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MITOCHONDRIAL DNA ANALYSIS OF SKELETAL REMAINS

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Abstract : Mitochondrial DNA analysis or mtDNA analysis is a valuable tool for the detailed examination of skeletal remains. While mtDNA analysis has to be used to identify people and provide information about maternal lineage, it may not be able to reveal particular details about an individual's personality or traits. DNA extraction from skeletal remains can be challenging due to the degraded and fragmented nature of the DNA. The control region, or Dloop, is a hypervariable non-coding area of mtDNA with large variable sites to identify individual or population differences. The mtDNA sequences are unchanging across time and are stable. So, there is a good chance that people with similar or identical mtDNA sequences had a common maternal ancestor. With the help of DNA sequencing technology, it is possible to determine the precise nucleotide order within a DNA molecule, yielding a comprehensive genetic profile for that individual.

IndexTerms - mtDNA analysis, Maternal lineage, Traits, Hypervariable, Nucleotide, Genetic profile

I. INTRODUCTION

Skeletal remains examination can provide a variety of difficulties for investigators. There is a chance of difficulty to identify victims, especially when bodies are burned, brokened pieces, sometimes seriously injured. Since, skeletal remains are regularly old, they may get become more worse, resulting in DNA damage. This may have more impact on the precision and accuracy of the str typing outcomes. Though the use of mtDNA sequencing, scientists may examine genetic variation both within and between groups, It has a great advantage on past population trends and patterns of human migration. Capillary electrophoresis can be used in the context of mtDNA sequencing to sort and examine mtDNA fragments produced throughout the sequencing procedure. Accurate nucleotide sequence determination of the mtDNA molecule is made possible by capillary electrophoresis, which enables high resolution separation of DNA fragments according to size and charge. The D-loop area, sometimes referred to as the control region, is the region that is usually targeted for study in mitochondrial DNA (mtDNA) sequencing. This region is helpful for population studies and genetic identification since it has non-coding sequences and is highly variable among individuals. (Niamh Nic Daeid et al. 2021 Sep 24).

Skeletal remains were found by two onlookers in 1996 near the Columbia River in Washington, USA. One of the oldest and most complete skeletons ever discovered in North America was revealed to be these bones. Kennewick Man's morphology led to the initial belief that he was of European heritage, but subsequent DNA study showed that he really belonged to an ancestral Native American group. Following the discovery, the scene was investigated by the Benton County Coroner's Office and local law enforcement. More skeletal remains were discovered strewn along the riverbank. They got in touch with archaeologists from the Burke Museum of Natural History and Culture at the University of Washington, which is located nearby, after realising the find might have historical significance. An extensive excavation of the site was carried out by archaeologists under the direction of forensic anthropologist Dr. James Chatters. In order to reconstruct the burial context, they meticulously removed the skeletal remains and related artefacts, recording the location of each bone and artefact.

The skeletal remains underwent thorough forensic examination by Dr. Chatters and his colleagues. They inspected the bones for evidence of illness, trauma, and other conditions that might have revealed details about the person's life and demise. The remains' ancient appearance was first noted, which prompted additional research and radiocarbon dating. Scientific and legal debates were aroused by the discovery of Kennewick Man. Under the terms of the Native American Graves Protection and Repatriation Act (NAGPRA), Native American tribes claimed the remains as their ancestors and asked to be returned for reburial. On the other hand, scientists contended that the remains were highly valuable from an archaeological and scientific standpoint. Kennewick Man's custody dispute was the subject of a protracted legal battle that resulted in numerous court decisions and appeals. In the meantime,

investigations into Kennewick Man's ancestry and origins, such as those involving DNA analysis and cranial morphology, confirmed his Native American ancestry.

Mothers only pass on their mother's mtDNA to their children, but nuclear DNA is inherited from both parents. Thus, understanding maternal ancestry, population migrations, and even specific genetic disorders can be gained through mtDNA analysis. Analysing and identifying genetic information encoded in a person's DNA is the main objective of DNA testing. All things considered, mtDNA sequencing is essential to many disciplines, including medical genetics, forensic identification, and the study of human evolution and population history.

Due to their gradual disintegration, highly degradable human body components like hair and nails do not contain viable DNA for analysis, making DNA typing impossible. In order to support legal procedures and guarantee that justice is done, forensic science provides objective analysis and evidence, which is essential to the medico-legal system. In forensic genetics, DNA samples from crime scenes, victims, suspects, and other pertinent sources are analysed in order to establish biological relationships, ascertain an individual's identity, and provide evidence for criminal investigations and court cases. Forensic genetics is a vital field that analyses DNA samples taken from remains in order to identify victims of mass disasters like aeroplane crashes or natural disasters. This procedure facilitates the return of remains to their native country and helps families find closure.

