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Ancient Human and Ice Age Predator DNA Recovered Directly from 22,000-Year-Old Cave Dirt in Spain

Sedimentary Ancient DNA * Paleogenomics * El Mir n Cave

For well over a century, reconstructing the story of prehistoric humans required finding physical human remains -- a fossilized tooth, a dense inner-ear bone , or a burial preserved in a limestone cavern. Because Paleolithic hunter-gatherers lived in small, mobile bands and rarely left behind intact skeletons, huge stretches of the Ice Age archaeological record contain stone tools and hearth charcoal but zero human bones. Now, an international research team led by evolutionary anthropologists Pere Gelabert, Victoria Oberreiter, and Ron Pinhasi at the University of Vienna, working with archaeologists Lawrence Guy Straus and Manuel Ram n Gonz lez Morales, has bypassed the fossil bottleneck entirely [1] . Reporting in Nature Communications , the team extracted 22,000-year-old human nuclear and mitochondrial DNA -- along with the genetic signatures of 28 animal species -- from tiny pinches of cave dirt weighing no more than a few grains of rice [1] .

1. How Cave Dirt Preserves a Hidden Genetic Library

The breakthrough relies on a technique known as sedimentary ancient DNA (sedaDNA) -- the recovery of microscopic genetic fragments directly from soil and mineral layers [1, 2] . Whenever humans or animals sheltered inside a cave thousands of years ago, they continuously shed microscopic biological traces into the floor: sloughed skin cells, hair follicles, sweat, blood from butchering prey, and bodily waste. Normally, bacteria and moisture rapidly destroy loose DNA in outdoor soil. Inside cool, alkaline limestone caves like El Mir n Cave in the Cantabrian Mountains of northern Spain, however, negatively charged DNA backbone molecules chemically bind to positively charged edges of clay and silicate mineral grains. Locked onto mineral surfaces and shielded from temperature swings, these ultra-short DNA strands (typically just 35 to 60 chemical letters long) can survive for tens of thousands of years.

To read those ancient genetic fragments without contamination from modern microbes or archaeologists, the team collected 36 sediment samples spanning five meters of excavated stratigraphy at El Mir n Cave, covering a timeline from more than 46,000 years ago (the era of Mousterian Neanderthals) through 14,000 years ago (the late Ice Age Magdalenian culture) [1] . Using specialized magnetic hybridization baits -- synthetic RNA molecules designed to act like molecular fishing hooks that snag only mammalian mitochondrial and human chromosomal DNA -- the researchers separated authentic Pleistocene DNA from billions of background soil-bacteria sequences. They verified the age of the DNA by measuring cytosine deamination , a predictable chemical weathering pattern in which the DNA letter C slowly decays into T at the frayed ends of genuinely ancient molecules [1, 2] .

2. Surviving the Ice Age Peak: The 22,000-Year-Old Solutrean Humans

Until this study, the oldest human genome recovered from El Mir n Cave came from the famous "Red Lady of El Mir n" , an ochre-stained burial of a woman who lived roughly 18,700 years ago during the Magdalenian period, after the worst of the Ice Age had already begun to ease [3] . What happened in northern Spain a few thousand years earlier -- right at the freezing peak of the Last Glacial Maximum (LGM , roughly 26,500 to 19,000 years ago), when massive ice sheets covered northern Europe and polar desert winds swept across France -- remained an open question because Solutrean-era human bones are exceedingly rare.

From Layer 106 of El Mir n Cave, radiocarbon-dated to between 21,800 and 21,100 calibrated years before present (~22,000 years ago) and containing stone weapon points but zero human bones or teeth , the team successfully captured both human mitochondrial DNA and over 150,000 nuclear single-nucleotide polymorphism (SNP) markers [1] . The chemical readouts revealed that the Solutrean occupants carried mitochondrial haplogroup U2'3'4'7'8'9 and belonged to the Fournol genetic cluster -- an ancient western European hunter-gatherer population first identified in pre-Ice Age France and Spain [1, 4] . Furthermore, comparing X- and Y-chromosome read ratios indicated that the sediment layer preserved DNA shed primarily by at least one male individual who likely had dark skin, dark brown hair, and brown eyes [1] .

