Chapter 20
Mitochondria in Anthropology and Forensic Medicine

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Abstract Mitochondria's role in crucial metabolic pathways is probably the first answer which comes to our minds for the question: what do these tiny organelles serve for? However, specific features of their DNA made them extremely useful also in the field of anthropology and forensics. MtDNA analyses became a milestone in the complex task of unraveling earliest human migrations. Evidence provided by these experiments left no doubts on modern humans origins pointing to Africa being our cradle. It also contributed to interpretation of putative ways of our dispersal around Asia and Americas thousands years ago. On the other hand, analysis of mtDNA is well established and valuable tool in forensic genetics. When other definitely more popular markers give no answer on identity, it is the time to employ information carried by mitochondria. This chapter summarizes not only current reports on the role of mitochondria in forensics and reconstruction of modern humans phylogeny, but also calls one's attention to a broad range of difficulties and constraints associated with mtDNA analyses.

Keywords Anthropology • Forensic genetics • mtDNA analysis • Phylogenetics

20.1 Mitochondrial DNA Polymorphism in Anthropology

Soon after deciphering the entire sequence of mitochondrial DNA (Anderson et al. 1981) this tiny molecule let us reveal more and more of its potential. Alas, it has one "drawback" among plenty of indisputable advantages – sometimes it cannot replicate itself perfectly. Though, this relatively high rate of mutation changes makes mtDNA a good source of knowledge about recent history of *Homo sapiens* spanning 150,000 years back or even more. The only marker that played almost as important role as mtDNA in unraveling our species phylogeny is Y chromosome.

Obviously it is not possible to fully reconstruct phylogeny of any species using a single DNA marker, yet realizing that today's variation reflects past events – although seemed a challenge at first – allowed drawing reasonable conclusions concerning genetic history of *Homo sapiens*.

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20.1.1 Introduction

While for most geneticists mtDNA sequence is fairly enough for subsequent inferences, phylogeneticist seeks for special features of the polynucleotide chain to firstly assign molecule under study to specific group, called haplogroup. Haplogroup is a cluster of closely related haplotypes that share mutations inherited from common ancestor. Term "haplotype", in turn, embraces all molecules that are characterized by the same order of nucleotides and fell into definite haplogroup. It is worth noting that mutations in mtDNA sequence are usually reported as deviations from the revised Cambridge Reference Sequence (rCRS, Andrews et al. 1999).

Skeleton of the world mtDNA phylogeny has been quite well reconstructed and most of the major haplogroups have been characterized quite deeply (Fig. 20.1). Nevertheless, any new haplogroup has to be named according to specific rules. Nomenclature of haplogroups is fixed and imposes using letters and numbers by turns beginning with a letter, for example U5a1b (Macaulay et al. 1999).

Mutual relationships of haplotypes can be depicted as trees – kind of graphs (as seen by mathematics) in which each pair of vertices is connected by exactly one straight path. Each path represents at least one mutation event. According to this theorem, haplotype can be found in almost every vertex and in every leaf (in case it has no offspring). Sometimes, when sequence under study has extremely high mutation rate, i.e. mtDNA control region, it is reasonable to present phylogeny in a form of cyclic graphs which are called networks by phylogeneticists. Their major attribute, which serves as a key factor for discriminating them from trees, is possibility of presenting various plausible simultaneous ways of evolution, including specific events like parallel and back-mutations.

Given a tree, one is able to estimate evolutionary age of each lineage and sometimes also its dispersal time. What has to be utilized is so called human mitochondrial molecular clock, which for general purposes might be treated as a direct assumption of mutation rate in certain mtDNA regions. There are several molecular clocks available, although no uniform calibration has been proposed, that would take both coding and non-coding regions into account. On the basis of human-chimp divergence time of 6.5 MYBP Mishmar et al. (2003) calculated mutation rate of 1 nucleotide change per 5,138 years that was probably most widely used so far. Its major drawback, however, is exclusion of influence of selection on shaping mtDNA diversity. Other authors (e.g. Kivisild et al. 2006) pointed out significant excess of non-synonymous mutations in younger mtDNA clades and therefore suggested counting only synonymous mutations for the calculations. According to their estimations one mutation event takes place in mtDNA coding region every 6,764 years, on average. Although the clock took effects of selection into account, it still didn't seem to be accurate enough since it excluded a significant portion of mtDNA genome from calculations. Problem of evident time-dependency of mutation rate was solved partially by Soares et al. (2009). Their calibration also utilizes human-chimp splitting time, yet is based on most recent fossil evidence. According to Soares et al. (2009), substitution rate in the whole molecule accounts to 1 per 3,624 years, while in coding region itself 1 mutation arises every 3,533 years.

