PALEOANTHROPOLOGY

U-Th dating of carbonate crusts reveals Neandertal origin of Iberian cave art

D. L. Hoffmann,[1]* C. D. Standish,[2]* M. García-Diez,[3] P. B. Pettitt,[4] J. A. Milton,[5] J. Zilhão,[6,7,8] J. J. Alcolea-González,[9] P. Cantalejo-Duarte,[10] H. Collado,[11] R. de Balbín,[9] M. Lorblanchet,[12] J. Ramos-Muñoz,[13] G.-Ch. Weniger,[14,15] A. W. G. Pike[2]†

The extent and nature of symbolic behavior among Neandertals are obscure. Although evidence for Neandertal body ornamentation has been proposed, all cave painting has been attributed to modern humans. Here we present dating results for three sites in Spain that show that cave art emerged in Iberia substantially earlier than previously thought. Uranium-thorium (U-Th) dates on carbonate crusts overlying paintings provide minimum ages for a red linear motif in La Pasiega (Cantabria), a hand stencil in Maltravieso (Extremadura), and red-painted speleothems in Ardales (Andalucía). Collectively, these results show that cave art in Iberia is older than 64.8 thousand years (ka). This cave art is the earliest dated so far and predates, by at least 20 ka, the arrival of modern humans in Europe, which implies Neandertal authorship.

The origin of human symbolism is a central concern of modern paleoanthropology (*1*). For the European Middle Paleolithic and the African Middle Stone Age, symbolic behavior has been inferred from the use, presumably for body adornment, of mineral pigments, shell beads, eagle talons, and feathers (*2*–*7*). Cave and rock art constitutes particularly impressive and important evidence for symbolic behavior (*8*), but little is known about the chronology of its emergence, owing to difficulties in precise and accurate dating (*9*).

Claims for Neandertal authorship of cave art have been made (*10*, *11*). However, ambiguities of indirect dating and uncertainty in distinguishing between natural and intentional modification (*12*, *13*) leave these claims unresolved. Recent technical developments enable the possibility of obtaining age constraints for cave art by U-Th dating of associated carbonate precipitates (*14*). This dating approach can provide robust age constraints while keeping the art intact. However, it is a destructive technique, in that a carbonate sample is required (albeit, a very small sample, typically <10 mg) and is taken not from the art itself but from the associated carbonates. The key condition is demonstrating an unambiguous stratigraphic relationship between the sample and the art whose age we wish to constrain. Dating of carbonate crusts formed on top of the art provides a minimum age (*15*). For art painted on top of carbonates (e.g., on flowstone walls, stalagmites, or stalactites), dating the underlying "canvas" provides a maximum age (*15*).

With this approach, the earliest results so far are for a hand stencil from Leang Timpuseng, Sulawesi (Indonesia), with a minimum age of 39.9 thousand years (ka) (*16*), and a red disc on the Panel of Hands in El Castillo, Cantabria (Spain), with a minimum age of 40.8 ka (*17*). Whereas the art in Sulawesi has been attributed to modern humans, the minimum age for the red disc in El Castillo relates to a point in time when it could be attributed to either Cantabria's first modern humans or the region's earlier Neandertal populations (*18*, *19*).

Here we report U-Th dating results of carbonate formations associated with rock art in three Spanish caves: La Pasiega (Cantabria), Maltravieso (Extremadura), and Doña Trinidad (or Ardales; Andalucía) (fig. S1) (*20*). Our criteria for sample selection and subsequent sampling strategy strictly followed previously described methods (*14*). The reliability of the U-Th dating results is controlled by quality criteria for the carbonate (*14*) as well as by the collection and analysis of multiple subsamples of a given crust.

