

Paleogenetic Networks of the Pleistocene: How Ancient DNA Reveals Long-Distance Connectivity and Regional Continuity Across Ice Age Europe

For much of the twentieth and early twenty-first centuries, paleoanthropologists and archaeologists wrestled with a fundamental paradox regarding the Upper Paleolithic of Europe. Between roughly 34,000 and 25,000 calibrated years Before Present (cal BP), a broad cultural horizon known as the Gravettian spanned the European continent from the Atlantic coast of the Iberian Peninsula to the Russian Plain. In the material record, the Gravettian appears remarkably cohesive. Foragers across thousands of kilometers manufactured similar backed flint blades, utilized comparable organic projectile technologies, and carved iconic portable female figurines—commonly referred to as Venus figurines—that shared striking stylistic conventions from the Pyrenees to Moravia. This widespread distribution of material traits suggested that Ice Age hunter-gatherers maintained extensive, continent-spanning communication networks across the mammoth steppe.

When the first large-scale paleogenomic datasets of Upper Paleolithic Europe were compiled over the past decade, however, a starkly different picture emerged. Rather than revealing a single pan-European population, ancient nuclear DNA indicated that Gravettian-associated populations were divided into at least two deeply diverged genetic lineages: an eastern and central group known as the Věstonice cluster, and a western and southwestern group designated as the Fournol cluster. Despite sharing a common archaeological label, individuals in France and Spain appeared genetically isolated from their contemporaries in Czechia, Austria, and southern Italy. This discrepancy raised a central question in human evolutionary biology: did the Gravettian represent a purely cultural phenomenon transmitted between isolated populations without biological mixing, or was the apparent genetic divide an artifact of sparse skeletal sampling?

Recent paleogenomic research published in *Science Advances* by Pere Gelabert, Ron Pinhasi, and an international consortium at the University of Vienna has resolved a critical piece of this puzzle. By combining newly sequenced nuclear genomes from rare Upper Paleolithic skeletal remains with autosomal human DNA extracted directly from cave sediments, the researchers demonstrated that eastern and western Gravettian groups were not strictly isolated. Instead, hunter-gatherers engaged in long-distance genetic exchange across Europe prior to the Last Glacial Maximum (LGM), even while maintaining thousands of years of local demographic stability in southwestern refugia and practicing sophisticated mating networks that prevented inbreeding.

1. The Upper Paleolithic Demographic Canvas: From Initial Dispersals to the Gravettian Divide

To understand the significance of East-West gene flow during the Mid-Upper Paleolithic, it is necessary to examine the multi-layered demographic history of *Homo sapiens* in Europe prior to 30,000 cal BP. Modern humans first dispersed into the European continent at least 45,000 years ago during the Initial Upper Paleolithic (IUP). However, genomic reconstructions indicate that these earliest pioneers did not form a simple, unbroken ancestral line leading to later Europeans.

Individuals from sites such as Zlatý kůň in Czechia (dated to roughly 45,000 cal BP) and Pestera cu Oase in Romania represent early divergences out of the basal non-African metapopulation that left no detectable genetic legacy in subsequent European populations. Shortly thereafter, another IUP wave—represented by approximately 45,000-year-old genomes from Bacho Kiro Cave in Bulgaria—introduced a lineage that possessed surprising genetic affinities to early East Asian populations, such as the 40,000-year-old Tianyuan individual from China. While the Bacho Kiro population was largely replaced in southeastern Europe, a fraction of its genetic heritage survived by mixing into populations moving westward.

Around 38,000 to 36,000 cal BP, a major demographic expansion carrying what geneticists term "West Eurasian Core" ancestry established the primary genetic foundation for subsequent Upper Paleolithic Europeans. This expansion split into distinct geographic trajectories across the continent:

- **The Eastern Lineage (Kostenki-Sunghir Branch):** Represented by genomes such as Kostenki 14 (roughly 38,000 cal BP) and the burial group from Sunghir in Russia (roughly 34,000 cal BP), this branch established the genetic baseline for hunter-gatherers on the East European Plain and exhibited almost no admixture from the earlier Bacho Kiro IUP groups.
- **The Western Lineage (Goyet Q116-1 Branch):** Associated with the Aurignacian techno-complex and exemplified by the 35,000-year-old Goyet Q116-1 individual from Belgium, this western branch derived the majority of its ancestry from the West Eurasian Core but retained approximately 17 percent ancestry derived from the older Bacho Kiro IUP lineage. This specific admixture proportion gave western Aurignacian populations a measurable shift in allele sharing toward East Eurasian groups compared to their eastern neighbors.

