TO THE BONE: DNA RESEARCH AND ARCHAEOLOGY

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Many a time, to talk about DNA is to ask for a blank look.
Therefore, in this contribution,[1] I will discuss DNA research in general, relating it to archaeology in particular. To begin with, DNA is the largest biomolecule in the world. If you enlarged a DNA string to be 4 cm wide, it would be long enough to encircle the earth, its core would be the size of an office building, and a proportionally enlarged human being would be as tall as our planet. If a chromosome were stretched to be 1 km, then research of DNA would have to be done on 0.05 mm.

An organism's total DNA content is called a genome. The human genome consists of approximately 3,000,000,000 base pairs. About 2% of human DNA encodes proteins, which determine the development of an individual's specific functions. Forensic research is concerned with non-coding DNA, also called nonsense or junk DNA. All higher eukaryotes (organisms in which a true nucleus is visible) are rich in repetitive DNA. Non-reproductive cells of human beings contain 46 chromosomes, each of which has a single linear molecule of double stranded ('tandem') DNA.

With the increasing co-operation of molecular biologists and archaeologists, a distinction has come about between ancient and modern DNA. The term ancient DNA refers to all DNA molecules originally belonging to ancient biological material. All other DNA molecules are called modern.[2] It should be noted that the definition, clear though it may seem, yields no hard and fast dividing line in time. Whether DNA molecules are ancient or modern varies with the archaeological time spheres involved.

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[1] Mr. J.J.M. Schepers is to be thanked for his version of the English text.

[2] The present definition, ruling out that modern DNA contaminating ancient biological material can be called ancient, is based on Brown/Brown 1992, 10: "The term 'ancient DNA' refers to DNA molecules that are preserved in ancient biological material."

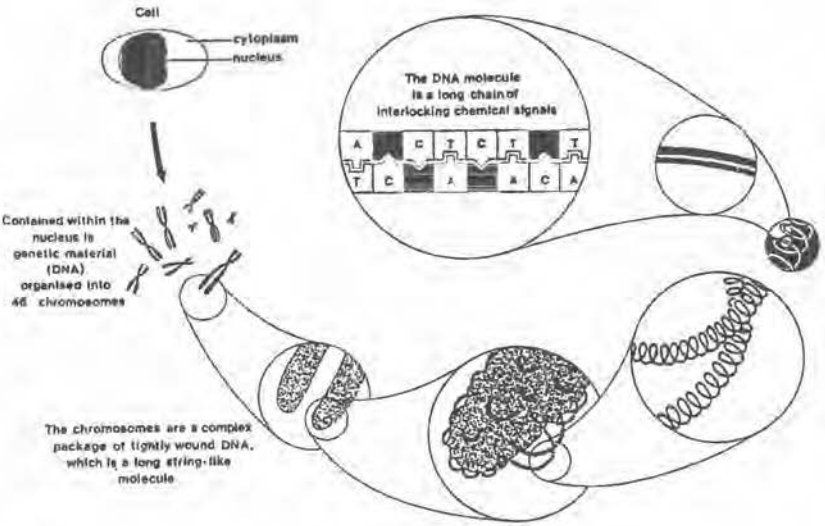

Fig. 1. Organization of DNA in cells.

The first step of any DNA analysis is to collect the samples. Where biologists and archaeologists meet, the latter bring in samples for the former to analyse. The archaeologists are interested in population migrations and diffusions, kinship relations between individuals and groups, and sex of individuals. As to animals and plants, they would like to find out the origins of domestic species, sex of individuals and such properties as meat yield, milk production and 'good bread making quality'.[3]
What information can be elicited from DNA extractions from excavated human or animal bones highly depends on their state of preservation. In turn, the state of preservation is conditioned by burial environment, by how long bones were in soil, and by their treatment during and after excavation. Standard archaeological techniques serve to minimize any mixing of and damage to skeletal material and to prevent contamination with modern DNA. Thus, when excavating, archaeologists have to stick to a protocol. In addition, it is imperative for bones to be handled with gloved hands or forceps. Excavated bones must be preserved very carefully. Careless washing, drying and storage while they are damp can quicken decay.
In my experience, it is practically impossible to establish DNA survival in a bone without subjecting it to a DNA test. The age of a bone hardly

[3] cf. Brown/Brown 1992, 18-19: "What can ancient DNA do for archaeologists?"

Fig. 2. Collection and delivery.

bears on its DNA content. Instead, I think, burial conditions will prove to be decisive.
In the lab, biologists extract DNA from the samples. To monitor any contamination, blank control samples (no bone, only chemical agents) are used in parallel tests.

Types of DNA Analysis
The human genome contains a large number of polymorphisms unique of each individual, unless he or she is one of an identical (monozygotic) twin. Monozygotic refers to the two coinciding, similar hereditary markers, which will show as a single peak. Heterozygotic will show as two peaks.

