DispatchDate: 22.08.2022 · ProofNo: 1875, p.1

nature ecology & evolution

ANALYSIS

https://doi.org/10.1038/s41559-022-01875-z

Check for updates

Merging morphological and genetic evidence to assess hybridization in Western Eurasian late Pleistocene hominins

K. Harvati[1,2] ✉ and R. R. Ackermann[2,3,4] ✉

Previous scientific consensus saw human evolution as defined by adaptive differences (behavioural and/or biological) and the emergence of *Homo sapiens* as the ultimate replacement of non-modern groups by a modern, adaptively more competitive group. However, recent research has shown that the process underlying our origins was considerably more complex. While archaeological and fossil evidence suggests that behavioural complexity may not be confined to the modern human lineage, recent palaeogenomic work shows that gene flow between distinct lineages (for example, Neanderthals, Denisovans, early *H. sapiens*) occurred repeatedly in the late Pleistocene, probably contributing elements to our genetic make-up that might have been crucial to our success as a diverse, adaptable species. Following these advances, the prevailing human origins model has shifted from one of near-complete replacement to a more nuanced view of partial replacement with considerable reticulation. Here we provide a brief introduction to the current genetic evidence for hybridization among hominins, its prevalence in, and effects on, comparative mammal groups, and especially how it manifests in the skull. We then explore the degree to which cranial variation seen in the fossil record of late Pleistocene hominins from Western Eurasia corresponds with our current genetic and comparative data. We are especially interested in understanding the degree to which skeletal data can reflect admixture. Our findings indicate some correspondence between these different lines of evidence, flag individual fossils as possibly admixed, and suggest that different cranial regions may preserve hybridization signals differentially. We urge further studies of the phenotype to expand our ability to detect the ways in which migration, interaction and genetic exchange have shaped the human past, beyond what is currently visible with the lens of ancient DNA.

Natural hybridization promotes evolutionary innovation, creating novel and diverse outcomes in subsequent generations, thereby providing a rich substrate on which selection can further act to shape evolutionary trajectories[1,2]. Since 2010, methodological advances allowing unprecedented, high-resolution insights into ancient genomes have provided increasing evidence for hybridization and resultant gene flow among late Pleistocene humans. Currently, indications for gene exchange include movement of genes from Neanderthals into early *Homo sapiens* (conventionally called 'early modern humans')[3–8], resulting in approximately 2–3% Neanderthal ancestry of non-African living modern humans[7]; as well as evidence that *H. sapiens* contributed to the Neanderthal gene pool as early as 150 to >200 thousand years ago (ka)[9,10]. Gene flow from Denisovans into the ancestors of modern Asian populations[11,12], from Neanderthals into Denisovans[13,14], and from some unknown hominin into Denisovans[13] has also been reported, and the genome of a first-generation descendant of a Neanderthal mother and a Denisovan father living ca. 90 ka was recently discovered[15]. Finally, genetic exchanges between ancient and recent lineages may have also occurred within Africa[9,16–21]. Taken together, these studies indicate that gene flow has been multidirectional, was much more common than previously appreciated by most (but see for example, ref. [22]), and may have been instrumental in structuring genetic diversity across our ancestral lineage over the last half a million years. Given the speed at which new discoveries and methodological breakthroughs are occurring, such as the retrieval of hominin DNA from cave sediments[14], our expectation is that such evidence will probably continue to accumulate in the future.

Gene flow among hominins has had variable effects, best documented over the last 100 K years. These include genetic evidence for some level of introgression affecting phenotypes in a beneficial manner, including those involved in immunity, spermatogenesis, adaptation to low-oxygen contexts, response to ultraviolet radiation and other traits[23–31] (but see ref. [32]). For example, Neanderthal genes affecting skin and hair phenotypes are retained in humans living today[27,31], suggesting that these genes might have been important in the dispersal and adaptation of people emerging from Africa and migrating into environments inhabited by Neanderthals. In other cases, gene exchange may have been detrimental. For example, the existence of chromosomal regions in living humans devoid of Neanderthal-derived alleles, such as the X-chromosome and genes related to testes and therefore reproduction[27,31], suggests that selection may have acted to purge these genes from descendants. Neanderthal alleles present in living people have also been associated with a range of phenotypes considered detrimental in modern (but not necessarily ancient) contexts, including depression, neurodevelopmental disorders, hypercoagulation, altered carbohydrate metabolism and addiction[27,29,33] (but see ref. [32]). A few recent studies suggest that Neanderthal-derived genetic variation also influences brain phenotypes[29,34,35] and susceptibility to infectious diseases[36,37].

