REVIEW

Ancient DNA: revisiting past through population genomics

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ABSTRACT

In the last two decades, advances in human population genetics & novel anthropometric approaches have played an essential role in understanding human population structure, their migration and adaptation toward a particular environment. Genetic haploid markers such as mitochondrial DNA (mtDNA), Y chromosomal DNA (NRY- non recombining region of Y chromosome) & 3 Dimensional morphometric (craniofacial measurement of human skull) have proven to be one of the best tools to learn about the prehistory as well as to test various models in the course of evolution of modern humans. Studies on the origins of modern humans in the Indian subcontinent has taken two different approaches – one, focusing on the archaeological record of the initial settlement of our species in South Asia, the other, pinpointing on the genetic diversity and evolutionary history of Indian populations. This paper would discuss the promise of ancient DNA research as a tool in understanding the past population dynamics of our ancestors in South Asia.

KEYWORDS: *Ancient DNA, Y Chromosome, mitochondrial DNA, Population genomics.*

Citation: Nagarjuna and Mustak. Ancient DNA: Revisiting Past Through Population Genomics. Polymorphism 2020; 5: 45-52.

INTRODUCTION

Ancient DNA is the genetic material from an extinct or dead species, which yields the genetic information that, can be used to reconstruct the population history of a given species using new technologies of DNA sequencing. With the advancement of next-generation sequencing technologies, we are in a position to recover the ancient DNA from the archaeological and paleontological remains and sequence the complete or partial genome. Initially, Bacterial cloning used to be done to amplify the ancient DNA fragments due to poor preservation of the samples and low concentration of DNA. Ancient DNA researchers also face severe problem is retrieving the authentic indigenous ancient DNA molecules due to environmental DNA contamination as most of the DNA reads consists of microbial or fungal contamination (Rizzi et al. 2012, Paabo 1985). The ancient DNA samples yield a very low copy number of indigenous DNA, which are usually degraded and fragmented. After the invention of PCR by Kary mulis in 1983, ancient DNA science started to blossom, but contamination of DNA reads was always a problem (Pruvost et al. 2005). Besides that, the laboratory environment also plays a crucial role in contamination, so guidelines were made to ensure perfection and avoid the contamination by laboratory environment. A series of guidelines to ensure the quality of ancient DNA data and reliability have been made and implemented worldwide (Scholz et al. 2000 and Bollongino et al. 2008). With time, these guidelines gradually formed into a more detailed and qualitative form, including important requirements. There are several international strict guidelines, which suggest that without the fulfillment of these criteria, the reliability and authenticity of ancient DNA data will remain uncertain (Rizzi et al. 2012 and Paabo et al. 2004). The enzymatic repair process within a living cell ensures the DNA repair, but after the death of the organism, cellular mechanisms shatter down, which often mislead the ancient DNA data and interpretation. As a result, the repair enzymes are not synthesized and the DNA is rapidly degraded by the lysosomal nucleases, the bacteria, fungi and other micro-organisms including insects feed on the dead matter and degrade the complex macromolecules into simpler molecules ultimately results a severe damage to DNA molecule. In rare cases, we can say that the DNA may overcome the degradation by getting absorbed on any matrix but still the DNA gets affected by some chemical processes which results in the degradation and fragmentation (Paabo et al. 2004).

The ancient DNA research completely transformed after the introduction of next-generation sequencing technologies, which enabled to produce huge genomic datasets in a short period of time. The sequencing results of the ancient DNA are now widespread; this became possible only because of next-generation sequencing. The first report of ancient DNA data was published and reported on 454 pyro-sequencing sequencing method, which revealed 13 million base pairs of an extinct Mammoth species (Miler et al. 2008, Hofreiter and Michael 2008). The ancient DNA from the Mammoth species was collected and extracted from single permafrost preserved bone, which yielded the first authentic ancient DNA whole-genome data using next-generation sequencing method. This report concluded that next-generation sequencing could be widely used for the ancient DNA. Besides that, the next-generation sequencing has made us more aware about the contamination in the ancient DNA extracts from modern human DNA. The most famous cases for contamination include the first reported ancient human DNA sequence in a 4,000year-old mummy (Shapiro and Hofreiter 2010).

