



Original Research Paper

MITOCHONDRIAL DNA PHYLOGEOGRAPHY AND HUMAN POPULATION GENETICS: INSIGHTS INTO MIGRATION, DEMOGRAPHY, AND EVOLUTION

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ABSTRACT

Mitochondrial DNA (mtDNA) has been widely used to trace maternal ancestry and investigate population structure, migration, and demographic changes. Its high copy number, predominantly maternal inheritance, and relatively rapid sequence evolution have also made it useful when DNA preservation is poor, including forensic and ancient samples. Much of what is known about the global distribution of maternal lineages initially came from control region sequences and haplogroup frequencies. Complete mitogenomes have improved the resolution of these studies. Modern sequencing has also changed the study of variations within individuals. Low-level heteroplasmy, once difficult to detect systematically, can now be examined across large datasets, raising new questions about maternal transmission, somatic mutations, and the influence of the nuclear genome on mitochondrial variation. In forensic genetics, the high copy number of mtDNA remains particularly useful when biological material is highly degraded, or nuclear DNA recovery is poor. At the same time, mtDNA reflects only a single maternal lineage and should not be considered a comprehensive account of population ancestry. Evolutionary interpretations can be influenced by factors like the founder effect, genetic drift, selection, heteroplasmy, NUMTs, and the calibration of molecular clocks. The analysis of mtDNA is being enhanced by integrating genome-wide and ancient DNA, archaeological findings, and supplementary data. The inclusion of data from underrepresented and indigenous groups, advancements in sequencing technology, and insights into mitonuclear interactions are expected to uncover aspects of maternal lineage that are not well-represented in existing mitochondrial reference datasets.

Keywords: Mitochondrial DNA, Evolution, Population Genetics, Phylogeography, Haplogroups, Human Migration, Paleogenomics

1. INTRODUCTION

Mitochondrial DNA (mtDNA) has been used extensively in human population genetics, molecular anthropology and phylogeography, and it also has an established role in forensic genetics. Several features account for this. Human mtDNA is predominantly maternally inherited, occurs in high copy number and accumulates sequence variation relatively rapidly. Recombination is considered absent or very limited compared with the nuclear genome. As a result, mitochondrial variants are transmitted largely along maternal lines, producing genealogical relationships that can be followed across populations (Giles et al., 1980). The mitochondrial genome itself is small: 16,569 base pairs containing 13 protein-coding genes, 22 transfer RNAs and two ribosomal RNAs (Anderson et al., 1981). Hundreds to

thousands of mtDNA copies may occur within a cell. This abundance becomes particularly useful when DNA is scarce or degraded.

Some of the earliest influential applications of mtDNA concerned human origins and dispersal. Cann, Stoneking and Wilson (1987) used mitochondrial variation to investigate relationships among human populations and provided evidence for a recent African ancestry of modern human maternal lineages. A much larger mitochondrial phylogeny has since developed. Mutations accumulated along maternal lineages are used to define haplogroups and increasingly finer sub-haplogroups, many of which show marked geographic structure. Their distributions have been examined in relation to dispersal out of Africa, the settlement of Eurasia, Oceania and the

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Received: 25th May, 2026; Revised: 6th July, 2026; Accepted: 28th July, 2026; Available Online: 29th August, 2026

(DOI): 10.70102/AEJ.2026.18.2.31

Americas, and later regional population movements. At smaller geographic scales, mtDNA has been used to investigate founder events, isolation, bottlenecks and relationships among neighbouring populations. These histories are specifically maternal. They may resemble patterns obtained from autosomal or Y-chromosomal data, but they do not necessarily have to.

High copy number has given mtDNA another practical advantage. In forensic and humanitarian investigations, mitochondrial sequences can sometimes be recovered from material in which nuclear DNA is too limited or degraded for conventional profiling. Hair shafts, old skeletal material, teeth and highly degraded remains are examples. Maternal relatives can also provide reference samples when direct identification is not possible. For these reasons, mtDNA has been used in missing-person investigations, disaster victim identification and the analysis of historical remains (Parson & Bandelt, 2007). Its discriminatory power is lower than that of autosomal STR profiling because maternally related individuals may share the same mitochondrial haplotype. It is therefore particularly useful when nuclear DNA recovery is poor or when maternal lineage itself is relevant to the question.

The type of information obtained from mtDNA has changed considerably with sequencing technology. Earlier studies relied heavily on restriction fragment analysis and Sanger sequencing of the control region. Complete mitogenomes provided substantially more phylogenetic information, while massively parallel sequencing made it practical to examine large numbers of mitochondrial genomes at greater depth (Parson & Bandelt, 2007; Parson et al., 2014). Ancient DNA changed the temporal scale of the field as well. Instead of inferring past maternal variation entirely from living populations, mitochondrial genomes can now be recovered from individuals who lived hundreds or thousands of years ago. Population continuity, lineage loss and migration can therefore be examined more directly, although genome-wide ancient DNA is generally required to reconstruct broader ancestry and complex admixture.

More sequence data have also exposed aspects of mitochondrial variation that were less visible in earlier population studies. Heteroplasmy is one example. Low-frequency mitochondrial variants can now be detected much more readily, and large datasets have shown variation associated with maternal transmission, tissue, somatic mutation and age. At the same time, deeper sequencing creates its own interpretative problems. Sequencing thresholds, contamination and nuclear mitochondrial DNA segments (NUMTs) can affect the identification of low-frequency variants. Selection and genetic drift further complicate population-level interpretation, and mitochondrial lineages may not follow the same history as the nuclear genome. Mitonuclear interactions have therefore become relevant

to questions that once treated the mitochondrial genome largely on its own.

This review considers how these developments have changed the use of mtDNA in studies of human populations. We begin with the biological and population-genetic properties of the mitochondrial genome, followed by mitochondrial phylogeny, global haplogroup diversification, phylogeography and demographic inference. Ancient DNA and the relationship between mitochondrial and genome-wide evidence are considered separately, along with developments in sequencing and analytical methods. Forensic applications are also discussed. Particular attention is given to heteroplasmy, selection, mitonuclear interactions and the limitations that arise when a single maternal genealogy is used to reconstruct population history.

