. Ancient DNA is thirteen years old. *Nature Biotechnology* 15: 855-858.

Austin CC. 1999. Lizards took express train to Polynesia. *Nature* 397: 113-114.

Austin JJ, Smith AB, Thomas, RH. 1997. Palaeontology in a molecular world: the search for authentic ancient DNA. *Tree* 12: 303-306.

Bailey JF, Henneberg M, Colson IB, Ciarallo A, Hedges REM, Sykes B. 1999. Monkey business in Pompeii—unique find of a juvenile Barbary Macacque skeleton in Pompeii identified using osteology and ancient DNA techniques. *Molecular Biol. Evolution* 16: 1410-1414.

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen K-H, Wallace DC. 1992. Southeast Asian mitochondrial DNA analysis reveals genetic continuity of Ancient Mongoloid migrations. *Genetics* 130: 139-152.

Balter M. 2005. Ancient DNA yields clues to the puzzle of European origins. *Science* 310: 964-965.

Balter M. 2007. Seeking agriculture's ancient roots. *Science* 316: 1830-1835.

Balter M. 2008. Ancient DNA from frozen hair may untangle Eskimo roots. *Science* 320: 1146-1147.

Balter M. 2009. Ancient DNA says Europe's first farmers came from afar. *Science* 325: 1189.

Balter M. 2010a. Ancient DNA from Siberia fingers a possible new human lineage. *Science* 327: 1566-1567.

Balter M. 2010b. Who are the Jews? Genetic studies spark identity debate. *Science* 328: 1342.

Bar-Gal GK, Khalaily H, Mader O, Ducos P, Horwitz LK. 2002. Ancient DNA evidence for the transition from wild to domestic status in Nealithhic goats: a case study from the site of Abu Gosh, Israel. *Ancient Biomolecules* 4: 9-17.

Baron H, Hummel S, Herrmann B. 1996. *Mycobacterium tuberculosis* complex DNA in ancient human bones. *Journal of Archaeological Science* 23: 667-671.

Bass WM. 1971. *Human Osteology: a Laboratory and Field Manual of the Human Skeleton*. Columbia: Missouri Archeological Society.

Beja-Pereira A, Caramelli D, Lalueza-Fox C, Vernesi C, Ferrand N, Casoli A, Goyache F, Royo LJ, Conti S, Lari M, Martini A, Ouragh L, Magid A, Atash A, Zsolnai A, Boscato P, Triantaphylidis C, Ploumi K, Sineo L, Mallagni F, Taberlet P, Erhardt G, Sampietro L, Bertranpetit J, Barbujani G, Luikart G, Bertorelle G. 2006. The origin of European cattle: evidence from modern and ancient DNA. *Proc. Natl. Acad. Sci. USA* 103: 8113-8118.

Bellwood P. 1991. The Austronesian dispersal and the origin of languages. *Scientific American* 165: 88-93.

Bellwood P & Dizon E. 2008. Austronesian cultural origin: out of Taiwan, via the Batanes Islands, and onward to Western Polynesia. Pages 23-39 in: Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Ben-Ari ET. 1999. Molecular biographies. *BioScience* 49: 98-103.

Binladen J, Wiuf C, Gilbert MTP, Bunce M, Barnett R, Larson G, Greenwood AD, Haile J, Ho SYW, Hansen AJ, Willerslev E. 2006. Assessing the fidelity of ancient DNA sequences amplified from nuclear genes. *Genetics* 172: 733-741.

Blench R, Ross M, Sanchez- Mazas A. 2008. Methodological issues: linking genetic, linguistic and archaeological evidence. Pages 3-19 in: Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology* 28: 495-503.

Bouman AS, Brown TA. 2002. Comparison between silica-based methods for the extraction of DNA from human bones from 18th to mid-19th century London. *Ancient Biomolecules* 4: 173-178.

Bower MA, Spencer M, Matsumura S, Nisbet RER, Howe CJ. 2005. How many clones need to be sequenced from a single forensic or ancient DNA sample in order to determine a reliable consensus sequence? *Nucleic Acids Research* 33: 2549-2556.

Bramanti B, Thomas MG, Haak W, Unterlaender M, Jores P, Tambets K, Antanaitis-Jacobs I, Haidle MB, Jankauskas R, Kind C-J, Lueth F, Terberger T, Hiller J, Matsumura S, Forster P, Burger J. 2009. Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science* 326: 137-140.

Braun M, Cook DC, Pfeiffer S. 1998. DNA from *Mycobacterium tuberculosis* complex identified in North-American, pre-Columbian human skeletal remains. *Journal of Archaeological Science* 25: 271-277.

Briggs AW, Good JM, Green RE, Krause J, Maricic T, Stenzel U, Lalueza-Fox C, Rudan P, Brajkovíc D, Kućan Ž, Gušić I, Schmitz R, Doronichev VB, Golovanova LV, de la Rasilla M, Fortea J, Rosas A, Pääbo S. 2009. Targeted Retrieval and analysis of five Neanderthal mtDNA genomes. *Science* 325: 318-321.

Briggs AW, Stenzek U, Johnson PLF, GreenRE, Kelso J, Prüfer K, Meyer M, Krause J, Ronan MT, Lachmann M, Pääbo S. 2007. Patterns of damage in genomic DNA sequences from a Neandertal. *Proc. Natl. Acad. Sci. USA* 104: 14616-14621.

Brown TA, Allaby RG, Brown KA, O'Donoghue K, Sallares R. 1994. DNA in wheat seads from European archaeological sites. *Experientia* 50: 571-575.

Burbano HA, Hodges E, Green RE, Briggs A, Krause J, Meyer M, Good JM, Maricic T, Johnson PLF, Xuan Z, Rooks M, Bhattacharjee A, Brizuela L, Albert FW, de la Rasilla M, Fortea J, Rosas A, Lachmann M, Hannon GJ, Pääbo S. 2010. Targeted investigation of the Neanderthal genome by Array-Based sequence capture. *Science* 328: 723-725.

Caccone A & Powell JR. 1989. DNA divergence among hominoids. *Evolution* 43: 925-942.

Cann RL. 2001. Genetic clues to dispersal in human populations: retracing the past from the present. *Science* 291: 1742-1748.

Cano RJ & Poiner HN. 1993. Rapid isolation of DNA from fossil and museum specimens suitable for PCR. *Biotechniques* 15: 433-435.

Capell A. 1962. Oceanic linguistics today. *Current Anthropology* 3: 371-428.

Caramelli D, Lalueza-For C, Vernesu C, Lari M, Casoli A, Mallegni F, Chiarelli B, Dupanloup I, Bertranpetit J, Barbujani G, Bertorelle G. 2003. Evidence for a genetic discontinuity between Neanderthals and 24,000-year-old anatomically modern Europeans. *Proc. Natl. Acad. Sci. USA* 100: 6593-6597.

Caramelli D, Milani L, Vai S, Modi A, Pecchioli E, Girardi M, Pilli E, Lari M, Lippi B, Ronchitelli A, Mallegni F, Casoli A, Bertorelle G, Barbujani G. 2008. A 28,000 years old Cro-Magnon mtDNA sequence differs from all potentially contaminating modern sequences. *PLoS ONE* 3: 1-4.