Crucially, this 22,000-year-old dirt genome bridges the gap between pre-glacial Gravettian hunter-gatherers and the 18,700-year-old Red Lady of El Mir n [1, 3, 4] . Rather than going extinct when glaciers advanced across Europe, southwestern European hunter-gatherers retreated into the sheltered valleys of the Franco-Cantabrian region -- an ecological safe haven or "refugium" -- and maintained continuous maternal and nuclear ancestry right through the coldest millennia of the Pleistocene [1, 4] .

| Cave Layer / Period | | Calibrated Age (Years BP) | | Human Remains in Layer | | Recovered Human DNA Signal | | Key Carnivore & Megafauna DNA Recovered | | Primary Paleobiological Finding |

| Layer 130 (Mousterian) | | 46000 | | None | | Trace archaic hominin mtDNA | | Cave bear (Ursus spelaeus), Cave hyena (Crocuta crocuta), Leopard (Panthera pardus) | | Carnivore denning dominates cave during brief Neanderthal visits |

| Layers 117-119 (Early Upper Paleolithic) | | 34000 | | None | | Basal Eurasian mtDNA fragments | | Asiatic dhole (Cuon alpinus), Gray wolf (Canis lupus), Red deer (Cervus elaphus) | | Confirmed Pleistocene range of Asiatic dhole in northern Iberia |

| Layer 106 (Solutrean / LGM Peak) | | 21500 | | None (Dirt only) | | Nuclear Fournol ancestry (150,000+ SNPs) & mtDNA U-branch | | Iberian ibex (Capra pyrenaica), Chamois (Rupicapra), Salmonid & ungulate prey | | Direct proof of continuous human survival in Iberia across the Ice Age peak |

| Layer 504 (Lower Magdalenian) | | 18700 | | "Red Lady" Burial | | GoyetQ2 / Fournol admixed Magdalenian lineage (mtDNA U5b) | | Domestic-lineage basal wolf, Red deer, Iberian ibex | | Genetic link between Solutrean cave dirt and post-LGM Magdalenian inhabitants |

3. Ghost Predators in the Dirt: Caucasian Leopards and Asiatic Dholes

Beyond tracking ancient humans, the cave dirt revealed a hidden ecosystem of Ice Age predators and hoofed prey that left virtually no physical bones in the excavated layers [1] . In standard archaeology, carnivores are chronically underrepresented because predators rarely die inside the caves where they sleep or drag carcasses, whereas human hunters actively bring in hundreds of deer and goat bones from their meals. By sequencing mitochondrial genomes from the same sediment samples, the team identified 28 mammal species spanning rodents, ungulates, and apex predators [1] .

Three carnivore discoveries stood out:

* The Asiatic Dhole (Cuon alpinus): Today, the dhole (or whistling wild dog) lives only in the forests and steppes of South, East, and Southeast Asia. Finding dhole mitochondrial DNA in El Mir n layers dating to more than 30,000 years ago -- despite zero dhole bones ever being identified among the cave's physical fossils -- confirms that pack-hunting dholes roamed the mountains of northern Spain alongside gray wolves during the Late Pleistocene [1] .

* Pleistocene Leopards (Panthera pardus) with Caucasian Ties: Sediment layers dating between 46,000 and 35,000 years ago yielded a nearly complete mitochondrial genome of an Ice Age European leopard. Surprisingly, family-tree comparisons showed that the Spanish leopard was more closely related to Pleistocene leopards from the Caucasus Mountains (such as Mezmaiskaya Cave) than to modern African leopards, pointing to a connected Eurasian population of cold-adapted leopards before their European extinction [1] .

* Spotted Cave Hyenas (Crocuta crocuta spelaea) and Cave Bears (Ursus spelaeus): In the oldest Mousterian layers, predator DNA strongly outnumbered human DNA, showing that Neanderthals used the cave only for brief, seasonal hunting camps while cave bears, hyenas, and leopards claimed the cavern as a den for most of the year. By the time Solutrean modern humans occupied Layer 106 around 22,000 years ago, large carnivore DNA vanished from the floor, replaced by dense human nuclear DNA and the DNA of hunted ibex and red deer [1] .

4. Why Sedimentary DNA Changes the Future of Archaeology

Together with the team's earlier recovery of 25,000-year-old human, wolf, and bison genomes from cave mud in Georgia's Satsurblia Cave [5] , the El Mir n Cave study proves that nuclear population genetics can now be performed on ordinary archaeological dirt [1, 5] . Because museums worldwide already store thousands of dated soil bags collected during past excavations, scientists can now revisit fossil-barren sites across Eurasia, Africa, and the Americas to map human migrations, predator extinctions, and ecological shifts without destroying a single rare bone.

References & Primary Sources

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