By the onset of the Gravettian period around 33,000 cal BP, these deep ancestral trajectories had crystallized into two geographically partitioned clusters. In central, eastern, and southern Europe—encompassing celebrated sites such as Dolní Věstonice and Pavlov in Czechia, Krems-Wachtberg in Austria, and Paglicci and Ostuni in Italy—populations belonged to the **Věstonice cluster**. Admixture graph modeling shows that the Věstonice cluster derived roughly 64 percent of its ancestry from the Sunghir-related eastern branch and 36 percent from the Goyet-related western branch, resulting in a diluted Bacho Kiro IUP signal of only about 6 percent. Conversely, in France and the Iberian Peninsula, Gravettian populations belonged to the **Fournol cluster** (named after the Fournol rockshelter in Lot, France, alongside

sites like Ormesson, La Rochette, and the Serinyà caves), which descended directly from the Goyet Q116-1 lineage and retained the higher 17 percent IUP genetic signature.

Paleogenetic Cluster / Lineage	Primary Geographic Distribution	Chronological Window (cal BP)	Associated Techno-Complexes	Key Ancestral Components and Characteristics
Bacho Kiro (IUP)	Southeastern Europe (Bulgaria)	~45,000 – 43,000	Initial Upper Paleolithic	Deep Eurasian branch sharing genetic drift with East Asian Tianyuan lineage; contributed minor ancestry to later western European groups.
Kostenki / Sunghir	East European Plain (Russia, Ukraine)	~38,000 – 33,000	Early Upper Paleolithic / Early Gravettian	Core West Eurasian ancestry; lacks Bacho Kiro IUP admixture; primary ancestral source for the Věstonice cluster.
Goyet Q116-1	Northwestern Europe (Belgium)	~35,000	Aurignacian	Core West Eurasian ancestry combined with roughly 17% Bacho Kiro IUP-related admixture; direct

				precursor to the Fournol cluster.
Věstonice Cluster	Central, Eastern, and Southern Europe (Czechia, Austria, Italy)	~33,000 – 26,000	Pavlovian / Eastern & Mediterranean Gravettian	Modeled as ~64% Sunghir-related and ~36% Goyet-related ancestry; completely disappears from Central Europe during the Last Glacial Maximum.
Fournol Cluster	Western and Southwestern Europe (France, Iberia)	~32,000 – 25,000	Western Gravettian	Direct continuation of the Goyet Q116-1 genetic profile; persists through the Last Glacial Maximum in Franco-Cantabrian refugia.
Goyet-Q2 / Magdalenian	Franco-Cantabria expanding into Central Europe	~23,000 – 14,000	Solutrean & Magdalenian	Post-LGM continuation of Fournol ancestry mixed with incoming Villabruna/Epi-Balkan hunter-gatherer gene flow.

Until the 2026 University of Vienna study, paleogeneticists observed a sharp boundary between the Fournol and Věstonice domains. Furthermore, because human skeletal preservation during

the Upper Paleolithic is notoriously fragmentary—often due to funerary practices that did not involve deep inhumation—the Franco-Cantabrian region suffered from a severe genomic data gap between 45,000 and 20,000 cal BP.

2. Bridging East and West: Genomic Evidence from Isturitz and Ormesson

To address the western European sampling gap, Gelabert and colleagues generated genome-wide nuclear data from three newly analyzed Gravettian skeletal specimens—recovered from Isturitz Cave in southwestern France, Chufin Cave in northern Spain, and Ostuni in southern Italy—alongside high-resolution sedimentary ancient DNA from El Mirón Cave in Spain.