The DNA methods chiefly used in Forensics are:
1 Variable Number of Tandem Repeats (VNTR). Length variation (nowadays falling out of forensic use, however). Here, the varying numbers of double stranded DNA of individuals are involved. One person, for instance, may have five blocks of a 1000 base pairs of a hereditary marker, whereas another may have eight.
2 Short Tandem Repeats (STR). Length variation. Here, the lengths of the blocks are e.g. 250 base-pairs.
3 Mitochondrial DNA (MtDNA). Sequence variation. In contrast to VNTRS, there are no repeats with MtDNA. Instead, the entire circular DNA, including all base pairs, is analysed and sequenced. The various strand patterns will show the differences.

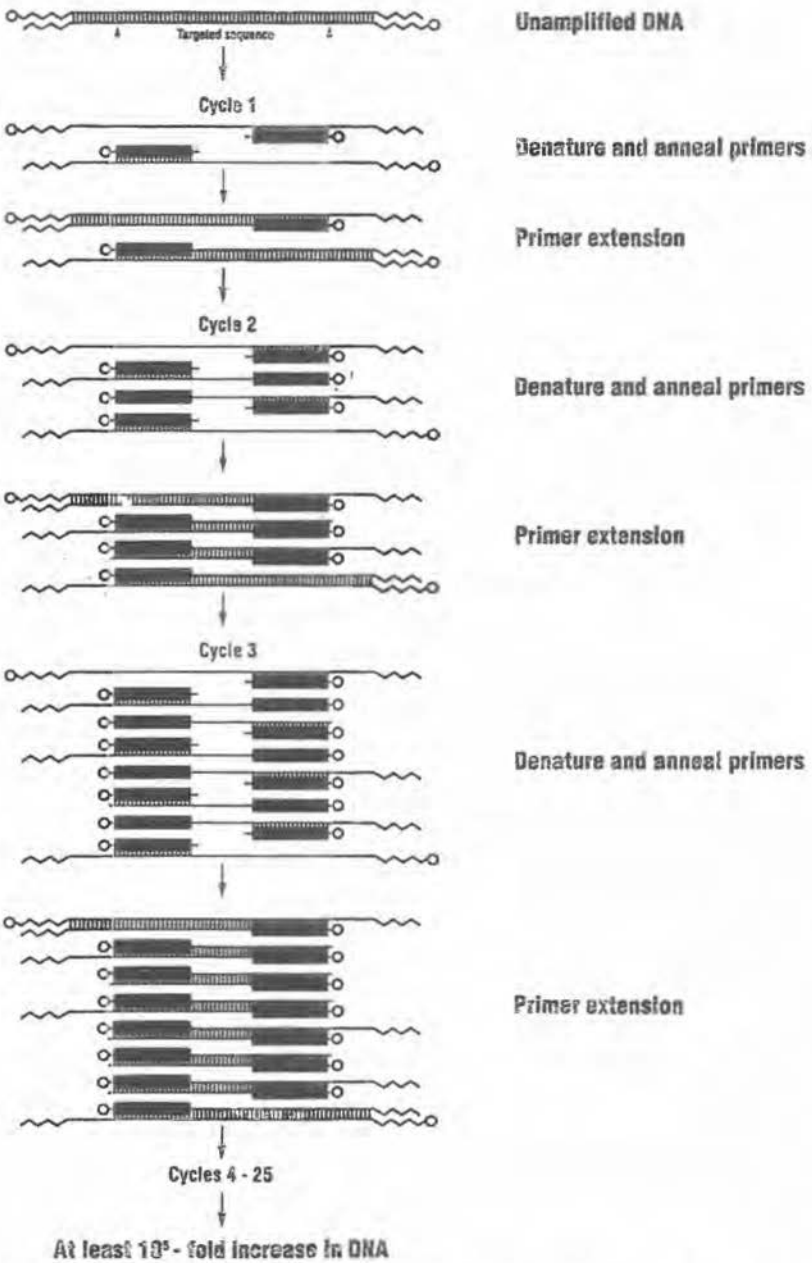

Fig. 3. DNA amplification (Polymerise Chain Reaction, PCR).

STR

The STR technique is widely used for purposes of archaeology. Analysing STR, the first step involves Polymerise Chain Reaction (PCR) amplification (Fig. 3). Owing to the small core units and the small number of repeats, STR alleles (alternative gene forms) usually are under 400 base-pairs. This facilitates amplification of degraded samples and samples with very low amounts of DNA. Most laboratories use the same DNA/STR markers. The primers flanking the amplified STR products are labelled with a fluorescent dye. The commonest method to detect STR's involves the use of polyacrylamide gels for the separation of the PCR-products. In one reaction, 6 DNA/STR markers can be amplified and put on to a gel. The markers won't overlap. Therefore, they can fairly easily be detected by making a laser(-beam) activate the fluorescent dye spotted by sensors (Fig. 4).