The geographical and socioeconomic divisions in Japanese society are emphasised by the dual structure model. It emphasises how wealth, opportunities, and resources are concentrated in urban areas while rural areas face depopulation, declining economies, and ageing populations. With its rich cultural legacy and ability to adapt to a variety of environments, the indigenous Jomon population represents an important period in Japan's prehistoric past and continues to contribute to our understanding of early East Asian human societies. In contrast to other developed nations, Japan still has a small immigrant population, but it is steadily increasing as a result of shifting demographic and economic conditions.

Evidence from archaeology and morphology in Japan sheds light on the nation's prehistoric past, patterns of human settlement, cultural evolution, and the physical traits of its initial occupants. Ancient burial mounds, known as "kofun," are connected to the Kofun period (250–538 CE) and can be found all over Japan. These colossal tombs, which are frequently fashioned like mounds or keyholes, hold artefacts, grave goods, and human remains that shed light on early Japanese society's religious beliefs, social structure, and customs surrounding funerals. Skeletal remains from burial sites and settlements have been discovered during archaeological excavations, and these remains offer important insights into the physical traits and general health of ancient Japan's residents. Researchers can better understand population movements, interactions, and adaptations over time by using anthropological studies of skeletal morphology, dental patterns, and genetic markers.

One of the most important tools in forensic investigations is DNA typing of bone samples, also referred to as forensic DNA analysis or forensic DNA typing. Nuclear DNA, which is located in cell nuclei, is the main subject of DNA analysis in bone samples. The increasing success of DNA typing in old bone samples is attributed to ongoing developments in forensic methodologies, sequencing technologies, and DNA extraction techniques.

Aged skeletal remains' mtDNA fragments are frequently amplified using polymerase chain reaction (PCR) amplification for analytical purposes. MtDNA testing on old skeletal remains sheds light on ancient population structures, demographic shifts, and genetic relationships, which is useful for population studies and anthropological research. Researchers can reconstruct population movements, find genetic traces of historical migrations, and look into how cultural and environmental factors affect genetic diversity by examining mtDNA variation in ancient skeletal remains. In DNA testing facilities, mtDNA comparisons between maternal relatives can be used as a quality control measure to guarantee the precision and dependability of mtDNA profiles. Examine the DNA sequencing data to compare the relatives' mtDNA or STR profiles. To determine genetic relatedness or maternal relationships, look for shared sequences or alleles.

Hypervariable regions are areas of the mitochondrial genome in human mitochondrial DNA (mtDNA) that show significant variation in sequence between individuals. Compared to other regions of the mitochondrial genome, these hypervariable regions accumulate mutations more quickly, making them especially helpful for genetic ancestry testing. The D-loop region, which includes the mtDNA's Hypervariable Regions 1 (HVR1) and 2 (HVR2), is frequently home to these C-rich sequences. (Masamune Kobayashi et al. December 2009).

Maternal inheritance is a possible mode of inheritance for heteroplasmy, with the amount of mutant mtDNA transferred from mother to child differing between generations. A lineage's degree of heteroplasmy may vary over time as a result of compensatory mechanisms, genetic drift, or selection.

With the use of NGS technologies, the complete mitochondrial genome can be sequenced, yielding detailed data regarding genetic variation, heteroplasmy, and population diversity. Through the use of NGS, forensic scientists can create mtDNA profiles from limited or deteriorated DNA samples, increasing the likelihood that they will find relevant evidence in criminal cases or investigations into missing persons. Sanger sequencing is still a dependable and popular method for sequencing short DNA fragments with high accuracy and precision, despite not having the same throughput as next-generation sequencing techniques. When identifying ancient human bone remains, combining the STR and mtSNP technologies can improve identification efforts' accuracy and dependability.

II. MtDNA SEQUENCING

Illumina created the MiSeq system, a portable, benchtop next-generation sequencing (NGS) platform. It is intended to deliver high-quality data output for smaller-scale sequencing projects, with flexibility, speed, and accuracy to suit various applications in clinical diagnostics, genomics research, and other domains. It might be necessary to fragment the DNA in order to obtain smaller, more manageable fragments for DNA sequencing.

Isolating the mitochondrial DNA from the sample of cells or tissues is the first step in the process. Several techniques can be employed for this, contingent on the kind of sample and the required level of mtDNA purity. Because of the extreme environmental conditions that bones can be exposed to and the limited quantity of DNA found in old or deteriorated samples, handling degraded DNA from bone fragments presents special challenges. The particular regions to be amplified in mitochondrial DNA (mtDNA)

sequencing rely on the objectives of the investigation or analysis. It can be challenging to isolate high-quality DNA suitable for sequencing from bone samples, particularly if they are old or deteriorated. Nonetheless, mtDNA can be extracted and sequenced from bone samples using cautious extraction and analysis methods.