Cattaneo C, Craig OE, James NT, Sokol RJ. 1997. Comparison of three DNA extraction methods on bone and blood stains up to 43 years old and amplification of three different gene sequences. *J. Forensic Sci* 42: 1126-1135.

Chan YL, Anderson CNK, Hadly EA. 2006. Bayesian estimation of the timing and severity of a population bottleneck from ancient DNA. *PLoS ONE Genetics* 2: 451-460.

Cherfas J. 1990. Ancient DNA: still busy after death. *Science* 253: 1354-1356.

Chou Q, Russell M, Birch DE, Raymond J, Bloch W. 1992. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Research* 20: 1717-1723.

Cipollaro M, Di Bernardo G, Galano G, Galderisi U. Guarino F, Angelini F, Cascino A. 1998. Ancient DNA in human bone remains from Pompeii archaeological site. *Biochemical and Biophysical Research Communications* 247: 901-904.

Clark GM & Whyte LS. 2003. Phylogeography and population history of the endangered golden sun moth (*Synemon plana*) revealed by allozymes and mitochondrial DNA analysis. *Conservation Genetics* 4: 719-734.

Cobb J. 2002. Ancient DNA recovered by a non-destructive method. *Ancient Biomolecules* 4: 169-172.

Collins MJ, Penkman KEH, Rohland N, Shapiro B, Dobberstein RC, Ritz-Timme S, Hofreiter M. 2009. Is animo acid racemization a useful tool for screening for ancient DNA in bone? *Proc. R.Soc. B* 276: 2971-2977.

Cooper A. 1994. DNA from museum specimens. Pages 149-165 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA*. New York: Sringer-Verlag.

Cooper A & Poinar HN. 2000. Ancient DNA: do it right or not at all. *Science*. 289: 1139.

Cooper A, Rambaut A, Macaulay V, Willersiev E, Hansen AJ, Stringer C. 2001. Human origins and ancient human DNA. *Science* 292: 1655-1656.

Cooper A, Rhymer J, James HF, Olson SL, McIntosh CE, Sorenson MD, Fleischer RC. 1996. Ancient DNA and island endemics. *Nature* 381: 484.

Crubézy E, Amory S, Keyser C, Bouakaze C, Bodner M, Gibert M, Röck A, Parson W, Alexeev A, Ludes B. 2010. Human evolution in Siberia: from frozen bodies to ancient DNA. *BMC Evolutionary Biology* 10: 25-41.

Culotta E. 2007. Ancient DNA reveals Neanderthals with red hair, fair complexions. *Science* 318: 546-47.

Currat M & Excoffier L. 2004. Modern humans did not admix with Neanderthals during their range expansion into Europe. 2004. *PLoS Biology* 2: 2264-2274.

Cyran KA & Kimmel M. 2010. Alternatives to the Wright-Fisher model: the robustness of mitochondrial Even dating. *Theoretical Population Biology* 78:165-172.

Dalton R. 2010a. Neanderthals may have interbred with humans. *Nature* online.

Dalton R. 2010b. European and Asian genomes have traces of Neanderthal. *Nature* online.

Deguilloux M-F, Pemonge M-H, Dubut V, Hughes S, Hänni C, Chollet L, Conte E, Murail P. 2011. Human ancient and extant DNA from the Gambier Islands (French Polynesia): evidence for an early Melanesian maternal contribution and new perspectives into the settlement of easternmost Polynesia. *American Journal of Physical Anthropology* 144: 248-257.

Delson E & Havarti K. 2006. Return of the last Neanderthal. *Nature* 443: 762-763.

Derenko M, Malyarchuk B, Grzybowsky T, Denisova G, Rogalla U, Perkova M, Dambueva I, Zakharov I. 2010. Origin and post-glacial dispersal of mitochondrial DNA haplogroups C and D in Northern Asia. *PLoS ONE* 5: 1-9.

Diamond JM. 1988. Express train to Polynesia. *Nature* 336: 307-308.

Dodge RD. 2010. A molecular approach to Neanderthal extinction. *Quaternary International* xxx: 1-11.

Doebley J. 2006. Unfallen grains: how ancient farmers turned weeds into crops. *Science* 312: 1318-1319.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research* 19: 4008-503.

Donoghue HD, Spigelman M, Zias J, Gernaey-Child AM, Minnikin DE. 1998. *Mycobacterium tuberculosis* complex DNA in calcified pleura from remains 1400 years old. *Letters in Applied Microbiology* 27: 265-269.

Doran GH, Dickel DN, Ballinger WE, Agee OF, Laipis PJ, Hauswirth WW. 1986. Anatomical, cellular and molecular analysis of 8,000-yr-old human brain tissue from the Windover archaeological site. *Nature* 323: 803-6.

Drancourt M, Aboudharam G, Signoli M, Dutour O, Raoult D. 1998. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proc. Natl. Acad. Sci. USA* 95: 12637-12640.

Dunn M, Terrill A, Reesink G, Foley RA, Levinson SC. 2005. Structural phylogenetics and the reconstruction of ancient language history. *Science* 309: 2072-2075.

Ellengren H. 1994. Genomic DNA from museum bird feathers. Pages 211-217 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA*. New York: Sringer-Verlag.

Endicott P, Ho SYW, Stringer C. 2010. Using genetic evidence to evaluate four paleoanthropological hypotheses for the timing of Neanderthal and modern human origins. *Journal of Human Evolution* 59: 87-95.

Endicott P, Sanchez JJ, Pichler I, Brotherton P, Brooks J, Egarter-Vigl E, Cooper A, Pramstaller P. 2009. Genotyping human ancient mtDNA control and coding region polymorphisms with a multiplexed single-base-extension assay: the singular maternal history of the Tyrolean Iceman. *BMC Genetics* 10: 29-42.

Ermini L, Olivieri C, Rizzi E, Corti G, Bonnal R, Soares P, Luciani S, Marota I, De Bellis G, Richards MB, Rollo F. 2008. Complete mitochondrial genome sequence of the Tyrolean Iceman. *Current Biology* 18: 1687-1693.

Erren TC, Cullen P, Erren M. 2007. Comparing Neanderthal and human genomes. *Science* 315: 1664.

Fabre V, Condemi S, Degioanni A. 2009. Genetic evidence of geographical groups among Neanderthals. *PLoS One* 4: 1-8.

Faerman M & Bar-Gal GK. 1998. Determining the sex of infanticide victims from the Late Roman era through ancient DNA analysis. *Journal of Archaeological Science* 25: 861-865.

Filon D, Faerman M, Smith P, Oppenheim A. 1995. Sequence analysis reveals a Beta-thalassaemia mutation in the DNA of skeletal remains from the archaeological site of Akhziv, Isreal. *Nature Genetics* 9: 861-865.

Finlayson C, Pacheco FG, Rodríguez-Vidal J, Fa DA, Gutierrez López JM, Pérez AS, Finlayson G, Allue E, Preysler JB, Cáceres I, Carrión JS, Jalvo YF, Gleed-Owen CP, Jimenez Espejo

FJ, López P, López Sáez JA, Riquelme Cantal JA, Marco AS, Guzman FG, Brown K, Fuentes N, Valarino CA, Villalpando A, Stringer CB, Martinez Ruiz F, Sakamoto T. 2006. Late survival of Neanderthals at the southernmost extreme of Europe. *Nature* 443: 850-853.

