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The Leonardo Project

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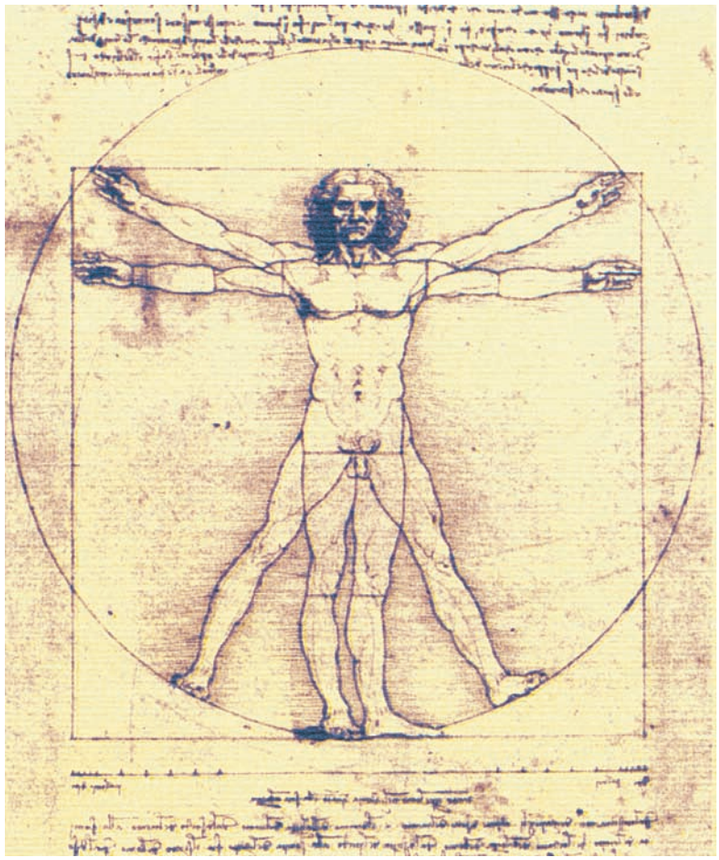

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On cover: Drawing by Leonardo da Vinci (1452-1519) - Study of the human body.

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Using New Anthropological and Biological Tools to Learn about Leonardo da Vinci

INTRODUCTION

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His life marked the rebirth of Western civilization, but no gravestone marks with certainty the final resting place of Leonardo da Vinci. Now using modern techniques, anthropologists and geneticists are seeking Leonardo's remains not simply to mark the spot but also to study and better understand his remarkable abilities and talents. The work underway resembles in complexity recent projects such as the exhumation and reburial of Leonardo's English contemporary, King Richard III, in March 2015 some 500 years after his death.

Like Richard, Leonardo was born in 1452, and like Richard he was given a Christian burial in a setting that underwent changes in subsequent years such that the exact location of the grave became lost. And this is where science and technology come to the aid of history.

In November 2014 an international team of specialists embarked on the first phase of a project to identify conclusively the alleged remains of Leonardo da Vinci at Amboise Castle in the Loire Valley southwest of Paris. They aim to compare DNA yet to be found there with the DNA of Leonardo's father and several close relatives whose remains are buried in Florence, and possibly his mother, in Milan. They hope to acquire an extensive enough genetic profile to understand better his abilities and visual acuity.

The project brings together experts from France, Italy, Spain, the United States, and Canada. Participating organizations include the Institut de Paléontologie Humaine in Paris, the International Institute for Humankind Studies in Florence, the Laboratory of Molecular Anthropology and Paleogenetics at the Biology Department of the University of Florence, Museo Ideale Leonardo Da Vinci in Vinci, J. Craig Venter Institute in La Jolla, California, Laboratory of Genetic Identification at the University of Granada, and the Rockefeller University in New York. Initial support comes from the Richard Lounsbery Foundation in Washington D.C.

Work proceeds along several fronts from Leonardo's family tomb in Florence, to Leonardo's presumed remains at Amboise Castle, and to Milan where there might be the remains of Leonardo's mother, Caterina. Team members are also pursuing traces of

her children by a later marriage. The study team plans to verify whether DNA extracted from the remains at Amboise Castle matches that of remains from Florence and possibly Milan, and traces taken from Leonardo's works of art, drawings, and notebooks and from possible descendants in the area of the town of Vinci, where he was born. Researchers in all these studies are adhering to the latest ethical guidelines for studies in human genomics. The project will verify Leonardo's physical characteristics from historical accounts, portraits and other images, and the cast of a skull attributed to Leonardo. To help further with identification, scholars will verify from historical accounts any illnesses, traumas, injuries, eating habits, and any other physical activities that are likely to have left biomechanical traces on Leonardo's bones (see King, this issue, pp. 133-147).