The next-generation sequencing techniques such as those offered by Roche (GS titanium sequencing), ABI (SOLiD), Helicos (Biosciences) and Illumina (Solexa). The HIseq instruments from Illumina are particularly suited to ancient DNA (aDNA) analysis as they are capable of sequencing billions of short DNA fragments, producing as large as 1Tb of sequence in a single run. After all such advances in technology, the recovery of the first complete animal genome of wooly mammoth was done in 2008, followed by sequencing of many other extinct iconic extinct and extant animal groups (Huynen et al. 2012). In order to study the aDNA, source of aDNA serves as an important template, source condition and source type decides the amount of available undamaged and less fragmented aDNA. Many of the studies have suggested various types of sources for taking the aDNA, but another factor is the availability of the source, which serves as another important aspect. Study reveals that the DNA material is present in the coprolites of animals, which can provide wonderful information of a species, which was alive once upon a time.

The advantage of taking coprolite, as a source is that coprolite not only contains the aDNA from organism's diet but also it possesses the DNA from the animal itself. In fecal waste, some amounts of gut cells and other cells are also found which gives the information about the organism. The benefit of using a coprolite is that it not only reveals the genetic information but also gives an idea of animal's diet, coprolites has helped a lot in characterizing diet of extinct species (Poinar et al. 1998). Almost 2,000 coprolite samples from the rock shelters and cave sites from across southern New Zealand were analyzed to understand the detailed account of information about the extinct animals and their dietary pattern (Hofreiter et al. 2000). Eggshells also proved a reliable source of aDNA, recently the endogenous DNA has been recovered from the inner surface of ancient avian eggshells to understand the species identification and phylogeny of the extinct avian species (Tyler 1957).

Some sources of data also reveal that ancient DNA can also be recovered from the fossilized feathers of the bird, which can provide wonderful phylogenetic information of the extinct or extant bird species. Moreover, hair proved to be an excellent source of aDNA in recent researches as hair structure makes it relatively possible to remove the contamination from the exogenous DNA, as the contamination is limited to the hair shaft of the keratin. Hair contains relatively higher amounts of endogenous DNA; most of the DNA is retrieved from the dividing cells within the hair bulb. After cell death, the DNA gets fragmented, yet it yields a reliable and sufficient amount of DNA (Huynen et al. 2012). Another approach for ancient DNA analysis is the relationships in evolutions of the extinct species; phylogenetic relationships are also clarified.

Various extinct animals are subjected to aDNA recovery such as quagga, marsupial wolf, moa, and mammoth, cave bear and ground sloth etc. A fascinating research on extinct hominin species, Neanderthals from European countries, revealed their hierarchy of existence and admixture events with modern humans about 40-50 thousand years ago. Overall ancient DNA technology is proving a very successful gate for the evaluation and experimentation of hidden agenda (Lalueza-Fox et al. 2000). The burial culture followed by various societies provides a great source of information on behalf of their aDNA. Genetic information reveals the homology between multiple classes and subclasses; it also helps in finding relationships with the present man on the genetic level thus, ancestral suggestions also become clear (Naomi 2011).

History of Ancient DNA

The study of ancient DNA originally dates back to thirty years with the extraction of DNA followed by sequencing. It was done for the first time from the endogenous cells of the quagga, a South African equid (Equus quagga quagga) which became extinct in the nineteenth century (Rizzi et al. 2012), positive clones were detected using a radiolabeled probe analysis was done on the basis of mitochondrial DNA of zebra. Another sample was taken from the Egyptian mummy (Paabo 1985). First experiments describing the conservation of genetic material in archaeological specimens was achieved through the germination of a 500 years old peanut seed (canna species). Till 80's the research on aDNA had not been flourished. A big success was achieved when it was found that aDNA cannot only be extracted from the conserved and dried tissue, but it can also be extracted from soft tissues.