It's crucial to remember that the degree of DNA damage and the efficiency of the extraction and analysis techniques used determine whether genetic analysis on burned bone fragments is successful. Applications such as population genetics, evolutionary studies, and forensic identification can benefit greatly from the information that mtDNA genotyping offers. Researchers can study genetic variations and relationships in populations or individuals by efficiently preparing bone tissue samples for mitochondrial DNA genotyping.

After being isolated, the mtDNA is broken up into manageable chunks and ready for sequencing. The research or diagnostic objectives, such as amplifying the whole mitochondrial genome, particular gene regions, or identifying known mutations, determine which primers are best. It's critical to take into account various aspects when choosing a PCR kit for mtDNA analysis, including the particular application, the target region or regions of interest, the assay's sensitivity and specificity, and its compatibility with subsequent methods like genotyping or sequencing. DNA polymerases with improved processivity and proofreading skills are commonly used in long-range PCR to guarantee accurate and effective amplification over longer DNA templates. Long-range PCR lowers the need for multiple PCR reactions and streamlines downstream processing by amplifying large genomic regions in a single reaction, which saves money and time in molecular biology workflows. In genomics, molecular biology, diagnostics, and other fields where long DNA sequences need to be amplified, long-range PCR is a useful technique for amplifying large DNA fragments (Mitchell M. Holland et al. June 1993). Approximative quantification can be obtained by comparison with known DNA concentrations or DNA size standards. To produce DNA fragments of a particular size range appropriate for sequencing library preparation, DNA fragmentation is required.

III. DNA EXTRACTION FROM SKELETAL REMAINS

There are commercial DNA extraction kits available that offer pre-made buffers and purification columns, making the process simpler. Through customisation of DNA isolation techniques to particular sample kinds and subsequent uses, scientists can procure superior DNA appropriate for an array of molecular biology methods. To prevent contamination during sample handling and processing, adhere to stringent laboratory protocols that include donning gloves, using disposable equipment, and maintaining aseptic techniques. In order to maximise contamination from outside sources and guarantee accurate results when sequencing bone fragments using mitochondrial DNA (mtDNA), sample decontamination is an essential step. It's crucial to remember that different protocols for decontaminating bone fragments may be needed based on things like the intended use, institutional policies, and legal requirements. Compared to earlier sequencing techniques, the amount of starting material needed for high-throughput sequencing techniques like next-generation sequencing (NGS), which are frequently employed in ancient DNA research, can be comparatively small. Because bone fragments include inhibitors and degradation factors, extra care must be taken when extracting DNA, particularly when sequencing mitochondrial DNA (mtDNA). Purify the mtDNA from nuclear DNA, RNA, proteins, and other impurities after DNA extraction.

The goal of skeletal DNA extraction techniques is to obtain DNA from skeletal remains for various purposes, such as forensic identification, archaeological research, and population genetics studies. The stable matrix of calcium phosphate minerals makes up the majority of bones. By forming a physical barrier, this mineralization aids in shielding DNA molecules from enzymatic degradation. Mild heating can be used to help break down proteins and disrupt cellular structures during DNA purification, releasing DNA in the process. However, keep in consideration that too much heat can damage DNA when working with bone samples. Thus, it's important to strike a balance between minimising DNA damage and effectively disrupting cellular structures. All things considered, it is possible to obtain complete DNA profiles from soft tissues; however, in order to obtain accurate results, sample quality, preservation, extraction techniques, and contamination control have to be carefully considered. Since exposure to high temperatures can cause DNA to degrade and fragment, creating DNA profiles from burned bodies can be difficult. Yet, depending on the degree of burning and the state of the DNA, it is still possible to extract DNA for analysis from burned remains, albeit with variable degrees of success. Molecular biology labs frequently use liquid nitrogen for a variety of purposes, such as pulverising or grinding samples in preparation for DNA extraction. In general, applying heat to bone samples during the DNA extraction procedure can improve the effectiveness and consistency of DNA recovery, especially for difficult samples like old or crumbling bones.

The efficiency and accuracy of this process have been greatly enhanced by recent developments in skeletal extraction techniques. These developments are intended to improve sensitivity, lower background noise, and raise the success rate of DNA profiling especially for difficult-to-probable sample types like low-copy-number or degraded DNA. Gaining insight into the intricate relationship between skeletal components and DNA yield is crucial to enhancing forensic procedures, raising the effectiveness of DNA profiling, and developing the discipline of forensic anthropology. Spongy and compact bones have different microstructural properties that can affect how well DNA is extracted.