Leonardo's path from Vinci to Amboise

Leonardo was born in Archiano, a village of Vinci in the province of Florence, Italy, perhaps on 15 April 1452. He was the illegitimate son of the notary Ser Piero di Antonio and the young Caterina, who later married Accattabriga di Piero del Vacca Buti. Leonardo was born in the same year that his father entered into an advantageous marriage with Albiera Amadori, who accepted and reared this healthy and lively boy. In 1457, his name appears in the family records of Antonio Vinci, Leonardo's grandfather, as "illegitimate son."

Near the end of his life, Leonardo accepted an invitation from the French king, Francis I, to leave Italy and to move to the castle of Cloux, near Amboise, with some of his students, where he held the position of "first painter, engineer, and architect of the King." There he was able to devote time to his studies and his projects without having to comply with contractual terms or to fulfill specific obligations.

On April 23, 1519, Leonardo dictated his last will and testament. He died in Cloux on 5 May 1519 (though according to some on 2 May of the same year), at the age of 67. In accordance with his wishes, his mortal remains were buried in the Chapel of Saint Florentin in Amboise Castle. But the burial deeds of the artist bear the date 12 August, which indicates an initial temporary burial of the remains of Leonardo, followed, over three months later, by a final burial in the chapel of Saint Florentin. Here he rested until 1802, when the chapel was demolished and some of the tombs were destroyed and their remains were lost.

Although many of Leonardo's biographies have assigned to his remains this unfortunate fate, in reality there are still many doubts. It is not known for certain whether Leonardo's grave was among those destroyed and scattered. Doubts emerged in 1863 with the excavation of the site where the chapel of Saint Florentin once stood. There between the foundations of the destroyed building, a stone coffin was discovered containing a skeleton with a large skull, judged to be "large enough to hold an exceptional brain" and not far from this coffin a slab with a badly deteriorated inscription, LEO DUS VINC, thought to indicate Leonardus Vincius (see King, this issue, pp. 133-147).

From the skull a cast was made for examination by phrenologists in Paris, but its present whereabouts are unknown. Moreover, the bones attributed to Leonardo, first placed in a basket, were lost, then recovered, and in September 1874 buried in the chapel of Saint-Hubert, in the castle of Amboise, where they should still be today. But though a memorial on the site bears Leonardo's name, an adjacent plaque casts doubt by claiming only "presumed remains."

Tracking Leonardo's relatives

Historical documents attest that during the years 1472-74, Leonard's father, Ser Piero di Antonio, purchased a tomb below the floor of the Badia Fiorentina, the Florentine abbey (Santa Maria Assunta), situated in the very heart of Florence across the street from the Bargello, now an art museum. The Badia Fiorentina is the oldest monastery in the city and was founded in 978 for monks who followed the Rule of St. Benedict. Today the Badia is home to the Fraternity of Jerusalem. Thus far, reliable documentary evidence shows that at least fourteen of Leonardo's blood relatives were laid to rest in the Badia, including Leonardo's father and adult half-brothers by his father's third and fourth wives, as well as another illegitimate brother by an unknown mother (see Leader, this issue, pp. 149-158).

Records in the Florence State Archives and national library have been particularly useful when trying to identify the exact location of tombs or burial sites, including Badia tomb registers compiled in the seventeenth century, and contemporaneous accounts written by the abbey's historian and abbot. These volumes generally contain not only information derived from both official and public documents but also details provided by the churches, therefore affording additional clues because the positions of the tombs are often referred to in relation to the internal features of the church at that time. This information assumes greater importance in light of the many transformations that the Badia has undergone over the course of centuries. We know that the monastery was expanded toward the end of the 1400s. In 1627 the orientation of the church was rotated by 90 degrees and the entrance became what had previously been the apse. The present entrance was opened in 1494. Moreover, in 1663 the floor of the Badia was completely repaved, and all of the tombstones were removed, leaving no physical indication of the former locations of the various tombs. In the absence of many of the church's original features mentioned in official records, Anne Leader (this issue, pp. 149-158) has reconstructed from documented sources the original plans and features of the church, and all ensuing transformations, to discover the likely location of Leonardo's family's remains.