2. BIOLOGICAL CHARACTERISTICS OF mtDNA AND THEIR POPULATION GENETIC SIGNIFICANCE

Mitochondrial DNA (mtDNA) is one of the many genetic markers employed in evolutionary and population genetic studies, and it has been especially informative due to its unique biological properties and evolutionary dynamics. Human mtDNA (human mitochondrial DNA) is a small circular genome of 16,569 base pairs located in the mitochondria. It is small but encodes 13 proteins involved in oxidative phosphorylation, 22 transfer RNAs, and 2 ribosomal RNAs that are required for mitochondrial function (Anderson et al., 1981). The evolutionary dynamics of the mitochondrial genome are influenced by its highly compact organization and the lack of introns. Another important region is the control region or displacement loop (D-loop), which contains regulatory elements for transcription and replication. The hypervariable regions HVR-I, HVR-II, and HVR-III in this region are useful markers for tracing maternal lineages and recent population history because they mutate relatively rapidly.

Along with the absence of recombination, another important feature of the mitochondrial genome is its higher substitution rate than that of most nuclear DNA, although rates vary considerably among sites and regions. Replication-associated errors and the distinctive replication and repair environments of mtDNA are thought to contribute to this pattern (Table 1). The high mutation rate provides the resolution required to examine recent demographic events, such as population expansion, founder effects, and migration patterns. mtDNA has been widely used for molecular dating to reconstruct key events in the history of human evolution, including the migration of modern humans out of Africa and to estimate divergence times across lineages (Soares et al., 2009).

However, when inferring temporal estimates, mutation rates vary between genomic areas and evolutionary timescales; the effects of purifying selection and rate heterogeneity should be considered. The small effective population size of mtDNA also has consequences for population genetics. mtDNA is haploid and maternally inherited; therefore, its effective population size is expected to be approximately 25% of that of autosomal DNA under neutrality (Birky et al., 1983). Consequently, mtDNA is particularly susceptible to genetic drift, and the distribution of haplogroups is strongly shaped by demographic factors such as bottlenecks, founder effects, and population subdivision. mtDNA is frequently more sensitive than many nuclear markers and thus provides a different, maternal-lineage-specific picture of the population structure. Nonetheless, it is crucial to approach demographic interpretations with caution, as drift can sometimes resemble migration or selection signals (Ballard & Rand, 2005). Moreover, recent progress in high-throughput sequencing has revealed that mitochondrial variation is more intricate than previously thought.

Traditionally, population genetics analysis relies on an individual's consensus mtDNA sequence. However, this approach does not encompass the full scope of mitochondrial variation. Numerous studies have demonstrated the existence of heteroplasmy, which implies that an individual may have multiple mtDNA variants that can arise through maternal transmission or somatic mutation (Stewart & Chinnery, 2015). The frequency of these variants may also change between generations as mtDNA passes through the mitochondrial bottleneck during oogenesis (Goto et al., 2011). Large-scale sequencing has shown that heteroplasmy is considerably more widespread than was apparent from earlier sequencing approaches. Analysis of more than 250,000 individuals, for example, identified distinct

patterns for heteroplasmic single-nucleotide variants and indels, with many single-nucleotide variants showing age-related somatic accumulation while a substantial proportion of indels showed maternal transmission (Gupta et al., 2023). Nuclear genetic variation is also associated with mtDNA copy number and heteroplasmy, providing further evidence that mitochondrial variation cannot be considered entirely independent of the nuclear genome. Heteroplasmy therefore adds another level of variation to mtDNA and needs to be considered when maternal transmission and population-level mitochondrial diversity are interpreted.

Although mtDNA has long been considered a mostly neutral genetic marker, mounting evidence suggests that natural selection may play a significant role in shaping mitochondrial variation. Purifying selection is thought to remove deleterious mutations in mitochondrial genes essential for energy production in cells, although some variants may be favored under specific conditions (Bazin et al., 2006). Geographic associations between particular mitochondrial lineages and environmental variables, including temperature and altitude, have led to proposals of local adaptation, although separating selective effects from the demographic history remains difficult. In addition, mitonuclear epistasis may affect organism fitness, supporting the idea that mtDNA variation is a marker of functional evolution and population history (Ballard & Rand, 2005). These properties explain both the value and limitations of mtDNA in population genetic research. Its maternal inheritance, mutation dynamics, and sensitivity to drift provide considerable resolution for reconstructing maternal population history; however, the resulting patterns must be interpreted alongside demographic processes, selection, and evidence from the nuclear genome (Ballard & Rand, 2005).

Table 1. Biological Characteristics of mtDNA Pertinent to Population Genetics

Feature	Description	Significance
Maternal inheritance	Predominantly transmitted through the maternal lineage	Traces maternal ancestry; enables reconstruction of maternal genealogical relationships
Lack of recombination	Most mtDNA is inherited as a linked haplotypic unit	Preserves phylogenetic structure and facilitates haplogroup reconstruction
High mutation rate	Higher sequence evolution than most nuclear DNA; substantial regional/site variation	Provides resolution for recent population differentiation and molecular dating
Small effective population size (N_e)	Haploid, uniparentally inherited genome; theoretical N_e =one-quarter that of autosomes under idealized assumptions	Greater sensitivity to drift, founder effects and population bottlenecks
High copy number	Hundreds-thousand copies/cell	Facilitates recovery from ancient, degraded and low-quantity samples
Small genome size	16,569 bp with high coding	Allows complete mitogenome

	density	sequencing and comparative phylogenetic analysis
Mitonuclear interactions	Mitochondrial function depends on coordinated mitochondrial and nuclear gene products	Selection on mtDNA may depend partly on the nuclear genomic background
Mitochondrial bottleneck	Reduction and subsequent expansion of transmitted mtDNA during the female germline	Can produce rapid intergenerational shifts in heteroplasmy frequencies

