

# Uncovering the Sources of DNA in the Turin Shroud

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The Shroud of Turin is a linen cloth almost five square meters in size that enveloped a man pieced in the side afterwards his death following crucifixion after being beaten, scourged and crowned with thorns. It is the most important relic of Christianity because the Catholic tradition identifies such burial cloth with that where the body of Jesus Christ was enveloped before being placed in a Palestine tomb about 2000 years ago.

Dust particles have been collected directly from the linen cloth of the Turin Shroud and also minutely vacuumed from the interspace between the Turin Shroud and the Holland Cloth sewn to it as reinforcement. All these specimens are derived from different filters corresponding to various areas of the Turin Shroud and some of the dust particles have been removed from the filters and supplied to scientists for investigative studies. Genetic analyses of DNA isolated from plant fibers and cell debris or human blood crusts deriving from samples of the Turin Shroud have been postulated several times. In the past decades, hundreds of pollen grains have been identified and classified in terms of genus and species, using both optical and scanning electron microscopes, in order to be compared with geographic areas in which the corresponding plants did originate and now live. Independent cytological observations have clearly demonstrated that the identified geographic areas are fully compatible with the supposed historic paths travelled by the Turin Shroud. In more recent years, molecular results have apparently supported the existence of human DNA. Nevertheless, the DNA as a whole of the Turin Shroud remains still uncovered.

Here we document novel and robust genetic data used for taxonomic identification of plant species on the basis of chloroplast DNA (cpDNA) barcodes and molecular reconstruction of human mitochondrial DNA (mtDNA) haplotypes by exploiting short DNA sequences recovered from pollen grains, cell debris and other minuscule organic specimens, such as plant-derived fibers and blood-like clots found into the dusts sampled in the Turin Shroud by STURP Members. In particular, the dust particles analyzed in this study

belong to different filters of the back of the Turin Shroud, also corresponding to the areas face, hands, buttocks and feet. Our main task dealing with the attribution of DNA residuals to living organisms or fossils of known genetic identity was engaged from a molecular point of view by isolating, amplifying, subcloning and sequencing specific regions of the genome. In particular, we focused our investigations on nuclear markers (rDNA intergenic transcribed spacers, ITS) and extra-nuclear markers, of both chloroplast (cpDNA barcodes RuBisCO or *rbcl*, *trnH-psbA*, and *trnL-intron*) and mitochondrial (mtDNA targets CYB, COI and NADH dehydrogenase-subunit 5 along with the hypervariable regions HVRI and HVRII, the so-called D-loop) origin for the analysis of plant and human microscopic residuals, respectively. Organic remnants restrained by the dusts of the Turin Shroud allowed us to extract nanograms of DNA that was then amplified by PCR assay and sequenced by NGS technology (*i.e.*, Roche GS FLX sequencing) in order to recover genic and intergenic markers suitable to identify plant species as well as human haplotypes.

Concerning the taxonomic entities of land plant species identified in this study, some of them are native to the Mediterranean countries and widespread in the Middle East regions, but some others have their center of origin in the Oriental Asia and Americas, and were not yet introduced in Europe during the Medieval period. It is worth mentioning that about 2/3 of the plant species, including herbaceous weeds and crops, and woody trees and shrubs, have origin and are spread in temperate and sub-tropical regions of the Eastern Europe, Middle-Eastern Asia and Northern Africa. Among the plant species of the New World, stands out the black locust (*Robinia pseudoacacia* L.), a tree of the family of Fabaceae native to the South-Eastern United States of America. If it is true that the quantitatively most abundant taxonomic entity belong to the genus *Picea* (mainly *Picea abies* (L.) H. Karst.), that includes spruce trees typically widespread in temperate and boreal forest regions of the northern hemisphere of our planet, it is also true that different species were identified as largely widespread in the

countries of the Mediterranean basin, like clovers (*Trifolium* spp.), ryegrasses (*Lolium* spp.) and plantains (*Plantago* spp.), and central Asia, such as forms of pear (genus *Pyrus*) and plum (genus *Prunus*) of the family of Rosaceae. In addition, we identified crop species largely grown by farmers and common in many agriculture systems, such as chicory (*Cichorium intybus* L.), common hop (*Humulus lupulus* L.), cucumber (*Cucumis sativus* L.) and grapevines (*Vitis* spp.), widespread in cultivation since a few centuries in a lot of countries worldwide. Our analysis allowed us to identify also certain species attributable to woody trees commonly present in forests, such as hornbeams (*Carpinus* spp.), walnuts (*Juglans* spp.) and willows (*Salix* spp.), whose centers of origin are mainly located in Central Asia and Eastern Europe, and areas of introduction in a worldwide scale are now extremely broad.