Finney B. 2007. Tracking Polynesian seafarers. *Science* 317: 1873-1874.

Forster P, Harding R, Torroni A, Bandelt H-J. 1996. Origin and evolution of Native American mtDNA variation: a reappraisal. *Am. J. Hum. Genet.* 59: 935-945.

Forster P & Matsumura S. 2005. Did early humans go north or south? *Science* 308: 965-966.

Fountain D, Ralston M, Higgins N, Gorlin JB, Uhl L, Wheeler C, Antin JH, Churchill WH, Benjamin RJ. 1997. Liquid nitrogen freezers: a potential source of microbial contamination of hematopoietic stem cell components. *Transfusion* 37: 585-591.

Fricker EJ, Spigelman M, Fricker CR. 1997. The detection of *Escherichia coli* DNA in the ancient remains of Lindow Man using the polymerse chain reaction. *Letters in Applied Microbiology* 24: 351-354.

Gamble C. 1999. The Paleoloithic Societies of Europe. Cambridge University Press. Cambridge.

Geigel EM. 2001. Inadequate use of molecular hybridization to analyze DNA in Neanderthal Fossils. *Am. J.Hum. Genet.* 68: 287.

Gibbons A. 1993. Genetics trace the DNA trail of the first Americans. *Science* 259: 312-313.

Gibbons A. 2009. Africans' deep genetic roots reveal their evolutionary story. *Science* 324: 575.

Gibbons A. 2011. A new view of the birth of *Homo sapiens*. *Science* 331: 392-393.

Gigli E, Rasmussen M, Civit S, Rosas A, de la Rasilla M, Fortea J, Gilbert MT, Willerslev E, Lalueza-Fox C. 2009. An improved method for endogenous DNA retrieval in contaminated Neanderthal samples based on the use of blocking primers. *Journal of Archaeological Science* 36: 2676-79.

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I. 2005. Assessing ancient DNA studies. *Trends in Ecology and Evolution* 20: 541-544.

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Research* 35: 1-10.

Gilbert MPT, Miller W, Schuster SC. 2008. Response to comment on "whole-genome shotgun sequencing of mitochondria from ancient hair shafts." *Science* 322: 857b.

Gilbert MTP, Tomsho LP, Rendulic S, Packard M, Drautz DI, Sher A, Tikhonov A, Dalen L, Kuznetsova T, Kosintsev P, Campos PF, Higham T, Collins MJ, Wilson AS, Shidlovskiy F, Buigues B, Ericson PGP, Germonpré M, Götherström A, Iacumin P, Nikolaev V, Nowak-Kemp M, Willerslev E, Knight JR, Irzyk GP, Perbost CS, Fredrikson KM, Harkins TT, Sheridan S, Miller W, Schester SC. 2007. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* 317: 1927-1930.

Goloubinoff P, Pääbo S, Wilson AC. 1993. Evolution of maize inferred from sequence diversity of an *Adh2* gene segment from archaeological specimens. *Proc. Natl. Acad. Sci. USA* 90: 1997-2001.

Gray RD, Drummond AJ, Greenhill SJ. 2009. Language phylogenies reveal expansion pulses and pauses in Pacific settlement. *Science* 323: 479-482.

Green RE, Briggs AW, Krause J, Prüfer K, Burbano HA, Siebauer M, Lachmann M, Pääbo S. 2009. The Neanderthal genome and ancient DNA authenticity. *The EMBO Journal*28: 2494-2502.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MHY, Hansen NF, Durand EY, Malaspinas AS, Jensen JD, Marques-Bonet T, Alkan C, Prüfer K, Meyer M, Burbano HA, Good JF, Schultz R, Aximu-Petri A, Butthof A, Höber B, Höffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan Ž, Gušic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, de la Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PLF, Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Pääbo S. 2010. A draft sequence of the Neanderthal genome. *Science* 328: 710-22.

Green RE, Krause J, Ptak SE, Briggs AW, Ronan MT, Simons JF, Du L, Egholm M, Rothbrg JM, Paunovic M, Pääbo S. 2006. Analysis of one million base pairs of Neanderthal DNA. *Nature* 444: 330-336.

Green RE, Malaspinas A-S, Krause J, Briggs AW, Johnson LF, Uhler C, Meyer M, Good JM, Marici T, Stenzel U, Prüfer K, Siebauer M, Burbano HA, Ronan M, Rothberg JM, Egholm M, Rudan P, Brajkovic D, Kućan Ž, Gušić I, Wikström M, Laakkonen L, Kelso J, Slatkin M, Pääbo S. 2008. A complete Neanderthal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* 134: 416-426.

Gugerli F, Parducci L, Petit RJ. 2005. Ancient Plant DNA: review and prospects. *New Phytologist* 166: 409-418.

Gunz P, Bookstein FL, Mitteroecker P, Stadlmayr A, Seidler H, Weber GW. 2009. Early modern human diversity suggests subdivided population structure and a complex Out-of-Africa scenario. *Proc. Natl. Acad. Sci. USA* 106: 6094-6098.

Haak W, Balanovsky O, Sanchez JJ, Koshel S, Zaporozhchenko V, Adler CJ, Der Sarkissian CSI, Brandt G, Schwarz C, Nicklisxh N, Dresely V, Fritsch B, Balanovska E, Villems R, Meller H, Alt KW, Cooper A, Consortium G. 2010. Ancient DNA from European Early Neolithic farmers reveals their Near East affinities. *PLoS Biology* 8: 1-16.

Haak W, Forster P, Bramanti B, Matsumura S, Brant G, Tänzer M, Villems R, Renfrew C, Gronenborn D, Alt KW, Burger J. 2005. Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. *Science* 310: 1016-1018.

Haensch S, Bianucci R, Signoli M, Rajerison M, Schultz M, Kacki S, Vermunt M, Weston DA, Hurst D, Achtman M, Varniel E, Bramanti B. 2010. Distinct clones of *Yersinia pestis* caused the Black Death. *PLoS Pathology* 6: 1-8.

Hagelberg E. 1994. Mitochondrial DNA from ancient bones. Pages 195- 204 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA.* New York: Sringer-Verlag.

Hagelberg E. 1995. Genetic affinities of prehistoric Easter Islanders: reply to Langdon. *Rapa Nui Journal* 9: 16-19.

Hagelberg E, Bell LS, Allen T, Boyde A, Jones SJ, Clegg JB. 1991. Analysis of ancient bone DNA: techniques and applications. *Philos. Trans. R. Soc. London* 333: 399-407.

Hagelberg E & Clegg JB. 1991. Isolation and characterization of DNA from archaeological bone. *Proc. R. Soc. Lond. B* 244: 45-50.

Hagelberg E, Cox M, Schiefenhövel W, Frame I. 2008. A genetic perspective on the origins and dispersal of the Austronesians: mitochondrial DNA variation from Madagascar to Easter Island. Pages 356-375 in: Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Hagelberg E, Quevedo S, Turbon D, Clegg JB. 1994. DNA from ancient Easter Islanders. *Nature* 369: 25-26.

Handt O, Höss M, Krings M, Pääbo S. 1994. Ancient DNA: methodological challenges. *Experientia* 50: 524-29.