20.1.2 MtDNA and the Origins of Modern Humans

It is widely accepted now that all modern humans derive from African populations. The most convincing proof was given by Alan Wilson's group (Cann et al. 1987) of the University of California, who used 12 restriction enzymes in their study to digest mtDNA taken from as little as 147 donors representing various contemporary populations – Americans (both of European and African origins), East Asia and New Guinea inhabitants and Australian Aborigines. Restriction mapping results allowed construction of a maximum parsimony phylogenetic tree (MP) depicting evolutionary relationships

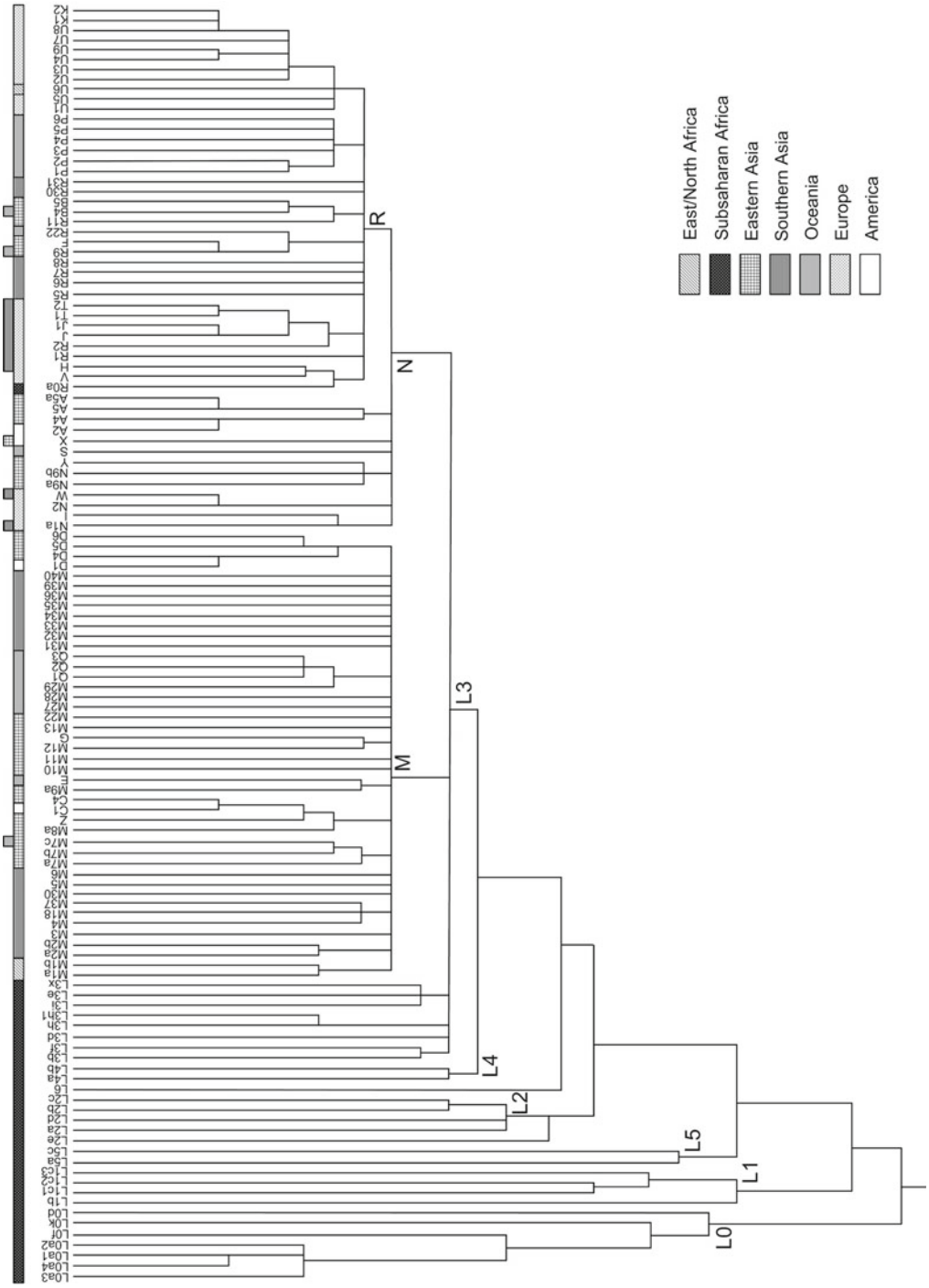

Fig. 20.1 Global mitochondrial DNA phylogenetic tree, with a basic branching pattern according to the updated tree of van Oven and Kayser (2009)