Discriminative power

Individual STR's do not have much discriminative power. In the case of multiple reactions, however, the discriminative power can be very high. Thus, an individually amplified STR has no more discriminative power than 1:100, whereas six collectively amplified STR's have a discriminative power (in this example) of 1:100 x 1:100 x 1:100 x 1:100 x 1:100 x 1:100.

Mitochondrial DNA (MtDNA)

A cell having one and only one core but many mitochondria, its mitochondrial DNA, unlike its core DNA (CDNA), has multiple copies. The mitochondria contain a circular/spiral DNA molecule for their specific genome. Unlike the nuclear genome, this genome is homozygous (containing either but not both members of at least one pair of alleles). The presence of multiple copies of MtDNA in every cell furthers the analysis of such degraded samples as DNA bones damaged by environment. MtDNA has only one parent (the mother). The mutation rate in MtDNA is very slow. Therefore, failing maternal near relatives (mothers etc.), to establish kinship, grandmothers, great aunts etc. come in for reliable references. The slow mutation rate yields makers to identify bodies found together, inasmuch as these may have been relatives. It also determines the use of MtDNA for criminal investigations, as lot of people have identical DNA profiles (Fig. 5).

Heteroplasmy, the fact that not each sample of a body has the same mt-DNA base-pair order, causes a problem. Luckily, the problem is not likely to arise with CDNA.

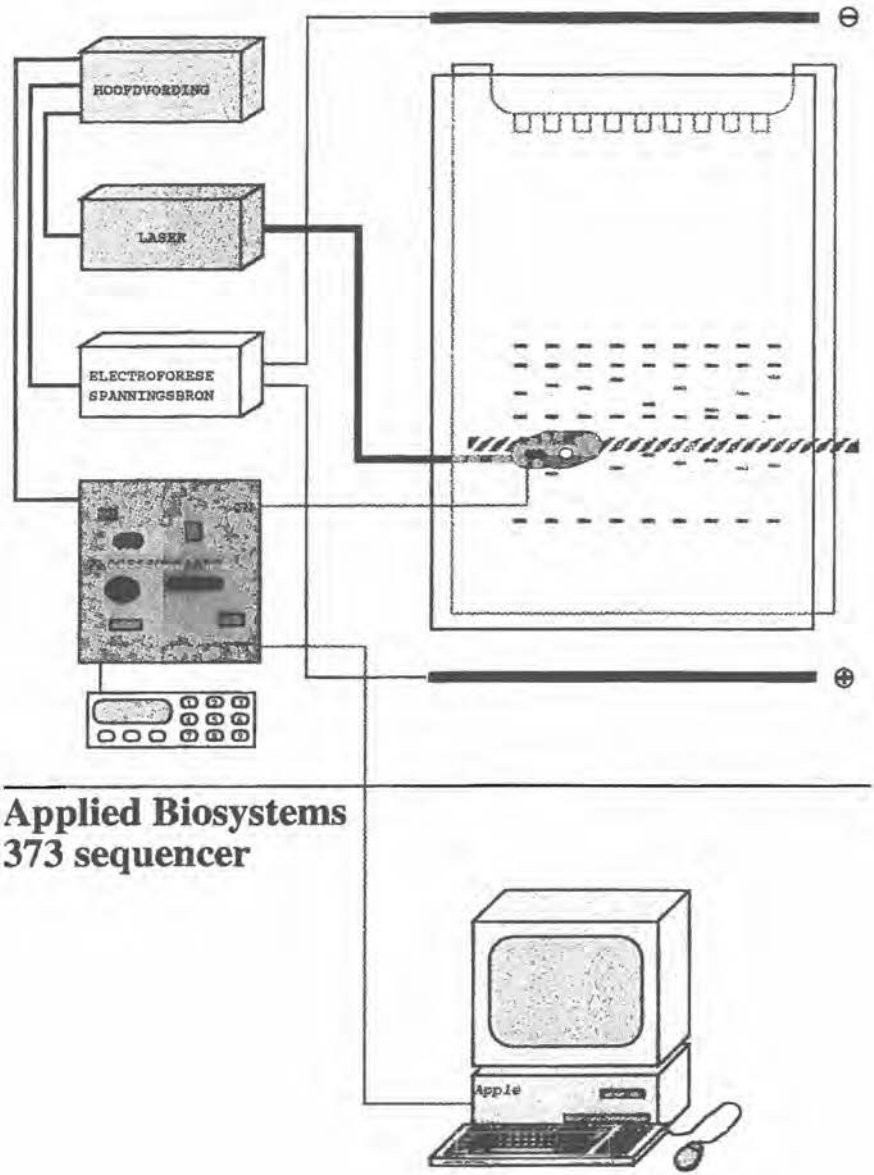

Fig. 4. Laser detection (Applied Biosystems 373 sequencer).