3. MITOCHONDRIAL DNA PHYLOGENY AND GLOBAL HAPLOGROUP DIVERSIFICATION

The maternal inheritance of mtDNA and its limited recombination allow mutations acquired along a lineage to be retained across generations. These mutations form the basis of the mitochondrial phylogeny (Parson & Bandelt, 2007). These trees divide human populations into haplogroups, lineages passed down maternally, defined by shared mutations, and characterized by their distinct geographic distributions. Whole mitochondrial genome sequencing and large-scale phylogenetic databases, such as PhyloTree, have greatly improved the resolution of haplogroup classification and divergence time estimates, thus enabling a much more detailed picture of human evolutionary history (Behar et al., 2012). According to coalescence-based analyses, all modern human mtDNA lineages are descended from a single ancestral mtDNA sequence known as "Mitochondrial Eve," who most likely lived in Africa between 150,000 and 200,000 years ago (Cann et al., 1987).

Some of the earliest branches of the human mtDNA tree are represented by the African macrohaplogroup L and its major sub-lineages (Behar et al., 2012). Their diversification reflects a long and complex history in Africa, rather than a single population expansion. Among these lineages, L3 occupies a particularly important position because the two major non-African branches, M and N, roughly 50,000-70,000 years ago, descended from the broader L3 radiation. Their emergence is closely associated with the dispersal of modern humans out of Africa, although the precise timing and routes of this process continue to be refined as genetic and archaeological evidence accumulates.

Outside Africa, most mitochondrial diversity falls within the major branches M and N and their many derived lineages. Their subsequent diversification is extensive. Macrohaplogroup M developed numerous regionally differentiated branches across South and East Asia and later contributed to the lineages found in Oceania and the Americas. Its particularly high diversity in South Asia has been interpreted as evidence of the early presence and long-term diversification of maternal lineages in the region (Kivisild et al., 2003). Macrohaplogroup N, including its major descendant R, also underwent substantial diversification and gave rise to lineages now

distributed across western Eurasia, South and East Asia, Oceania, and the Americas.

A new study on haplogroup R30 recently studied a mix of 190 modern and ancient samples across South Asia, and a new basal branch, R30c, was discovered, while the date of R30 was estimated to be 50 Kya, suggesting a deep history of this lineage within the Indian subcontinent (Desai et al., 2026).

Its distribution was also non-uniform. R30b was associated with older individuals in northern India, whereas much of the diversity within R30a and the newly identified R30c was concentrated in southern India. In some relatively isolated populations, specific branches appear to have expanded locally. Such founder patterns have been reported among the Vedda of Sri Lanka, the Uru Kurumban of southern India, and populations from the Lakshadweep Islands. Interestingly, early diverging R30 lineages were identified farther east, including in Thailand and Indonesia. The authors interpret these lineages as evidence of old maternal connections between South and Southeast Asia (Desai et al., 2026).

R30 is the only maternal lineage; therefore, its history cannot describe the dispersal of an entire population. However, it does show why looking below the level of broad macrohaplogroups such as M, N, and R can be informative. Regional migrations, isolation, and founder events may become visible only after a lineage is divided into finer branches. The R30 phylogeny is a recent example of this approach, although the study is currently available as a preprint, and its proposed dates and dispersal patterns require comparison with additional ancient and modern mitogenomes.

The present distribution of these lineages was not the result of a continuous expansion. Founder events, population isolation, later migrations, and repeated episodes of population growth altered haplogroup frequencies after the initial dispersal from Africa. Some maternal lineages remained geographically restricted, whereas others spread over large areas. This mixture of continuity and movement is what gives mtDNA much of its phylogeographic value, but it also means that the present location of a haplogroup should not automatically be equated with its place of origin.

Haplogroups H, U, J, T, K and V occur widely in European and western Eurasian populations, although

their present distributions reflect different periods of population history rather than a single expansion. Several of these lineages have been discussed in relation to postglacial population movements and the later spread of farming populations from the Near East into Europe (Richards et al., 2000). South Asia shows a different pattern. Macrohaplogroup M is highly diverse in the region and contains a number of deeply branching lineages, supporting a long history of maternal diversification within South Asia (Kivisild et al., 2003). In East and Southeast Asia, major branches derived from M and N include haplogroups such as A, B, C, D, F and G, while lineages including P, Q and S form important parts of the mitochondrial diversity of Oceania and Australia.

The settlement of the Americas produced another distinct pattern. Most Indigenous American maternal lineages belong to founder branches derived from haplogroups A, B, C and D, together with X2a in parts of North America. These lineages ultimately have affinities with populations of northeastern Asia and Siberia and entered the Americas through Beringia during the Late Pleistocene. Complete mitogenome sequencing has since divided the broad A, B, C and D classifications into more specific American founder subclades, including A2, B2, C1 and D1, allowing individual maternal expansions to be examined at considerably finer resolution (Achilli et al., 2008).

The geographical distribution of mtDNA haplogroups can be used to study migration routes, founder effects, population expansion, mixing processes, and geographical isolation. Genetic data can be used with broader reconstructions of human history, as haplogroups tend to disperse according to paleoenvironmental and archaeological evidence. Moreover, coalescent-based mtDNA methods provide an important molecular clock for estimating the timing of migration events, population expansions, and lineage splits (Soares et al., 2009). However, caution should be exercised when interpreting mtDNA phylogenies.

However, haplogroup distribution should be considered as records of lineage history rather than a direct map of population movement. A lineage may expand, disappear locally, or change substantially in frequency without the same change occurring across the rest of the genome. Modern distributions can also obscure these earlier patterns. Ancient DNA has made this particularly clear, revealing cases of maternal lineage continuity and population turnover that could not have been recognized from present-day samples alone. Hence, mtDNA phylogenies are effectively informative when their geographical and temporal distributions are combined and interpreted along with archaeological evidence, ancient DNA, and genome-wide data.