between all haplotypes. Two deepest roots in this tree were representing mtDNA of exclusively African origin and a mixture of African and non-African lineages. Surprisingly, most significant diversity was noted among purely African lineages. These observations were interpreted as a strong evidence supporting so called "out of Africa" hypothesis suggesting that *H. sapiens* is of African origin. Performing molecular dating of the tree has shown, that the last common ancestor of the all mtDNA sequences under study (so called "mitochondrial Eve") must have lived in Africa about 140,000–200,000 years ago. Many subsequent mtDNA analyses confirmed Wilson's conclusions (Vigilant et al. 1991; Mishmar et al. 2003; Macaulay et al. 2005). Global mtDNA tree can be divided into two daughter branches, L0 and L1'5 (Behar et al. 2008). The L1'5 branch encompasses sub-Saharan L3 that is dated 50,000–84,000 years back (Macaulay et al. 2005; Kivisild et al. 2006). Two macrohaplogroups, M and N, deriving directly from L3 cover all mtDNA molecules noted in all populations beyond Africa. Part of N haplogroup evolved into another numerous and also very divergent haplogroup – R.

Phylogenetic tree reconstructed on the basis of single nucleotide polymorphisms (SNPs) found on non-recombining part of Y chromosome (NRY) has a topology that highly resembles the one created for mtDNA. They both support the 'out of Africa' hypothesis, which assumes gene pool of *Homo sapiens* was dramatically reduced as a result of founder effect.

20.1.3 Earliest Human Migrations from Africa to Eurasia

The time and routes of earliest migrations of our ancestors from Africa to Eurasia were most widely reconstructed on the basis of mtDNA analyses. All non-African mtDNA lineages are classified as belonging to one out of two major macrohaplogroups M and N – both deriving from sub-Saharan L3 haplogroup. West Eurasian mitochondrial genomes fall almost exclusively into macrohaplogroup N. On the contrary, mtDNAs of inhabitants of East Eurasia belong both to M and N macrohaplogroups, which originate most probably from East Africa (Quintana-Murci et al. 1999; Kivisild et al. 2003; Metspalu et al. 2004). They reached Asia via so called southern route, during earliest waves of migration of *Homo sapiens sapiens* taking place ca. 70,000 years ago. Existence of the southern route, conducted from East Africa through Bab-el-Mandeb and southern part of Arabian Peninsula (Yemen), through tropical coasts of the Indian Ocean to Southeastern Asia and Oceania, is supported mostly by the M macrohaplogroup phylogeny. It's important to notice that its highest diversity and frequencies are noticed in India, while in the Near East and Southwest Asia it is very scarce (Kivisild et al. 2003; Metspalu et al. 2004; Sun et al. 2006; Chandrasekar et al. 2009). Distribution of frequencies and diversity of macrohaplogroup M indicates that existence of the ca. 45,000 years ago so called northern route of Eurasian colonization, presumed to lead along the Nile river, through Sinai to the Near East, is less plausible. Evolutionary ages of M and N haplogroups are similar – 50,000–70,000 years, respectively (Soares et al. 2009). Since all mtDNA lineages descending from African L3 haplogroup in India belong to M and N haplogroups that are of similar age, one may expect all founder lineages of both macrohaplogroups reached Eurasia via southern route during first migrations of *Homo sapiens* from Africa. First division into west- and east-Eurasian took place in the region between the Indus Plain and territories encompassing southwestern Asia (Metspalu et al. 2004). Haplogroup R arose from macrohaplogroup N relatively early (Kivisild et al. 2003). In eastern Eurasia it was divided into B and R9 and in western Eurasia – into HV, TJ and U clades. This scenario is reflected by the results of entire mtDNA genomes sequencing experiments encompassing some populations inhabiting regions adjacent to the southern route. For example, results of entire mtDNA genomes sequencing from indigenous peopling of Malaysia, named *Orang Asli,* showed existence of two haplogroups M21 and M22 of advanced age (ca. 57,000 years ago). Its appearance is limited almost exclusively to *Orang Asli* and a few Thailand inhabitants (Macaulay et al. 2005). Similar geographical specificity of some mtDNA clades, derived directly from M, N and R haplogroups, was spotted during full mtDNA sequencing experiments among