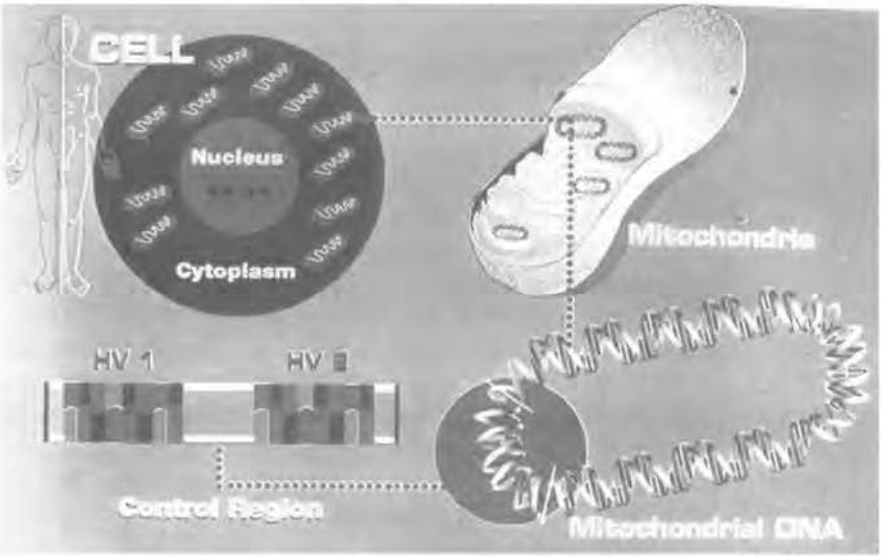

Fig. 5. Mitochondrial DNA (MtDNA).

In the absence of archaeological case studies, the successful use of MtDNA along maternal lines is best exemplified by the identification of the Romanovs. For the identification, the sequences from the bones recovered from the grave were compared with the sequence of a blood

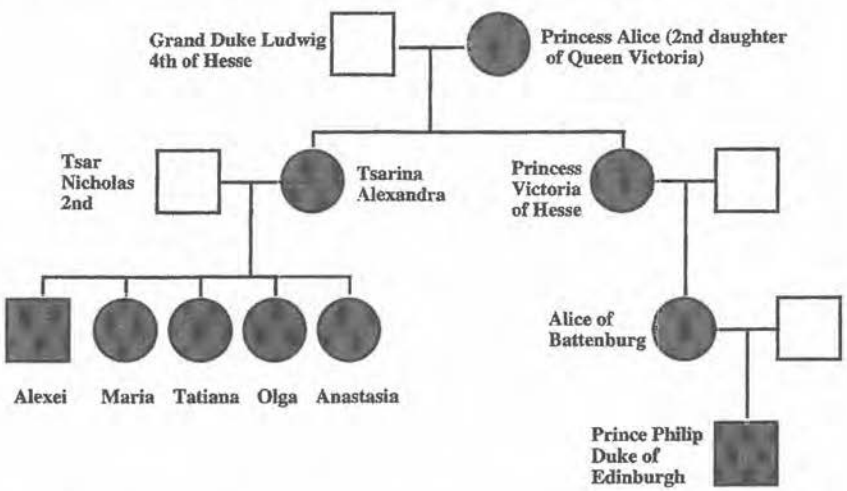

Fig. 6. Schematic representation of the identification of Czar Nicolas II.

sample from the Duke of Edinburgh. The Duke is matrilineally related to the mother of Czar Nicolas II. The Czar was identified by comparison of two maternal lines (Fig. 6)

Interpretation of results

Following DNA analysis, there are generally two strategies:

1 The sample(s) do not match the reference sequence. This means exclusion. No further analysis is required.

2 The sample(s) match the reference sequence. This means inclusion. Now, the significance of the match is to be established. This is done by measuring the frequency of each component of the profile in a given population and by analysing unrelated individuals of the population. An analysis of a 100 or more people will serve the purpose.[4] The bone material of a large metropolis is required to apply this strategy to ancient DNA.

To conclude with, the results of DNA research may shed a new light on such issues as population migration and yield population statistics of single groups, e.g. the organisation of necropoleis. All the more so if archaeologists ask the right questions for biologists to answer, and if the answers are put in proper perspective. Therefore, let us keep the interdisciplinary wheels well oiled.

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[4] Unfortunately, the analysis involves a statistic method beyond the scope of this article.