To extract high-quality DNA from bone fragments, efficient techniques for DNA extraction are essential. Commonly employed techniques include phenol-chloroform extraction, silicabased column purification (e.g., using commercial kits), or magnetic bead-based methods (Joanna Jakubowska et al. 2012). In forensic and archaeological contexts, polymerase chain reaction (PCR) is frequently utilised to sequence mitochondrial DNA (mtDNA) from bone fragments. This technique allows for the analysis of old or deteriorated DNA samples. Every PCR run includes controls to check for contamination and gauge PCR efficiency. The PCR products are purified to get rid of unincorporated primers, nucleotides, and enzymes after PCR amplification. When sequencing bone fragments using mitochondrial DNA (mtDNA), PCR inhibitors can present serious difficulties, especially when working with old or deteriorated DNA samples. In order to overcome the obstacles presented by PCR inhibitors, careful sample preparation, optimised DNA extraction techniques, and altered PCR conditions are necessary for the successful sequencing of bone fragments'

mitochondrial DNA (mtDNA). Selecting DNA extraction kits made especially to reduce inhibitor co-extraction and optimise DNA yield from difficult samples, like bone fragments, is crucial when working with PCR inhibitors in DNA extraction.

IV. ANALYSIS OF DNA

The study of an organism's DNA to determine its genetic composition, variability, and functions is known as genetic analysis. In forensic science, genetic testing is used to determine biological relationships or to identify individuals during legal investigations. The majority of an organism's genetic material is found in its nuclear DNA, but mitochondrial and chloroplast DNA have different inheritance patterns and are essential to photosynthesis and energy production, respectively. Different regions of nuclear DNA in the human genome are used for different genetic analysis and research purposes.

DNA analysis can take many different forms, each with its own set of techniques and applications. DNA analysis of bone samples can be useful in forensic identification, archaeology, and anthropology. Precautions must be taken at every stage of the extraction process to guarantee the integrity of the DNA, avoid contamination, and maximise purity and yield. Analysis of the generated DNA profiles identifies genetic markers or alleles at particular loci. When DNA profiles match, it is most likely that the samples came from the same person or biological source. Single nucleotide polymorphisms, or STRs, are frequently employed genetic markers in forensic DNA analysis (SNPs). (Sayed A M Amer et al. 2017). DNA profiles from samples of evidence are compared to reference samples that are known, such as databases containing information on known people, suspects, or victims. DNA profile matching can be used to establish identity or link suspects to crime scenes.

The analysis of forensic DNA is extremely important for many areas of criminal investigations, court cases, and the legal system. DNA databases containing profiles from crime scene evidence, unidentified human remains, and missing persons are frequently kept up to date by forensic laboratories. By comparing DNA profiles, these databases assist forensic scientists in identifying victims, establishing connections between people, and connecting crimes.

V. MtDNA ANALYSIS

Cells' mitochondria contain mitochondrial DNA, or mtDNA. Compared to nuclear genomes, mitochondrial genomes are usually smaller, circular, and have fewer genes. Because mitochondrial DNA is only inherited from the mother, it can be used to trace maternal lineages in genetic studies like population genetics and ancestry testing. In most organisms, including humans, mitochondrial DNA (mtDNA) is inherited through a uniparental pattern, specifically maternal inheritance. One can study evolutionary relationships among species, populations, and individuals using mtDNA because it evolves more quickly than nuclear DNA and is not as constrained by evolutionary rules. MtDNA analysis can aid in the identification process in mass disaster scenarios where traditional methods of identification may be difficult owing to extensive tissue damage or mingling of remains.

DNA repetitive sequences known as STRs are made up of tandemly repeated motifs that are usually two to six base pairs long. It was once thought that, in contrast to nuclear DNA, which frequently undergoes recombination during meiosis, mtDNA is exclusively inherited by the mother and does not undergo recombination. Nonetheless, certain research has indicated the potential for recombination events to transpire in mtDNA, although with extremely low frequencies and specific conditions. It has been stated that different mtDNA molecules within a cell may occasionally undergo recombination-like events that result in the creation of unique mtDNA genotypes. Although it's not as often used as nuclear DNA, mitochondrial DNA (mtDNA) can be used in forensic investigations to determine an individual's age at death. Relationships between people can be examined using mitochondrial DNA (mtDNA) analysis, especially those involving mothers. Since mtDNA is inherited maternally and does not recombine, it is passed from mothers to their progeny essentially unaltered. Nevertheless, mtDNA analysis can still be helpful in forensic investigations by offering extra proof to support or refute the identity of the deceased, particularly if there are direct maternal relatives to compare with.

Since the late 1980s, mitochondrial DNA (mtDNA) analysis has been applied to a wide range of fields, such as population genetics, evolutionary biology, forensic investigations, and medical diagnostics. The human mtDNA genome is a circular molecule with 16,569 base pairs in average. The control region, sometimes referred to as the displacement loop (D-loop), is one of the most frequently examined regions since it houses the regulatory elements necessary for transcription and replication of mtDNA (Kim et al. 2013). Since the control region varies greatly between people, it can be helpful in determining maternal lineages and ancestry. On the other hand, NGS technologies greatly sped up sequencing while lowering costs, making it possible for researchers to sequence transcriptomes, epigenomes, and entire genomes more quickly.

