The Denisova discovery: Ancient genomics shed new light on human origins

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An international team of scientists made headlines at the end of last year when they used genetic evidence to show that an ancient people, once living in the Altai Mountains of southern Siberia, were distant cousins of the Neanderthals and contributed to the modern human genome before their extinction.

The discovery is a triumph of modern genomics and decades of publicly funded science research in the United States and elsewhere, which has led to the sequencing of the human genome and promises to revolutionize our understanding of evolution, disease, and global genetic diversity.

While geneticists and paleontologists at the Max Planck Institute in Leipzig, Germany, at Harvard and at MIT spearheaded the work, there were also substantial contributions by scientists throughout the United States, Spain, Canada, Russia, and China. Ancient DNA specialist Svaante Pääbo, alongside evolutionary biologists and geneticists David Reich, Richard Green and Johannes Krause, were among the researchers leading the work.

Denisova cave is located in the Altai Mountains of southern Siberia, and has been an important site of human occupation for over 250,000 years. Early humans often sought out caves as sources of shelter and protection as they dispersed, repeatedly, from Africa into Eurasia and beyond. Like a number of other sites, Denisova cave is important because stone tool technology suggests that different peoples occupied the site at different times toward the end of the Pleistocene age (2.6 million to 10,000 years ago), as modern *Homo sapiens* began to disperse from Africa and generally replace other, older populations.

The scientists wanted to know if human remains in the cave, 30,000-50,000 years old, were Neanderthals or modern humans, and so extracted ancient DNA from a phalanx bone, or digit, to map its DNA onto known human and Neanderthal genomes. Surprisingly, the Denisovans proved to be distinct from both humans and Neanderthals known to have been living elsewhere in Eurasia at the time. Instead, researchers found that the Denisovans were distant cousins of the Neanderthals from Europe, apparently having branched off from other Neanderthal populations shortly after Neanderthals branched from modern humans, 300,000-450,000 years ago.

Unlike their Neanderthal cousins in Europe, who contributed an estimated 1-4 percent of genomic material among modern humans living throughout Eurasia, the Denisovans did not contribute to the DNA of these populations. Perhaps most surprisingly however, before their disappearance from southern Siberia the Denisovans did contribute approximately 4-6 percent of modern Melanesian DNA.

This finding adds to a series of others in the last decade, some genetic and some paleontological, which paint an increasingly complex picture of human origins within Africa and beyond. Future work will investigate the distribution of Denisovans across Asia, the context of their mixing with modern humans leaving Africa, and the possible existence of other archaic populations that may have contributed to the modern human genome.

Human genetics in the 21st century

While the Denisova discovery enriches our understanding of human evolution, it does not overthrow but rather contributes to a consensus among human geneticists regarding the remarkable genetic similarity of human individuals and populations around the globe.

Humans are unusually genetically homogeneous as a species, a result of our recent, common origin from small populations in Africa in the past 150,000-200,000 years. Populations of chimpanzees or other primates separated by rivers or other barriers are often genetically distinct from one another, interbreeding very little and maintaining separate populations despite their close proximity. Human populations, by contrast, are remarkably identical despite their distribution across distant regions of the earth.

Early studies in the 1970s, since corroborated by modern sequencing methods, showed that 85-90 percent of genetic diversity within the entire human species is contained within any given group. By contrast, only 10-15 percent of human genetic variation distinguishes one group from another, or is exclusive of certain groups. If all people on earth had their DNA at a specific location sequenced, and 100 genetic variants were found in the human species at that location, what these studies show is that 85-90 of those variants would be found in any given population. Only very few variants would be found in one population and not in another.

Decades of research into DNA sequencing methods have generated ongoing, international projects to document human genetic diversity. Among these projects are the Human Genome Diversity Panel (HGDP), the International HapMap Project, and the Centre d'Etudes du Polymorphisme Humain (CEPH). These projects sequence entire genomes or portions of genomes in thousands of individuals across the globe in an effort to understand diversity, relatedness, migration and settlement histories, and vulnerability to disease in different populations.