Australian Aborigines and Papua New Guinea inhabitants (Ingman and Gyllensten 2003). Age and geographical specificity of the abovementioned haplogroups indicate that they are regional relicts of earliest migrations of modern humans. According to these facts it seems obvious that no matter if first colonizers chose northern or southern route, they must have reached the Indian Ocean coasts soon after leaving Africa. Some authors assume that there was one wave of migration leading through the southern route which made all three M, N and R haplogroups reach Eurasia. On the basis of molecular dating of these clades (TMRCA) in the region between India and Australia it is assessed that first colonizers got to India, Eastern Asia (China) and Australia 66,100 ± 5,700, 64,500 ± 3,800 and 63,400 ± 5,200 years ago, respectively. Assuming that route of migration adjacent to the Indian Ocean coasts numbered ca. 12,000 km it can be estimated that the speed of dispersal of modern humans came to 0.7–4 km per year. Population which left Africa about 60,000 years ago probably wasn't numerous and consisted of as little as 500–2,000 women (Macaulay et al. 2005).

It seems that the results concerning scenario of colonization of Eurasia, as revealed by entire mtDNA sequencing, are consistent with the data obtained from population analyses based on the nonrecombining part of chromosome Y (NRY). Vital for the inference is the observation of C, D and F haplogroups of Y chromosome in populations of South Asia. It is probable that founders of these three clades reached Eurasia as a result of single migration event via the southern route. However, some authors suggest that beside the earliest wave of migration along southern route, which brought C and D clades to Europe, second wave also existed – one that led north, across the Near East, which took haplogroup F to West and East Eurasia (Richards et al. 2006).

One should be aware, however, that these discrepancies in opinions concerning Eurasia colonization events may be only apparent. Colonizing of west Eurasia, which according to soma anthropologists, archeologists and geneticist was carried out along northern route from Africa, across Sinai and the Near East, could be the result of early modification of the chosen direction of migration by some group of founders, who finally took the southern route. This hypothesis is supported by similar age of N and R mtDNA haplogroups in India and Europe (66,100 ± 5,700 and 66,300 ± 5,600 years, respectively) (Macaulay et al. 2005). In compliance with this scenario, ancestors of contemporary Europeans would be this group of colonizers, which after having left Africa changed the route and sidetracked into north-western direction. Wanderers were restrained from further conquests by the harsh weather conditions, yet finally entered Near East and Europe 45–50 k years ago. There is also an evidence of back-migrations from the Near East into North Africa, based on the presence of haplogroups U6 and M1 in North and East Africa (Olivieri et al. 2006).

20.1.4 Time and Routes of the Americas Colonization in the Light of mtDNA Testing

Perfect climatic and topological conditions for conquering America by the modern humans were noticed in a period of time between 30,000 and 13,000 years ago due to existence of mainland between Siberia and Alaska. If first colonizers took a chance of migrating across the Bering Strait, they would run into unexpected obstacle, which blocked the main route into the continent for the vast part of Pleistocene. This barrier was an ice-cap lingering huge part of the North America. In the times between 55 and 30,000 years ago and about 13,500 y.a. the abovementioned obstacle was probably cut with the ice-free corridor between today's Yukon River, through Canada reaching Montana state. This piece of human-friendly ground could serve the colonizers as the promenade into America (Jobling et al. 2004).

Last decade was abound with intense genetic analyses which aimed in searching for Asian territories, which were associated with entering the New World. So far, most advanced research concerning mtDNA is currently made with maximum resolution – on the entire mitochondrial genome sequencing level.