The centerpiece of the trans-European connectivity finding comes from **Isturitz Cave** (Pyrénées-Atlantiques) in the French Basque Country. Strategically situated in the foothills of the western Pyrenees along the Arberoue Valley, Isturitz is a massive karstic tunnel that functioned as one of the premier cultural aggregation sites of the Upper Paleolithic. Excavations at Isturitz have yielded rich stratigraphies spanning the Aurignacian, Gravettian, Solutrean, and Magdalenian periods, including dozens of bird-bone flutes, engraved slate plaques, and exotic raw materials transported from hundreds of kilometers away. From a Gravettian layer at Isturitz dated to approximately 30,000 cal BP, researchers analyzed a single human bone belonging to an adult female. This specimen yielded the oldest genome-wide dataset of an anatomically modern human recovered to date from southwestern Europe. When the research team projected the Isturitz woman's single nucleotide polymorphisms (SNPs) onto a principal component analysis (PCA) alongside other Upper Paleolithic Eurasian genomes and evaluated her allele-sharing profiles using outgroup and admixture *f*-statistics, they discovered an unexpected genetic profile.

Rather than clustering purely with other western European individuals of the Fournol cluster, the Isturitz genome occupied an intermediate position between the western Fournol cluster and the central-eastern Věstonice cluster. Quantitative ancestry modeling demonstrated that while the majority of the Isturitz woman's genome derived from the local western European lineage (Fournol/Goyet Q116-1 related), she carried a substantial, statistically robust proportion of ancestry derived from the central and eastern European Věstonice lineage.

To determine when this biological mixing occurred, the researchers examined the length distribution of ancestral chromosomal segments within the Isturitz woman's genome. When two distinct populations interbreed, the resulting offspring inherit intact chromosomes from each parental group. In every subsequent generation, meiotic recombination breaks these chromosomes into progressively shorter blocks of alternating ancestry. By measuring the average genetic length of the Věstonice-derived and Fournol-derived chromosomal tracts and applying a standard human recombination clock (assuming a generation interval of roughly 28 to 30 years), the team calculated that the admixture event took place relatively recently before the woman lived—specifically around **32,500 to 31,500 years ago**.

Crucially, the Isturitz woman was not an isolated anomaly. When the researchers re-evaluated previously published genomic data from an early Gravettian individual at **Ormesson** (Les

Bossats, Seine-et-Marne) in the Paris Basin of northern France—who lived roughly 31,000 years ago—they identified a parallel, albeit slightly weaker, signal of Věstonice-related ancestry embedded within a predominantly Fournol genetic background.

Integrating Genetics with Lithic and Symbolic Exchange Networks

The detection of Věstonice ancestry in southwestern and northern France around 32,000 to 30,000 cal BP transforms our understanding of Gravettian mobility. The physical distance between the core Věstonice settlements in Moravia and Lower Austria (such as Dolní Věstonice and Krems-Wachtberg) and Isturitz Cave in the western Pyrenees exceeds 1,300 kilometers. Even if gene flow occurred via a stepping-stone model across central Germany or the northern Alpine foreland rather than a single direct migration of individuals, it proves that social networks were permeable to biological movement over vast expanses of sub-glacial terrain. This genetic pulse aligns remarkably well with long-standing archaeological observations:

1. **Exotic Lithic Procurement:** At Isturitz, Gravettian toolmakers utilized high-quality flint originating from the Bergeracois region of the Dordogne (over 200 kilometers to the north) as well as Chalosse flint from the Landes region and radiolarites from the southern side of the Pyrenees in modern-day Spain. In central Europe, Pavlovian groups routinely moved flint and radiolarite over distances of 200 to 400 kilometers from southern Poland and the Danube basin.
2. **Marine Shell Ornaments:** Perforated fossil and contemporary marine shells originating from both the Atlantic coast and the Mediterranean Sea were traded deep into the continental interior, appearing in burials and habitation layers along the Rhine and Danube corridors.
3. **Shared Acoustic and Artistic Traditions:** Isturitz is famous for yielding over twenty bone flutes crafted primarily from vulture radii, dating from the Aurignacian into the Gravettian. This musical tradition mirrors the bone and ivory flutes recovered from the Swabian Jura in southwestern Germany (such as Geißenklösterle and Hohle Fels), indicating an enduring corridor of cultural transmission linking the Danube headwaters, the Rhone-Saône corridor, and the Pyrenees.