Handt O, Krings M,Ward RH, Pääbo S. 1996. The retrieval of ancient human DNA sequences. *Am. J. Hum. Genet.* 59: 368-376.

Handt O, Richards M, Trommsdorff M, Kilger C, Simanainen J, Georgiev O, Bauer K, Stone A, Hedges R, Schaffner W, Utermann G, Sykes B, Pääbo S. 1994. Molecular genetic analysis of the Tyrolean Ice Man. *Science* 264: 1775-1778.

Hänni C, Brousseau T, Laudet V, Stehelin D. 1995. Isopropanol precipitation removes PCR inhibitors from ancient bone extracts. *Nucleic Acids Research* 23: 881-882.

Hansen AJ, Willerslev E, Wiuf C, Mourier T, Arctander P. 2001. Statistical evidence for miscoding lesions in ancient DNA templates. *Mol. Biol. Evol.* 18: 262-265.

Hauswirth WW, Dickel CD, Lawlor DA. 1994. DNA analysis of the Windover population. Pages 104-121 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA.* New York: Sringer-Verlag.

Hauswirth WW, Dickel CD, Rowold DJ, Hauswirth MA. 1994. Inter- and intrapopulation studies of ancient humans. *Experientia* 50: 585-591.

Helgason A, Lalueza-Fox C, Ghosh S, Sigurõardóttir S, Sampietro ML, Gigli E, Baker A, Bertranpetit J, Árnadóttir L, Þorsteinsdottir U, Stefánsson K. 2009. Sequences from first settlers reveal rapid evolution in Icelandic mtDNA pool. *PLoS ONE Genetics* 5: 1-10.

Herrera KJ, Somarelli JA, Lowery RK, Herrera RJ. 2009. To what extent did Neanderthals and modern humans interact? *Biol. Rev.* 84: 245-257.

Herrmann B & Hummel S, eds. 1994. *Ancient DNA.* New York: Sringer-Verlag.

Higuchi R, Bowman B, Freiberger M, Ryder OA, Wilson AC. 1984. DNA sequences from the quagga, an extinct member of the horse family. *Nature* 312: 282-84.

Hodgson JA & Disotell TD. 2008. No evidence of a Neanderthal contribution to modern human diversity. *Genome Biology* 9: 206.1-206.7.

Hofkin BV, Wright A, Altenbach J, Rassmann K, Snell HM, Miller RD, Stone AC, Snell HL. 2003. Ancient DNA gives green light to Galápagos land iguana repatriation. *Conservation Genetics* 4: 105-108.

Hofreiter M. 2008. Paleogenomics. *C. R. Palevol* 7:113-124.

Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, Possnert G, Pääbo S. 2000. A molecular analysis of ground sloth diet through the last glaciations. *Molecular Ecology* 9: 1975-1984.

Hofreiter M, Serre D, Poinar HN, Kuch M, Pääbo S. 2001. Ancient DNA. *Nature Reviews* 2: 353-359.

Holden C. 2004. Rats tell Polynesian story. *Science* 304: 1742.

Holden C. 2006. It's Neandertal time. *Science* 313: 279.

Horai S, Hayasaka K, Murayama K, Wate N, Koike H, Nakai N. 1989. DNA amplification from ancient human skeletal remains and their sequence analysis. *Proc. Japan. Acad. Ser. B.* 65: 229-233.

Höss M. 2000. Neanderthal population genetics. *Nature* 404: 453-54.

Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S. 1996. DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Research* 24: 1304-07.

Höss M, Pääbo S. 1993. DNA extraction from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research* 21: 3913-3914.

Hummel S, 1991. Y-chromosome-specific DNA amplified in ancient human bone. *Naturwissenschafen* 78: 266-267.

Hummel S. 2003. *Ancient DNA typing: Methods, Strategies and Applications*. New York, Springer-Verlag Berlin Heidelberg.

Hummel S & Herrmann B. 1994a. General aspects of sample preparation. Pages 59-68 in: Herrmann B & Hummel S, eds. *Ancient DNA*. New York: Sringer-Verlag.

Hummel S & Herrmann B. 1994b. Y-chromosomal DNA from ancient bones. Pages 205-210 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA*. New York: Sringer-Verlag.

Hummel S, Nordsiek G, Herrmann B. 1992. Improved efficiency in amplification of ancient DNA and its sequence analysis. *Naturwissenschaften* 79: 359-360.

Hurtley S. 2010. Kissing Cousins. *Science* 328: 663.

Hurtley S& Szuromi P. eds. 2006. Neanderthal Metagenomics. *Science* 314: 1045.

Jin J-H, Tyler-Smith C, Kim W. 2009. The peopling of Korea revealed by analysis of mitochondrial DNA and Y-chromosome markers. *PLoS ONE* 4: 1-10.

Jorde LB. 1985. Human genetic distance studies: present status and future prospects. *Annual Review of Anthropology* 14: 343-373.

Kaestle FA & Horsburgh KA. 2002. Ancient DNA in anthropology: methods, applications, and ethics. *Yearbook of Physical Anthropology* 45: 92-130.

Kaestle FA & Smith DG. 2001. Ancient mitochondrial DNA evidence for prehistoric population movement: the Numic Expansion. *American Journal of Physical Anthropology* 115: 1-12.

Kafri R, Springer M, Pilpel Y. 2009. Genetic Redundancy: New Tricks for Old Genes. *Cell* 136: 389-392.

Kahn P & Gibbons A. 1997. DNA from an extinct human. *Science* 277: 176-178.

Keyser-Tracqui C, Crubézy E, Ludes B. 2003. Nuclear and mitochondrial DNA analysis of a 2,000-year-old necropolis in the Egyin Gol Valley of Mongolia. *Am. J. Hum. Genet.* 73: 247-260.

Kim K, Kim K-Y, Jeon E, Togloom A, zcho Y-O, Lee M-S, Lkhagvasuren G, Choi J-H, Tumen D, Park AJ, Kim K-C, Park K-W, Kim J-H, Noh M, Yoo K-J, Lee K-H. 2008. Technical note: improved ancient DNA purification for PCR using ion-exchange columns. *American Journal of Physical Anthropology* 136: 114-121.

Kim NY, Lee HY, Park MJ, Yang WI, Shin K-J. 2011. A genetic investigation of Korean mummies from the Joseon Dynasty. *Mol. Biol. Rep.* 38: 115-121.

Kirch PV & Green RC. 1992. History, phylogeny, and evolution in Polynesia. *Current Anthropology* 33: 161-186.

Kittles RA, Bergen AW, Urbanek M, Virkkunen M, Linnoila M, Goldman D, Long JC. 1999. Autosomal, mitochondrial, and Y-chromosomal DNA variation in Finland: Evidence for a male-specific bottleneck. *American Journal of Physical Anthropology* 108: 381-399.

Klein RG. 2003. Whither the Neanderthals? *Science* 299: 1525-1527.

Kolman C, Tuross N. 2000. Ancient DNA analysis of human populations. *American Journal of Physical Anthropology* 111: 5-23.

Krause J, Fu Q, Good JM, Viola B, Shunkov MV, Derevianko AP, Pääbo S. 2010. The complete mitochondrial genome of an unknown hominin from souther Siberia. *Nature* 464: 894-897.