La Pasiega is part of the Monte Castillo cave art complex, a World Heritage Site that also includes the caves of El Castillo, Las Chimeneas, and Las Monedas. Together, these caves show continued human occupation throughout the past 100 ka. At La Pasiega, the rock art comprises mainly red and black paintings, including groups of animals, linear signs, claviform signs, dots, and possible anthropomorphs (*21*). Maltravieso was episodically used by hominin groups during the past 180 ka (*22*); it contains an important set of red hand stencils (~60), which form part of a larger body of art that includes both geometric designs (e.g., dots and triangles) and painted and engraved figures (*23*). Ongoing excavations have shown that Ardales was occupied in the Middle and Upper Paleolithic. Its walls feature an impressive number (>1000) of paintings and engravings in a vast array of forms, including hand stencils and prints; numerous dots, discs, lines, and other geometric shapes; and figurative representations of animals, including horses, deer, and birds (*24*).

We obtained U-Th ages for 53 samples removed from 25 carbonate formations stratigraphically

Fig. 1. Red scalariform sign, panel 78 in hall XI of La Pasiega gallery C. This panel features the La Trampa pictorial group (*21*). (**Inset**) Crust sampled and analyzed for a minimum age (64.8 ka), which constrains the age of the red line. See (*20*) for details.

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[1] Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany. [2] Department of Archaeology, University of Southampton, Avenue Campus, Highfield Road, Southampton SO17 1BF, UK. [3] Faculty of Humanities and Social Sciences, University of Isabel I, Calle de Fernán González 76, 09003 Burgos, Spain. [4] Department of Archaeology, Durham University, South Road, Durham DH1 3LE, UK. [5] Ocean and Earth Science, University of Southampton Waterfront Campus, National Oceanography Centre Southampton, European Way, Southampton SO14 3ZH, UK. [6] University of Barcelona, Departament d'Història i Arqueologia (SERP), Carrer de Montalegre 6, 08001 Barcelona, Spain. [7] Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain. [8] Centro de Arqueologia da Universidade de Lisboa (UNIARQ), Faculdade de Letras, Campo Grande, 1600-214 Lisboa, Portugal. [9] Prehistory Section, University of Alcalá de Henares, Calle Colegios 2, 28801 Alcalá de Henares, Madrid, Spain. [10] Centro de la Prehistoria/Cueva de Ardales, Avenida de Málaga, no. 1, 29550 Ardales (Málaga), Spain. [11] Quaternary-Prehistory Research Group, I-PAT Research Group, D. G. Bibliotecas, Museos y Patrimonio Cultural, Junta de Extremadura, Spain. [12] CNRS, Roc des Monges, 46200 St. Sozy, France. [13] Departamento de Historia, Geografía y Filosofía, Universidad de Cádiz, Avenida Gómez Ulla s/n, Cádiz, Spain. [14] Neanderthal Museum, Talstraße 300, 40822 Mettmann, Germany. [15] Institute of Prehistory, University of Cologne, Cologne, Germany.
*These authors contributed equally to this work.
†Corresponding author. Email: a.w.pike@soton.ac.uk

