Mitochondrial DNA Variation in Ancient and Modern Humans

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INTRODUCTION

Mitochondrial DNA (mtDNA) has been used extensively in recent years as a tool for the study of human evolutionary history. The human mtDNA genome is a closed circular molecule of approximately 16,569 base pairs in length (Anderson *et al.* 1981) located in the cellular cytoplasm. It is relatively simple, containing only 37 genes with no introns, and little other non-coding DNA besides the 1 Kb (Kilobase) control region. The simplicity and rapid rate of evolution of mtDNA make it suitable for phylogenetic studies over recent time scales, while the presence of thousands of copies of mtDNA in each cell favours their survival in compromised biological samples. The mtDNA genome is inherited in a strictly maternal fashion and evolution occurs by the accumulation of mutational changes through generations. The very high rate of accumulation of mutations (mtDNA evolves on average about 10 times faster than nuclear DNA) means that deleterious mtDNA mutations are important in human disease and ageing (Wallace 1995). It also means that there are numerous harmless mutations, either silent or in non-coding regions, which can provide convenient genetic markers for the reconstruction of human evolutionary history.

The study of the patterns of genetic diversity in modern human populations can help reconstruct past events, such as ancient migrations, population expansions, and bottlenecks. All humans have ancestors, so each person carries in his or her genes a record of their past history. However, the patterns of genetic diversity in present-day populations may occasionally obscure past events, for instance in the case of multiple migrations, recent invasions, or demic collapse resulting from infectious diseases or genocide. In order to achieve an accurate understanding of what happened in the past it becomes necessary to rely on additional information provided by historical reports or the archaeological record. Nevertheless, with the development of powerful techniques for DNA analysis, notably the polymerase chain reaction, the possibility of recovering genetic information directly from the bones or mummified remains of ancient peoples promises to provide exciting new insights into past historical events. The application of novel molecular biology methods to the analysis of compromised biological samples has been particularly relevant for forensic identification and has also helped to increase the rate of acquisition of valuable population genetic data.

MITOCHONDRIAL DNA AND HUMAN EVOLUTION

A notable example of the use of genetic markers, and specifically mtDNA, for the reconstruction of human evolutionary history was the 1987 study by the late Allan Wilson and colleagues at the University of California in Berkeley (Cann *et al.* 1987). These workers used high resolution restriction mapping of mtDNA from humans of different geographical origins to generate data to test the two contrasting models for the

evolution of anatomically modern humans. In the first of these models, known as the regional continuity model, modern humans are thought to have evolved in parallel from archaic *Homo erectus* in different parts of the world, with enough gene flow between populations of different continents to ensure that modern *Homo sapiens* evolved into a single biological species. The opposing hypothesis, known as the Out of Africa model, agrees with the view that archaic humans spread throughout the Old Word, but proposes that anatomically modern humans originated solely in Africa and eventually replaced the different species of archaic humans in the course of a second, more recent, expansion from Africa. Before this study was published, most palaeontologists tended to support a middle view between the two extremes and containing elements of both theories, but the mtDNA data, frequently misunderstood or overinterpreted, caused a deep polarization of views (Wolpoff 1989).

Wilson and colleagues observed markedly little variation in modern human mtDNA types, suggesting a recent common origin, as well as more variation in African mtDNA types than anywhere else, consistent with the view that African lineages are the oldest and had more time to accumulate mutations. Phylogenetic analysis by maximum parsimony suggested that all modern mtDNAs could be traced to a single individual, known as the African Eve, who lived in Africa about 200,000 years ago. The term African Eve, suggesting a single female ancestor for modern humans, undoubtedly contributed to some the controversy that greeted the results (Lewin 1987; Wainscoat 1987). Further studies by the Wilson group, based on sequencing of the hypervariable control region of mtDNA, confirmed that the coalescence time (origin of the single common ancestor) of the maximum parsimony tree was about 200,000 years ago, and that the deepest branches of the tree were in Africa, giving further support to the Out of Africa model (Vigilant *et al.* 1991). This model also agreed with the conclusion reached by the studies by Wainscoat *et al.* (1986) of β-globin gene variation in different human populations.

Although later studies have questioned the statistical validity of the mitochondrial DNA tree (Templeton 1992), genetic studies on modern human populations have generally revealed more variation in Africa than elsewhere. A recent restriction enzyme analysis of mtDNA variation revealed the most ancient of all the continent-specific lineages in African populations (Chen *et al.* 1995). On balance, although there are still many problems in the interpretation of phylogenetic data, the DNA evidence seems to point to Africa as the place of origin of modern humans.