Research has shown how early humans left Africa, how different regions were populated, and how ancient populations interacted with one another. The mtDNA variation of the Indian subcontinent is indicative of its high genetic diversity. Research has demonstrated that historical migrations, interactions, and population movements have shaped the complex genetic structure found in Indian populations. In comparison to populations on the mainland, organisms inhabiting islands frequently have smaller populations and are more vulnerable to genetic drift. Since mtDNA is more abundant and better preserved in ancient remains than nuclear DNA, it is frequently used in phylogenetic analyses of ancient humans. Comparisons between various historical eras and geographical locations are possible thanks to

phylogenetic analysis of ancient and modern populations. Because of its higher mutation rate, mtDNA is especially helpful for researching population dynamics and recent evolutionary events.

This distinct inheritance pattern has consequences for how mitochondrial diseases develop and spread. Most mitochondrial illnesses are inherited from mothers, which means that mothers pass them on to their children. However, variables like the particular mutation, the degree of heteroplasmy, and the existence of nuclear genetic modifiers can affect the severity and penetration of mitochondrial diseases.

VI. DNA DATABASES

DNA profiles in databases are generally sets of genetic data that are kept organised for forensic or scientific reasons. DNA databases are useful for many things, such as identifying missing people and solving crimes as well as advancing science and developing better medical procedures. A variety of legal frameworks, regulatory policies, and ethical guidelines that aim to strike a balance between the protection of privacy and individual rights and the pursuit of justice oversee the use of DNA databases in the forensic community. Relational database structures are common in DNA databases, where each DNA profile is linked to metadata including the subject's name and birthdate, sample collection details (date and location), and any pertinent case information (crime scene details, case number). By giving law enforcement agencies an effective tool for identifying suspects, solving crimes, and apprehending offenders, CODIS plays a crucial role in forensic investigations in the US and other nations. Indexing DNA databases facilitates fast searching and profile comparison.

The Central Forensic Science Laboratory (CFSL) in India founded the NDDI, which houses DNA profiles from convicted criminals, suspects, and crime scenes. Its goal is to support law enforcement organisations in their efforts to identify and solve criminals. DNA samples can be sent to the NDDI database by authorised personnel for analysis and database inclusion. Authorised users can perform searches and analyses to compare DNA profiles from crime scene evidence with profiles belonging to known individuals once the profiles have been stored in the NDDI database. Unauthorised access to DNA profiles may also result in privacy violations or unethical or discriminatory use of genetic data. It's critical to strike a balance between the defence of civil liberties, individual privacy rights, and ethical principles and the possible advantages of DNA databases in crime solving.

In 1995, the United Kingdom established the first large-scale DNA database (Lauren Wilson. 2023). DNA profiles need to be entered into the database according to certain procedures, which should include data security and accuracy verification. Since the analysis of STRs forms the foundation of many forensic DNA databases, switching to SNPs might necessitate modifications to analysis protocols and database architecture. Commercial DNA databases, like those provided by 23 and Me and Ancestry DNA, enable people to learn more about their genetic heritage and establish connections with relatives through shared DNA. These developments have made DNA analysis workflows more scalable and efficient, enabling the quick creation and processing of DNA profiles in forensic labs and other environments.

Profiles from forensic databases, law enforcement organisations, academic institutions, and commercial DNA testing businesses may be included in the database. DNA databases are kept up to date for a variety of uses by numerous nations worldwide, such as forensic investigations, criminal justice, medical research, and genealogy projects. The national and regional DNA data banks have been the focus of the Indian government's efforts. Genetic counsellors are medical professionals with training in genetics and counselling who can offer individuals and families support and advice on DNA databases and genetic testing. Medical experts may help with DNA evidence analysis, person identification, and expert witness testimony in court cases in forensic settings. Deceased individuals may provide anthropological data, such as age, sex, ancestry, and physical characteristics. DNA databases containing the personal information of deceased individuals can be utilised to support forensic inquiries related to cold cases, mass casualties, and murders.

VII. MITOCHONDRIAL VARIATION IN HUMAN POPULATION

In genetic studies, mitochondrial DNA, or mtDNA, is frequently used to track population migrations and maternal ancestry. Researchers can create phylogenetic trees and understand the evolutionary history of human populations and other organisms by comparing mtDNA sequences from different individuals. Researchers have been able to extract and sequence mtDNA from ancient samples, such as fossils and archaeological remains, has a great advancements in mtDNA sequencing technology. Comparing the sequences of individual mitochondrial genomes within a population or between populations can help identify polymorphic sites in mtDNA sequencing data. A "common minor allele" in mitochondrial DNA (mtDNA) sequencing is a variation of a specific mtDNA nucleotide position that is found in a population at a lower frequency than the predominant allele at that position.