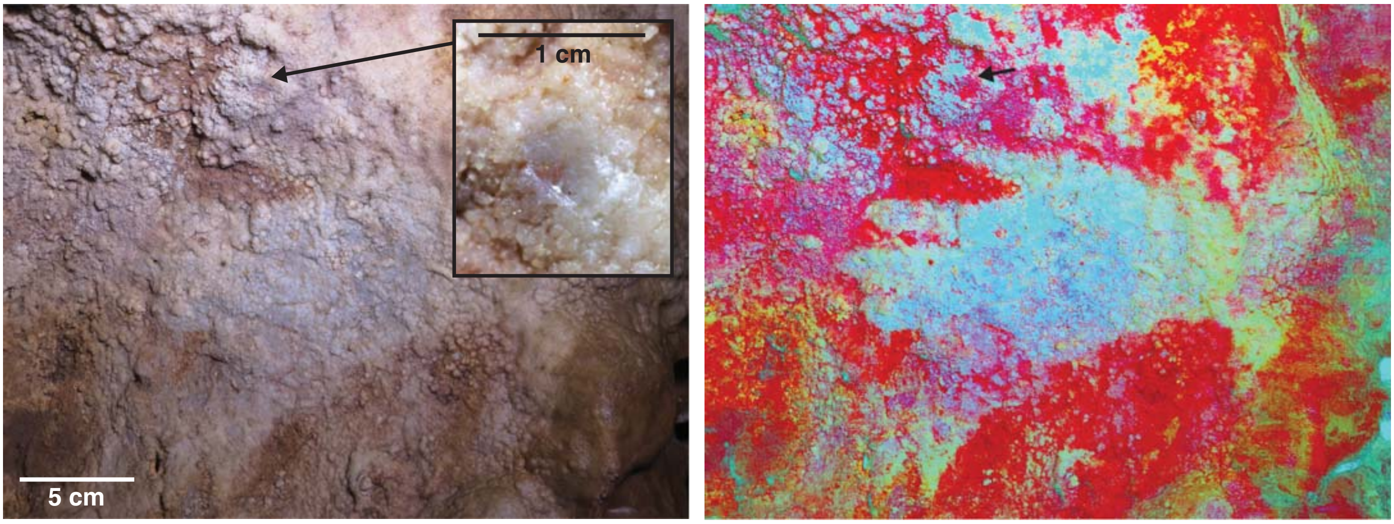

Fig. 2. Hand stencil GS3b in Maltravieso cave (minimum age 66.7 ka). (**Left**) Original photo. The inset shows where the overlying carbonate was sampled for MAL 13. (**Right**) Same picture after application of the DStretch software (*25*) (correlation LRE 15%, auto contrast) to enhance color contrast. See (*20*) for details.

related to paintings in these caves. The full details of our methods and data are described in the supplementary materials (*20*). Here we present and discuss the results that are most meaningful for the antiquity of the art.

In La Pasiega gallery C (fig. S2), a cauliflower-type carbonate formation on top of a red scalariform sign [panel 78 of hall XI (Fig. 1) (*20*)] yielded U-Th dates for three subsamples (outer, middle, and inner) that increase in age with depth—that is, toward the pigment layer. They provide a minimum age of 64.8 ka (sample PAS 34) (Table 1) (*20*) for the sign.

In Maltravieso (fig. S7), we dated samples from five locations on various carbonate formations overlying the same red hand stencil (motif GS3b) (Fig. 2) (*20*). Carbonate deposits almost completely obscure this hand stencil, making it difficult to see with the naked eye and challenging to record by conventional photography. Figure 2 therefore also shows a version of the photographic documentation after we used the DStretch software (*25*) to enhance the image. For subsamples in all locations, the expected depth-age consistency was confirmed. The oldest date provides a minimum age of 66.7 ka (MAL 13) (Table 1) (*20*) for the hand stencil.

In Ardales (fig. S9), we dated layers of five carbonate curtains from three areas of the cave (II-A, II-C, and III-C) that had been painted red. In three cases we were able to obtain both maximum and minimum ages by dating samples from immediately underneath the pigment and from carbonate that subsequently formed on top. These age pairs constrain one or more episodes of painting to between 48.7 ka and 45.3 ka ago (ARD 14 and 15), 45.5 ka and 38.6 ka ago (ARD 26 and 28), and 63.7 ka and 32.1 ka ago (ARD 6 and 8) (Table 1) (*20*). A further two samples yielded minimum ages of 65.5 ka (ARD 13) (Fig. 3), indicating an earlier episode of painting, and 45.9 ka (ARD 16), consistent with the other episodes (fig. S42) (*20*).