Such an understanding is provided by analysis of segments of varying genetic code called single-nucleotide polymorphisms (SNPs, pronounced "snips"), repetitive microsatellite sequences, DNA copy number variations (CNVs) and duplications or deletions of genes and other segments. SNPs are small variants of base pairs within the DNA sequence, which act like letters in the genetic code. Microsatellites, by contrast, are approximately of word length, and genes, which are long strings of coding base pairs, are like short manuals describing how to build proteins, the machinery of the cell.

In 2008, geneticists Li and Absher from Stanford and the University of Michigan used data collated from HGDP and CEPH to reconstruct global human relatedness patterns and migration routes from Africa. The study, examining 650,000 DNA sites in almost 1,000 individuals from 50 human populations, illustrates how early humans peopled the world in a sequential series of dispersals beginning in sub-Saharan Africa and continuing on to North Africa, the Middle East, Europe, South and Central

Asia, East Asia, Oceania and America.

While the geneticists were able to distinguish between sub-Saharan Africans and individuals outside Africa, their analyses did not reconstruct "racial groups" previously recognized for political or social purposes. For instance, almost all Middle Eastern individuals have enormous contributions of DNA variants that are found in higher frequencies in Africa, Europe, Central and South Asia, reflecting mixed ancestry and continued gene flow across continents.

Importantly, Li and Absher also found that 85-95 percent of genetic variation found among all people is common to all groups, while only a small amount of variation can be used to distinguish one group from another.

Despite efforts by corporations to copyright genetic material, and privatize or profit from methods developed through publicly funded research, dozens of complete and annotated genomes from living organisms around the world are now freely availably online, and contribute to the burgeoning field of genomics.

Ancient DNA and Denisova

The discovery at Denisova is an example of how genetic analysis has been applied not only to living populations, but also to fossil specimens. The similarities in physiology and form between closely related species have prevented scientists from determining, previously, whether populations outside Africa like the Neanderthals mixed with modern populations migrating from Africa 150,000 to 200,000 years ago.

The "multiregional" model of human evolution argued for large-scale mixing and continuous gene flow between populations outside and within Africa, both before and after modern human migrations that supposedly replaced Neanderthals. In this sense, multiregionalists like Professor Milford Wolpoff at the University of Michigan claimed that Neanderthals never wholly disappeared, but rather became incorporated into the populations leaving Africa and settling the Middle East and Europe.

The "Out of Africa" or "Recent African Origin" model of human evolution, ultimately supported by most scientists in the field, claimed that human populations leaving Africa in recent history did replace Neanderthals and other archaic populations throughout Eurasia, citing African technological innovations in the middle to late Stone Age, and similar osteological characteristics like "gracile" or finer features seeming to unite all modern humans to the exclusion of Neanderthals.

Mitochondrial analysis of all living humans and extraordinary work to extract mitochondrial DNA from Neanderthal remains gave support in recent decades to the African Origin model of evolution. This work showed all living humans to have a recent common mitochondrial ancestor, nicknamed "Eve," who lived in Africa about 200,000 years ago; Neanderthal mitochondrial lineages are by contrast distinct from modern humans.

But this evidence could not rule out the possibility that Neanderthals had mixed with modern humans and left nuclear, but not mitochondrial DNA among their living descendents.

Extracting and sequencing DNA from fossils is notoriously difficult. The human genome is 3 billion base pairs long, and is contained within each cell of the body in 23 volumes called chromosomes. The chromosomes themselves range from 50 million to 250 million base pairs long each, and altogether hold about 23,000 genes that code for proteins, the machinery of our cells. But when organisms die, their many base pairs quickly break down: Neanderthal remains typically offer DNA in small fragments an average of 50 base pairs in size, difficult to retrieve and analyze. Further complicating any study of ancient DNA are the myriad species of bacteria that colonize our bodies when we die, and leave their genomes alongside our own. The DNA retrieved from most ancient bones are 95-99 percent bacterial, with a small remainder belonging to the organism we mean to study.

A new form of DNA sequencing, however, has given scientists the ability to analyze ancient DNA as never before. Called "pyrosequencing," the technique uses millions of microscopic beads, each functioning as a DNA copying factory, to faithfully preserve and amplify even severely damaged DNA. Innovations by Svaante Pääbo's team in Leipzig have furthermore enabled the researchers to successfully capture only fossil DNA, and avoid contamination by other sources.