Concurrent with this search, another investigation was underway using ground penetrating radar, the noninvasive tool used to find the remains of Richard III beneath a parking lot and those of Miguel de Cervantes in a long-lost crypt (http://www.bbc.com/news/world-europe-31869746). Geological technologists from the University of Siena

scanned the floor of the Badia early in 2015 and found electromagnetic anomalies consistent with that of tomb chambers (see Minucci & Colonna, this issue, pp. 159-168). Their survey, in concert with analysis of historical records by Anne Leader on behalf of the International Institute for Humankind Studies, supports a request for permission to excavate and expose the remains of Leonardo's relatives for exhumation and DNA analysis in the second phase of the project.

The remains of Leonardo's mother present an alternative route to identifying his remains by DNA analysis, but that route is obscure. Annotations found in Leonardo's notebooks refer to expenses incurred for the treatment and funeral of "Caterina," an otherwise unidentified woman who died on 26 June 1494 in the parish of Santi Nabore e Felice in Milan. However, there is no indication of the exact place of burial, neither are there bibliographical sources to suggest that its whereabouts have ever been investigated.

Museo Ideale Leonardo Da Vinci and the J. Craig Venter Institute, on another track, are advancing Leonardo's genealogy from historical sources to construct a family tree with Progeny software. Given that the Y-chromosome DNA haplogroups (inherited through the paternal line) and the mitochondrial DNA haplogroups (inherited through the maternal line) are the same from generation to generation except for any mutational events that may have occurred, it may be possible to trace surviving relatives from the paternal line and the maternal line to obtain DNA samples for eventual comparison to the genetic material obtained from exhumed remains or pieces of art. The Museo Ideale Leonardo Da Vinci will also seek to locate possible descendants of Leonardo near the town of Vinci (see Vezzosi, this issue, pp. 169-189) so that the team can compare their genetic profiles to DNA obtained from Leonardo's remains.

Artwork and fingerprints

It is well known that Leonardo used his fingers along with his brushes while painting, some prints of which have remained, and so it could be possible to find cells of his epidermis mixed with the colors. The Leonardo Project seeks to verify whether fingerprints obtained from Leonardo's paintings, drawings, and notebooks can be compiled and eventually attributed to him. A more general objective is to verify whether DNA extracted from traces taken from Leonardo's works of art and manuscripts are consistent with DNA extracted from identified remains.

In January 2015, the International Institute for Humankind Studies opened discussions with the laboratory in Florence where Leonardo's *Adoration of the Magi* has been undergoing restoration for nearly two years to explore the possibility of analyzing dust from the painting for possible DNA traces. In preparation for such analysis, a team from the J. Craig Venter Institute will examine contemporaneous paintings from a private collection to develop and calibrate techniques for DNA extraction and analysis. If human DNA is obtained from Leonardo's work and sequenced, the genetic material can then be

compared with genetic information from skeletal or other remains that may be exhumed in the future.

Experts in forensic genomics plan to design inhibition and sensitivity experiments to study different pigments and resins used in Leonardo's day to determine if the chemicals and materials of which these paints and resins are composed are likely to allow for successful amplification and detection of trace human DNA.

A conservative approach

The analyses necessary to identify the remains of Leonardo must be as conservative as possible. The enterprise must not arrive at the identification of his remains, only to have destroyed what was left. Therefore, any analysis that could possibly result in the destruction or impairment of even minimal skeletal portions must be evaluated beforehand both qualitatively and quantitatively to assess the importance of the contribution that such an analysis is likely to provide for the investigation.

With this precaution in mind, work will proceed in phases, beginning with obtaining permission for excavation to access remains at the Badia Fiorentina. Similar excavations have been done several times before, recently at the Medici Chapels in Florence to unearth members of the Medici family. Historical study is needed to reconstruct events that may have affected the remains of Leonardo, from the place of his alleged death to their present location. Already information has been gathered from literature about Leonardo's physical characteristics, from age at death to stature, size, degree of robustness, and other physical and physiognomic traits. For example, Leonardo would have been exposed either in his studio or his workshops to lead, mercury, and other chemicals that would leave lasting traces (see King, this issue, pp. 133-147).