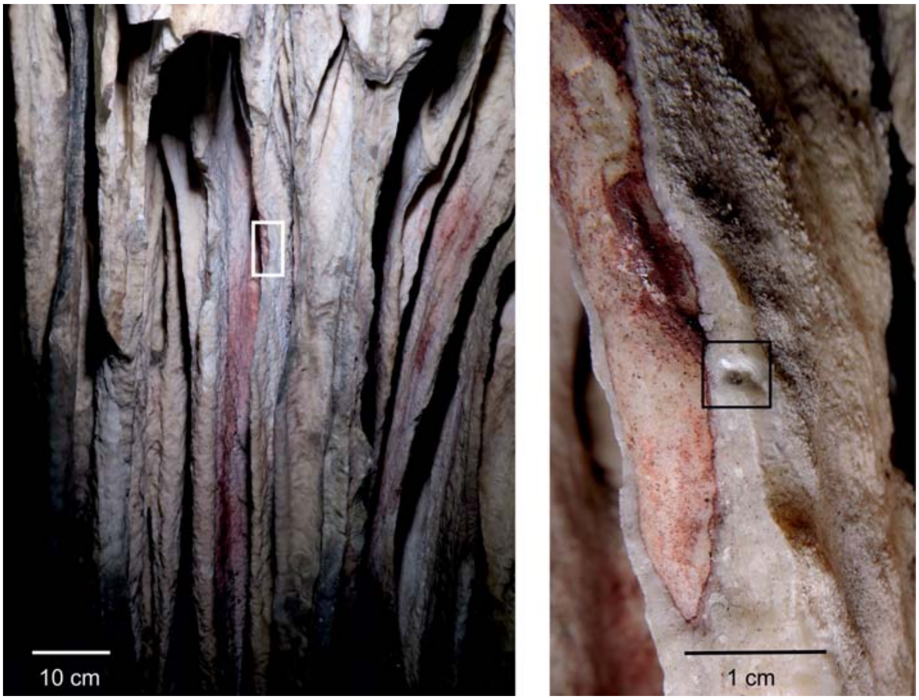

Fig. 3. Speleothem curtain 8 in section II-A-3 in Ardales cave with red pigment, painted before at least 65.5 ka ago. (**Left**) Series of curtains with red paint on top, partially covered with later speleothem growth. The white rectangle outlines the area shown at right. (**Right**) Detail of curtain 8. The black square indicates where carbonate, overlying the red paint, was sampled for ARD 13. See (*20*) for details.

Criteria for reliable minimum (or maximum) ages (*14*) were met by all samples. The oldest minimum ages from the three caves are consistent and, at 64.8 ka or older for each site, substantially predate the arrival of modern humans in Europe, which has been variously estimated at between 45 ka and 40 ka ago (*26*, *27*). Our dating results show that cave art was being made at La Pasiega, Maltravieso, and Ardales at least 20 ka before that. In this age range, Iberia was exclusively populated by Neandertals, as indicated by numerous diagnostic osteological remains, including articulated skeletons (*28*, *29*). The implication is, therefore, that the artists were Neandertals.

All examples of early cave art dated thus far were created in red pigment, and comprise dots, lines, disks, and hand stencils (*30*). This is a

Table 1. U-Th results of samples discussed in the text. More details and additional results can be found in table S4 (*20*). All ratios are activity ratios. Analytical errors are at the 95% confidence level. Spl ID, sample identifier.