3.1 *Phylogeography and Human Migration*

Phylogeography connects the evolutionary relationships among mitochondrial lineages with their geographic distributions. In simple terms, it asks not only how maternal lineages are related, but also where they occur and how they may have reached those regions. When information on lineage age is added, these distributions can be used to investigate population expansion, migration, isolation, and founder events. mtDNA has been particularly useful in this context, although the geographic distribution seen today is the result of many demographic processes acting over thousands of years and should not be read as a direct map of ancient migration.

Genetic evidence suggests that *Homo sapiens* originated in Africa, as all non-African mtDNA lineages can be traced to haplogroup L3 and its descendant macrohaplogroups M and N (Cann et al., 1987). Between 50,000 and 70,000 years ago, modern humans migrated from Africa to Eurasia. The high diversity and deep branches of macrohaplogroup M in South and Southeast Asia contribute to the proposal of an early southern dispersal, possibly involving movement through Arabia and along parts of the Indian Ocean rim (Macaulay et al., 2005). This idea is attractive because it offers an explanation for the relatively early appearance of modern humans across southern Asia and eventually Sahul. However, the route is difficult to reconstruct using mtDNA alone. Present-day distributions have been altered by later population movements and lineage loss, and much of the Pleistocene coastline is submerged. Rather than a simple division between a southern route represented by M and an inland route represented by N (Behar et al., 2012), mitochondrial evidence is better viewed as part of a broader picture in which early Eurasian populations dispersed and subsequently diversified across several regions.

The settlement of the Pacific provides another well-known example of mitochondrial phylogeography, although its interpretation has changed considerably as more complete mitochondrial genomes have become available. Haplogroup B4a1a1a, commonly known as the “Polynesian motif,” was initially used as an important maternal marker for the Austronesian expansion through Island Southeast Asia and into the Pacific (Trejaut et al., 2005). The demographic history represented by this lineage appears to be more complicated. Analysis of more than 1,300 B4a1a1 mitogenomes suggests that the lineage ancestral to the Polynesian motif reached Near Oceania before the later expansion associated with Austronesian-speaking and Lapita populations. Its subsequent spread into Remote Oceania appears to have involved founder events and rapid island colonization, rather than a simple maternal migration directly from Taiwan. Thus, the linguistic expansion of Austronesian languages and the movement of maternal lineages were

related, but they did not necessarily follow the same demographic histories (Table 2).

The peopling of the Americas is another case in which mtDNA made an early and important contribution to the migration models. Several founding maternal lineages, particularly subclades A, B, C, and D, together with X2a in North America, connect Indigenous American populations with ancestral populations from Northeast Asia. Patterns of mitochondrial diversity also contribute to the Beringian standstill model, in which the ancestors of Indigenous Americans remained isolated for a period in or around Beringia before expanding into the Americas (Tamm et al., 2007).

Ancient DNA has added considerable detail. The initial movement south of the continental ice sheets was followed by rapid dispersal and early population branching, whereas later histories differed from one region to another. Continuity occurred in some areas, while admixture or population replacement occurred in others. Genome-wide ancient DNA has therefore made the peopling of the Americas look less like a single migration followed by straightforward expansion and more like a sequence of movements, separations, and local demographic histories. The mitochondrial founder lineages remain informative, but they represent only the maternal part of a much larger history.

Mountain ranges, deserts, oceans, and other physical barriers have influenced the distribution of mitochondrial lineages. Genetic divergence among regions has been

affected by endogamy, language boundaries, and societal systems. Such effects are particularly relevant in isolated island populations, the deep population structure across Africa, and the genetic divergence between caste and tribal groups in South Asia (Atkinson et al., 2022; Chaubey et al., 2007; González-Santos et al., 2022; Singh et al., 2022).

The strength of mtDNA phylogeography lies in the connection between ancestry, time, and place. A mitochondrial lineage can sometimes preserve evidence of an old founder event or migration long after other aspects of the population have changed. However, the same property can also be misleading. A haplogroup may become common through genetic drift, disappear from a region, or move between populations through subsequent gene flow. Therefore, the geographic range of a lineage today is not necessarily the geographic range it had when it first arose.

Hence, mtDNA is rarely interpreted as evidence in isolation. Archaeology can provide dates and cultural context; ancient DNA shows which lineages were present at a particular place and time, and genome-wide data reveal ancestry that a single maternal lineage cannot capture. Linguistic and paleoenvironmental evidence may add another layer. When these sources agree, the migration models become considerably stronger. When they do not, the disagreement itself can reveal population replacement, sex-biased migration, or cultural transmission without the large-scale movement of people.

Table 2. Geographical distribution of the main human mtDNA haplogroups.

Haplogroup	Geographic Distribution	Evolutionary Significance
L0	Primarily sub-Saharan Africa, especially southern Africa	Among the deepest branches of modern human mtDNA diversity
L1	Primarily Central and parts of West/East Africa	Deep African maternal diversification
L2	Widespread in Africa	Regional African diversification; some subclades expanded during later demographic movements
L3	Africa, with descendants extending outside Africa	Ancestral radiation associated with the branches leading to M and N
M	South, East and Southeast Asia; Oceania; Americas through derived branches	Major non-African macrohaplogroup with extensive regional diversification
N	Widely distributed outside Africa	Major non-African macrohaplogroup; ancestral to numerous Eurasian and Oceanian lineages
R	Eurasia, South Asia and Oceania	Major descendant of N with extensive diversification across Eurasia
H	Predominantly Europe and western Eurasia	Important for studies of European population history and postglacial demographic change
U	Europe, West and South Asia	Contains deep western Eurasian branches with complex Palaeolithic and later histories
J	Europe, Near East and West Asia	Associated with West Eurasian population history; some subclades expanded during Neolithic and later population movements
B4a1a1a	Remote Oceania; related branches in Island Southeast Asia/Near Oceania	Important for studying Pacific settlement and founder events; demographic interpretation has been revised with complete mitogenomes

3.2 *mtDNA Population Structure and Demographic History*

Population bottlenecks and founder effects are two of the most significant demographic events that can be identified by mtDNA variation. The diversity of mtDNA changes when there is a growth, decline, or shift in the population size, leading to either a loss or rise in the frequency of certain variants and haplotypes. These changes in frequency are an important tool for studying population genetics and demographic changes. Its relatively small N_e is particularly sensitive to genetic drift (Birky et al., 1983). A signature can be observed in the mtDNA faster than in the nuclear DNA because of its high mutation rate, and it can be studied as an indication of a significant demographic event. However, strong drift also means that changes in mtDNA cannot always be translated directly into changes in population size.