Kraus J, Orlando L, Serre D, Viola B, Prüfer K, Richards MP, Hublin J-J, Hänni C, Derevianko AP, Pääbo S. 2007. Neanderthals in central Asia and Siberia. *Nature* 449: 902-904.

Krings M, Capelli C, Tschentscher F, Geisert H, Meyer S, von Haeseler A, Grossschmidt K, Possnert G, Paunovic M, Pääbo S. 2000. A view of Neanderthal genetic diversity. *Nature Genetics* 26: 144-146.

Krings M,Geisert H, Schmitz RW, Krainitzki H, Pääbo S. 1999. DNA sequence of the mitochondrial hypervariable region II from the Neanderthal type specimen. *Proc. Natl. Acad. Sci. USA* 96: 5581-5585.

Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M, Pääbo S. 1997. Neanderthal DNA sequences and the origin of modern humans. *Cell* 90: 19-30.

Lalueza-Fox C, Gigli E, de la Rasilla M, Fortea J, Rosas A. 2009. Bitter taste perception in Neanderthals through the analysis of the TAS2R38 gene. *Biol. Lett.* 5: 809-811.

Lalueza-Fox C, Gigli E, de la Rasilla M, Fortea J, Rosas A, Fortea J, Rosas A, Bertranpetit J, Krause J. 2008. Genetic characterization of the ABO blood group in Neanderthals. *BMC Evolutionary Biology* 8: 342-346.

Lalueza-Fox C, Krause J, Caramelli D, Catalano G, Milani L, Sampietro ML,Calafell F, Martínez-Maza C, García-Tabernero A, de la Rasilla M, Fortea J, Pääbo S, Bertranpetit J, Rosas A. 2006. Mitochondrial DNA of an Iberian Neanderthal suggests a population affinity with other European Neanderthals. *Current Biology* 16: R629-R630.

Lalueza-Fox C, Römpler H, Caramelli D, Stäubert C, Catalano G, Hughes D, Rohland N, Pilli E, Longo L, Condemi S, de la Rasilla M, Fortea J, Rosas A, Stoneking M, Schöneberg T, Bertranpetit J, Hofreiter M. 2007. A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. *Science* 318: 1453-1455.

Lalueza-Fox C, Rosas A, Estalirrich A, Gigli E, Campos PF, García-Tabernero A, García-Vargas S, Sánchez-Quinto F, Ramírez O, Civit S, Bastir M, Huguet R, Santamaría D, Gilbert MTP, Wllerslev E, de la Rasilla M. 2011. Genetic evidence for patrilocal mating behavior among Neanderthal groups. *Proc. Natl. Acad. Sci. USA* 108: 250-253.

Lalueza-Fox C, Sampietro ML, Caramelli D, Puder Y, Lari M, Calafell F, Martínez-Maza C, Bastir M, Fortea J, de la Rasilla M, Bertranpetit J, Rosas A. 2005. Neanderthal Evolutionary Genetics: Mitochondrial DNA data from the Iberian Peninsula. *Molecular Biology & Evolution* 22: 1077-1081.

Lalueza-Fpx C, Sampietro ML, Gilbert MPT, Castri L, Facchini F, Pettener D, Bertranpetit J. 2004. Unravelling migrations in the steppe: mitochondrial DNA sequences from ancient Central Asians. *Proc.R. Soc. Lond. B* 271: 941-947.

Lambert DM, Ritchie PA, Millar CD, Holland B, Drummond AJ, Baroni C. 2002. Rates of evolution in ancient DNA from Adelie Penguins. *Science* 295: 2270-2272.

Lari M, Rizzi E, Milani L, Corti G, Balsamo C, Vai S, Catalano G, Pilli E, Longo L, Condemi S, Giunti P, Hänni C, De Bellis G, Orlando L, Barbujani G, Caramelli D. 2010. The *Microcephalin* ancestral allele in a Neanderthal individual. *PloS ONE* 5: 1-6.

Larson G, Albarella U, Dobney K, Rowley-Conwy P, Schibler J, Tresset A, Vigne J-D, Edwards CJ, Schlumbaum A, Dinu A, Bălăçsescu A, Dolman G, Tagliacozzo A, Manaseryan N, Miracle P, Van Wijngaarden-Bakker L, Masseti M, Bradley DG, Cooper A. 2007.Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proc. Natl. Acad. Sci, USA* 104: 15276-15281.

Larson G, Liu R, Zhao X, Yuan J, Fuller D, Barton L, Dobney K, Fan Q, Gu Z, Liu X-H, Luo Y, Lv P, Anderson L, Li N. 2010. Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. *Proc. Natl. Acad. Sci. USA* 107: 7686-7691.

Lassen C, Hummel S, Herrmann B. 2000. Molecular sex identification of stillborn and neonate individuals. *Anthropologischer Anzeiger* 58:1-8.

Laursen L. 2009. Neanderthal census reveals diversity. *Nature online* doi:10.1038/news.2009.70Morgan J. 2008. First complete Neanderthal genome sequenced. *Nature online* doi10.1038/news.2008.1026

Lawler A. 2010. Beyond *Kon-Tiki*: did Polynesians sail to South America? *Science* 328: 13441347.

Ledford H. 2007. Some Neanderthals were red-heads. *Nature online* doi.1038/news.2007.197

Leonard JA, Wayne RK, Wheeler J, Valadez R, Guillén S, Vilá C. 2002. Ancient DNA evidence for Old World origin of New World dogs. *Science* 298: 1613-1616.

Lindahl T. 1993. Instability and decay of the primary structure of DNA. *Nature* 362: 709-715.

Liu H, Prunolle F, Manica A, Balloux F. 2006. Geographically explicit genetic model of worldwide human-settlement history. *The American Journal of Human Genetics* 79: 230-237.

Loo JH, Trejaut J, Chu C-C, Lin M. 2008. Mitochondrial DNA diversity of Tao-Yami and Batan Islanders: relationships with other Taiwanese aborigines. Pages 349-355 in: Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Lum JK & Cann RL. 1998. mtDNA and language support a common origin of Micronesians and Polynesians in Island Southeast Asia. *American Journal of Physical Anthropology* 105: 109-119.

Manderscheid EJ & Rogers AR. 1996. Genetic admixture in the late Pleistocene. *American Journal of Physical Anthropology* 100:1-5.

Manica A, Amos W, Balloux F, Hanihara T. 2007. The effect of ancient population bottlenecks on human phenotypic variation. *Nature* 448: 346-348.

Matheson CD & Loy TH. 2001. Genetic sex identification of 9400-year-old human skull samples from Çayönü Tepesi, Turkey. *Journal of Archaeological Science* 28: 569-575.

Matisoo-Smith E & Allen JS. 2001. Name that rat: molecular and morphological identification of Pacific rodent remains. *International journal of Osteology* 11: 34-42.

Metisoo-Smith E & Robin JH. 2004. Origins and dispersals of Pacific peoples: evidence from mtDNA phylogenies of the Pacific rat. *Proc. Natl. Acad. Sci. USA* 101: 9167-9172.

Melchoir L, Kivislild T, Lynnerup N, Dissing J. 2008. Evidence of authentic DNA from Danish Viking age skeletons untouched by humans for 1,000 years. *PLoS ONE* 3: 1-8.