The convergence of Věstonice genes and diverse material culture at Isturitz strongly supports the archaeological hypothesis that the cave functioned as a periodic macro-band aggregation hub. In low-density foraging landscapes, dispersed bands of 25 to 50 people must periodically gather at predictable seasonal bottlenecks—such as river fords or mountain passes where migrating herds of reindeer (*Rangifer tarandus*), horse (*Equus ferus*), and steppe bison (*Bison priscus*) funnel through—to exchange raw materials, perform communal rituals, share ecological information, and find marriage partners outside their immediate kin group. Isturitz, commanding the western pass around the Pyrenees toward the Cantabrian coast, was a geographical nexus where western Fournol groups and eastern-shifted groups met and interbred.

3. Southwestern Refugia and Deep Continuity: Skeletal and Sedimentary DNA in Iberia

While the Isturitz genome illustrates the dynamic horizontal movement of genes across Europe

before the glaciers reached their peak, the second major pillar of the 2026 study demonstrates profound vertical continuity—the persistence of a single regional gene pool across thousands of years in northern Spain.

As global temperatures plummeted toward the Last Glacial Maximum (roughly 26,500 to 19,000 cal BP), ice sheets expanded across Scandinavia, the British Isles, and the Alps. Vast stretches of central Europe and the northern European plain turned into arid polar deserts swept by dust storms, rendering them largely uninhabitable for year-round human occupation. Consequently, the Věstonice genetic cluster in central Europe suffered a local extinction; post-LGM genomes from central Europe show no continuity with the earlier Pavlovian mammoth hunters. Instead, human populations contracted into southern climatic refugia, most notably the Franco-Cantabrian region (spanning southwestern France and the Cantabrian coast of northern Spain), the Italian Peninsula, and the Balkans.

To test how the western Fournol lineage weathered these climatic oscillations in northern Iberia, Gelabert and colleagues examined human remains from **Chufin Cave** (Riclonés, Cantabria) and cave sediments from **El Mirón Cave** (Ramales de la Victoria, Cantabria).



At **Chufin Cave**, a site celebrated for its deep red-ochre parietal dot paintings and Paleolithic engravings overlooking the Nansa River valley, the researchers sequenced genome-wide data from a Gravettian individual. Unlike the Isturitz woman on the northern flank of the Pyrenees, the Chufin genome showed unadmixed western European Fournol ancestry, matching the genetic profile observed in earlier and contemporary Iberian and southern French individuals

(such as those from the Serinyà caves in Catalonia). This confirmed that the core Fournol lineage was firmly established along the Cantabrian coastal strip during the Gravettian.

The Methodological Breakthrough of Autosomal Sedimentary DNA at El Mirón

Tracing what happened to this Cantabrian population during the height of the glacial freeze—a cultural period known in southwestern Europe as the **Solutrean** (roughly 24,500 to 22,000 cal BP)—has historically been hampered by the extreme scarcity of Solutrean human skeletons. While the Solutrean is famous for its pressure-flaked laurel-leaf flint points, osseous eyed needles, and base-camp hearths, very few diagnosable human bones have survived from this critical 2,500-year window, leaving only a 23,000-year-old individual from Cueva del Malalmuerzo in southern Andalusia to represent the entire Iberian Peninsula.

To bypass the absence of fossils in northern Spain, the Vienna team turned to **sedimentary ancient DNA (sedaDNA)** at **El Mirón Cave**, a massive cavity perched above the Asón River valley in eastern Cantabria. Throughout the Upper Paleolithic, humans and carnivores occupied El Mirón, continuously shedding microscopic biological traces into the cave floor through sloughed skin cells, hair, blood, sweat, feces, and microscopic bone dust generated during tool manufacture and butchery. Over millennia, extracellular DNA molecules bind electrostatically to clay minerals and humic acids within the soil matrix, which shields the nucleic acids from complete enzymatic degradation by soil microbes.

While earlier sedaDNA studies successfully recovered mitochondrial DNA (mtDNA) from Denisova Cave and Neanderthal sites, mitochondrial DNA is inherited solely down the maternal line and behaves as a single genetic locus, offering limited power to resolve complex population mixtures. Recovering **autosomal (nuclear) DNA** from cave dirt is orders of magnitude more difficult: nuclear DNA exists in only two copies per cell compared to hundreds or thousands of mitochondrial copies, and human DNA represents a tiny fraction (often less than 0.01 percent) of the total metagenomic DNA in a soil sample, which is overwhelmingly dominated by bacteria, fungi, plants, and cave-dwelling mammals.