Taken together, the genetic evidence so far indicates that gene flow played an important role in shaping the evolutionary fate of

[1]Paleoanthropology section, Senckenberg Centre for Human Evolution and Palaeoenvironment, Institute for Archaeological Sciences, Eberhard Karls Universität Tübingen, Tübingen, Germany. [2]DFG Centre for Advanced Studies 'Words, Bones, Genes, Tools', Eberhard Karls Universität Tübingen, Tübingen, Germany. [3]Human Evolution Research Institute, University of Cape Town, Cape Town, South Africa. [4]Department of Archaeology, University of Cape Town, Cape Town, South Africa. ✉e-mail: katerina.harvati@ifu.uni-tuebingen.de; becky.ackermann@uct.ac.za

our lineage[38], although its exact effects appear to vary considerably across time, population and environmental/geographical context.

Hybridization in extant primates and other mammals and its relevance to hominins

Although the genetic evidence for hybridization in hominins has shifted the prevailing narrative about human origins over the past decade, there was already a growing realization before this (for example, see refs. [39,40]) that its role may have been underappreciated based on an increasing understanding of its prevalence across other mammals, including primates. We now know that approximately 10% of animal species produce hybrids, and occasionally 'phylogenetic hotspots' occur in which hybridization rates in animals exceed those seen in plants[41,42]. Within mammals, hybridization occurs across a wide range of lineages, including (but not limited to) a number of large-bodied terrestrial groups such as bovids[43–45], bears[46,47], cats[48,49], canids[50–54] and primates (see below). These studies have provided compelling evidence that gene flow impacts the evolutionary trajectory of large-bodied mammals, acting as a particularly strong force for accelerating evolution in novel or changing environmental contexts[1,2], a scenario that resonates with the narrative of human origins.

Non-human primates are arguably the most relevant models for human evolution, and there is considerable evidence for hybridization in the wild within all the major lineages at both specific and intraspecific levels, including strepsirrhines[55,56], American monkeys[57–61] and Afro-Eurasian monkeys[62–66]. Among these, perhaps the best studied are baboons (genus *Papio)*, which have also repeatedly been put forth as models for human evolution[40,67]. The six recognized baboon species (or 'allotaxa'; see ref. [40]) have parapatric ranges, with natural hybridization recorded between the species that are most phylogenetically distant (*Papio ursinus* vs *P. cynocephalus*), morphologically distinct (*P. ursinus* vs Kinda baboons) and behaviourally different (*P. hamadryas* vs *P. anubis*)[68]. Like our own genus *Homo*, *Papio* is the evolutionary product of a radiation that began in non-forested regions of tropical Africa around 2 million years ago (Ma); both genera have inhabited similar regions in Africa and been subject to comparable climatic fluctuations.

Hybridization has also occurred among our closest primate relatives, the apes (Superfamily Hominoidea). It is well-documented among the small-bodied apes[69,70], and there are also genetic signatures of gene flow both among subspecies[71] and between species[72] of great apes. One percent of the central chimpanzee genome has been shown to derive from the bonobo[72], indicating two ancient hybridization events comparable to the admixture seen between *H. sapiens* and Neanderthals.

Hybridization can have a wide range of effects on anatomy, behaviour and speciation[73], but it is in its interplay with adaptation that its impact may be most powerful. However, the impact of adaptive introgression can differ even among closely related taxa. For example, in chimpanzees the regions of adaptive introgression are subspecies-specific (for example, regions involving male reproduction versus immune system)[74]. As a species-specific example, the region around the FOXP2 locus is devoid of introgression in humans[75,76], but not in either chimpanzees[77] or bonobos[78].