During severe population decline, rare maternal lineages may simply disappear, and overall diversity can be reduced. A founder event is slightly different from a bottleneck. Here, only a small group contributes to the formation of a new population, carrying only part of the mitochondrial diversity present in the source population. By chance, a lineage that was uncommon in the original population may become frequent in the new population. Such occurrences have been noted in small populations that are genetically isolated, as well as during the colonization of new geographic areas. These trends may continue well beyond the initial founder event.

When populations expand, it frequently results in the development of short branches that diverge from common ancestors, forming a star-like configuration in the mtDNA network. This study may also reveal a surplus of variants that are either relatively new or uncommon. Tajima's D compares different estimates of sequence diversity to test departures from the standard neutral model (Tajima, 1989), whereas Fu's F_s is particularly sensitive to patterns that can occur during population growth (Fu, 1997). Negative values of these statistics are often discussed as evidence of demographic expansion. However, these are not proofs. Fu (1997), for example, showed that population growth and genetic hitchhiking can produce similar polymorphism patterns. Therefore, selection and population structure must be considered before a demographic explanation is accepted.

The population structure creates its own problems. Human groups do not exchange genes equally, even when they live in the same broad geographic regions. Sometimes, the reason is obvious: an ocean, a mountain range, or a large geographic distance. In other cases, the boundaries are social. Endogamy, language, and residence practices can restrict maternal gene flow for many generations. This is particularly relevant in South Asia, where neighboring communities may have quite

different demographic histories despite their geographic proximity.

Several statistics are commonly used to describe this variation, but they measure different aspects. Haplotype diversity describes the probability that two sampled mitochondrial sequences are different, whereas nucleotide diversity reflects the average sequence difference between the two. F_{ST} was used to examine the differentiation between populations. Variation can also be partitioned at different hierarchical levels using analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and applied to mtDNA data. None of these measures, by itself, reconstructs the demographic history. They first tell us how diversity is distributed in the world.

Population structure may also be mistaken for changes in population size. If genetically differentiated groups are combined and analyzed as though they formed a single randomly mating population, the resulting genealogy may not represent the history of any one of those groups. The reverse problem occurs when a present-day population contains maternal lineages that were introduced by earlier migrations. What appears to be an ancient demographic signal from that population may have actually originated elsewhere.

Coalescent approaches attempt to look further back in time. Instead of considering only haplogroup frequencies or diversity statistics, they used the genealogy of sampled sequences to estimate how the effective population size changed in the past. The Bayesian Skyline Plot (BSP), become widely used because it does not require population history to follow a fixed model of growth or decline (Drummond, 2005). Changes are inferred across different intervals of the genealogy, and related approaches, such as the Extended Bayesian Skyline Plot, provide additional flexibility.

However, a skyline plot should not be interpreted as a census curve. It estimates the genealogical effective population size, which is not the same as the census size. Sampling, mutation rate calibration, migration, and population structure can all change the shape of the reconstructed trajectory.

Miller et al. (2018) provided a useful demonstration of this issue. This study presented an analysis of 1700 individual complete mtDNA sequences and used extended BSP to study demographic histories across several geographical regions. Surprisingly, the expected population increase associated with the Neolithic transition was not consistently observed. However, many of the inferred changes were considerably older. The authors suggested that some of these patterns could reflect changes in population structure or demographic events that occurred elsewhere, with the signal later being carried into present-day populations through migration and mixing processes. They referred to these

inherited traces as demographic “ghost signals”(Miller et al., 2018).

This makes sampling particularly important. A small dataset can miss rare maternal lineages, and sampling from only one locality may make a diverse population appear unusually homogeneous. Dates bring another source of uncertainty because they depend on the mutation rate and calibration. Selection adds another complication. None of these problems make mtDNA unsuitable for demographic analysis, but they do limit how literally its signal can be read (Bazin et al., 2006).

There is also a biological limit that cannot be overcome by sequencing: mtDNA follows one maternal genealogy. It records the history of mothers through mothers, not the ancestry of the entire genome.

4. ANCIENT DNA AND PALEOGENOMICS: TEMPORAL INSIGHTS INTO HUMAN POPULATION HISTORY

Paleogenomics and ancient DNA (aDNA) have transformed the study of human population history by providing direct genetic information on ancient populations. Unlike studies that examine only modern genetic variation (Slatkin & Racimo, 2016), ancient mitochondrial DNA (mtDNA) provides a time perspective that opens the possibility of investigating migration, population continuity, demographic change, and extinction events. mtDNA was the first genetic marker that could be successfully retrieved from ancient specimens and is still widely used for haplogroup assignment, maternal lineage reconstruction, and comparisons between ancient and modern populations because of its high copy number, compact genome, and relatively good preservation in archaeological remains (Hofreiter, 2001).

Advances in next-generation sequencing (NGS), targeted enrichment, contamination control procedures, and bioinformatic tools have greatly benefited the retrieval of complete mitochondrial genomes from highly compromised samples. This has permitted high-resolution reconstruction of evolutionary and demographic histories (Hofreiter, 2001). The amount of information that can be recovered has increased considerably since then. Ancient samples are usually short and damaged, and modern human contamination during sample handling can be easily mistaken for endogenous DNA. Hence, additional factors such as damage pattern, fragment length, and independent replication were considered during the formal analysis. In some cases, mitochondrial DNA can be recovered even when no human bone is available for analysis. Sedimentary DNA from Denisova Cave, for example, has been used to detect Denisovan, Neanderthal, and modern human mtDNA in different archaeological layers, providing evidence for repeated hominin occupation of the site (Vernot et al., 2021).