The result has been a revolution in ancient DNA studies, and the complete sequencing of the Neanderthal genome using multiple Neanderthal remains across Europe.

A draft sequence of the Neanderthal genome published last year provided strong evidence that Neanderthals interbred with modern humans in the Middle East as they dispersed from Africa, perhaps 50,000-80,000 years ago. After screening dozens of Neanderthal remains for the presence of DNA, scientists chose three from Vindija Cave in Croatia from which to extract bone powder and, ultimately, ancient DNA.

The scientists consistently found Neanderthals to be slightly more closely related to Eurasians than to sub-Saharan Africans, suggesting that approximately 1-4 percent of modern Eurasian DNA is inherited from Neanderthals. This Neanderthal contribution to the modern human genome, which is substantial, may imply only a small amount of interbreeding; a variety of historical scenarios describing modern human and Neanderthal interactions are compatible with the available data.

The sequencing of Denisovan remains at the end of last year provides further evidence for interbreeding between dispersing populations of modern humans, and archaic populations inhabiting Eurasia.

Previous sequencing of the mitochondrial genome, using DNA from a finger bone, found Denisovans to be the descendants of an ancient population that split apart from a lineage common to both Neanderthals and modern humans. This meant that humans and Neanderthals were more closely related to one another than to Denisovans, our ancient cousins. This discovery fueled widespread interest because it implied that descendents of our ancient ancestors *Homo erectus* or *Homo heidelbergensis*, having dispersed from Africa a million years ago or even before, persisted in Eurasia until 30,000 years ago.

Homo erectus and *heidelbergensis* were descendants of ancient *Australopithecines* like "Lucy," who, after evolving in Africa, then dispersed into the Middle East, Europe and Asia between 800,000 and 2 million years ago. *Homo heidelbergensis* is thought to have given rise to both modern humans in Africa and to Neanderthals in Europe; it was not anticipated that a third lineage, the Denisovans, might have also evolved and then remained in southern Siberia until quite recently.

Using fossils from the Atapuerca Caves in Spain, Dminisi Cave in Georgia and Mojokerto in Indonesia, scientists have long known that ancestors like *Homo erectus* may have dispersed from Africa as early as 2 million years ago. Like the discovery of the "hobbit" remains found on the Indonesian island of Flores, however, the sequencing of Denisovan mitochondrial DNA raised the possibility that ancient ancestors left Africa and persisted, possibly without interaction with later Neanderthals or modern humans, all the way into the late Stone Age.

Now, complete sequencing of the Denisovan nuclear genome has shown that these individuals were in fact distant cousins of Neanderthals, and more closely related to them than to us. A tooth in Denisova cave, which the researchers interpret as being larger than those found in modern humans or Neanderthals, appears to corroborate what has been revealed by DNA: the Denisovans belonged to a population anciently diverged from Neanderthals.

But the sequencing of the Denisovan genome reveals an extraordinary

twist: while Neanderthals and Denisovans shared a common lineage hundreds of thousands of years ago, in the last 50,000 years the Denisovans appear to have contributed to a significant portion—about 5 percent—of the modern human genome among Melanesians in southeast Asia and the southwest Pacific.

It is unclear whether the Denisovans contributed to the modern human genome because they inhabited a swath of territory extending down into southeast Asia, or whether modern peoples dispersing from Africa may have passed through southern Siberia.

Constructing plausible historical scenarios for such an interaction is complicated by the difficulty of determining the precise timing of Denisovan occupation in the Altai Mountains. Caves are notoriously complex geologically because they house unusual patterns of water flow, sediment deposition and animal presence over many millennia. Radiocarbon dating of animal bones altered by human tools suggest at least two periods of occupation at Denisova: one 50,000 years ago or older, and another only 15,000-30,000 years ago.

The sequencing of the Denisovan genome illustrates the remarkable advances in genetic technology and international scientific collaboration that are advancing the field of biology. More than 150 years after Darwin unveiled his theory of evolution by natural selection, scientific discoveries continue to transform our understanding of evolutionary processes and of our own origins. We may look forward to many new, and perhaps unexpected revelations concerning human evolution as the field of ancient genomics flourishes in the 21st century.