Melchoir L, Lynnerup N, Siegismund HR, Kivisild T, Dissing J. 2010. Genetic diversity among ancient Nordic populations. *PLoS ONE* 5: 1-9.

Merriwether DA, Friedlaender JS, Mediavilla J, Mgone C, Gentz F, Ferrell RE. 1999. Mitochondrial DNA variation is an indicator of Austronesian influence in Island Melanesia. *American Journal of Physical Anthropology* 110: 243-270.

Merriwether DA, Rothhammer F, Ferrell RE. 1994. Genetic variation in the New World: ancient teeth, bone, and tissue as sources of DNA. *Experientia* 50: 592-601.

Minogue K. 2010. Mummies for medicine. *Science* 3300: 1027.

Mitchell D, Willerslev E, Hansen A. 2005. Damage and repair of ancient DNA. *Mutation Research* 571: 265-276.

Miyamoto MM & Goodman M. 1990. DNA systematic and evolution of primates. *Annual Review of Ecology and Systematics* 21: 197-220.

Morgan J. 2008. First complete Neanderthal genome sequenced. *Science online* doi.1038/news.2008.1026.

Mulligan CJ. 2006. Anthropological applications of ancient DNA: problems and prospects. *American Antiquity* 71: 365-380.

Nerlich AG, Haas CJ, Zink A, Szeimies U, Hagedom HG. 1997. Molecular evidence for tuberculosis in ancient Egyptian mummy. *Lancet* 350: 1404.

Noonan JP, Coop G, Kudaravalli S, Smith D, Krause J, Alessi J, Chen F, Platt D, Pääbo S, Pritchard JK, Rubin EM. 2006. Sequencing and Analysis of Neanderthal genomic DNA. *Science* 314: 1113-1118.

Nordborg M. 1998. On the probability of Neanderthal ancestry. *Am. J. Hum. Genet.* 63: 1237-40.

Ohashi J, Naka I, Tokunaga K, Inaoka T, Ataka Y, Nakazawa M, Matsumura Y, Ohtsuka R. 2006. Brief communications: mitochondrial DNA variation suggests extensive gene flow from Polynesian ancestors to indigenous Melanesiansin the Northwestern Bismarck Archipelago. *American Journal of Physical Anthropology* 130: 551-556.

Olivieri C, Ermini L, Rizzi E, Corti G, Bonnal R, Luciani S, Marota I, De Bellis G, Rollo F. 2010. Characterizatin of nucleotide misincorporation patterns in the Iceman's mitochondrial DNA. *PLoS ONE* 5: 1-9.

Oppenheimer SJ & Richards M. 2001. Slow boat to Melanesia? *Nature* 410: 166-167.

O'Rourke DH, Carlyle SW, Parr RL. 1996. Ancient DNA: methods, progress, and perspectives. *American Journal of Human Biology* 8: 557-571.

O'Rourke DH, Hayes MG, Carlyle SW. 2000. Ancient DNA studies in physical anthropology. *Annu. Rev. Anthropol.*29: 217-42.

Ovchinnikov IV, Götherström A, Romanova GP. Kharitonov VM, Lidén K, Goodwin W. 2000. Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 404: 490-493.

Ovchinnikov IV & Kholina OI. 2010, Genome gigging: insight into the mitochondrial genome of *Homo*. *PLoS ONE* 5: 1-11.

Pääbo S. 1985a. Molecular cloning of ancient Egyptian mummy DNA. *Nature* 314:644-45.

Pääbo S. 1985b. Preservation of DNA in ancient Egyptian mummies. *J. Arch. Sci.* 12: 411-17.

Pääbo S. 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proceedings of the National Academy of Science USA* 86: 1939-1943.

Pääbo S. 1999. Human evolution. *Trends in Cell Biology* 9: M13-M16.

Pääbo S, Gifford JA, Wilson AC. 1988. Mitochondrial DNA sequences from a 7,000-year –old brain. *Nucleic Acids Research* 16: 9775-9787.

Pääbo S, Poinar H, Serre D, Jaenicke-Després V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M. 2004. Genetic analysis from ancient DNA. *Annu. Rev. Genet.* 38: 645-679.

Palmirotta R, Verginelli F, Di Tota G, Battista P, Cama A, Caramiello S, Capasso L, Mariani-Costantini. 1997. Use of multiplex polymerase chain reaction assay in the sex typing of DNA extracted from archaeological bone. *International journal of Osteoarchaeology* 7: 605-609.

Pawley A & Ross M. 1993. Austronesian historical linguistics and cultural history. *Annual Review of Anthropology* 22: 425-459.

Pennisi E. 2002. Ancient DNA untangles evolutionary paths. *Science* 295: 2197.

Pennisi E. 2007. No sex please, we're Neanderthals. *Science* 316: 967.

Pennisi E. 2009. Sequencing Neanderthal mitochondrial genomes by the half-dozen. *Science* 325: 252.

Petchey F, Spriggs M, Leach F, Seed M, Sand C, Pietrusewsky M, Anderson K. 2011. Testing the human factor: radiocarbon dating the first peoples of the South Pacific. *Journal of Archaeological Science* 38: 29-44.

Pilgrim KL, Boyd DK, Forbes SH. 1998. Testing wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *The Journal of Wildlife Management* 62: 683-689.

Poinar HN, Kuch M, Sobolik KD, Barnes I, Stankiewicz AB, Kuder T, Spaulding WG, Bryant VM, Cooper A, Pääbo S. 2001. A molecular analysis of dietary diversity for three archaic Native Americans. *Proc. Nat. Acad. Sci. USA* 98: 4317-4322.

Prüfer K, Stenzel U, Hofreiter M, Pääbo S, Kelso J, Green RE. 2010. Computational challenges in the analysis of ancient DNA. *Genome Biology* 11: R47-61.

Pusch CM & Bachmann L. 2004. Spiking of contemporary human template DNA with ancient DNA extracts induces mutations under PCR and generates non-authentic mitochondrial sequences *Molecular Biology and Evolution* 21: 957-964.

Rafi A, Spigelman M, Stanford J, Lemma E, Donoghue H, Zias J. 1994. DNA of *Mycobacterium leprae* detected by PCR in ancient bone. *International Journal of Osteoarchaeology* 4: 287-290.

Reich D,Green RE, Kircher M, Krause J, Patterson N, Durand EY, Viola B, Briggs AW, Stenzel U, Johnson PLF, Maricic T, Good JM, Marques-Bonet T, Alkan C, Fu Q, Mallick S, Li H, Meyer M, Eichler EE, Stoneking M, Richards M, Talamo S, Shunkov MV, Derevianko AP, Hublin J-J, Kelso J, Slatkin M, Pääbo S. 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* 468: 1053-1060.

Relethford JH. 2001. Absence of regional affinities of Neanderthal DNA with living humans does not reject multiregional evolution. *American Journal of Physical Anthropology* 115: 95-98.

Relethford JH. 2008. Genetic evidence and the modern human origins debate. *Heredity* 100: 555-63.

Ricaut F-X, Kolodesnikov S, Keyser-Tracqui C, Alekseev AN, Crubézy E, Ludes B. 2006. Molecular genetic analysis of 400-year-old human remains found in two Yakut burial sites. *American Journal of Physical Anthropology* 129: 55-63.