Some of the most striking results have come from archaic humans. Mitochondrial genomes recovered from Neanderthal remains established maternal lineages that were distinct from those of living humans. The Denisova Cave material produced an even greater surprise for researchers. A mitochondrial genome recovered from a small finger bone represents a deeply divergent hominin lineage that was not known from morphology alone. Subsequent nuclear genomic analysis established Denisovans as a sister group of Neanderthals and revealed a much more complicated history of interaction between archaic and modern humans (Krause et al., 2010; Reich et al., 2010). Therefore, ancient DNA did more than place old samples on an existing mitochondrial tree. In some cases, populations whose genetic history could not be reconstructed from living people alone were revealed.

The same principle applies to modern human populations. Present-day genetic diversity is only what survives. Lineages might have vanished due to genetic drift, migration, or the replacement of populations, leaving minimal evidence among current inhabitants of the same areas. Ancient DNA offers insights into historical events; this has been especially valuable in Europe, where paleogenomic research has uncovered significant population changes linked to the advent of agriculture and subsequent movements during the Bronze Age. Genome-wide analyses from Eastern and Southern Africa have also shed light on demographic shifts over the past few millennia (Lipson et al., 2022), which could not have been achieved using only modern DNA samples.

However, ancient DNA has limitations; it is not uniformly available worldwide, and its preservation is entirely dependent on environmental conditions. Humidity and high temperatures cause DNA degradation, making it difficult to access. Geographical regions such as Africa, South Asia, and other warm regions are still under-represented and less well-known. Sampling is uneven within regions, and the individuals recovered from archaeological sites cannot automatically be assumed to represent the entire population that lived there. For mtDNA, there is an additional constraint: even a well-preserved ancient mitogenome follows only one maternal lineage. Complex admixture and population replacement are therefore much better resolved when mitochondrial evidence is considered along with genome-wide ancient DNA.

Consequently, ancient DNA is most useful not as a replacement for evidence from living populations but as a way of testing it against genetic observations from the past. A lineage inferred from modern DNA samples to have lived in a region for thousands of years can be verified using ancient DNA; however, ancient samples can tell a different story. As the geographical and temporal representation of ancient samples from underrepresented areas increases with technological

development, this comparison between ancient and modern samples should provide a more detailed picture of maternal demographic history.

4.1 Ancient mtDNA and Genome-wide Ancient DNA: Complementary Views of Population History

Ancient mtDNA and genome-wide ancient DNA do not provide the same perspective of the past. The mitochondrial genome follows a maternal lineage. In contrast, genome-wide data contain information inherited through many ancestors and can therefore reveal population relationships and admixture that cannot be reconstructed from mtDNA alone (Stoneking & Krause, 2011). This difference became particularly evident as ancient nuclear genomes began to accumulate. Population histories that appeared relatively simple from maternal lineages were sometimes found to involve repeated migration, mixture, and population replacement.

However, mtDNA has not become redundant. There is a practical reason for this: many copies of the mitochondrial genome are present in a cell, whereas only two copies of each autosomal locus are usually present. In poorly preserved material, mitochondrial sequences may be recovered when genome-wide nuclear coverage is very low or absent. This advantage was particularly important during the early years of ancient DNA research, when much of the available information came from short mitochondrial fragments (Hofreiter, 2001). Complete ancient mitogenomes can now be used to assign samples to fine maternal subclades and to determine whether a lineage persisted in a region, appeared at a particular time, or disappeared from later populations.

The maternal inheritance of mtDNA also sets a clear boundary on what can be inferred from this. A mitochondrial sequence represents one maternal line and cannot be considered a summary of an individual's total ancestry. This is important when migration is sex-biased. Male-mediated movement, for example, may introduce substantial Y-chromosomal and autosomal ancestry into a population without producing a similar change in its mitochondrial lineages. Female-biased movement can produce different patterns. Such differences have been observed in human populations and are useful in their own right, particularly when migration, marriage practices, or other forms of social organization are being considered. In these cases, the disagreement between mtDNA and nuclear markers is not necessarily a problem with the data. These markers may simply record different parts of the same demographic history.

Genome-wide ancient DNA becomes particularly important when the question concerns admixture is involved. Because information is obtained across the nuclear genome, ancestry derived from different source populations can be identified, and in many cases, its proportion and timing can be modelled. This represents a major change in paleogenetics. Archaic admixture

provides an obvious example: mitochondrial sequences establish distinct Neanderthal and Denisovan maternal lineages, but nuclear genomes reveal that these groups also contributed to the ancestry of modern human populations (Slatkin & Racimo, 2016). The maternal tree alone could not have shown this history.

This is why ancient mtDNA continues to be useful alongside genome-wide ancient DNA. Complete mitogenomes can follow the appearance, persistence, or loss of particular maternal lineages over time, including in samples where nuclear DNA is poorly preserved. Genome-wide data answer a broader set of questions and are better suited to identifying complex admixture and ancestry from multiple source populations. The two do not need to produce identical histories. A maternal lineage may continue to exist even as the surrounding nuclear ancestry undergoes significant changes, or it might vanish due to genetic drift, despite the persistence of some broader population ancestry. Consequently, analyzing these patterns collectively can uncover instances of population replacement, migration influenced by gender, or the disappearance of lineages, which would be challenging to detect using only mtDNA. Ancient nuclear DNA has not made mitochondrial data obsolete; instead, it has offered an improved context for understanding the significance of a maternal lineage.

5. METHODOLOGICAL ADVANCES IN mtDNA ANALYSIS

Human mtDNA was initially studied using restriction fragment length polymorphisms (RFLPs), followed by Sanger sequencing, particularly of HVR-I and HVR-II in the control region of mtDNA. These relatively short sequences generated much of the early information on mitochondrial haplotypes and their geographic distributions (Cann et al., 1987). However, only part of the mitochondrial genome was examined, and closely related lineages were not always separated. Once complete mitogenomes become available, additional variants from the coding region can be included. Haplogroups could then be assigned at finer levels, new sublineages were recognized, and more sequence information became available for phylogenetic and molecular dating analyses (Parson & Bandelt, 2007). This difference was particularly noticeable within common haplogroups. The information was useful but limited. For example, two individuals carrying the same control region haplotype may still differ at several positions elsewhere in the mitochondrial genome.