To overcome this signal-to-noise barrier in a **22,000-year-old Solutrean layer** at El Mirón, the researchers employed high-throughput shotgun sequencing combined with targeted hybridization capture enrichment:

- **Massive Sequencing Depth:** Generating usable autosomal data from the El Mirón sediment required sequencing over **1.15 billion raw DNA reads**, illustrating both the immense computational scale and the technical hurdles of sedimentary paleogenomics.
- **Hybridization Capture Probes:** The team washed the extracted sedimentary DNA libraries over synthetic single-stranded RNA/DNA baits designed to specifically bind to over one million known variable positions (SNPs) across the human nuclear genome, washing away microbial and non-target environmental DNA.
- **Authentication via Taphonomic Damage Filtering:** Because modern human laboratory contamination is a constant hazard when working with trace ancient human DNA, the bioinformatic pipeline strictly filtered the captured reads. Authentic ancient DNA exhibits characteristic post-mortem chemical damage—specifically **cytosine deamination** at the overhanging single-stranded ends of short DNA fragments (typically 35 to 60 base pairs

in length), where cytosine bases lose an amine group and convert into uracil, which sequencing machines read as thymine (manifesting as a high frequency of Cytosine-to-Thymine substitutions at the 5-prime end of the read).

- **Competitive Metagenomic Mapping:** Because mammals such as cave bears (*Ursus spelaeus*), hyenas, wolves, and ungulates also inhabited El Mirón, DNA fragments that are evolutionary conserved across mammals could falsely map to the human reference genome. The researchers utilized competitive mapping algorithms against a comprehensive database of 28 mammalian taxa identified in the cave, discarding any sequence read that matched faunal genomes as closely as, or better than, the human genome.

Through this rigorous pipeline, the team successfully isolated approximately **14,000 authentic human autosomal SNPs** directly from the 22,000-year-old Solutrean dirt at El Mirón—without a single macroscopic bone or tooth present in the sample.

When analyzed alongside skeletal genomes, the 22,000-year-old El Mirón sedimentary nuclear genome clustered squarely within the western European **Fournol cluster**. This finding provides definitive proof of long-term population continuity in northern Spain right through the coldest peak of the Last Glacial Maximum. Furthermore, El Mirón Cave is famous for a later burial discovered in 2010: the "Red Lady of El Mirón," an adult woman buried in red ochre staining around 18,700 cal BP during the Lower Magdalenian period. By linking the Gravettian genome at nearby Chufin Cave (~27,000 cal BP), the Solutrean sediment DNA at El Mirón (~22,000 cal BP), and the Magdalenian "Red Lady" of El Mirón (~18,700 cal BP), paleogeneticists can now trace an unbroken thread of Fournol-derived human occupation in the Cantabrian mountains spanning more than 10,000 years. As the ice sheets retreated after 19,000 cal BP, descendants of this Franco-Cantabrian refuge population—carrying what is known in post-LGM terminology as the **Goyet-Q2 / Magdalenian ancestry profile**—expanded northward and eastward out of Iberia and southwestern France to repopulate central Europe.

4. Kinship, Exogamy, and Mating Practices in Gravettian Italy: The Ostuni Mother and Fetus

If Ice Age Europeans maintained genetic continuity in regional refugia while periodically exchanging genes over hundreds of kilometers, how did they organize their immediate local communities to ensure biological survival? Because Upper Paleolithic hunter-gatherers lived in small, dispersed bands with low overall population density, they faced a persistent evolutionary risk: **inbreeding depression**. If individuals consistently mated with first or second cousins within a closed local band, the accumulation of deleterious recessive mutations would rapidly compromise fertility and infant survival.

The 2026 University of Vienna study sheds remarkable light on this question through the genomic analysis of **Ostuni1b**, a Gravettian specimen from the Grotta di Santa Maria di Agnano near Ostuni in Apulia, southern Italy.

Excavated in 1991, the Ostuni burial is one of the most poignant archaeological discoveries of the European Paleolithic. Dated to approximately 27,800 to 27,300 cal BP, the grave contained the skeleton of a roughly 20-year-old woman (**Ostuni1**) who died in the late third trimester of

pregnancy (estimated at 31 to 33 weeks of gestation), with the delicate skeletal remains of her unborn fetus (**Ostuni1b**) preserved *in utero* within her pelvic cavity. The woman had been buried in a flexed position, surrounded by hundreds of perforated *Cyclope neritea* marine shells covering her head and wrists, red ochre pigment, horse and aurochs teeth, and flint tools.