Rogan PK & Salvo JJ. 1990. Study of nucleic acids isolated from ancient remains.*Yearb. Phys. Anthropol.* 33:195-214.

Rohland N, Hofreiter M. 2007a. Comparison and optimization of ancient DNA extraction. *Biotechniques* 42: 343-352.

Rohland N, Hofreiter M. 2007b. Ancient DNA extraction from bones and teeth. *Nature Protocols* 2: 1756-1762.

Rohland N, Siedel H, Hofreiter M. 2004. Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. *Biotechniques* 36: 814-820.

Rollo F, Ubaldi M, Ermini L, Marota I. 2002. Ötzi's last meals: DNA analysis of the intestinal contents of the Neolithic glacier mummy from the Alps. *Proc. Nat. Acad. Sci. USA* 99: 12594-12599.

Rollo F, Venanzi FM, Amici A. 1994. DNA and RNA from ancient plant seeds. Pages 218-236 in: Herrmann B & Hummel S, eds. 1994. *Ancient DNA*. New York: Sringer-Verlag.

Römpler H, Dear PH, Krause J, Meyer M, Rohland N, Schöneberg T, Spriggs H, Stiller, M, Hofreiter M. 2006. Multiplex amplification of ancient DNA. *Nature Protocols* 1: 720-728.

Ross M. 2008. The integrity of the Austronesian language family: from Taiwan to Oceana. Pages 161-181 in: Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Ruano G, Fenton W, Kidd KK. 1989. Biphasic amplification of very dilute DNA samples via 'boster' PCR. *Nucleic Acids Research* 17: 5407.

Salo WL, Aufderheide AC, Buikstra J, Holcomb TA. 1994. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. *Proc. Natl. Acad. Sci. USA* 91: 2091-2094.

Sampietro ML, Lao O, Caramelli D, Lari M, Pou R, Marti M, Bertranpetit J, Lalueza-Fox C. 2007. Paleogenetic evidence of Neolithic spreading into Europe. *Proc. R. Soc. B* 274: 2161-2167.

Sanchez-Mazas A, Blench R, Ross MD, Peiros I, Lin M. eds. 2008. Past human migrations in East Asia. New York & Abingdon: Routledge.

Schultz Shook BA & Smith DG. 2008. Using ancient mtDNA to reconstruct the population history of Northeastern North America. *American Journal of Physical Anthropology* 137: 14-29.

Science Supplement. 1927. The Neanderthal Man. *Science* 66: 1715.

Seager RD. 1990. 'Eve' in Africa: human evolution meets molecular biology. *The American Biology Teacher* 52: 144-149.

Serre D, Langaney A, Chech M, Teschler-Nicola M, Paunovic M, Mennecier P, Hofreiter M, Possnert G, Pääbo S. 2004. No evidence of Neanderthal mtDNA contribution to early modern humans. *PLoS Biology* 2: 313-317.

Shearin NL, King EJ, O'Rourke DH. 1989. DNA preservation in Precolumbian remains from the American Southwest. *Human Evolution* 4: 263-270.

Shi H, Zhong H, Peng Y, Dong Y-L, Qi X-B, Zhang F, Liu L-F, Tan S-J, Ma RZ, Xiao C-J, Wells RS, Jin L, Su B. 2008. Y-chromosome evidence of earliest modern human settlement in East Asia and multiple origins of Tibetan and Japanese populations. *BMC Biology* 6: 45-54.

Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. 1993. A systematic approach to the sampling of dental DNA. *J. Forensic Sci* 38: 1194-1209.

Smith CI, Chamberlain AT, Riley MS, Cooper A, Stringer CB, Collins MJ. 2001. Not just old but old and cold? *Nature* 410:771-72.

Smith P & Kahila G. 1992. Identification of infanticide in archaeological sites: a case study from the Late Roman-Early Byzantine periods at Ashkelon, Israel. *Journal of Archaeological Science* 19: 667-675.

Speller CF, Kemp BM, Wyatt SD, Monroe C, Lipe WD, Arndt UM, Yany DY. 2010. Ancient mitochondrial DNA analysis reveals complexity of indigenous North American turkey domestication. *Proc. Natl. Acad. Sci. USA* 107: 2807-2812.

Spencer M & Howe CJ. 2004. Authenticity of ancient-DNA results: a statistical approach. *Am. J. Hum. Genet.* 75: 240-250.

Stone AC, Milner GR, Pääbo S, Stoneking M. 1996. Sex determination of ancient human skeletons using DNA. *American Journal of Physical Anthropology* 99: 321-238.

Stoneking M. 1995. Ancient DNA: How do you know when you have it and what you can do with it? *Am. J. Hum. Genet.* 57: 1259-1262.

Storey AA, Spriggs M, Bedford S, Hawkins SC, Robins JH, Huynen L, Matisoo-Smith E. 2010. Mitochondrial DNA from 3000-year old chickens at the Teouma site, Vanuatu. *Journal of Archaeological Science* 37: 2459-2468.

Suzuki K, Takigawa T, Tanigawa K, Nakamua K, Ishido Y, Kawashima A, Wu H, Akama T, Sue M, Yoshihara A, Mori S, Ishii N. 2010. Detection of *Mycobacterium leprae* DNA from archaeological skeletal remains in Japan using whole genome amplification and polymerase chain reaction. *PLoS ONE* 5: 1-8.

Szabo K. 2008. Review: From Southeast Asia to the Pacific: Archaeological perspectives on the Austronesian expansion and the Lapita Cultural Complex. *Archaeol. Oceania* 43: 128.

Tattersall I. 1995. The Fossil Trail: How We Know What We Think We Know About Human Evolution. Oxford University Press. New York & Oxford.

Tattersall I. 1999. The Last Neanderthal: The Rise, Success, and Mysterious Extinction of Our Closest Human Relatives. Nevraumont Publishing Company. New York.

Terrell JE. 2009. Review: Oceanic explorations: Lapita and Western Pacific settlement. *Archaeol. Oceania* 44: 42.

Thomas R. 1996. Novel mitochondrial DNA insertion polymorphism and its usefulness for human population studies. *Human Biology* 68: 847-854.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Vullo CM, Wallace DC. 1993. Asian affinities and continental radiation of the four founding Native American mtDNAs. *American Journal of Human Genetics* 144:1835-1850.

Trinkaus E & Shipman P. 1993. The Neanderthals: Changing the Image of Mankind. Alfred A Knopf. New York.

Underdown S. 2008. A potential for Transmissible Spongiform Encephalopathies in Neanderthal extinction. *Medical Hypotheses* 74: 4-7.

Van Blerkom LM. 2003. Role of viruses in human evolution. *Yearbook of Physical Anthropology* 46: 14-46.

Vilar MG, Kaneko A, Hombhanje FW, Tsukahara T, Hwaihwanje I, Lum JK. 2008. Reconstructing the origin of the Lapita Cultural Complex: mtDNA analysis of East Sepik Province, PNG. *J. Hum. Genet.* 53: 698-708.

Vives S, Gilbert MT, Arenas C, Gigli E, Lao O, Lalueza-Fox C. 2008. Statistical analysis of post mortem DNA damage-derived miscoding lesions in Neanderthal mitochondrial DNA. *BMC Research Notes* 1: 40-45.