After migration through Bering Strait was visualized, may scientists naturally paid their attention to Central Siberia populations, scarcely studied so far. In fact in the 1990s it was stated that the variety of mtDNA pool of Native Americans constitutes some part of diversity seen in Central-Asian and Siberian populations – in all three linguistic groups of American Indians (Amerindian, Na-Dene and Eskimo-Aleut) only four main founder mtDNA haplogroups were noted – A, B, C and D – and all that are also seen in Asia (Wallace 1995). Undoubtedly, these sought-after "founder territories" for the New World must be located somewhere in Central Asia and/or Siberia. The fifth founder clade seen among Native Americans is X – one that is spotted almost exclusively among some north Amerindian groups (i.e. Ojibwa speaking Algonquin language) and Navajo belonging to Na-Dene linguistic group (Brown et al. 1998). What characterizes the X haplogroup, and particularly its subclade X2 is its wide range of occurrence in Europe (Reidla et al. 2003). On the contrary, not much is known about its existence in Asian populations. Therefore, quite interesting was the discovery of its occurrence in southern Siberian populations – among Altaians and Buryats. On this basis it has been suggested that Siberian haplogroup X could have been one of the Asian founders for Paleoindian mtDNA pool (Derenko et al. 2001). Subsequent analyses of coding region sequence of mtDNA using PCR-RFLP technique and entire genome sequencing have shown that Altai, who were assigned to X haplogroup, belong to its subclade X2e, which seems much different from X2a typical for Native Americans (Reidla et al. 2003; Derenko et al. 2007). The latter hasn't been found in any Eurasian population so far.

Number and time periods of the first *Homo sapiens* migrations from Asia to the New World are still a matter of heated discussion. First hypothesis assumed existence of three waves corresponding to three main linguistic groups of Native Americans. Yet, it hasn't been proven to be true according to data obtained from various genetic markers analyses. Arguments against existence of these three waves come i.a. from mtDNA analyses, which demonstrate no correlation between genetic structure of Native Americans and their affinity to linguistic groups. More light has been shed on this issue by the mtDNA experiments held just 2–3 years ago.

Thanks to entire mtDNA genomes sequencing, phylogeny of Native American (A-D) haplogroups could be resolved in detail, distributions of frequencies of certain haplogroups were analyzed and their evolutionary age was assessed. It turned out that indigenously American origin characterizes only subclades A2, B2, C1 and D1 and their evolutionary age is very close (about 20,000 years), what suggests that they reached deeper American lands in just one wave of migration from Beringia (Tamm et al. 2007; Achilli et al. 2008). Until now, it is not clearly explained what was the number of founder haplotypes for these haplogroups that took part in colonizing the America. Each of the haplogroups could have been represented by single haplotype (its founder node) or multiple haplotypes – as suggested for C1 haplogroup, which is said to have three founder haplotypes among the New World colonizers (Achilli et al. 2008).

Irrespective of the number of founder haplotypes, molecular dating results (TMRCA) for A2, B2, C1 and D1 indicate that these haplogroups were brought to the North American continent about 19,000 years ago, right after the last glacial maximum (LGM). The data gathered so far support the hypothesis of an early settlement of modern humans in America, preceding Clovis culture. mtDNA sequencing results are absolutely consistent here with archeological data which points to arrival of modern humans to the South America (Monte Verde, Chile) at least 12,500 years ago.

20.1.5 Colonization of Europe as Revealed by mtDNA Analysis

After having left Africa, modern humans manned all the remaining continents and genetic drift in populations inhabiting geographically distant areas led to development of a kind of subdivision (structure) of the global population. It is reflected by the results of molecular variance analyses (AMOVA)

performed for data coming from various genetic markers. For example, for autosomal biallelic loci (single nucleotide polymorphisms, SNPs), about 91% of diversity is observed within population, whereas as little as 9% is seen between main continental groups of populations (Li et al. 2008). For non-recombinant markers (mtDNA, NRY) part of diversity that is observed between continents is considerably higher (12–52%), what is a result of smaller effective population size (1/4 as compared to autosomal loci) and related increased impact of genetic drift favoring formation of stratification in global population (reviewed by Jobling et al. 2004). It is worth to notice that this structure is more evident in the case of Y chromosome than in mtDNA (about 12–22% and 16–52% between continents, respectively). To some extent, this difference follows patrilocality that is responsible for higher genetic distances between populations as inferred from Y chromosome analyses than mtDNA.

While intercontinental differences are easily noticed in a variety of markers, differences between populations inhabiting the same continent are definitely harder to be spotted. One of the existing exceptions is a clear structure of African populations (Tishkoff et al. 2009). The autosomal gene pool in Europe is homogeneous but at the same time small genetic differentiation that is present between subpopulations (an average of 0.17% of the total genetic variance) is characterized by a significant correlation between genetic and geographic distance (Lao et al. 2008).