Specimen / Sample	Archaeological Site & Region	Approximate Age (cal BP)	Sample Material	Cultural Attribution	Primary Genetic Findings
Isturitz Individual	Isturitz Cave, Basque Country, SW France	~30,000	Skeletal element (Adult Female)	Early / Mid Gravettian	Oldest genome-wide data in SW Europe; predominantly Western (Fournol) ancestry with significant Eastern (Věstonice) admixture dated to ~32,500–31,500 cal BP.
Ormesson Individual	Les Bossats, Ormesson, Northern France	~31,000	Skeletal element (Previously sequenced, re-analyzed)	Early Gravettian	Predominantly Fournol genetic background carrying a detectable minor signal of Věstonice-related eastern gene flow.
Chufin Individual	Chufin Cave, Cantabria,	~27,000 – 25,000	Skeletal element	Western Gravettian	Unadmixed Western European

	Northern Spain				(Fournol) genetic profile; demonstrates deep regional stability in northern Iberia prior to the LGM.
El Mirón Sediment	El Mirón Cave, Cantabria, Northern Spain	~22,000	Cave sediment (>1.15 billion reads; ~14,000 SNPs)	Solutrean (LGM Peak)	Autosomal nuclear DNA recovered without bones; proves Fournol ancestry persisted uninterrupted in the Cantabrian refugium during the glacial maximum.
Ostuni1 & Ostuni1b	Grotta di Santa Maria di Agnano, Apulia, Italy	~27,600	Maternal skeleton (Ostuni1) & <i>in utero</i> fetus (Ostuni1b)	Mediterranean Gravettian	Confirmed mother-son relationship (male fetus); both belong to the Věstonice cluster; low Runs of Homozygosity (ROH)

					show parents were not closely related.
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While the maternal genome (Ostuni1) had been partially sequenced in earlier foundational studies—placing her within the southern branch of the **Věstonice cluster** alongside individuals from Grotta Paglicci—the fetus (Ostuni1b) had never been genomically characterized. Gelabert and colleagues successfully extracted and sequenced nuclear DNA from the fragile fetal remains.

First, chromosomal sex determination—evaluated by comparing the ratio of sequencing reads mapping to the X and Y chromosomes relative to the autosomes—established that **Ostuni1b was biologically male**. Kinship analysis confirmed the expected first-degree maternal-offspring relationship between Ostuni1 and Ostuni1b, sharing exactly 50 percent of their autosomal alleles identical by descent.

More importantly, because a child inherits half of its genome from its mother and half from its father, sequencing both the mother (Ostuni1) and her son (Ostuni1b) allowed geneticists to computationally reconstruct the genetic profile of the **unsequenced father** and evaluate the genetic relatedness between the two parents. This analysis operates through two complementary genomic metrics:

1. **Paternal Ancestry Reconstruction:** By subtracting the maternal alleles at shared heterozygous and homozygous loci in the fetus, researchers examined the population affinities of the paternal haplotypes. The results indicated that Ostuni1b's father belonged to the exact same southern Italian **Věstonice genetic cluster** as the mother. Unlike Isturitz, there was no recent long-distance exotic admixture in this specific coupling; both parents drew their ancestry from the regional Apulian/Mediterranean Gravettian gene pool.
2. **Runs of Homozygosity (ROH):** To determine whether the mother and father were close biological relatives (such as siblings, cousins, or avuncular pairs), the researchers scanned the fetal genome for Runs of Homozygosity—continuous stretches of the diploid genome where the maternal copy and the paternal copy are completely identical because both parents inherited the exact same chromosomal segment from a recent common ancestor.
 - In human population genetics, **short ROH tracts** (typically 4 to 8 centimorgans in length) reflect "background relatedness"—meaning the parents were not close family members, but belonged to a population with a small overall effective population size where everyone shares distant ancestors dozens of generations back.
 - Conversely, **long ROH tracts** (exceeding 20 to 50 centimorgans, summing to hundreds of megabases across the genome) are the unmistakable signature of recent consanguinity, such as first-cousin or incestuous unions.