As a general comment, most of the plant DNA sequences recovered from the total genomic DNA isolated from the Turin Shroud belong to taxonomic species native to and spread in the Old World. Based on the data obtained so far, we can assume that the Turin Shroud was likely displayed in, or in very close contact with, different types of natural and anthropological environments. The large variety of plant forms (*i.e.* herbaceous plants and woody trees, with agricultural and/or forest interest, used as food source and ornamental purposes, etc.) is compatible with highly diversified geographic ranges, whose areas of radiation may vary from the farms of the cultivated plains to the forests of the uncontaminated mountains. Similarly, on the basis of the known ages of introduction of certain plant species from Northern and Southern America, and Eastern Asia, we can assert that the Turin Shroud may have followed a highly variable temporal path. In fact, some species have their center of origin and diversification in areas around the Mediterranean basin, including North Africa, and most of these species were widely distributed throughout Europe before Christ age. Other species among those identified in the Turin Shroud, however, were introduced to Europe not before the XVI century, after the discovery of America by Christopher Columbus (for example, the species of the genus *Robinia* and some others of the Solanaceae family). Finally, fruit plants of the genera *Prunus* and *Pyrus*, belonging to the Rosaceae family, are necessarily deriving from Central Asia and Middle East regions, and they were likely introduced in the countries bordering the Mediterranean sea from the XIII century onwards, after the travels of Marco Polo.

On the basis of the analyses performed so far in the Turin Shroud, we can state to have ascertained the

presence of human mtDNA characterized by distinct haplogroups, including R0 and R8, U2 and U5, several H haplotypes (*e.g.*, H1, H2, H3, H13 and H33), and few L3 and M haplotypes. Although the bioinformatic analysis of genomic DNA sequences deriving from the Turin Shroud is still in progress, basic information can be inferred from these haplogroups putatively identified up to now. The haplogroup R0 occurs frequently in the Arabian Plateau, especially in local populations of Saudi Arabia and Pakistan, with its highest frequency in Yemen (38%) and smaller frequency in North-East Africa, including Horn of Africa, Anatolia, Iranian Plateau and Dalmatia, whereas the highest frequency of haplogroup R8 occurs mainly towards the East India. Concerning U subgroups, they are widely distributed across Western Eurasia, North Africa and South Asia. In particular, the branch U5 of the haplogroup U is known to be very ancient, and it is considered among the oldest and most common in Europe (11%), while the haplogroup U2 is most common in South Asia, but is also found in low frequency in Central and West Asia, as well as in Europe. The haplogroup H, for which several subgroups were identified in the Turin Shroud, is by far the most frequent in Europe. H1 is very common in the Western regions of Europe, but also among the populations of North Africa including the Berbers. The haplogroup H13 is known to be rare in Europe and West Asia, being also present in the Caucasus. Finally the M haplogroups are typical of Asia, while L3 is very common in Northern Africa and it is also found in Southern Europe.

The lineage of a haplotype, in this case understood as the combination of allelic variants – based on single and informative nucleotide polymorphisms – along a segment of mitochondrial DNA (*i.e.* D-loop), reconstructed by using our data does not reflect neither the total genetic variation of the ancient populations, as with the genetic drift some variants of the mitochondrial DNA may have been lost, nor the current geographical locations, as with the gene flow as a consequence of migration of individuals among populations of different countries some variants of the mitochondrial DNA may have been mixed in modern populations. The fact remains that the haplotypes deciphered up to now from the human DNA found in the Turin Shroud are all related to haplogroups that belong to ethnic groups of Europe, North Africa and Middle East, hence especially populations who live in countries that overlook the Mediterranean basin and in regions of the very large Arabian plateau, spanning from the Iberian Peninsula to Anatolia and Caucasus to the Horn of Africa.

In conclusion, results from this study are consistent with the presence of several plant species according to cpDNA barcodes and distinct human mtDNA haplogroups. Overall DNA data were compared with historical information to verify whether the geographic areas of origin and distribution of land plant species (embryophytes) and

human mitochondrial haplogroups are coherent with the proposed temporal and spatial paths of the Turin Shroud. Our experimental findings and additional clues pose a further difficulty to those who postulate a central European origin and a historical interval corresponding to the Middle Ages of the Relic.