Mitochondrial DNA pool of Europeans is even more homogenous (Simoni et al. 2000; McEvoy et al. 2004). As a result of early founder analyses of mtDNA in the Near East in Europe is was shown that the majority of mtDNA haplogroups in Europe shared a common regional ancestry from the Upper Paleolithic and Neolithic (Richards et al. 2000). The pioneer colonization of the Upper Paloolithic might have been marked by the most ancient haplogroups in Europe, U8 and U5, dated back to ca. 50,000 and 30,000 years, respectively (Soares et al. 2010; Malyarchuk et al. 2010). During the Last Glacial Maximum (LGM, between 19,000 and 22,000 YBP) human populations became concentrated in refugial areas in the western Caucasus and southern European peninsulas. Thus, re-colonisation of the continent from southern refugia in the wake of the major warming phase after 15 kya appears to be a major concept to explain the mtDNA diversity of the present-day Europeans. Recent analyses of the complete mitochondrial genomes point to the postglacial expansion times for most of the lineages spreading from south-west Europe. This re-expansion time frame is reflected mainly in the age of haplogroups V, H1 and H3 which all appear to have originated in south-west Europe and date to 11–11.5 kya—the end of the Younger Dryas glacial relapse (Soares et al. 2010). Near Eastern Neolithic contribution to the mtDNA pool of Europeans is probably minor and includes selected subclusters of haplogroups J and K, namely, J2a1a and K2a, both dating to 8–9 kya within Europe (Soares et al. 2010). One may predict that future analyses of complete mitochondrial genomes accompanied by dating with improved mtDNA clock will shed more light on the archeogenetics of Europe.

20.2 Mitochondrial DNA as a Tool in Forensic Investigations

20.2.1 Introduction

Solving many criminal cases is dependent on determining the origin of material evidence by the means of molecular biology techniques. Individual identification is a multidisciplinary field, gathering most exciting achievements of molecular biology, genetics, informatics, genomics and many more under just one name. The techniques that are currently in use in forensics, are mostly based on DNA analyses, so it is not surprising why so much emphasis is put on development of these.

The real breakthrough in forensic genetics took place when in vitro amplification of DNA was proposed. PCR technique revolutionized virtually all fields of experimental biology, but it is not an

exaggeration to say that for forensics the new era began. It finally turned possible to draw DNA profiles from tiny amounts of biological stains and individual DNA identification, thanks to its improved (in order of several magnitudes) sensitivity, became first-line method in solving criminal cases where any biological trace is involved. Nevertheless, scientific aspects of individual identification are still evolving.

One may expect that the commonly used autosomal microsatellite (STR) profiles obtained from DNA analysis would be sufficient for individual identification in all cases, yet quite surprisingly it is not true. In certain cases, i.e. when it comes to maternity testing or the collected material is degraded or available from hair shaft only, the only hope for forensics expert is utilizing mtDNA analyses.

20.2.2 Mitochondrial DNA Analyses in Forensics

Although autosomal miniSTRs and single nucleotide polymorphism (SNP) loci are very effective tools in identification of most types of specimens, they face some restrictions when analyzed material spent some time in unfavorable conditions making nuclear DNA degrade. However, degraded DNA is not a deadlock anymore, as mitochondrial DNA testing works quite efficiently even if material underwent major damage.

Mitochondrial genome has some specific features making it more resistant than nuclear DNA. First of all, it is of circular shape, which ensures its small vulnerability to exonucleases that usually quite easily degrade DNA chains. Secondly, it is present in each somatic cell in high numbers – each mitochondrion contains up to 11 mtDNA particles (Cavalier et al. 2000), while there are 1,000–100,000 mitochondria in each cell (depending on its type) (Lightowlers et al. 1997). Therefore, it is more likely to survive under unfavorable conditions than nuclear DNA. Human mtDNA is inherited along maternal lineage and undergoes no recombination. Therefore, when used in identification, it doesn't allow pointing to a single person, but rather a group of relatives, descending from a single woman. Nevertheless, even if it is not as discriminating as set of microsatellite autosomal loci, it has other interesting yet unique attribute – it is quite clearly distinguishable between populations representing various continents and even specific regions. Therefore, in many cases mtDNA analysis results may serve as an important information concerning biogeographic ancestry of the donor.