How does gene exchange manifest itself in skeletal morphology?

Genetic evidence for the effect of gene flow on the hominin skeleton remains limited, despite its importance in linking the genetic and fossil record, as well as potentially understanding the functional implications of skeletal variation. Recent studies suggest that Neanderthal-derived genetic variation influences shape variation in the crania and brains of Europeans living today[34,35]. In particular, Neanderthal ancestry was found to be associated with a more Neanderthal-like, elongated cranial and endocranial shape in these Europeans, including morphology of the occipital and parietal regions, as well as differences in brain morphology[34,35].

Genetic evidence aside, some researchers have proposed hybrid individuals in the human fossil record on the basis of their morphology. Such proposed hybrids include Lagar Velho 1[79], Mladeč 5 and 6 (ref. [80]), Cioclovina 1 (ref. [81]), Peștera cu Oase 1 (refs. [82,83]) and 2 (refs. [82,84]), Skhul IV and V[84], Vindija[85], Klasies River Mouth[86], Jebel Irhoud and Mugharet el 'Aliya in North Africa[86,87] and others[88–90]. However, these hypotheses have generally not been possible to test, and the hybrid status of these specimens has been disputed[91–93] or considered inconclusive[94,95]. This was mainly due to the lack of clear expectations about hybrid morphology that could be empirically applied to the fossil record (but see refs. [84,96–99]), but also because of the problem of equifinality, as phenotypes consistent with hybridization, especially 'intermediate' morphology, may also be produced through other processes, most importantly by the retention of primitive features. In the face of these shortcomings, admixed status has almost exclusively been recognized on the basis of genetic evidence (as discussed above). However, such evidence is limited in many respects. For example, the application of ancient DNA is constrained due to preservation issues, which can vary from site to site and specimen to specimen but become particularly severe as we move further back in time or into warmer climates. Additionally, knowledge derived from comparisons among extant genomes can only provide partial insight into the past, given the extinction of many ancient lineages. Therefore, evidence for hybridization present in the skeletal phenotype remains essential to the interpretation of the fossil record, as it can help us to locate such potential events in time and place, and particularly within lineages for which we do not have a genetic record.

The taxon for which most empirical evidence for the effects of hybridization on the skeleton is available is baboons. Studies of baboons have revealed visible perturbations in dental and sutural formation at high frequencies in early-generation inter-specific hybrids, as well as atypical expression of some dental traits[1,96,97], suggesting that hybridization breaks down the coordination of early development, although this does not appear to meaningfully affect fitness[84,97,100]. These results are consistent with what is seen in the skeletal anatomy of hybrids in other mammalian lineages, including ungulates[43], rodents[101], and most recently canids[102], although they manifest somewhat differently in each taxon. Hybrid baboons also have, on average, crania that are larger than an intermediate value between their parents[96,97,103], with some measurements that are extreme relative to both parents. The production of extreme hybrid phenotypes, or transgressive phenotypes, outside of the range of both parental taxa (in a negative or positive direction) is called transgressive segregation[104], and in the case of the mammals mentioned above could include atypical traits as well as extreme size/shape.

Large cranial size in hybrids (relative to either a parental midpoint value, or the mean of the largest parents) has also been identified in mice[98,105–110] (as well as tamarins[111]). Inter-subspecific mouse hybrids (F1s, F2s and backcrossed individuals) are typically as large as or larger than the larger parent taxon, with associated size-related shape changes[98,110]. They also tend to have cranial and mandibular shape variation that is somewhat intermediate to that of the parents, but more closely resembling the smaller parent (Fig. 1), with high levels of heterosis in certain features such as molar length[98,110]. Later generations (F2, B2) are more variable than first-generation hybrids, with backcrosses expectedly moving towards the shape of the parent taxon with which they are hybridizing. These patterns hold for crosses of taxa that hybridize in the wild but have low levels of gene flow and low hybrid fertility; for taxa that hybridize in the wild and produce successful offspring; and for taxa that are geographically separated in nature but nevertheless hybridize under laboratory conditions[98,110], making them robust across different