Spl ID	Site and description	^{238}U (ng/g)	^{230}Th/^{232}Th	^{230}Th/^{238}U uncorrected	^{234}U/^{238}U uncorrected	Age uncorrected (ka)	Age corrected (ka)
PAS 34a	Pasiega C, no. 78, cauliflower-type carbonate on top of red line of scalariform motif, minimum age	289.29 ± 9.06	32.82 ± 0.21	1.5149 ± 0.0106	3.7694 ± 0.0082	52.52 ± 0.47	51.56 ± 1.09
PAS 34b	As above	215.56 ± 7.43	28.28 ± 0.19	1.5453 ± 0.0121	3.6744 ± 0.0094	55.53 ± 0.56	54.36 ± 1.39
PAS 34c	As above	178.31 ± 8.31	7.25 ± 0.07	2.0348 ± 0.0213	3.4591 ± 0.0092	85.79 ± 1.28	79.66 ± 14.90
MAL 13 surface cleaning fraction	Maltravieso, cauliflower-type carbonate layer overlying hand stencil GS3b, minimum age	117.2 ± 1.99	12.47 ± 0.16	0.4639 ± 0.0068	1.1872 ± 0.0328	53.32 + 2.30 or − 2.13	41.68 + 2.44 or − 2.29
MAL 13A	As above	142.69 ± 3.39	37.50 ± 0.57	0.6067 ± 0.0123	1.2024 ± 0.0305	74.86 + 3.78 or − 3.41	70.08 + 3.82 or − 3.37
ARD 6	Ardales, red paint on curtain formation, II-C-8, carbonate from underlying curtain, maximum age	511.42 ± 6.38	34.95 ± 0.14	0.4661 ± 0.0021	1.0459 ± 0.0021	64.09 ± 0.44	62.97 ± 0.69
ARD 8	Ardales, red paint on curtain formation, II-C-8, carbonate from overlying curtain, minimum age	297.21 ± 2.89	145.58 ± 1.06	0.2703 ± 0.0018	1.0477 ± 0.0024	32.51 ± 0.26	32.35 ± 0.27
ARD 13A	Ardales, red paint on curtain formation, II-A-3 curtain 8, minimum age	1229.61 ± 25.84	152.83 ± 1.14	0.3661 ± 0.0033	1.0385 ± 0.0033	47.33 + 0.57 or − 0.56	47.13 + 0.56 or − 0.57
ARD 13B	As above	331.54 ± 13.53	42.59 ± 0.58	0.4878 ± 0.0073	1.0369 ± 0.0234	69.09 + 2.93 or − 2.62	68.13 + 2.96 or − 2.62
ARD 14A	Ardales, red paint on curtain formation, II-A-3 curtain 6, carbonate from underlying curtain, maximum age	684.76 ± 13.29	395.03 ± 4.91	0.3683 ± 0.0063	1.0379 ± 0.0029	47.72 + 1.05 or − 1.02	47.64 + 1.07 or − 1.03
ARD 15A	Ardales, red paint on curtain formation, II-A-3 curtain 6, carbonate from overlying curtain, minimum age	1696.03 ± 53.88	337.14 ± 3.63	0.3584 ± 0.0050	1.0374 ± 0.0025	46.15 + 0.81 or − 0.82	46.06 + 0.81 or − 0.77
ARD 15B	As above	667.98 ± 37.85	152.07 ± 3.27	0.3467 ± 0.0110	1.0347 ± 0.0061	44.45 + 1.79 or − 1.82	44.25 + 1.78 or − 1.77
ARD 16A	Ardales, red paint on curtain formation, II-A-3 curtain 5, carbonate from overlying curtain, minimum age	313.84 ± 5.88	58.92 ± 0.74	0.3317 ± 0.0044	1.0323 ± 0.0051	42.23 + 0.74 or − 0.72	41.75 ± 0.77
ARD 16B	As above	250.2 ± 4.29	84.25 ± 0.84	0.3628 ± 0.0050	1.0314 ± 0.0051	47.23 + 0.85 or − 0.83	46.86 + 0.85 or − 0.92
ARD 16C	As above	227.59 ± 28.55	56.70 ± 2.84	0.3690 ± 0.0213	1.0227 ± 0.0342	48.79 + 4.26 or − 4.00	48.23 + 4.43 or − 4.10
ARD 26A	Ardales, red paint visible as a line on cross section of a broken curtain, between III-C-3 and III-C-2, carbonate from overlying curtain, minimum age	564.64 ± 13.56	1004.53 ± 20.81	0.3243 ± 0.0099	1.0502 ± 0.0203	40.20 + 1.84 or − 1.69	40.17 + 1.73 or − 1.77
ARD 26B	As above	532.37 ± 14.02	985.93 ± 24.33	0.3258 ± 0.0112	1.0496 ± 0.0113	40.45 + 1.82 or − 1.70	40.42 + 1.79 or − 1.78
ARD 28A	Ardales, red paint visible as a line on cross section of a broken curtain, between III-C-3 and III-C-2, carbonate from underlying curtain, maximum age	520.54 ± 8.11	4626.61 ± 188.57	0.3379 ± 0.0192	1.0458 ± 0.0124	42.48 + 3.09 or − 2.91	42.47 + 3.07 or − 2.97

restricted and nonfigurative set of subjects and could represent the extension of Neandertal body art to the external world. Regardless of whether concentrations of color, dots, disks, and linear motifs can be conceived as symbolic, hand stencils (which, unlike positive hand prints, cannot be created by accident) require a light source and previous selection and preparation of the coloring material—evidence of premeditated creation. Because a number of hand stencils seem to have been deliberately placed in relation to natural features in caves rather than randomly created on accessible surfaces (*31*), it is difficult to see them as anything but meaningful symbols placed in meaningful places.