Lack of recombination in mtDNA causes certain haplotypes to get fixed in population and mutations are the main source of diversity. Quite luckily, mutations occur there ten times more frequently, on average, that in nuclear genome (Brown et al. 1979, 1982). High rate of mutation is the main cause of high mtDNA diversity, that is noticed in human population, which in turn makes mtDNA highly useful tool in forensic genetics. Clear-cut differences are spotted when examining evolution rate in various mitochondrial DNA regions. They occur most frequently per unit of time in so called hypervariable regions of the D-loop – HVSI in region 16,024–16,400, HVSII between 44 and 340 nucleotide and HVSIII from 438 to 576 nucleotide. The highest mutation rate is seen in HVSI region (Brandstätter et al. 2004; Lutz et al. 1998).

In forensic genetics mtDNA analyses are performed usually when other methods are expected to or already failed, for example to identify human remains especially when they are old, skeletal remains or when the only available material is a hair deprived of bulb. It seems useful also in Disater Victims Identification (DVI) teams work, where it serves as a mean of determination of maternal kinship.

How it works? Specific fragments of mtDNA (usually hypervariable regions HVSI and HVSII) are amplified in PCR reaction followed by sequence analysis on automatic analyzers. Sequences obtained are tagged with haplotypes variants as a result of comparison with Anderson's reference sequence (rCRS) (Anderson et al. 1981; Andrews et al. 1999). If the expert observes conformity of the haplotype extracted from material evidence and from the suspect, he or she performs statistical analyses on

the basis of population databases consisting of thousands of records representing haplotypes from persons unrelated in maternal lineage. The more seldom in the database the haplotype from evidence is seen, the more likely it actually belongs to the suspect. However, the strength of mtDNA haplotype frequency estimations is still limited by the size of the current forensic databases, especially for rare mtDNA sequences that have not been observed in databases. For rare types, the apparent frequency in the database will underestimate the true population frequency (Holland and Parsons 1999). Therefore, the painstaking efforts are currently being made to increase the size of control region population datasets and their geographical coverage (Pereira et al. 2004; Behar et al. 2007).

It is worth noting that forensic mtDNA analysis has recently been expanded by development of minisequencing strategies enabling haplogroup assignment of casework and reference samples. These could be extremely helpful in the preselection of samples deriving from suspects and crime scenes in high-volume cases (Brandstätter et al. 2006). Several systems of this kind, based on typing of haplogroup-specific SNPs have been proposed – e.g. the one allowing dissection of the most frequent in Europe haplogroup H into its subclades (Brandstätter et al. 2006) or multiplex minisequencing reaction for genotyping 32 selected coding region SNPs representing main and derived branches of the East Asian and Native American mtDNA phylogeny (Alvarez-Iglesias et al. 2007). In the near future one may envisage the introduction of complete genome sequencing in forensics, accompanied by a development entire mtDNA genome reference population data suitable for forensic comparisons (Irwin et al. 2010).

It is widely known that not only human genetic material is found on crime scene. When species of the donor of DNA has to be determined, mtDNA testing can be a method of choice. The point is to get to know *cytb* sequence. Why this particular one? It has been shown that *cytb* gene is characterized by high level of diversity between species and remains quite uniform in animals belonging to the same species. Therefore, simple PCR followed by sequencing reaction and aligning to *cytb* gene sequence entries in nucleotide databases using the program BLAST let expert witnesses answer questions concerning donor species with high confidence (Parson et al. 2000).

20.2.3 Quality Control

One of the absolutely most important issues in forensic genetics is appropriate and strict quality control. Although it is not that obvious at a first glance, molecular phylogenetics has a lot to do when it comes to assessing value of the data obtained and in forensics in general.

Undoubtedly when an attorney provides forensics expert with material evidence, he or she is usually interested not in evolutionary history but the identity of the donor of DNA to be found. Nevertheless, getting to know haplogroup's affinity of the donor, makes it possible to check the correctness of the results of the DNA analyses – to some extent. It is particularly important if the biological material is old and degraded and the analyses have to be performed in several time-consuming steps – each providing only a subset of the total DNA sequence. Good Laboratory Practice (GLP) justifiably forces experts to work in two independent groups yet nobody has a right to assume, that it would be enough for assuring top class analyses.

Haplotype which is obtained during the analyses has to make some "phylogenetic sense" – in other words, it has to conform to some haplogroup. All the mutations that are found have to be interpreted with known evolutionary events in expert's mind. If there are some deviations like presence of diagnostic mutations typical for two or more haplogroups in one specimen, it should arouse suspicion. In most cases such result arises as a consequence of contamination of the sample. In fact, experienced geneticist should be able to predict almost entire mtDNA sequence on the basis of its small fragment. If his/her predictions are inconsistent with the actual results, analysis should be repeated.