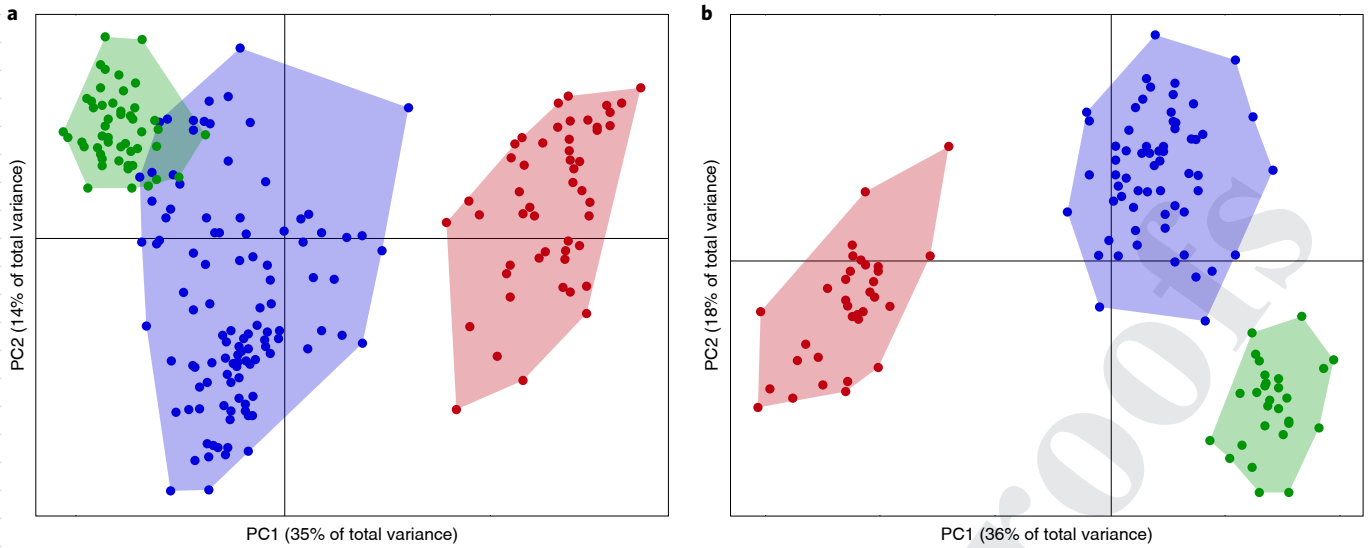

Fig. 1 | a,b, Principal components analyses of mouse crania (**a**) and mandibles (**b**) from laboratory bred mice. Images redrawn with permission from ref. [110] (Figs. 7.2.1 and 7.2.2). Red and green represent parent taxa *Mus musculus castaneus* and *M. m. musculus*, that hybridize successfully in the wild across a large hybrid zone in China and Japan. Blue represents a pooled sample of first (F1), second (F2) and unidirectionally (with *M. m. musculus*) backcrossed (B1) inter-subspecific hybrids.

scenarios of contact and hybrid fitness. Importantly, the fact that these various mammal models show similar patterns for hybridization across both species and subspecies provides a robust model for assessing its impact in taxa where the specific status is debated, such as Neanderthals and *H. sapiens* (see below).

The studies above have focused on skulls. Unfortunately, considerably less information exists on the effects of hybridization on the postcranial skeleton, outside of the observation from a number of previous studies in mice and primates that hybrids generally exhibit both longer limbs and increased body size relative to parents[84,98,105,106,109,112–114]. A new study of macaques suggests that effects of admixture on the pelvis may be relatively small, possibly due to functional or developmental constraints, or relatively minor divergence of the parent taxa (in this case, at or possibly below the subspecies level)[115].

Do late Pleistocene Western Eurasian humans fit the morphological predictions of a hybrid sample?

Although current genetic evidence indicates that hybridization occurred repeatedly among Pleistocene hominins, there have been few efforts to link this genetic evidence to morphological evidence from the fossil record itself, despite such a link being key for ascertaining the status and relevance of the bulk of the fossil record (for which genetic data are not available). This is further exacerbated by the fact that our ability to extrapolate from genotype to skeletal phenotype is currently very limited. While it is true that some individuals that show genetic evidence of admixture have limited morphology[15], making establishing these links difficult, this is not the case for other specimens. This lack of discourse between morphology and genetics is detrimental to understanding the dynamics of human evolution in the late Pleistocene.