In the Ostuni1b fetal genome, the researchers observed an absence of long ROH segments that would indicate close-kin mating. Even though both the mother and father belonged to the same regional Věstonice population in southern Italy, they were **not closely related**. This finding mirrors genomic patterns previously observed at the 34,000-year-old site of **Sunghir** in Russia, where four co-buried individuals exhibited low recent inbreeding despite living in a sparse Arctic environment. Together, Ostuni1b and Sunghir demonstrate that Upper Paleolithic foragers maintained institutionalized social mechanisms—specifically **band exogamy** and fluid residential mobility—designed to avoid mating with close relatives. Even when a regional population remained genetically cohesive over millennia, local bands actively exchanged marriage partners across a wider regional meta-population (comprising hundreds of individuals spread across multiple neighboring bands), effectively keeping inbreeding depression at bay while preserving cultural and genetic stability.

5. Methodological Synthesis: How Paleogenomics Reconstructs Prehistoric Mobility

The discoveries at Isturitz, Chufín, El Mirón, and Ostuni illustrate how modern paleogenomics has evolved from simple maternal lineage tracking into a high-resolution quantitative science capable of resolving fine-scale social and demographic processes. Several analytical frameworks work in concert to generate these insights from degraded Pleistocene molecules.

Analytical Technique	Underlying Biological Principle	Application in Upper Paleolithic Research	Specific Insight Gained in the 2026 Study
Outgroup & Admixture f-Statistics	Measures shared genetic drift (allele frequency correlations) between test populations relative to deep outgroups or source proxies.	Distinguishes whether two populations form a simple clade or require gene flow from an external third source.	Identified that the Isturitz and Ormesson individuals possess excess allele sharing with Central/Eastern European Věstonice populations relative to pure Western Fournol groups.
Linkage Disequilibrium / Recombination Dating	Meiotic recombination breaks down contiguous chromosomal blocks inherited	Estimates the number of generations elapsed between an admixture event and the death of	Dated the pulse of Eastern (Věstonice) into Western (Fournol) gene flow observed in the Isturitz woman to

	from admixing source populations at a predictable rate per generation.	the sequenced individual.	approximately 32,500–31,500 cal BP.
Hybridization Capture on Sedimentary DNA (sedaDNA)	Synthetic oligonucleotide baits selectively bind target human SNP loci out of complex environmental soil extracts.	Enables nuclear genome-wide ancestry reconstruction in archaeological strata lacking skeletal fossils.	Recovered ~14,000 human SNPs from over 1.15 billion reads in a 22,000 cal BP Solutrean layer at El Mirón Cave, proving LGM population survival.
Runs of Homozygosity (ROH) Profiling	Quantifies the length and total genomic coverage of identical-by-descent chromosomal segments inherited from both parents.	Differentiates between small ancestral population size (short ROH) and recent consanguineous mating (long ROH).	Demonstrated via the Ostuni1b fetus that Gravettian parents in southern Italy belonged to the same genetic cluster but avoided close-kin inbreeding.

Deconstructing Admixture Modeling and Recombination Clocks

When geneticists evaluate whether a newly sequenced individual like the Isturitz woman belongs purely to the Western Fournol cluster or carries Eastern Věstonice admixture, they rely on formal statistical tests of tree-ness, commonly referred to as **f-statistics** (and implemented in modeling suites such as *qpAdm* and *qpGraph*).

In descriptive terms, imagine comparing the frequency of hundreds of thousands of genetic variants across four groups: a deeply diverged baseline population (such as an African group that does not share specific Eurasian genetic drift), a Western Gravettian reference group (Fournol), an Eastern Gravettian reference group (Věstonice), and the test individual (Isturitz). If Isturitz descended purely from the Fournol group without outside contact, any genetic variants that drifted to higher frequency specifically within the Věstonice lineage after the East-West split should be absent in Isturitz. However, when researchers tallied these shared variants across the genome, they found a statistically significant excess of allele sharing between Isturitz and Věstonice. By comparing the magnitude of this excess against the baseline divergence between the two clusters, two-way admixture models can estimate the precise percentage of the genome derived from each ancestral source.