When permissions are obtained, physical anthropological analysis can begin with the exhumation of bone samples from remains thought to be those of Leonardo and his direct blood relatives. Investigators will extract DNA to assess whether its quality is sufficient for further forensic analysis and genetic interpretation. Radiometric dating of the finds by carbon-14 and other methods would show whether the indicated time of death corresponds with the established date of the bones. Research must verify whether the physical characteristics reported of Leonardo, such as his left-handedness, physical strength, and handsome appearance, are compatible with those ascertained from the study of skeletal remains, such as sex, stature, and facial features. Further, is evidence found from the bones compatible with Leonardo's reported illnesses, injuries, and eating habits? (e.g. Leonardo is thought to have followed a vegetarian diet for much of his life). Finally, superposition of radiographic and photographic images will help to assess any similarities between the facial skeleton and Leonardo's portraits and any other iconographic representations, such as the skull cast.

In conjunction with approaches from physical anthropology, the project can proceed to DNA analysis for comparison of the genetic profiles obtained from samples extracted from the alleged bones of Leonardo and those of his relatives. If DNA analysis yields a definitive identification, then conventional and computerized techniques might reconstruct the face of Leonardo from the real and virtual models of the skull and from the possible death mask, reportedly in Paris, made at the time of Leonardo's passing. If found, the death mask could also yield precious DNA.

With regard to Leonardo's artwork, investigators must identify and obtain permission to examine paintings that have retained fingerprints and notebooks that may have retained his skin cells. Then a crucial question is whether traces of DNA remain or whether restoration measures and the passage of time have obliterated all evidence of Leonardo's touch.

"Vissi d'arte"

Indeed it is Leonardo's art and creative genius that inspire the search for his bones, and it is to the history of art and the creative process that the search could leave an important legacy. Leonardo's reputation as a painter rests on a handful of paintings, while other works were not completed or were planned and not begun. Experts debate the authenticity of works attributed to Leonardo, such as a portrait of Isabella D'Este, lost for 500 years before it was purportedly identified in a private collection in 2013 (http://www.theguardian.com/artanddesign/2013/oct/04/leonardo-da-vinci-lost-portrait-isabella-deste) or the so-called *Bella Principessa*, identified as by Leonardo in 2008, but claimed to be a forgery in 2015 (http://theartnewspaper.com/news/news/could-leonardo-s-bella-principessa-be-greenhalgh-s-bolton-sally-/). DNA samples matched to others found in Florence (or Milan or Amboise) could serve as a reference standard for authenticating disputed or unidentified works of Leonardo. Scrutiny of Leonardo's art may also improve approaches to other forensic DNA studies.

Studying DNA traces in Leonardo's artwork could improve techniques for extracting and sequencing DNA from other centuries-old works of art, and associated methods of attribution. Art forgery is a multi-billion-dollar industry that depends on falsifying or confusing the attribution of artworks to their creators. Authentication of contemporary artworks, just as for Leonardo's, sometimes depends on consensus among interested parties rather than more definitive means. What if artists and dealers had at their disposal an inexpensive, indelible, and tamper-proof label with which to identify their work? Such a label developed by the University of New York at Albany makes use of synthetic DNA to create a genetic signature that permeates a work of art, invisible and undetectable until needed (http://www.nytimes.com/2015/10/13/arts/design/developing-dna-as-a-standard-for-authenticating-art.html?_r=0). It is an idea that Leonardo, who famously invented a sort of shorthand writing in reverse perhaps to protect his written ideas from theft, would embrace.

Summary

The search for Leonardo's death mask and remains at Amboise Castle, for the remains or traces of his family members in Florence, Vinci, and Milan, and for traces of his DNA in his works is fraught with difficulty. Matching Leonardo's DNA to that of his family presents puzzles that are minutely specific to their history and circumstances, but the tools the investigators use are generic and broadly applicable. We stand to gain not only greater historical knowledge of Leonardo but possibly a reconstruction of his genetic profile, which could provide insights into other individuals with remarkable qualities. The last Plantagenet king of England and the author who gave us Don Quixote are two whose places in history are somewhat better documented now through recent anthropological study. Is Leonardo the next?

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