Complete mitogenome sequencing has improved this resolution. Mutations from the coding region can now be considered together with control-region variation, allowing maternal lineages to be placed more precisely within the mitochondrial phylogeny. Haplogroup assignment improved, and previously unresolved branches were divided into finer subclades. Complete sequences also provide considerably more information

for phylogenetic and molecular dating analyses than the control region alone (Parson & Bandelt, 2007). This was an important shift, as mitochondrial studies were moving from a small, highly variable part of the molecule to the entire maternal genome.

Massively parallel sequencing (MPS), often referred to as next-generation sequencing, has changed the scale again. A large number of mitochondrial fragments can be sequenced simultaneously and at considerable depths. As a result, it is possible to produce entire mitogenomes for numerous individuals within a single experiment, even when dealing with samples that have fragmented DNA. This capability has proven especially beneficial in forensic and ancient DNA studies, where the molecules available might already be brief. When mitochondrial DNA constitutes just a minor portion of the extracted DNA, targeted methods are frequently employed. Multiplex PCR enhances specific mitochondrial regions through amplification, while hybridization capture employs probes to extract mitochondrial fragments from sequencing libraries. Capture-based approaches have become especially useful for highly degraded materials because very short endogenous fragments can still be recovered.

Greater sequencing depth has also changed the way heteroplasmy is studied. Sanger sequencing is generally suited for detecting relatively prominent mixtures of mitochondrial variants, but low-frequency heteroplasmy is much harder to distinguish reliably from the background signal. MPS produces several independent reads across the same position. Minor variants can therefore be detected at much lower frequencies, provided that sequencing errors, strand bias, contamination, and analytical thresholds are carefully controlled. This distinction is important. A low-frequency alternative base is not necessarily a biological heteroplasmy. The confidence of the call depends on the read depth, sequence quality, library preparation, and criteria used during variant calling. Modern sequencing has consequently increased sensitivity but has also made quality control more demanding.

Increased sequencing depth does not imply that every mitochondrial-like read should be accepted as mtDNA. Some mitochondrial sequences have been transferred to the nuclear genome during evolution and remain as NUMTs. Reads originating from these regions may map back to the mitochondrial reference sequence, particularly when the homologous regions are short. They may also be amplified together with the genuine mtDNA. This problem is more noticeable when very low-frequency heteroplasmy is being investigated, as a small number of NUMT-derived reads can resemble a minor mitochondrial allele. Therefore, mapping quality, read depth, and distribution of reads across both strands are checked during variant calling, and suspected NUMTs may require additional filtering. The same caution applies to the contamination. This is particularly

important for ancient DNA and other low-template samples, where a relatively small amount of introduced DNA may contribute to an appreciable fraction of the recovered sequence.

Long-read sequencing is also being used for mtDNA analysis. Short-read sequencing reconstructs the mitogenome from many overlapping fragments, whereas long-read platforms can sequence much larger stretches of the same mitochondrial molecule. Oxford Nanopore and PacBio sequencing have both been applied to mitochondrial genomes, although their use is not yet as routine as that of short-read MPS. One useful feature is the phasing. When two variants occur on the same sufficiently long read, they can be linked directly to the same molecule instead of inferring their relationship from separate short reads. This may be useful when several heteroplasmic sites are present in a single sample. Longer reads can also help evaluate sequences that might otherwise be confused with NUMTs. However, there are practical limitations. Ancient DNA, for example, is already highly fragmented; therefore, the main advantage of producing very long reads is largely lost in severely degraded material. Therefore, long-read sequencing is more immediately useful for well-preserved DNA and particular questions involving mitochondrial haplotypes, structural variation, or heteroplasmy than as a general replacement for existing short-read methods.

6. LIMITATIONS, CONTROVERSIES, AND CHALLENGES IN mtDNA STUDIES

The exact property that makes it excellent for reconstructing maternal genealogy also limits its interpretation; it only represents one maternal line. It is one of the many haplogroups that contribute to the maternal lineage of an individual's ancestry. Therefore, migration and admixture may alter the autosomal ancestry of a population without producing any significant change in the maternal lineage, specifically when the movement is sex-biased. The reverse can also occur in some cases. Therefore, a mitochondrial phylogeny should not be read as a genealogy of the entire population (Ballard & Rand, 2005).

Genetic drift adds further complications. The relatively small effective population size of mtDNA means that maternal lineages can be lost or become common by chance, particularly in small populations and after founder events (Birky et al., 1983). A haplogroup that is frequent today was not necessarily equally frequent in the past. Its present geographic distribution does not necessarily identify its origin. Population movement, lineage extinction, and later founder effects can all reshape the distribution. Sampling bias can worsen the picture. Regions represented by hundreds of mtDNA samples will naturally reveal rarer and more basal lineages than populations represented by only a few samples.

Neutrality cannot always be assumed. Many demographic analyses have treated mitochondrial variation largely within a neutral framework; however, functional variants in the mitochondrial genome are exposed to selection. Purifying selection removes many deleterious mutations, particularly from functionally constrained positions, whereas positive selection has also been proposed for some mitochondrial variants and haplogroups (Galtier et al., 2009). This is important because selection can change lineage frequencies without a corresponding demographic event. An apparent expansion, reduction in diversity, or geographic difference may therefore contain both demographic and selective components. Separating these two is not always easy.

Molecular dating has a similar problem. There is no single mitochondrial rate that can be applied to every dataset and timescale. Rates estimated from pedigrees, within-human phylogenies and deeper evolutionary comparisons can differ, and different parts of the mitochondrial genome do not evolve at identical rates. Purifying selection is one of the reasons for this difference. Soares et al. (2009) showed that correcting for its effect changes the estimates obtained from complete human mitogenomes and proposed a time-dependent mitochondrial molecular clock. The dates of historical demographic events should also be treated with caution and with knowledge of the calibration used (Soares et al., 2009).