Wall JD & Hammer MF. 2006. Archaic admixture in the human genome. *Current Opinion in Genetics and Development* 16: 606-10.

Wall JD & Kim SK. 2007. Inconsistencies in Neanderthal genomic DNA sequences. *PLoS Genetics* 3: 1862-1866.

Ward R & Stinger C. 1997. A molecular handle on Neanderthals. *Nature* 388: 225-226.

Wayne RK, Leonard JA, Cooper A. 1999. Full of sound and fury: the recent history of ancient DNA. *Annual Review of Ecology and Systematics* 30: 457-477.

Weaver RF. 2008. Molecular Biology, fouth edition. McGraw-Hill Companies Inc, New York.

Weaver TD & Roseman CC. 2005. Ancient DNA, late Neanderthal survival, and modern-human-Neanderthal genetic admixture. *Current Anthropology* 46: 677-683.

White TD. 1991. Human Osteology. San Diego: Academic.

Willerslev E & Cooper A. 2005. Ancient DNA. *Proc. R. Soc. B.* 272: 3-16.

Williams SR, Chagnon NA, Spielman RS. 2002. Nuclear and mitochondrial genetic variation in the Yanomamö: a test case for ancient DNA studies of prehistoric populations. *American Journal of Physical Anthropology* 117: 246-259.

Wilmshurst JM, Hunt, TL, Lipo CP, Anderson AJ. 2011. High-precision radiocarbon dating shows recent and rapid initial human colonization of East Polynesia. *Proc. Natl. Acad. Sci. USA* 108: 1815-1820.

Wilson MR, Polanskey D, Butler J, DiZinno JA, Replogle J, Budowle B. 1995. Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. *Biotechniques* 18: 662-669.

Wolinsky H. 2010. History in a single hair. *EMBO reports* 11: 427-429.

Wolpoff MH, Hawks J, Caspari R. 2000. Multiregional, not multiple origins. *American Journal of Physical Anthropology* 112: 129-136.

Wolpoff MH, Mannheim B, Mann A, Hawks J, Caspari R, Rosenberg KR, Frayer DW, Gill GW, Clark G. 2004. Why not the Neanderthals? *World Archeaology* 36: 527-46.

Woodward SR, King MJ, Chiu NM, Kuchar MJ, Griggs CW. 1994. Amplification of ancient nuclear DNA from teeth and soft tissues. *PCR Methods and Applications* 3: 244-247.

Xie CZ, Li CX, Cui YQ, Zhang QC, Fu YQ, Zhu H, Zhou H. 2007. Evidence of ancient DNA reveals the first European lineage in Iron Age Central China. *Proc. R. Soc. B* 274: 1597-1601.

Xing J, Witherspoon DJ, Ray DA, Batzer MA, Jorde LB. 2007. *Yearbook of Physical Anthropology* 50: 2-19.

Yang DY & Watt K. 2005. Contamination controls when preparing archaeological remains for ancient DNA analysis. *Journal of Archaeological Science* 32: 331-336.

Yang DY, ENG B, Waye JS, Dudar JC, Saunders SR. 1998. Technical note: Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* 105: 539- 543.

Yang H, Goldenberg EM, Shoshani J. 1997. Proboscidean DNA from museum and fossil specimens: an assessment of ancient DNA extraction and amplification techniques. *Biochemical Genetics* 35: 165- 179.

Yngvadottir B. 2007. Insights into modern diseases from our distant evolutionary past. *European Journal of Human Genetics* 15: 603-606.

Zhang F, Su B, Zhang Y-p, Jim L. 2007. Genetic studies of human diversity in east Asia. *Phil. Trans. R. Soc. B.* 362: 987-995.

Ziert H, Hummel S, Herrmann B. 1996. Amplification of human short tandem repeats from medieval teeth and bone samples. *Human Biology* 68: 185.

Zilhão J. 2006. Neanderthals and moderns mixed, and it matters. *Evolutionary Anthropology* 15: 183-195.

Zischler H, Geisert H, von Haeseler A, Pääbo S. 1995. A nuclear 'fossil' of the mitochondrial D-loop and the origin of modern humans. *Nature* 378: 489-492.

Glossary of Terms not Explained in Text

Δ261 deletion: a deletion of a guanine at position 261. It is what differentiates the O allele from the A allele in the ABO blood type.

Amelogenin gene: a gene that codes for a protein and is found in developing tooth enamel. It differs in a 112bp fragment between the X and Y chromosomes, and can be used for molecular sex determination.

Array-Based Sequence Capture: a technique used to sequence DNA from a DNA library. It utilizes several micro-arrays simultaneously, making it possible to sequence large amounts of DNA relatively quickly. The micro-arrays carry hundreds of thousands of probes that 'capture' DNA from the library on glass slides.

Assay: a molecular biology technique to look at an organism's molecular activity.

Buffer: a solution used to mollify the pH of a mixture that assists with PCR.

Control region: part of the mitochondrial DNA. It is a non-coding region and thus mutates more rapidly and is useful in distance, phylogeny, and migration studies, among others.

D-loop: comprises the control region along with promoter regions.

Denatured: The separation of two strands of DNA.

Depurination: the process by which a purine (adenine or guanine) is removed by hydrolysis. Amino acid racemization uses this to estimate the probability that a specimen contains useful DNA.

Dimerization: the result of the formation of two protein chain. This can interfere with PCR.

EDTA: ethylenediaminetetraacetic acid. It binds metal ions and is used as part of the buffer for PCR.

Endogenous DNA: DNA from the specimen.

Exogenous DNA: DNA from an outside source. A contaminating DNA.

Haplogroup: a group of phylogenetically related haplotypes.

Haplotype: a combination of alleles at a locus that identifies a certain group.

HVRI: Hypervariable Region I. Part of the D-loop of mitochondrial DNA that contains nucleotide repeats. Low resolution hypervariable region.

HVRII: Hypervariable Region II. Part of the D-loop of mitochondrial DNA that contains nucleotide repeats. High resolution hypervariable region.

numt: Mitochondrial (mt) DNA sequences that have inserted themselves in the nuclear (nu) genome sometime during the genome's evolution. They are useful as 'fossils' of past mitochondrial DNA. They are also referred to as 'insertions'.

Overhanging ends: The ends of a single strand of DNA that extends farther to the 5' or 3' end than its paired stand of DNA after it has been cut.

Paleogenomics: The sequencing and analysis of a substantial portion of either the mitochondrial or nuclear genome of an extinct species.

PCR: Polymerase Chain Reaction. Amplification of a region of DNA using primers that flank the region and repeated cycles of DNA polymerase action. The most common technique used to amplify aDNA.

Potassium salt: A salt used in the buffer to balance the pH.

Primer: A small piece of RNA that provides the free end needed for DNA replication.

Proteinase K: an enzyme that prevents nucleases from degrading DNA. It is used in the buffer.

Pyrosequencing: a method of DNA sequencing that tracks the nucleotides as they are incorporated into the DNAsequence by detecting the release of pyrophosphate.
Reagent: a compound used to start the PCR reaction.
Target sequence: The piece of DNA that one is attempting to amplify and sequence.
Tris-HCL: an organic compound that helps balance the pH of the buffer and inhibits protein enzymes.