MITOCHONDRIAL DNA POLYMORPHISMS AS ANTHROPOLOGICAL MARKERS

While these studies have helped clarify the general patterns of recent human evolution, more detailed studies of human populations of different geographic locations are required to address specific anthropological questions. In recent years much work has been done on the analysis of human mtDNA variation by restriction mapping and DNA sequencing to help shed light on the patterns of migrations of humans in different geographical regions, such as Africa, Europe, the Pacific and the Americas.

The Pacific area was one of the first regions of the world to be studied in detail by molecular anthropologists as it was settled relatively recently and provides an excellent scenario for testing models of human colonization. Although Australia and Papua New Guinea (PNG) were probably occupied as early as 50,000 years ago, the remote archipelagos of eastern Polynesia were only settled for the first time by humans in the last 1,000 years (Bellwood 1989). By measuring the degree of genetic diversity in

present day populations of PNG and assuming that this diversity had accumulated since the first human settlement, the Wilson group had a convenient method to calibrate the mitochondrial DNA clock. Unfortunately, this calibration assumed that all the variation in PNG had arisen since the first settlement and disregarded the effects of multiple migrations, problems inherent in most attempts to determine the rate of evolution of mtDNA. Estimates of the rate of mutation of mtDNA in humans have a very wide margin of error . This causes significant problems for the interpretation of relatively recent events in human prehistory, such as the settlement of the Pacific and the New World (Stoneking 1993).

One of the first anthropologically useful mtDNA markers to be identified was a deletion of 9 base pairs (9-bp) in a small non-coding region between the genes for cytochrome oxidase II and lysil transfer RNA (Wrischnik *et al.* 1987). The 9-bp deletion was observed at relatively high frequencies (5-40%) in individuals of Asian origin and was later found to be present in Polynesians at frequencies reaching fixation (100%). The occurrence of the 9-bp deletion throughout Asia and the Pacific, and its fixation in Polynesians, has been presented as evidence of the ultimately Southeast Asian ancestry of the Polynesians. This interpretation of the data is supported by the fact that the deletion is absent or virtually absent in Australia and in the highlands of PNG, areas which are presumably inhabited by the descendants of the first Australoid and Papuan settlers of the Pacific (Hertzberg *et al.* 1989; Stoneking & Wilson 1989).

Although the 9-bp deletion was originally thought to occur only in populations of Asian origin, including native Americans, it has subsequently been observed in African populations. It is now known that the deletion has occurred independently in different parts of the world, and that this region of the mtDNA genome is particularly sensitive to mutation. We have detected both the deletion of one of the 9-bp motifs as well as a 9-bp triplication in several European individuals.

In recent studies, we have observed the 9-bp deletion in the following Pacific populations, *inter alia*:

Eastern Polynesia:	Tahiti	96%
Central Pacific:	Samoa	100%
	Tonga	88%
	Fiji	66%
PNG Coast		42%
PNG Highlands		4%
Island SE Asia	Taiwan	35%
	Borneo	33%

Sequence data from the hypervariable region of mtDNA of Circum-Pacific populations have revealed further mtDNA polymorphisms that seem to be present exclusively in Polynesians (Hagelberg & Clegg 1993; Lum *et al.* 1994; Melton *et al.* 1995). This Polynesian mtDNA type is characterized by the 9-bp deletion and by base substitutions at positions 16,189, 16,217, 16,247 and 16,261 of the control region of the mtDNA genome. The Polynesian mtDNA type seems to derive from an ancestral type present in Asia and characterized by the 16,189 substitution and the 9-bp deletion. A second mutation occurred on this background, characterized by the additional substitution at 16,217. This mtDNA type is present in Taiwan and Borneo and also in the Americas (Schurr *et al.* 1990; Ballinger *et al.* 1992; Horai *et al.* 1991; our unpublished

observations), reflecting the Asian origin of the populations of the New World. The additional two substitutions 16,247 and 16,261 appear eastwards in the Pacific, with the Polynesian type accounting for about 80% of individuals sampled. The Polynesian mtDNA motif is also present at high frequency in Malagasy, showing that migrations from Southeast Asia did not only lead to the colonization of Oceania in the east, but also to Madagascar in the west (Soodyall *et al.* 1995).

