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Microremains from El Mirón Cave human dental calculus suggest a mixed plant–animal subsistence economy during the Magdalenian in Northern Iberia

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ABSTRACT

Despite more than a century of detailed investigation of the Magdalenian period in Northern Iberia, our understanding of the diets during this period is limited. Methodologies for the reconstruction of Late Glacial subsistence strategies have overwhelmingly targeted animal exploitation, thus revealing only a portion of the dietary spectrum. Retrieving food debris from calculus offers a means to provide missing information on other components of diet. We undertook analysis of human dental calculus samples from Magdalenian individuals (including the "Red Lady") at El Mirón Cave (Cantabria, Spain), as well as several control samples, to better understand the less visible dietary components. Dental calculus yielded a diverse assemblage of microremains from plant, fungal, animal and mineral sources that may provide data on diet and environment. The types of microremains show that the individuals at El Mirón consumed a variety of plants, including seeds and underground storage organs, as well as other foods, including possibly bolete mushrooms. These findings suggest that plant and plant-like foods were parts of her diet, supplementing staples derived from animal foods. As faunal evidence suggests that the Magdalenian Cantabrian diet included a large proportion of animal foods, we argue here for a mixed subsistence pattern.

1. Introduction

Although the Magdalenian in France, Belgium and Germany is commonly characterised as the period of the 'reindeer hunters', this was not the dominant dietary pattern in other European regions. The subsistence of this period in general focused heavily on the hunting of large game, supplemented by fishing and fowling (Álvarez-Fernández, 2011). In the coastal environments along the Atlantic Coast of Northern Iberia, faunal profiles are dominated by ibex (*Capra pyrenaica*), and red deer (*Cervus elaphus*), with smaller proportions of horse (*Equus* sp.), bison (*Bison* sp.), chamois (*Rupicapra rupicapra*), salmon (*Salmo salar*) and shellfish (e.g. Marín-Arroyo, 2009; Straus, 1977; Straus and González Morales, 2012). Red deer and ibex were the most commonly hunted species, perhaps due to their abundance, large size and gregarious behaviour.

However, there is little information on the use of vegetal resources in Northern Iberia. Plant remains including seeds from oak (*Quercus* sp.), hazelnut (*Corylus* sp.), raspberry (*Rubus* sp.), soft-grass (*Holcus* sp.), and chenopods (*Chenopodium* sp.) were recovered during flotation of Lower Magdalenian sediments from El Juyo Cave. Yet they are only tenuously associated to the occupation of the site, and may instead have been introduced by natural processes (Freeman et al., 1988). The only direct evidence for plant consumption in the region comes from a dental microwear study

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carried out on two human teeth found in the Magdalenian levels of Rascaño Cave, roughly 20 km from El Mirón (Guerrero and Lorenzo, 1981). Microwear indicated that these individuals had mixed diets, and possibly even tending toward a degree of reliance on plant foods. This scarcity of evidence occurs despite there being a strong theoretical basis for a reliance on plant foods in some Upper Palaeolithic Iberian diets (e.g. Jones, 2009; Haws, 2004; Owen, 2002; Zaatari and Hublin, 2014), although it is likely that major variation was present within Iberia's complex topography and ecosystem. Even where plant biomass is low, plant consumption may have allowed circumvention of the energy ceiling created from relying on high protein ungulates (Speth, 2010). For this reason, it is important to expand our knowledge to the full range of diet.

El Mirón Cave provides a rare opportunity to look for direct evidence of plant use in Cantabrian Iberia, due in part to the wealth of information about the local and regional environments at the time of the Magdalenian burial, which is the subject of our study. The immediate environment around the site during the Lower Magdalenian was an open landscape with a low biomass of vegetal food. It was a hilly area dominated by heath, grasses and dwarf shrubs, with only some scattered pine (*Pinus* sp.), and rare juniper (*Juniperus* sp.) and birch (*Betula* sp.) (Straus et al., 2013). Pollen analysis of a sediment sample from the burial layer itself reveals a very cold open and only moderately humid landscape at the time of the burial, with some pines but a high percentage of chenopods (Iriarte-Chiapusso et al., 2015). Yet with the large ranging behaviour seen in this time period (Langley and Street, 2013), a Magdalenian hunter-gatherer likely had access to a much wider range of resources than what was found in the near vicinity. Available trees that may have been targeted as a source of nutritional and non-nutritional plant resources include Scots pine (*Pinus sylvestris* L.), birch (*Betula* sp.) and rarer temperate trees species such as oaks (*Quercus* sp.), hazel (*Corylus avellana* L.), linden (*Tilia* sp.) and elder (*Sambucus* sp.). These temperate species would have been found during periods of climatic amelioration (Carrión et al., 2010), but would have been confined to the narrow lowland coastal belt. The limited isolated areas where trees and damp meadows persisted could have supported several calorie-rich plant foods (e.g. mast of oak, hazelnut) and a wealth of other forms of vegetal foods such as fleshy, starch-rich rhizomes (e.g. *Bistorta vivipara* L. Delarbre, *Argentina anserine* L.). These plants resources (oak mast, hazelnut, *Bistorta officinalis*, *Argentina anserina*) are all foods whose consumption is well documented by recent Eurasian and North American northern populations (e.g. Cuenca-Bescós et al., 2009; Kuhnlein and Turner, 1991).