The human consensus sequence provides insights into the variability and diversity of mtDNA sequences within the human population, whereas the rCRS is the standard reference for mtDNA research (Robert W. Carter. 2007 May). The study of genetic variation within the mitochondrial genome and the reconstruction of evolutionary relationships among various mtDNA sequences are two aspects of the haplotype and phylogenetic analysis of normal mitochondrial DNA (mtDNA). Numerous mitochondrial diseases are linked to mutations in the mtDNA.

Mothers pass on mutations in their mtDNA sequences to their offspring. Information regarding population divergence, genetic diversity within and between populations, and human migration patterns can be obtained through the analysis of mtDNA mutations. In a phylogenetic tree, branches stand for evolutionary lineages or lineages. Haplogroups are used in mitochondrial DNA (mtDNA) sequencing to categorise people into broad ancestral groups according to patterns of genetic variation found in their mtDNA (Dan Mishmar et al. November 15, 2002). Researchers connect past migration routes by studying various types of evidence, including archaeological findings, linguistic patterns, genetic data, and other historical records.

Genetic variation in human populations refers to variations in individual and group DNA sequences. Certain mtDNA haplogroups have a long evolutionary history in humans and may be more common in particular populations as a result of past population divergences and migrations.

VIII. RESULT AND DISCUSSION

RESULT

The nucleotide sequence of the mtDNA can be found using Sanger sequencing or more recent techniques like next-generation sequencing (NGS). Processing bones in order to extract DNA may be necessary, depending on the state of the skeletal remains. The Sanger sequencing technique, commonly referred to as chain termination sequencing, is used to carry out the sequencing reaction. Specialised techniques are needed for DNA extraction from deteriorated remains in order to maximise DNA recovery and reduce contamination. After the complete mtDNA genome is acquired, it can be examined for a number of reasons, including population genetics, evolutionary biology, genetic variation, and forensic identification.
Gathering skeletal remains from Vietnam War-related sites, including battlegrounds, crash sites, and locations where soldiers were stationed. Using methods like next-generation sequencing (NGS) or Sanger sequencing, depending on the quantity and quality of the available DNA.
During archaeological excavations at the Caddo Mounds site in 1932, seven burials were found. Burials found at the Caddo Mounds site have shed important light on the burial customs, way of life, and cultural practices of the Caddo people in the seventeenth century (Angie Ambers et al. March 2021). Skulls missing from anthropological studies of Caddo burials could also be the result of biassed sampling or insufficient skeletal remains recovered during archaeological digs. Researchers can use molecular biology methods like DNA sequencing and polymerase chain reaction (PCR) amplification to extract and analyse mitochondrial DNA (mtDNA) from skeletal remains found in Caddo burials. Researchers can assign individuals to specific maternal lineages and geographic populations by comparing mtDNA sequences from Caddo burials to reference databases of known mtDNA haplogroups.
Yayoi period human skeletal remains from the Doigahama site were analysed using mitochondrial DNA (mtDNA) (Kazunari Igawa et al. 2009). Archaeologists carefully remove the remains from the site to ensure there is no contamination or damage. The mtDNA extracted from the skeletal remains is sequenced using next-generation sequencing technologies. Yayoi period human skeletal remains from the Doigahama site were subjected to mitochondrial DNA analysis, which sheds light on the genetic heritage, demographic makeup, and migration patterns of the ancient Japanese people during this crucial epoch of cultural change.

In these literature studies, analysis of bone fragments were done in mass disasters, war and archaeological excavations. The data shows a clear report of prehistoric times when human remains are found from a place. When analysing bone fragments for geographic information, one must look at the elemental concentrations and isotopic composition of the skeletal remains to determine the origins and movements of the individual (J.S Sehrawat et al. 28 April 2022). By analysing these bone fragments, it is possible to identify timing of human occupation, population dynamics, cultural changes, and interactions with the environment.
Approaches for mtDNA analysis used in the reviewed studies, including data analysis techniques, sequencing methods, and PCR amplification.

8.1 Data analysis techniques

We introduce haplogroups, Certain sets of genetic variations, or mutations, in the mitochondrial genome, inherited maternally and passed down through the generations, characterise haplogroups. In population genetics research, haplogroups are utilised to evaluate genetic diversity, population structure, and relationships.
Genetic diversity, structure, and relationships among ancient populations are evaluated through population genetics analysis. Determining genetic clusters, calculating admixture proportions, and determining migration patterns are all part of population structure analysis.
To determine the evolutionary relationships between mtDNA sequences, phylogenetic reconstruction is used. Phylogenetic reconstruction is frequently used in population genetics to examine genetic data and clarify the evolutionary history of populations or species. Based on genetic information, phylogenetic reconstruction offers a strong framework for examining evolutionary relationships and processes, enabling researchers to deduce the origins and diversification of biological entities.