This cave painting activity constitutes a symbolic behavior by definition, and one that is deeply rooted. At Ardales, distinct episodes over a period of more than 25 ka corroborate that we are not dealing with a one-off burst but with a long tradition that may well stretch back to the time of the annular construction found in Bruniquel cave, France (*32*), dated to 176.5 ± 2.1 ka ago. Dating results for the excavation site at Cueva de los Aviones, Spain (*2*), which place symbolic use of marine shells and mineral pigments by Neandertals at >115 ka ago (*33*), further support the antiquity of Neandertal symbolism.

Cave art such as that dated here exists in other caves of Western Europe and could potentially be of Neandertal origin as well. Red-painted draperies are found at Les Merveilles (France; panel VII) (*34*) and El Castillo (Spain), whereas hand stencils and linear symbols are ubiquitous and, when part of complex superimpositions, always form the base of pictorial stratigraphies. We therefore expect that cave art of Neandertal origin will eventually be revealed in other areas with Neandertal presence elsewhere in Europe. We also see no reason to exclude that the behavior will be equally ancient among coeval non-Neandertal populations of Africa and Asia.

The authorship of the so-called "transitional" techno-complexes of Europe, which, like the Châtelperronian, feature abundant pigments and objects of personal ornamentation, has long been the subject of debate (*35*, *36*). Direct or indirect (via acculturation) assignment to modern humans has been based on an "impossible coincidence" argument—that is, the implausibility that Neandertals would independently evolve the behavior just at the time when modern humans were already in or at the gates of Europe. By showing that the Châtelperronian is but a late manifestation of a long-term indigenous tradition of Neandertal symbolic activity, our results bring closure to this debate.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/359/6378/912/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S42
Tables S1 to S4
References (*37*–*51*)

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STRUCTURAL BIOLOGY

Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation

Youngjin Kim and Jue Chen*

The multidrug transporter permeability (P)–glycoprotein is an adenosine triphosphate (ATP)–binding cassette exporter responsible for clinical resistance to chemotherapy. P-glycoprotein extrudes toxic molecules and drugs from cells through ATP-powered conformational changes. Despite decades of effort, only the structures of the inward-facing conformation of P-glycoprotein are available. Here we present the structure of human P-glycoprotein in the outward-facing conformation, determined by cryo–electron microscopy at 3.4-angstrom resolution. The two nucleotide-binding domains form a closed dimer occluding two ATP molecules. The drug-binding cavity observed in the inward-facing structures is reorientated toward the extracellular space and compressed to preclude substrate binding. This observation indicates that ATP binding, not hydrolysis, promotes substrate release. The structure evokes a model in which the dynamic nature of P-glycoprotein enables translocation of a large variety of substrates.

Cancer cells develop resistance to chemically diverse compounds, a phenomenon known as multidrug resistance (MDR). To improve the effectiveness of chemotherapy, many laboratories have searched for mechanisms that account for MDR. In 1973, Keld Danø demonstrated that the reduced drug accumulation in tumor cells was energy dependent (*1*). In 1976, by labeling cell-surface carbohydrates, Juliano and Ling identified a glycoprotein enriched in colchicine-resistant cells but not in wild-type cells (*2*). The protein was named the permeability (P)–glycoprotein (Pgp) because it was thought to confer drug resistance by making the cellular membrane less permeable (*3*). Ten years later, the genes responsible for MDR in human, mouse, and hamster (named MDR genes) were cloned (*4*–*6*), and it was shown that the protein product of the *mdr1* gene was indeed Pgp (*7*).

Pgp is an adenosine triphosphate (ATP)–binding cassette (ABC) transporter, which uses the energy from ATP hydrolysis to pump substrates across the membrane. It contains two transmembrane domains (TMDs) and two cytoplasmic nucleotide-binding domains (NBDs) (Fig. 1A). Pgp is expressed in many membrane "barriers" of the body, including the blood-brain barrier, gastrointestinal tract, kidney, liver, ovary, and placenta (*2*, *8*–*11*).

Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.
*Corresponding author. Email: juechen@rockefeller.edu