To investigate plant and other, less-understood foods consumed by the Magdalenian population in Northern Iberia, we analysed dental calculus from the human burial at El Mirón. Analysis of dental calculus is an emerging archaeobotanical method that can provide evidence on specific plant taxa and organs consumed during life (e.g. Armitage, 1975; Boyadjian et al., 2007; Henry et al., 2014; Horrocks et al., 2014; Salazar-García et al., 2013; Mickleburgh and Pagan-Jimenez, 2012), as well as information on other foods that produce morphologically distinct microremains, such as fungal spores and diatoms. Dental calculus forms due to precipitation of salivary calcium phosphate on the bacterial biofilm that covers the teeth, and therefore acts as a mineralised reservoir of the oral environment, including food fragments (Warinner et al., 2014: Power et al., 2014; Kucera et al., 2011; Boyadjian et al., 2007; Dobney and Brothwell, 1986). Studies of calculus in living populations demonstrate that the preserved food remains can reflect the known diet, though its coverage may be highly stochastic and it is constrained to foods where microremains are present. The dental calculus record may also reflect oral processing of non-dietary plants as well as plants introduced to the mouth through the consumption of prey animal stomach contents (Buck and Stringer, 2013), but is expected to overwhelmingly preserve staple foods. The results from calculus analysis have been used to complement those from other methods of dietary reconstruction such as microwear (e.g. Estalrrich et al., 2011) and stable isotopic analysis (e.g. Salazar-García et al., 2014a).

2. Methods

We examined the dental calculus from the Lower Magdalenian human burial in Level 504, also known as the “Red Lady”, to better understand the diet of this individual. The burial is from an adult female, who was deliberately interred (Straus et al., 2013). We also included in the study an isolated human molar from younger deposits dated to the late Lower or early Middle Magdalenian deposits (level 105 in Square W7). We first used scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) to study the elemental composition of a dental calculus sample, and to look for *in-situ* microremain inclusions on the surface of the calculus. Then, we performed optical microscopy on separate human dental calculus samples, along with control samples from faunal calculus, sediment and bone, looking for preserved microremains.

2.1. Calculus sampling

We collected dental calculus from four human teeth in the Laboratorio de Evolución Humana, Universidad de Burgos, Spain (Table 1). Three of the samples were from the “Red Lady” (samples eM1-3), and only one sample was from the isolated tooth of the second Magdalenian individual (sample eM5). The sampled teeth had a thick pristine band of hard and darkly stained supragingival calculus situated on the enamel surface. Each calculus band that was to be sampled was carefully photographed. The sampling surface was gently brushed clean with a sterile dry toothbrush. Then, a dental scaler was used to remove small areas of calculus (~4 mm^2) onto aluminium foil (Table 1). Each sample was transferred a microcentrifuge tube and weighed with a microbalance (Mettler Toledo MX5). Finally, the teeth with the remaining *in situ* calculus were photographed. We also took a comprehensive variety of control samples to ensure replicability (Weyrich et al., 2015); one sample of plastic storage foam, eight of animal calculus from isolated fauna teeth, two of trabeculae exposed from aDNA sampling of the centre of a human phalange, and five of sediment from four localities near to where the human remains were recovered.

2.2. Electron microscopy analysis

We conducted SEM-EDX analysis at University College Dublin's Nano-Imaging and Materials Analysis Centre (NIMAC) in Dublin, Ireland. One sample (eM2) was examined to check if the sample chemically matched dental calculus, as well as to visualise microremains that might have been trapped on the sample's exterior. The calculus sample was mounted on a stub using double-sided carbon tape, and sputter coated with gold for 20 s using an Emitech K575X Sputter Coating Unit to prevent surface charging by the electron beam. The calculus was mounted so that the exterior surface was visible. We then examined the sample using a FEI Quanta 3D FEG DualBeam (FEI Ltd, Hillsboro, USA) SEM attached to an EDAX ED APOLLO XV Silicon Drift Detector with a 5–10 kV accelerating voltage. The EDX created chemical maps of all elements.

2.3. Optical microscopy analysis

The remainder of the human calculus from the two individuals, fauna calculus and sediment samples were examined using optical