8.2 Sequencing methods

In mtDNA analysis, PCR-amplified fragments of the mitochondrial genome are sequenced using fluorescently labeled dideoxynucleotides (ddNTPs) to terminate DNA synthesis at each nucleotide position. Using DNA polymerase and a combination of deoxynucleotide triphosphates (dNTPs) and a tiny amount of dideoxynucleotide triphosphates (ddNTPs), new DNA strands complementary to the template DNA are created during the sequencing reaction. The pattern of bands on the gel is analysed to determine the DNA sequence. The accuracy, dependability, and capacity to sequence relatively long DNA fragments are wellknown attributes of Sanger sequencing.
NGS platforms like PacBio, Ion Torrent, and Illumina can be used to sequence particular regions of interest or the entire mitochondrial genome in mtDNA analysis. NGS platforms use various sequencing-by-synthesis techniques to ascertain the DNA fragments' nucleotide sequences within the clusters. Following sequencing, bioinformatics tools and software are used to process the raw data produced by the NGS platform and turn the raw signals into DNA sequences.

8.3 PCR Amplification

PCR amplification is very crucial MtDNA sequencing because mitochondrial DNA is present in cells in low copy numbers, PCR amplification is crucial for mtDNA sequencing because it enables researchers to selectively amplify the target regions of interest for sequencing, even from small or damaged samples.

8.4 Haplogroup Diversity

Geographically, haplogroup diversity differs, with some haplogroups being more common in particular areas or populations. Researchers can reconstruct historical population dynamics and monitor changes in haplogroup diversity over time by analysing ancient DNA from skeletal remains found in archaeological sites. Haplogroup assignments based on mtDNA analysis can be very helpful in forensic genetics when determining the maternal ancestry of unknown individuals.

8.5 Regional Variation

Regional variation in genetic diversity in mtDNA profiles can be caused by a variety of factors, including genetic drift, population size fluctuations, migration, and geographic isolation. Understanding the historical migration routes and colonisation patterns can be gained by tracking the distribution of ancestral haplogroups.

8.6 Temporal changes

Through the analysis of mtDNA diversity across various archaeological or historical eras, researchers can monitor shifts in population demographics and genetic makeup over time. More resolution and accuracy can now be achieved when studying temporal changes in mtDNA profiles thanks to advancements in analytical techniques and DNA sequencing technologies.

DISCUSSION

MtDNA is inherited from mothers, it is a perfect tool for determining maternal lineages and gaining insight into the demographic history of human populations via maternal lineage. This makes it possible for scientists to examine ancient human populations' genetic diversity and demographic makeup directly from their remains. Researchers can deduce ancient migration routes, colonisation events, and interactions between human populations by comparing mtDNA sequences from various historical periods and geographical locations. It can be difficult to recover intact DNA sequences due to fragmentation and chemical modifications caused by DNA degradation over time. Ancient DNA analysis is highly susceptible to contamination with contemporary DNA, especially in lab settings where modern DNA can be introduced during DNA extraction, PCR amplification, and sample processing. Many of the difficulties involved in analysing MtDNA from ancient bone fragments can be resolved and trustworthy insights into the history and evolution of the human population can be produced by researchers by putting strong protocols for sample handling, DNA extraction, contamination control, and data analysis into place.

Depending on a number of variables, including burial customs, preservation settings, and historical contexts, the human skeletal remains from the Dohigama sites in Japan and the remains discovered during the Vietnam War may show both similarities and differences. Anthropological examination of both sets of remains would ascertain details like age at death, sex, stature, ancestry, and signs of trauma or illness. In both situations, it is crucial to comprehend the cultural background of the funerals. These two sets of remains are historically significant and could help us comprehend historical occurrences and human experiences.

The Vietnam War remains are linked to a particular conflict in Southeast Asia, whereas the Dohigama sites in Japan are ancient burial grounds that date back thousands of years. The degree of deterioration experienced by the Vietnam War remains could vary depending on the surrounding environment, the manner of burial, and the post-mortem procedures. Given their age, the skeletal remains from the Dohigama may have experienced various taphonomic processes over time. The Vietnam War remnants could consist of people who lost their lives in battle, as POWs, or as innocent bystanders. The skeletal remains found in Dohigama may be those of people who passed away from illnesses, mishaps, or natural disasters. The identification of individuals from Vietnam War remains may be impeded by incomplete records, commingling of remains, and difficulties in obtaining DNA samples. The preservation, age determination, and context interpretation of the Dohigama skeletal remains could pose difficulties. Their importance for comprehending human history and offering perceptions into past lives and events is what unites them.

