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Palaeogenetic research at the El Sidrón Neanderthal site

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SUMMARY

El Sidrón (Asturias, north of Spain) is a subterranean karstic system, where the remains of a contemporaneous social Neanderthal group dated to about 49,000 years ago have been being excavated since their accidental discovery in 1994. Due to the particular preservation conditions of this site, all individuals identified so far have preserved DNA, and the anticontamination measures implemented during the excavation have made palaeogenetic studies possible on all individuals. The El Sidrón samples provide unique information on the kinship relationships and on the internal genetic diversity of Neanderthal groups, thus yielding for first time empirical data for the generation of demographic models of these extinct humans. Moreover, the exceptional preservation of some bone samples has allowed the retrieval of nuclear genes associated with some phenotypic traits involved in pigmentation, blood group, language or taste perception, as well as a significant fraction (0.1%) of the nuclear genome. A future project on Neanderthal genomic diversity could be based on at least some of the El Sidrón specimens.

1. History of the discovery

El Sidrón cave has been known from the beginning of the XXth century. In 1938, during the Spanish Civil War (1936–1939), several republicans took refuge in the cave, hiding from the advancing Nationalist troops. A young woman (called Olvido Otero Gonzalez) from a neighbouring village was shot while bringing food to the republicans and bled to death at the main cave entrance. She was buried close nearby (hence the name of the entrance, "Tomb's entrance"). The insurrects blew up the main entrance with dynamite but the republicans managed to flee from the cave through other minor entrances (de la Rasilla et al., 2011).

During the last decades of the XXth century, the cave was explored by speleologists from different organizations from Asturias and was quite a popular destination partly because of its intricacy and the beauty of the landscape. The place was placed under Partial Natural Reservation in 1995, to help preserve the environment and also to protect some endemic bats and beetles that lived in the system.

On March 23rd, 1994, three cave explorers from Gijón, Carlos Armando, Juan José and Francisco Javier del Río, found two human hemimandibles exposed at the surface layer within a small lateral gallery (thereby called "Galería del Osario" or "Ossuary Gallery"). Thinking they could belong to dead republican partisans, they called the Guardia Civil, who came into the cave three days later and extracted a few more bones with picks and shovels. All these skeletal remains were sent to the court in Infiesto and from there to the Instituto Anatómico Forense in Madrid (April 1994). The experts there concluded the mandibles most likely belonged to Neanderthals, while a posterior report written by an external paleoanthropologist concluded they were modern humans. The remains were sent back to Asturias, to the Consejería de Cultura, that commissioned yet another report to Antonio Rosas and Emiliano Aguirre (Museo Nacional de Ciencias Naturales de Madrid). The remains were definitely identified as Neanderthals and a preliminary analysis published in a Spanish journal in 1999 (Rosas and Aguirre, 1999). In the meantime, however, unidentified sackers came to the site and removed around 1 m^3 of the sediment, probably also extracting some bone remains. In fact, an anonymous letter and a photo sent to the local newspaper, La Nueva España, around that time showed what superficially looked like a Neanderthal skullcap. The exact nature and current location of this remain is unknown, although the letter was sent from the UK.

Systematic excavations, founded by the Consejería de Cultura of the Asturias Autonomous Government, started at the site in 2000, and were conducted each season until the present, with the sole exception of the year 2003. In the field, the excavations were directed by Professor Javier Fortea (University of Oviedo) until his death on October 1st 2009. His collaborator on the project, Marco de la Rasilla, also from the University of Oviedo, has since then continued the excavation. The paleontological analysis has been directed by Antonio Rosas (Museo Nacional de Ciencias Naturales, Madrid) while the paleogenetic analysis has been directed by Carles Lalueza-Fox (Institute of Evolutionary Biology, Barcelona). Once

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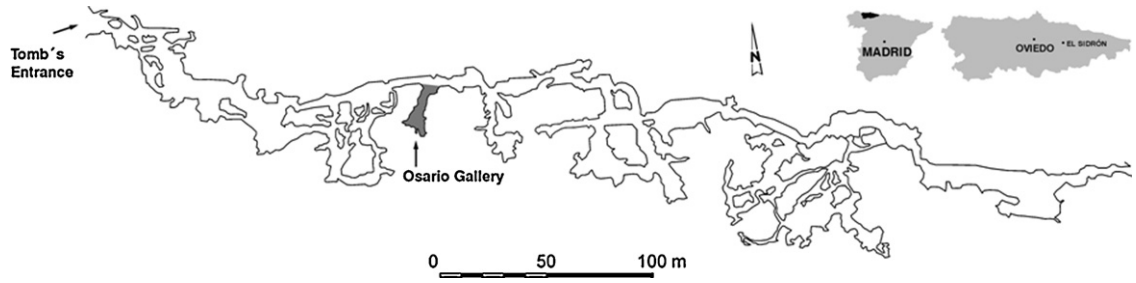

Fig. 1. Map of El Sidrón karst system. Tomb's entrance: main entrance into the system; Osario Gallery (Ossuary Gallery): lateral galley where the Neanderthal remains have accumulated.

investigations are complete, the El Sidrón collection will be housed permanently at the Archaeological Museum in Oviedo, Asturias, while an interpretation centre will be constructed at the town of Villamayor, near the site.