Heteroplasmy means that an individual does not always carry a single mtDNA sequence. More than one mitochondrial variant may be present, sometimes at very different frequencies, and these frequencies can vary between tissues or change during maternal transmission (Stewart & Chinnery, 2015). This variation is partly lost when a consensus sequence is used for population and phylogenetic analyses, since variants below the chosen threshold are usually not represented. The threshold is important. A variant reported as low-level heteroplasmy in one study may fall below the cutoff used in another. Haplogroup assignment is generally based on the dominant mitochondrial sequence, but low-frequency variants become much more relevant when maternal transmission, recent mutations, or changes in heteroplasmy over time are being studied.

Not all apparent mitochondrial variants are of mitochondrial origin. NUMTs can be amplified or mapped as mitochondrial sequences and may be mistaken for genuine variants, particularly when low-frequency heteroplasmy is being studied. Contamination creates similar difficulties in low-template and ancient materials. Ancient DNA has the additional problem of molecular degradation: only a fraction of the original DNA may survive, and preservation differs greatly among environments and archaeological sites. Therefore, strict authentication and appropriate sequence filtering

are part of the interpretation, not simply laboratory precautions.

Mitochondrial and nuclear histories may also disagree. Such mito-nuclear discordance can arise through sex-biased migration, admixture, differences in effective population size, and genetic drift; selection on mitochondrial or interacting nuclear loci may contribute in some situations as well (Toews & Brelsford, 2012). The cause should not be assumed based on discordance alone. Human population genomic analyses, for example, have detected mitonuclear linkage disequilibrium, but the associations were generally weak and did not provide evidence for unusually strong species-wide maintenance of associations between mtDNA and nuclear genes encoding mitochondrial proteins (Sloan et al., 2015). The demographic history of populations is still important.

None of these issues make mtDNA unsuitable for population genetic studies. They define what can be reasonably asked of it. mtDNA is particularly informative about maternal lineage history, including lineage continuity, founder events, maternal population structure, and some forms of sex-biased demographic change. Genome-wide data address ancestry at different scales, whereas ancient DNA can show whether lineages inferred from present-day samples were actually present at earlier points in time. Archaeology and historical evidence add yet another kind of information. Mitochondrial results can be misleading, mainly when the history of one maternal lineage is allowed to stand for the history of an entire population.

7. FUTURE DIRECTIONS IN mtDNA RESEARCH

Despite the swift expansion of genomic datasets, there are still significant gaps in the study of human mtDNA. Areas like Africa, South Asia, Oceania, and numerous indigenous communities are not adequately represented in the worldwide mitochondrial dataset. Ensuring these groups are properly included and conducting thorough sampling in regions already represented could uncover rare or previously unidentified maternal lineages and potentially introduce new haplogroups.

Advancements in deep sequencing have shown that low-level heteroplasmy can be hidden below conventional sequencing thresholds. Long-read approaches add another layer by allowing variants separated by larger distances to be examined on the same DNA molecule and by improving the detection of structural changes. These methods still have technical limitations, particularly for low-frequency heteroplasmy, and will require careful validation as their use expands.

Another direction is the study of mtDNA in the nuclear background. Mitochondrial function is largely reliant on proteins encoded by nuclear DNA, yet population studies frequently analyze mitochondrial lineages in isolation. Mitochondrial dysfunction has also been associated with

alterations in cellular metabolism and muscle-cell function, highlighting the importance of considering mitochondrial activity when linking molecular variation with cellular phenotypes (Wang et al., 2024). Integrating mtDNA with genome-wide data allows for the separation of maternal lineage from overall population ancestry and aids in identifying mitonuclear interactions on a population level. By using transcriptomic, proteomic, and metabolomic analyses, researchers can determine if mitochondrial variants or specific mitonuclear combinations correlate with observable differences in cellular function, rather than solely depending on geographic distribution as proof of adaptation. Recent experimental studies also show that mitochondrial activity and oxidative stress can affect cellular responses under physiological and metabolic stress conditions (Fu et al., 2024).

8. CONCLUSION

mtDNA remains one of the most widely used markers for tracing the maternal population history. Early studies used mitochondrial variation to establish relationships among maternal lineages and contributed to the reconstruction of major human dispersals, including the African ancestry of modern humans (Cann et al., 1987). The field now operates at a different resolution. Complete mitogenomes have separated lineages that could not be distinguished from control region sequences alone, and ancient DNA has allowed some of these lineages to be observed directly in past populations. Deep sequencing has added variation within individuals to this picture. Heteroplasmy can be inherited, change during transmission, differ among tissues, and accumulate with age. It can no longer be treated as an occasional complication of mitochondrial sequencing.

However, the history contained in the mitochondrial genome is limited. It follows a maternal line. Drift can remove lineages, founder events can increase their frequency, and selection may alter patterns that would otherwise be interpreted as demographic patterns. Even the age assigned to a lineage depends partly on the mutation rate, calibration, and evolutionary model used. Technical problems, including NUMTs and the detection of low-frequency heteroplasmy, add to this uncertainty. For these reasons, mtDNA is most informative when the question concerns maternal history rather than when it is used as a proxy for the ancestry of an entire population. Nuclear and ancient genomic data can show whether the same history is visible elsewhere in the genome, and archaeology and historical evidence may support a demographic interpretation or point in another direction. Mitochondrial sampling remains sparse in some populations. Filling these gaps may add lineages to the existing phylogeny or alter relationships that currently appear settled. mtDNA has not lost its place in human evolutionary research, but the way its evidence is interpreted has become more careful.

AUTHOR CONTRIBUTIONS

Conceptualization and formal analysis- RM, CD, AKS, and GC; investigation, HST, SB, and RK; writing-original draft preparation, RM, GC and AKS; writing-review and editing, RVS, AKS, TD, DK and RK. All authors have read and agreed to the published version of this manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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