When conventional methods prove to be unfeasible or inadequate in the event of a mass disaster, such as aeroplane crashes, terrorist attacks, or natural disasters, mtDNA analysis can help identify the victims. Forensic scientists can match DNA evidence to specific individuals, establish familial relationships, and uncover crucial leads in criminal investigations and cases involving missing persons by examining genetic markers found in forensic DNA profiles, such as single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs). In relation to genetic privacy, data sharing, and equity in genomic research and healthcare, population genetics studies bring up moral, legal, and social issues. Anthropologists reconstruct the evolutionary pathways leading to the emergence of modern humans by examining fossil remains, artefacts, and environmental contexts. This process sheds light on our evolutionary history and biological adaptations. Skeletal remains are examined by forensic anthropologists to determine biological traits like ancestry, age at death, sex, stature, and distinguishing features. In cases of homicide, large-scale disasters, or missing people, this information aids law enforcement organisations, medical examiners, and families by assisting in the identification of the deceased, determining the cause of death, and bringing closure to the family.

IX. CONCLUSION

Skeletal analysis's ultimate objective in forensic situations is frequently person identification. Before mtDNA analysis was a standard tool in forensic casework, several more years would pass. Isotope analysis on skeletal remains can yield important details about the diet, place of origin, and occasionally even lifestyle of the individual.

With the integration of advancements in multiple scientific fields, human identification technologies have undergone significant evolution over time. People have been identified in a variety of forensic contexts, such as missing persons cases, mass disasters, and historical investigations, thanks to the use of mtDNA loop regions in forensic DNA analysis. A popular platform for next-generation sequencing (NGS) that makes high-throughput sequencing of DNA samples possible is the Illumina MiSeq system.

Comprehending taphonomic elements like interment customs, surrounding circumstances, and post-depositional disruptions can aid in interpreting the Dohigama sites' archaeological context and integrity. The genetic connections amongst various Yayoi period populations can be ascertained through mtDNA diversity analysis. It can be used to determine the degree of genetic affinity or isolation between different genetic clusters or population subgroups. The eating patterns and means of subsistence of the people interred at the site can be deduced from isotope analysis of dental enamel or bone collagen.

Through genetic data comparison with contemporary populations and other archaeological sites, researchers are able to piece together historical population histories, patterns of migration, and genetic continuity or discontinuity over time. Juvenile skulls may have exposed growth lines signifying periods of rapid growth or unfused cranial sutures, while adult skulls typically have fully fused sutures. There are additional age-related hints found in the size and shape of the skull. An analysis of skeletal pathologies, including infections, degenerative joint diseases, fractures, and signs of nutritional deficiencies, sheds light on the general state of health and disease burden among adult Yayoi people. Skeletal trauma patterns can also provide information about workplace dangers and interpersonal violence.

The individual's haplogroup and genetic affinity are ascertained by comparing the mtDNA sequences extracted from the cranium sample with databases of human mitochondrial genomes that are currently available. In order to preserve as much of the skeleton as possible while still providing sufficient genetic material for analysis, the sample size is usually small.

Researchers can learn more about ancient populations and their interactions by comparing mtDNA from various samples. This helps to unravel the intricate story of human history. Aged bones, especially those from archaeological sites, are more difficult to analyse because of a number of factors, including contamination, deterioration, and preservation problems. In genetic analysis, mtDNA and nDNA analysis play complementary roles. While nDNA provides more comprehensive insights into individual traits, genetic disorders, and population diversity, mtDNA is frequently used for population genetics and maternal lineage tracing

X. FUTURE ADVANCEMENTS

Advances in forensic genomics have made it possible to predict physical characteristics from DNA evidence, including hair, eye, and facial morphology colour. The feasibility and reliability of mtDNA sequencing from skeletal remains have been greatly improved by advances in PCR amplification techniques, giving forensic scientists and archaeologists the ability to extract genetic information from ancient and degraded DNA samples that holds great genetic value. Using phylogenetic analysis, NGS and MPS data can be utilised to investigate the evolutionary relationships between various mitochondrial haplogroups and populations. Using phylogenetic analysis, Next Generation Sequencing (NGS) and Massive Parallel Sequencing (MPS) data can be utilised to investigate the evolutionary relationships between various mitochondrial haplogroups and populations (High-throughput sequencing platforms like Illumina, Ion Torrent, and PacBio are able to process many samples at once because of automation. Reliability of mtDNA sequence data is increased and detection of low-frequency variants is made more accurate by increasing the depth of coverage during NGS. Researchers can lower sequencing errors and reliably identify true genetic variants in the sample by sequencing the same region more than once. Although the price of mtDNA sequencing can vary based on the technology used and the coverage depth, it is typically less expensive than nuclear genome sequencing. All things considered, these developments keep improving the potential of forensic DNA analysis, aiding in the investigation of crimes, clearing the guilty, and providing comfort to the bereaved and their loved ones.

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