2. Geological structure of the cave and origin of the assemblage

From a geological perspective, the cave lies in the so-called Surco of Oviedo-Infiesto, composed of Mesozoic and Tertiary sediments with a natural barrier in the north consisting of the Sueve massif and in the south by the Picos de Europa mountain range. The cave measures 3700 m in total length, including all secondary galleries, with a total difference in altitude of 4.67 m. The main cave is basically a pressure tube of a subterranean river (the river Pando, which emerges later on) that runs for about 600 m in a mainly NNW to SSE direction (Fig. 1). The main gallery can be dangerous due to rising water levels during several months in the year; hence its name "Galería del río" ("River Gallery"). There are also some lateral galleries that run NE–WS to N–S in 50–100 m intervals. The Ossuary Gallery, where the bones are found, is located 220 m from the cave's main entrance (Fig. 1). It is a fairly small gallery, only 28 m in length and with a maximum width of 12 m. Its position slightly above the main gallery precludes the entrance of the water into the gallery itself. The archaeological assemblage consisted of >1800 human bone samples and >400 lithic tools (Santamaría et al., 2009).

The sediments in the Ossuary Gallery have been studied using a geological mapping based on stratigraphic cross-sections every 33 cm. Five main stratigraphic units (I–V) can be defined. Almost all the bones have been retrieved in an area of about 6 m^2 belonging to unit III, which consists of a massive debris flow deposit, composed of a chaotic mixture of gravels and mud. The deposition of these materials was most likely associated with a massive flooding event, possibly caused by a storm with heavy rainfall. It seems that the skeletal and archaeological material entered the gallery through one of the vertical shafts located in the centre of the Ossuary Gallery. The remains were dragged down from a higher level, either from the surface or from an undiscovered upper gallery. It seems that there was a rock shelter in the surface that was probably eroded and disappeared during the last glacial maximum. Therefore, the primary site for El Sidrón assemblage cannot be studied.

The El Sidrón site has been dated by different methods, including AMS ^{14}C, U/Th, OSL, ESR and AAR. Initial ^{14}C ages (Lalueza-Fox et al., 2005) were possibly underestimates due to contamination of the samples with modern carbon. Filtration of the contaminant carbon with the ninhydrin protocol produced more reliable dates of circa 49,000 years ago, which are in agreement with those obtained with the other dating methods (De Torres et al., 2010). Dates from different materials along the gallery are concordant with a synchronous accumulation event of the Neanderthal bones within the site.

Many of the recovered bones display un-equivocal signs of anthropic activities that have been associated with cannibalism at other Neanderthal sites as well as in modern humans. Among the signs found are cut marks, percussion pitting, inner conchoidal scars, adhering flakes, internal vault release and typical breakage patterns. Cut marks have been detected in some cranial vaults, mandibles and postcranial elements. Immature skull bones (frontal, temporal, parietal) show a higher frequency of cut marks than adult skulls, possibly indicating skinning processes. It seems clear that many, if not all, of the El Sidrón individuals were intentionally defleshed by other Neanderthals (because at that time no other hominin species were present in Europe).

At least 18% of the lithic tools can be refitted to few silex cores (maybe only two or three), indicating that knapping activities took place at the primary location of the assemblage (Santamaría et al., 2009). The spatial projection of the lithic refittings confirms this hypothesis and demonstrates the existence of a debris-flow cone that caused the current distribution of both the archaeological and the paleontological materials. Several additional lines of evidence confirm the synchronicity for the origin of El Sidrón assemblage. First, the osteological preservation of the bone surface shows little or no evidence of weathering or erosion. Second, the bones do not display tooth marks originating from large carnivores or other evidence of scavenging activities, suggesting they were exposed on the surface for a very short time. Third, apart from the Neanderthal bones and the lithic artefacts, there is an almost complete absence of other materials, such as animal bones. And fourth, some skeletal specimens, such as partial thoracic cages or foot bones, are still in anatomical connection, indicating limited *postmortem* displacement of the remains (Rosas et al., 2006).

Right now, the most plausible hypothesis for the origin of the assemblage is that a whole Neanderthal family group was killed and eaten by another group under unknown circumstances. The fact that the silex of the tools used come from a nearby location (few kilometres away) suggests that at least the other group had knowledge of the local environment and raw materials (de la Rasilla et al., 2011).

3. Paleogenetic analysis

3.1. Mitochondrial DNA lineages

In 2004, two teeth were sampled for DNA analysis, taking advantage of the fact that they had been already drilled at the root for dating purposes. After being extracted and analysed in Barcelona, one short mtDNA fragment yielded positive results on May 19th 2004 for the sample labelled SD-441. The short 47 bp DNA sequence showed six Neanderthal diagnostic positions, including a G at nucleotide position 16,258, shown previously to be polymorphic in Neanderthals. The paper reporting these results was published in 2005 (Lalueza-Fox et al., 2005). Although the finding could only confirm that Iberian Neanderthals (at the Western most edge of