Once admixture is confirmed, dating the contact relies on the mechanics of **chromosomal crossover during meiosis**. During the formation of sperm and egg cells, homologous chromosomes pair up and swap segments. Because recombination occurs randomly along the length of a chromosome at an average rate of roughly one crossover per 100 megabases (defined as one Morgan) per generation, long intact stretches of "Eastern" DNA sitting inside a "Western" genome act like a ticking molecular hourglass.

If an individual is the direct grandchild of an Eastern migrant, their Eastern chromosomal segments will be massive, spanning tens of millions of base pairs. If the interbreeding occurred 50 generations (roughly 1,400 years) prior, those segments will have been chopped by 50 rounds of meiosis into short, dispersed islands of Eastern alleles. By measuring the decay curve of linkage disequilibrium—the probability that two genetic markers located at a specific genetic distance along a chromosome share the same ancestral origin—researchers determined that the Věstonice ancestry in the 30,000-year-old Isturitz woman entered her lineage roughly 50 to 80 generations before her birth, pinpointing the contact window to between 32,500 and 31,500 cal BP.

6. Reconciling the Archaeological and Genetic Records of the Ice Age

The synthesis of these genomic and methodological advances forces a substantial revision of how anthropologists conceptualize human society during the Upper Paleolithic. For decades, researchers tended to gravitate toward one of two polarized models:

1. **The Pan-European Cultural Highway Model:** Rooted in classical typology, this view treated the Gravettian as a single, unified civilization where people, goods, and genes flowed effortlessly across the continent, creating a homogeneous cultural landscape from Portugal to Russia.
2. **The Isolated Regional Cluster Model:** Emerging from the initial wave of paleogenomics between 2016 and 2023, this view emphasized the stark genetic boundary between the Fournol and Věstonice clusters, suggesting that European hunter-gatherers were partitioned into isolated, territorial populations that rarely crossed major geographic barriers like the Rhine or the Alps, and that similarities in stone tools or Venus figurines represented superficial cultural convergence or low-level down-the-line copying.

The data from Isturitz, Ormesson, Chufín, El Mirón, and Ostuni demonstrate that the reality was far more nuanced—and far more resilient—than either extreme. Ice Age Europe was structured as a **multi-tiered metapopulation network** that balanced deep regional continuity with periodic long-distance connectivity:

- **At the Local and Micro-Regional Scale (10 to 150 km):** As shown by the Ostuni1 mother and Ostuni1b fetus in southern Italy, hunter-gatherers lived in small bands that belonged to a coherent regional gene pool. To prevent the lethal consequences of inbreeding within small residential groups, these bands practiced strict exogamy, exchanging marriage partners with neighboring bands across their local ecological province.
- **At the Regional Refugium Scale (150 to 500 km):** As demonstrated by the skeletal

genome from Chufin Cave and the 22,000-year-old sedimentary nuclear DNA from El Mirón Cave, regional lineages like the Fournol cluster were remarkably resilient. Anchored in rich topographic sanctuaries like the Franco-Cantabrian region—where steep river valleys sheltered woodland microclimates and diverse marine and montane food resources—these populations persisted in place for over 10,000 years, surviving the worst climatic deterioration of the Last Glacial Maximum without being wiped out or replaced.

- **At the Continental Macro-Scale (500 to 1,500+ km):** As revealed by the genomes from Isturitz and Ormesson, these stable regional populations were never hermetically sealed. Across the open mammoth steppe and along major riverine corridors before the LGM, individuals and groups periodically moved over vast distances, linking the central European Věstonice world with the western European Fournol world. Major aggregation hubs like Isturitz Cave served as cultural and biological crossroads where distant foraging networks intersected, exchanging not only exotic flint, marine shells, and artistic motifs, but also DNA.

Looking ahead, the successful recovery of 14,000 autosomal SNPs from a 22,000-year-old sediment layer at El Mirón Cave marks a pivotal turning point for paleoanthropology. Because over 95 percent of all excavated Paleolithic sites lack preserved human bones, sedimentary nuclear DNA—especially when paired with emerging paleoproteomic techniques like Zooarchaeology by Mass Spectrometry (ZooMS)—unlocks the ability to track human population movements across every well-stratified cave on Earth. As researchers apply these high-coverage sedimentary capture methods to earlier Aurignacian and Initial Upper Paleolithic layers across Eurasia, the silent soil of prehistoric caves will continue to reveal how early modern humans navigated, connected, and survived across the Pleistocene world.