

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	The CT and microCT data were inspected and segmented using Avizo 7.1 (Visualization Sciences Group, Mérignac). The obtained meshes were then processed in Geomagic Studio v.2014.3.0 (3D Systems, Rock Hill, USA) to remove artifacts. VTK mesh visualization was performed in the open-source software ParaView v.5.6.0 (Kitware). Digital image stacks were manipulated for tests in ImageJ 1.54m 5.
Data analysis	The shape analysis was performed using Deformetrica v.4.3 (http://www.deformetrica.org/) open-source software. Statistical analyses were performed in Rstudio v.2023.9.0.463 for R v.4.3.1 using the following packages: RToolsForDeformetrica v.0.1, stats, Morpho v.2.11, dispRity v.1.7.0. Code is available in the Supplementary Code 1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

- All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
 - A description of any restrictions on data availability
 - For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All the raw shape data and additional information needed to replicate the analyses are available through the Supplementary Data 1-3. Accession codes and

identifiers are specified for each of the analyzed individuals in the Supplementary Material.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The sex of the individuals was reported when available in the metadata associated with the CT and microCT scans or taken from the literature in the case of fossil specimens. Information about sex can be found in Supplementary Data 2.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The evolutionary history of the Neanderthal clade is well understood thanks to the available genetic and morphological data, yet several aspects remain unclear. This is the case of the processes that contributed to shaping the distinctive morphology found in Neanderthals. Particularly, there is no general consensus about whether these evolutionary processes happened in a continuous or discontinuous manner. In this study we investigated the phenotypic variation shown by semicircular canal and vestibule shape in large sample constituted by individuals attributed to different stages of Neanderthal evolution (namely, Sima de los Huesos, Krapina, and late Neanderthals). Thanks to the strong phylogenetic signal embedded in these inner ear structures, their morphological diversity was used here as a proxy to infer the underlying variability. The pattern of variation was also compared to that shown by a European population of modern humans, which are known to have undergone to a bottleneck event in their recent evolutionary history. The morphological analyses relied on a diffeomorphic approach, which is known to outperform both linear measurement- and landmark-based analyses. Different scenarios were put at test and our result allowed us to identify a significant reduction in morphological disparity around the start of the Last Glacial Period, which we interpreted as a hint for the presence of genetic bottleneck event in the late stage of the Neanderthal evolution. The consequences of such a late bottleneck, later than when originally conceived by previously published evolutionary models, for our understanding of Neanderthal evolution were discussed in view of the evidence available from the fossil record.
Research sample	The sample included the microCT and CT scans of 32 individual pertaining to different groups customarily considered as belonging to the Neanderthal clade. More in detail, we included 13 specimens collected at the Sima de los Huesos Middle Pleistocene site; 10 individuals from the Krapina cave (late Middle Pleistocene); 9 late Neanderthals, with a broad geographical distribution (Europe and West Asia) and covering most of the Late Pleistocene (between 70000 to 40000 years before present). The morphology of these individuals was compared to that of a sample composed by modern humans (2 fossil specimens and 18 extant humans) with a European distribution.
Sampling strategy	The inspected sample was conceived to constraint as much as possible the variation of Middle Pleistocene groups (i.e., Sima de los Huesos and Krapina) relative to late Neanderthals, so that the possible presence of a drop in the latter could be even more easily identified. The number of modern humans included in the analysis was balanced to be similar to that of the Neanderthal clade individuals.
Data collection	The 3D models of the specimens were obtained through segmentation of X-ray computed tomography or micro-computed tomography scans. All the scans of Sima de los Huesos individuals (as well as some modern human individuals) were performed by M.C.V., I.M., R.Q., and J.L.A. at the University of Burgos. Krapina CT and microCT scans were downloaded from Neanderthal Museum digital archive (https://archiv.neanderthal.de/data/), as well as some of the late Pleistocene Neanderthals. Scans of the remaining late Neanderthals and the fossil modern humans were provided by the National Museum of Natural History of Paris to A.U. All the models were segmented by A.U. (some of the multiple times) and verified by I.M. and M.C.V.
Timing and spatial scale	Given that all the investigated specimens were either dry crania/temporal bones or fossils, and that we limited our investigation to the bony labyrinth, the timing and spatial scale of the data collection is not affecting the analysis performed in the current study.

Data exclusions	No available data relevant for the study was excluded from the analysis.
Reproducibility	The methods employed in this work have been previously tested and variously published, and fully demonstrated to be reliable and replicable. All the raw shape data and code are provided too ensure repeatability. Replicates of segmentation and alignment were performed to exclude biases related with sample preparation.
Randomization	To ensure that the results were not dependent from sample size, disparity metrics were computed on the bootstrapped distributions of the groups. The effect of sample size on morphological variation measurements was further assessed by including a rarefaction of the groups to the minimum group size.
Blinding	No blinding was necessary in this study since it does not involve any clinical trial with participants and groups.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	All Sima de los Huesos individuals were recovered from cave infillings at the Sierra de Atapuerca paleoanthropological site (Burgos, Spain). Krapina specimens were recovered from cave infillings of the Krapina site (Krapina, Croatia). Late Neanderthal specimens have been recovered from several sites: La Ferrassie individuals come from cave sediments of the homonymous site found close to Savignac-de-Miremont (France); La Chapelle-aux-Saints individual was recovered at the Bouffia Bonneval cave at La Chapelle-aux-Saints (France); La Quina specimen was found at the namesake rock shelter (France); Amud specimens were recovered from the homonymous cave found in Upper Galilee (Israel); and the Kebara specimen was extracted from cave sediments at the Kebara cave in the Ramat HaNadiv preserve (Israel). Fossil Homo sapiens were excavated at the sites of Abri-Cro-Magnon and Laugerie-Basse (Les Eyzies-de-Tayac-Sireuil, France). Modern human individuals have a European provenance: specimens labeled CSJ have been recovered from the ossuary of the Cementerio San Jose (Burgos, Spain); the individual labeled Sep was excavated from an archeological site at Segovia (Spain); individuals labeled EMBR are included in the embryological collection of the University Paul Sabatier III (Toulouse, France); individuals labeled F were extracted from the publication by Wimmer et al. (2019), who performed analyses on anonymized Swiss individuals donated to the Institute of Anatomy, University of Bern (Switzerland).
Specimen deposition	Sima de los Huesos cranial material is curated at the Centro Mixto de Evolución y Comportamiento Humanos (Universidad Complutense de Madrid - Instituto de Salud Carlos III). Krapina specimens are curated at the Croatian Natural History Museum (Zagreb, Croatia). The specimens from La Ferrassie, La Chapelle-aux-Saints, La Quina, Abri-Cro-Magnon and Laugerie-Basse are housed at the Muséum national d'Histoire Naturelle (Paris, France). The Kebara and Amud individual are curated at the Tel Aviv University. Modern human individuals are curated at: University of Burgos, Spain (CSJ and Sep); the embryological collection of the University Paul Sabatier III Toulouse, France (EMBR); Institute of Anatomy, University of Bern, Switzerland (F).
Dating methods	We relied on well-established chronological data extracted from the literature. No new dates were published in the present study.
	☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.
Ethics oversight	No ethical approval was required for the present study as all the specimens were already available from previously published research articles.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A