Forensic genetics are usually familiar with phylogenetics and use this knowledge in verification of the data *a posteriori* (Bandelt et al. 2001; Salas et al. 2007). Nevertheless, many older mtDNA databases created in Europe and USA contained some data that in the light of subsequent phylogenetics analyses turned out to be partially incorrect and had to be wiped off (Yao et al. 2004; Bandelt et al. 2004).

20.2.4 Databases

Publicly accessible DNA databases are of great importance in forensic genetics. They allow making statistical inferences on the results generated by automated sequencers. Definitely, the most reliable and most widely used mtDNA haplotypes' database is EMPOP (www.empop.org) maintained by The European DNA Profiling Group (EDNAP). It is an initiative towards gathering control region haplotypes from various world's populations in just one place and making them as credible as possible by harsh quality control procedures (Parson et al. 2004).

The first release of EMPOP, that turned online in October 2006, contained 16121 (Dec 2011) haplotypes (Parson and Dür 2007). The second release (EMPOP2, available from May, 2010) includes more than 5,000 new mtDNA haplotypes from more than 30 populations and thus contains now 10,970 haplotypes. The database is divided into two separate parts – first, "forensic", containing data obtained by collaborating forensic laboratories and second, "literature", built up with reliable data from literature. All haplotypes included in forensic part were submitted in the form of raw database files subsequently assembled into consensus sequences. For each new haplotype sequence of the both strands had to be confirmed and further verified by three separate scientific teams also by the means of phylogenetics. Similar testing concerns literature data, that undergoes *a posteriori* check for correctness before being published.

EMPOP database, although managed by Institute of Legal Medicine (GMI), Innsbruck Medical University, fruits from partnership of several forensic laboratories, that are into mtDNA analyses and passed special verification procedures.

Yet, EMPOP is not only well-shaped collection of haplotypes but also bioinformatics tools allowing to check any population dataset. One of the available tools is Network – an algorithm created by mathematicians that allows clear, graphical presentation of numerical input data. In fact, it serves as a mean to verify correctness of the data (Bandelt and Dür 2007).

20.2.5 Casework Examples

It has already been mentioned here that mtDNA plays a crucial role in individual identification especially if the available evidence seems to be degraded, destroyed by fire or simply old. Geneticists that cooperate with DVI teams are often performing mtDNA analyses as this is the only hope for obtaining satisfactory results. Humankind history is full of mass disasters like terrorist attack on WTC, tsunami in Indonesia or earthquake on Haiti, to name only a few. Sometimes identification by recognition by family member is not possible – this in an open gate for DNA testing. Probably the most extensive mass identification was held by ICMP (International Commission on Missing Persons) in former Yugoslavia (Huffine et al. 2001). It was assessed that in Bosnia and Herzegovina there were over 30,000 missing persons. ICMP had to create a network of fully functional forensic laboratories that would cope together with this huge identification challenge. Considerable part of human remains were bare scattered bones – material unsuitable for testing on the basis of nuclear DNA. In this cases the only choice was to perform mtDNA analyses and compare the results with the before-created database of genetic profiles of families seeking their missing relatives.

MtDNA testing can be also a useful tool for identification of historical remains. In 2005 at Frombork Cathedral (Poland) remains of a man were exhumed from area proximal to the altar Nicolaus Copernicus was responsible for during his life (Bogdanowicz et al. 2009). All the remains – three molar teeth and femurs – had the same mtDNA haplotype. In case of Copernicus it was very hard to find descendants, whose DNA could serve for comparative purposes. Nevertheless, an attempt was made to search for other biological material that would help answer the question whether what have been found are the remains of famous astronomer. In collections of Museum Gustavianum in Uppsala (Sweden) there was a book, "Calendarium Romanum Magnum", which was presumed to belong to Copernicus for the vast part of his life. It was a subject of thorough examination that let scientists reveal several hairs caught between the pages. MtDNA analysis gave surprising results – the haplotype found was identical to the one from remains from cathedral! It is very likely that these remains belong to Nicolas Copernicus as the haplotype observed was noticed only four times in the EMPOP1 database – once in Denmark and three times in Germany (Bogdanowicz et al. 2009).

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