The invention of PCR just made the aDNA science possible from being very difficult, because of the limited amount of sample and no information regarding the analysis, the aDNA science has not progressed much. After PCR invention this science progressed and great findings were done on this basis. Various specimens of organisms were obtained and analyzed which yielded the desired results. After that time, it was known that the survival of aDNA in the tissues is possible for several millions of years, whereas in some cases DNA can survive for about 100,000 years. After some time, it was observed that the obtained results possess contamination, so various protocols were maintained, which enabled the prevention of contamination as discussed above (Rym 2011).

Role of Ancient DNA in modern genomics

The intervention of aDNA provides a lot of scope for the modern aDNA biotechnology, from knowing the complete history of the extinct animal to the most advanced part of recreating the extinct species. Scientists relied on the ancient species on the basis of physiological characteristics till the invention of aDNA techniques, till aDNA technology was not known the obtained ancient samples gives an idea on the basis of their shape, size and different parts of the body if available. After the intervention of aDNA techniques, a lot of information became available just by sequencing the obtained sample, no matter in which amount the sample is available. But this became possible only after the invention of PCR, which enabled the cloning of obtained aDNA in large quantities for experimentation. Beforehand when aDNA technology was not available, it was quite difficult to determine accurately the phylogeny of the animal, the protein it secretes and the habitat including diet. Now, we are in a condition to know a lot about the extinct deceased species such as the proteins it secretes, food and most important thing its relation with the present species. With the help of the genes obtained from the extinct samples, the proteins are remade using the genes, which reveal great information about the ancient species.

The effect of aDNA research is so flourishing now that the untold facts, which were unknown about the extinct species, can now be known easily, some of the facts include its phylogeny and genetics. This is a great step ahead in the recreation of the extinct species and rebuilding the lost diversity, which could be a priceless gift to the nature, from which once it was taken away by human interference.

Different strategies are present to elaborate on the benefits; sometimes, the history of the disease can be traced by the careful examination of the aDNA. The best-suited example to the point is the research of Antonio cascis et all; he demonstrated that the tuberculosis was present in the 18th and 19th-century slaves, and from here, it headed to America. The bones were collected, in which most of them were badly degraded. On sequencing it was found that they contain the sequences of mycobacterium tuberculosis complex; thus it got confirmed that mycobacterium tuberculosis was present in the old world as well as new world widely. The study also suggests that a lot of people were brought from Africa to Brazil as slaves, all the samples were collected from pretos novos (new blacks) cemetery (PNC: 1769- 1830) (Jaeger et al. 2013). Ancient DNA, however, is also very useful to study the phytogeography and its effect on animals, many studies revealed great results that how the old-world animals were different from the new world in their phylogeny due to the climatic conditions.

Recently, a study on the phytogeography on the red fox revealed that there was no significant difference in the old red foxes and the modern red foxes, even when the samples were collected from the diversified places of earth this was the great breakthrough, and it suggests that we cannot predict the changes in the phylogeny of animals for future on the climatic changes, the result could be anything. The theory of change of phylogeny with varied climatic conditions does not apply for all the species or organisms (Teacher et al. 2011). Evolution of genomes is a great point of curiosity among the scientists, which reveals how the genes are evolved with time. aDNA has helped a lot in the study of such genes by comparing the genes with present relatives of that species. Various genes are tested in vivo or invitro and the protein they secrete can be known with function. Various methods are available which helps in evaluation of the genes, they reveal enormous knowledge, which includes the evolution and function and thus gives a great breakthrough about the dwellings and surroundings of the organism, which was once alive (Rai et al. 2020 and Robbins et al. 2009). Various techniques are available for the evaluation of aDNA and these techniques are widely adapted to evaluate how these organisms live and adapt to the environment. Trans genetics is used to induce a gene into the transgenic model organism (transgenic mice), and the secreted protein is then evaluated with the help of various techniques (Pask et al. 2008).