Here we explore how insights derived from genetics and model organisms might be applied to the interpretation of the human fossil record. In particular, we examine the patterns of variation in cranial shape across the late middle to late Pleistocene, interpreted in conjunction with published genetic and non-metric phenotypic evidence for hybridization. The latter evidence consists of sutural and dental developmental anomalies comparable to what has been observed in comparative studies on hybridization and its effects on the phenotype. Even though admixture between hominin lineages has been demonstrated outside of Western Eurasian contexts, we focus specifically on Neanderthals and early *H. sapiens*, that is, hominins from Western Eurasia and Africa, to limit the scope of this inquiry. In recognizing the important limitations of species concepts and their application to the fossil record, as well as long-standing disagreements on Neanderthal alpha taxonomy, we avoid the term *Homo neanderthalensis*, while using *H. sapiens* to refer to extant humans and their ancestors in the late middle and late Pleistocene, following recent literature[116–118]. Consistent with current consensus, we consider these taxa to represent distinct lineages evolving in large part independently (see for example, ref. [116]) and be best viewed as anatomically distinctive but reproductively compatible 'allotaxa'[40]. We consider the following questions: (1) Do late Pleistocene Eurasian *H. sapiens* as a sample match the anatomical expectations, based on mammalian comparative data, for a Neanderthal-early *H. sapiens* hybrid population spanning multiple generations? (2) Among individuals for whom genetic data are available, does a higher level of Neanderthal ancestry co-occur with Neanderthal-like morphology or with developmental abnormalities? (3) How does hybrid status manifest itself in different aspects of cranial shape and size, and are these skeletal indicators useful predictors of admixture in samples where no genetic evidence is available?

Our analyses include late middle to late Pleistocene (roughly MIS 7-2) Neanderthal and *H. sapiens* specimens from Europe, Africa and the Middle East (Extended Data Table 1 and Fig. 2). We consider the Neanderthal sample as one of the 'parental' (unhybridized) populations. Because of the poor representation of penecontemporaneous African early *H. sapiens*, a pooled sample of ancient and recent sub-Saharan Africans, expected to have no or minimal Neanderthal ancestry (see ref. [9]), referred to as 'African *H. sapiens*', is used as a proxy for early *H. sapiens* anatomy and as the second 'parental' population. Our analyses included a few specimens with uncertain attribution or incomplete morphology (Omo 2, Eliye Springs, Apidima 1); these were not assigned to a group and were labelled and discussed separately. All other individuals are referred to as Eurasian *H. sapiens*. As such, they are potentially admixed and are the primary focus of this study. Three datasets

(hemimandible, posterior cranial profile, face) designed to capture typical Neanderthal/*H. sapiens* morphology routinely used for taxonomic identification[119] were investigated using principal components analysis (PCA). A shape index was developed by calculating an axis between the mean Neanderthal and mean African *H. sapiens* shapes and projecting all Eurasian *H. sapiens* onto it[120]. For the Eurasian *H. sapiens* sample, we also compiled data on non-metric skeletal abnormalities and percentage Neanderthal ancestry, where known from the literature, and integrated them in our plots. Results are presented in Figs. 3–5.

Empirical research on hybridizing mammalian taxa predicts that an admixed sample should contain: (1) individuals with 'mixed' or intermediate morphologies, (2) individuals with developmentally atypical traits and/or (3) individuals that are transgressive in shape or size relative to the parental taxa[1], resulting in hybrid populations that are more diverse than parental groups. In assessing potential hybridization between early *H. sapiens* and Neanderthals, however, we must keep in mind some important differences from studies on model organisms, which focus primarily on first- or early-generation hybrids. Neanderthal to early *H. sapiens* introgression occurred at low levels and asymmetrically, and our Eurasian *H. sapiens* sample certainly comprises mostly later-generation hybrids. Therefore, not all specimens in this sample are expected to be admixed, and those that are will probably have substantially greater African than Neanderthal ancestry components. Furthermore, our analyses focus on specific aspects of skull anatomy and therefore differ from model organism studies that generally examine size/shape of overall cranial morphology or key non-metric traits. Crucially, an important complicating factor is equifinality, that is, that similar morphologies can result from different processes, and that some of the predictions outlined above for admixture may also result from other evolutionary processes. These potentially include the retention of primitive features, or convergence due to selection for specific phenotypes under particular environmental conditions.