MtDNA control region polymorphisms associated with the 9-bp deletion. The Polynesian mtDNA type (type i) derives from an ancestral type that can be traced back to Asia:

i)	16 189	16 217	16 247	16 261
ii)	16 189	16 217		16 261
iii)	16 189	16 217		
iv)	16 189			

Interestingly, we have observed the Polynesian mtDNA type in bone samples from prehistoric sites in Polynesia, although we failed to detect the polymorphisms in several bones from archaeological sites in Melanesia and the central Pacific that were occupied by supposedly proto-Polynesian Lapita settlers (Hagelberg & Clegg 1993). This would tend to argue against the simple scenario of a recent expansion of people from island Southeast Asia into Polynesia and would support the contention that the earlier settlers of island Melanesia may have expanded gradually into the central Pacific before the arrival of the Polynesians (Terrell 1986). Further research on ancient bone samples is needed before this question can be settled. This and other problems relating to the migration patterns of ancient peoples can only really be addressed using direct genetic evidence based on ancient DNA.

We recently carried out analyses on prehistoric skeletal remains from two archaeological sites in Easter Island to help resolve the long-standing question of the origin of the prehistoric Easter Islanders (Bahn & Flenley 1992). Twelve ancient individuals were analysed and in every case we detected the 9-bp deletion and the Polynesian-specific base substitutions, confirming the Polynesian ancestry of the original inhabitants (Hagelberg *et al.* 1994). Despite these results, evidence from archaeology and botany seems to suggest a certain amount of contact between Polynesia and South America. We are now embarked on a new ancient DNA project to investigate the genetic origins of the prehistoric inhabitants of the Pacific coast of South America.

ANCIENT DNA STUDIES

The field of ancient DNA was born in 1984 with the report by Wilson's group of the cloning of DNA from the skin of an extinct quagga (Higuchi *et al.* 1984). Initially, developments were slow due to the technical problems associated with the analysis of ancient DNA sequences, but the subject has enjoyed rapid growth in the last few years (three international conferences on ancient DNA studies have taken place, and the first issue of a journal dedicated to ancient DNA research will be published in Spring 1996), as well as an unprecedented amount of attention from the news media.

From early on, the polymerase chain reaction (Saiki *et al.* 1985) became the technique of choice in ancient DNA studies, as it permits the amplification of a specific DNA fragment from degraded organic samples, even in the presence of vast amounts of microbial DNA and other contaminants, and avoids some of the sequence artefacts associated with DNA cloning (Pääbo & Wilson 1988; Pääbo *et al.* 1989). Ancient DNA studies have been burdened from the start by technical problems, including the difficulty of extracting usable DNA from some types of tissues, contamination by modern DNA, and PCR inhibition by unknown compounds in the tissue extracts. Despite this, DNA has been extracted from plant and insect tissues of considerable antiquity (DeSalle & Grimaldi 1994) and there is even a debated claim of DNA isolated from dinosaur bones (Woodward *et al.* 1994).

Because of the interest generated by ancient DNA research, scientists are pressed to produce exciting results on very old and exotic materials, and much of the research has been of a headline-catching type, rather than of real scientific interest. Some research groups have shied away from work on human remains because of the contamination problems. Samples can become contaminated by people handling the bones or by small amounts of human DNA in common dust, as well as by the sequences generated in previous PCR reactions. Whereas in animal studies one can rely on phylogenetic inference for checking the validity of the results, in the case of human studies it may be difficult to distinguish between a genuine DNA sequence and one resulting from contamination (Hagelberg & Clegg 1991; Hagelberg 1994).

The forensic identification of human remains became an early target of ancient DNA techniques, as well as providing a way to test the validity of the analyses by comparing DNA extracted from the remains with DNA from close relatives of a presumed victim. Bone DNA typing by analysis of polymorphic nuclear microsatellite DNA has been used successfully in a number of forensic cases (Hagelberg *et al.* 1991; Jeffreys *et al.* 1992; Gill *et al.* 1994). In addition, despite the difficulty in calculating likelihood ratios based on mtDNA data, the analysis of mtDNA can be applied usefully to the identification of samples like single hairs, or skeletal remains that are too old or decayed to permit the amplification of single-copy nuclear sequences (Higuchi et al. 1988; Stoneking *et al.* 1991; Ginther *et al.* 1993; Holland *et al.* 1993).

To conclude, mtDNA provides a unique tool for the study of the genetic relationships of ancient and modern human populations, as well as for forensic identification. Although the strictly maternal inheritance of mtDNA means that it is highly susceptible to genetic drift and any results must be interpreted with caution, the relative simplicity of mtDNA and its usefulness in ancient DNA research will undoubtedly help maintain its important role in anthropology and forensic science.

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