Ancient DNA research also plays a vital role in the assessment of genomic diversity and migration from across the world. These migrations can be traced only by the genetic evaluation of present population with aDNA of past population. Thus, it gives a clear insight about how the man travelled and settled, to which ethnicity he belongs and what was the place for his habitation (origination) (Harney et al. 2018, Narasimhan et al. 2019, Rai et al. 2014, Shinde et al. 2019 and Thangaraj and Rai 2019).

The process is simple and involves the finding the ancient remains and sequencing the aDNA that is available, then evaluating the data after comparison with different parameters. Thus, the diversity can be known well of its origination and migration (Henn et al. 2012 and Der 2013). Various studies show various aspects of genetics; a study reveals that the decay of nuclear DNA is twice in comparison with the mitochondrial DNA. This determines the half-life of the DNA, the fragmentation rate was recorded and illustrated when the genome will be degraded and fragmented (Allentoff et al. 2012).

Ancient DNA in population Genetics

In 2018 researchers sequenced five new genomes from Neanderthals living between 39,000 and 47,000 years ago (Hajdinjak et al. 2018), which gave us insights into the structure of their genetic diversity. We also learned that as a result of interbreeding with each other, modern humans and Neanderthals conferred genes implicated in resistance to each other's viruses (Enard D and Petrov AD 2018). one of the most exciting discoveries was the publication of the genome of a 90,000-year-old girl from Denisova Cave in the Altai Mountains in Siberia. Her genome revealed that she was a first-generation descendant of a mating between a Neanderthal woman and a Denisovan man (Slon et al. 2018). Her mother's lineage was most closely related to a population of Neanderthals from Europe rather than other Neanderthals from the same cave, hinting at a long-distance migration of Neanderthals. Finding her suggests that such encounters may have been more common than previously suspected, and not just confined to Neanderthal-human pairings; it is known that there were likely at least two interbreeding events between Denisovans and modern humans (Browning et al. 2018).

These findings helped contribute to a major theme of human evolutionary studies: an emphasis on understanding the biological consequences of genetic variants that we inherited from our Neanderthal and Denisovan ancestors. In a special issue of Current Opinion in Genetics and Development focusing on the genetics of human origins, Dannemann and Racimo reviewed recent research pertaining to the adaptive benefits (and drawbacks) from archaic hominin DNA in our genomes (Dannemann and Racimo 2018"). And in another paper, Wolf and Akey reviewed the state of our knowledge about modern human-archaic hominin admixture more generally (Wolf and Akey 2018), focusing on questions that we still do not have the answers. Therefore, aDNA plays a crucial role in revealing the past through molecular mechanisms.

Hurdles in Ancient DNA research

The aDNA is fragmented in majority, fragmentation results from the post mortem chemical processes. The length of the most aDNA samples ranging from 250bp – 300bp. Degradation of the DNA starts within hours or days by the effect of endonucleases, which is a consequence of autolysis after the death of the organism. Nature and its agents do degradation process slowly, radiation interferes with the bases, and the complex sugar-phosphate bonds present in DNA. On the other hand, hydrolysis causes the deamination of bases, depurination and depyrimidination; these processes cause a great loss in the stability of DNA, resulting in fragmentation and degradation of DNA.

Another factor, which plays a key role in the degradation process, is the environment, DNA degradation process greatly varies with environment. Some conditions are recorded which promote the preservation of the aDNA such as aridity, low temperature, neutral or slightly alkaline PH, coating of some minerals such as hydroxyapatite on the bone prevents the degradation of DNA from the bone. Most importantly, microorganisms play a major role in the degradation of DNA; some degrade through consumption and conversion into simpler forms, whereas others excrete some secretions, which result in damage of aDNA. The first problem, which is common for a very old aDNA sample, is the result we get i.e. aDNA in a very fragmented form and it becomes a hectic task to arrange and recover the whole of the DNA from the available sample (Rym 2011).

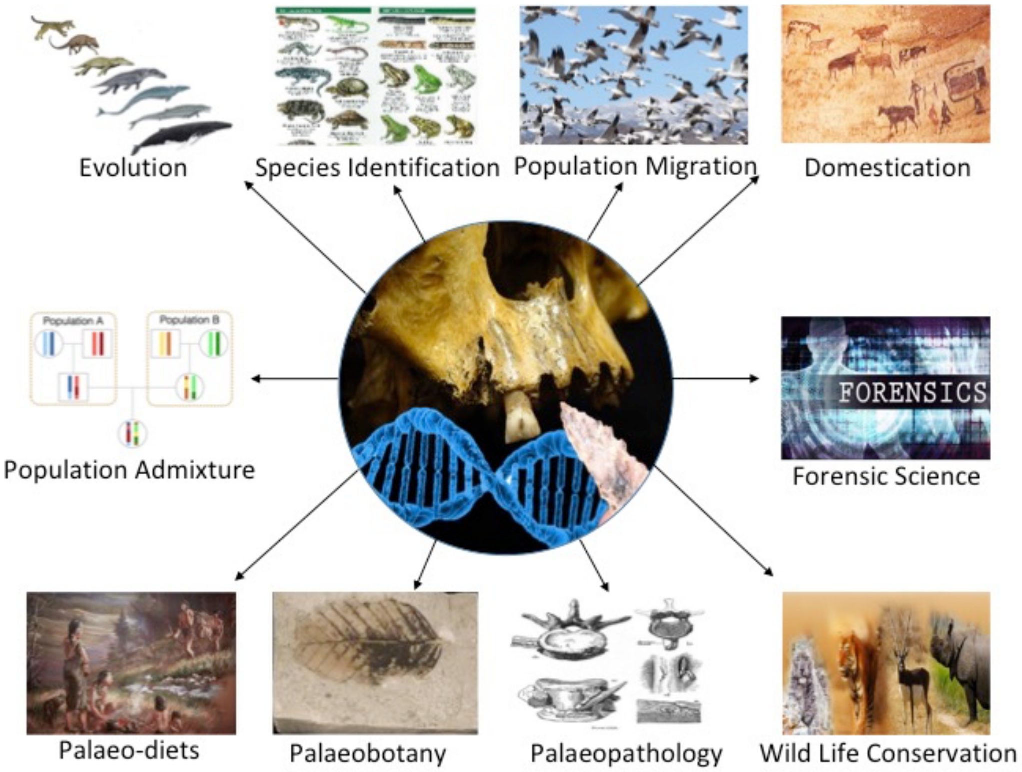

Fig. Ancient DNA research and scope

CONCLUSION

aDNA serves as the best tool to trace the history on the basis of DNA, thereby conferring the highest accuracy of data also it sheds light on today's population structure with respect to migration and admixture, thereby making it a very important tool for the study of population genomics. This field is booming with more accuracy and problems, which were there earlier, are now resolved up to a great extent. This technology possesses the power to rewrite the whole history with undeniable facts based on aDNA, technological advancements have helped in reshaping the potential of this technology. Considering the rich prehistoric records and high population diversity in Indian subcontinent, ancient DNA research in India provides an excellent opportunity to trace back the human population history of South Asians.

Acknowledgements

The authors cordially thanks to Dr. Niraj Rai for his constant support in completing this manuscript and reviewers for their valuable time and effort.

Conflict of interest statement

The authors have declared to have no competing interests.

Authors' contributions

PN conceived the idea and wrote the manuscript. MSM provided intellectual inputs.

Source of Funding

This research did not receive any specific funding.

Declaration of originality

The authors have declared that the content published in this review is original and has not been copied from other sources without appropriate citation.

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