Results

In the hemimandible analysis, Eurasian *H. sapiens* broadly conform to our expectations for a hybridized sample. They occupy shape space intermediate to Neanderthals and African *H. sapiens* in the PCA plot (PC1-2, 44.9% of total variance; Fig. 3). This is a similar pattern to that observed in mouse subspecific hybrids relative to parental lineages (Fig. 1). However, although broadly intermediate, the Eurasian *H. sapiens* sample falls largely outside the convex hulls of either Neanderthals or African *H. sapiens*, with several of these transgressive specimens also plotting outside the 95% confidence ellipses of either 'parental' sample. This includes all individuals with genetic or morphological signatures of hybridization. The Eurasian *H. sapiens* sample is also partly intermediate, although closer to the African *H. sapiens* in centroid size and in the shape index. However, neither the percentage of Neanderthal genetic ancestry, where known, nor the incidence of developmental abnormalities appear to follow a clear relationship with Neanderthal-like morphology or with the shape index values. A case in point is the Oase 1 mandible. This individual is known to have approximately 10% Neanderthal ancestry—equivalent to a Neanderthal ancestor four to six generations previously[4,121]—and is the earliest generation Neanderthal-modern human hybrid currently known. Oase 1 shows very large overall size (one of the two largest *H. sapiens* mandibles in centroid size in our sample) and megadont lower third molars[83,122], consistent with its hybrid status. Yet its mandibular shape index value is less Neanderthal-like than other specimens with known smaller Neanderthal genetic components (Fig. 3). Indeed, Muerii 1 (although there is no genome data available on Muierii 1, it may represent the same individual as Muierii 2 with 5.2% Neanderthal ancestry[4]), Oberkassel 2 and Dolní Věstonice 16 fall closest to Neanderthals in the mandibular shape index.

A similar pattern is shown by the posterior cranial profile analysis PCA plot (PC1-2, 81.2% of total variance; Fig. 4). Although the separation between the African *H. sapiens* and Neanderthal convex hulls is smaller than in the hemimandible analysis (largely due to the position of Omo 1), overlap in the 95% confidence ellipses of the two 'parental' taxa is similarly limited. The Eurasian *H. sapiens* sample is again intermediate between Neanderthals and African *H. sapiens*, but here it shows much more overlap with both 'parental' convex hulls and 95% confidence ellipses, and especially with the African sample, indicating that a large proportion of these Eurasian specimens display *H. sapiens*-like shape, while some are more Neanderthal-like (and some intermediate).

This dataset essentially investigates a single, albeit very important, feature—the outline of the posterior part of the cranium in lateral view. A rounded cranium is considered a derived feature for modern humans, and recent work has linked a relatively reduced globularity of the parietal and occipital bones in modern Europeans to Neanderthal genetic ancestry and even to the presence of specific Neanderthal alleles[35]. Our shape index of the posterior cranial profile, encompassing the midsagittal outline of the parietal region and the upper occipital, might reasonably be considered as a proxy for an important aspect of the 'globularization' index calculated in ref. [35]. The overall observed pattern of separation between our Neanderthal and African *H. sapiens* samples is consistent with that described in ref. [35], except for Omo 2 and to a lesser extent, Omo 1. These specimens differ from all other Africans in that they plot within the Neanderthal convex hull (Omo 2) or within the region of overlap of the African *H. sapiens* and Neanderthal 95% confidence ellipses (Omo 1). Omo 2, the only African specimen overlapping with Neanderthals and showing a Neanderthal-like shape index, may represent an archaic lineage rather than early *H. sapiens* (see for example, ref. [116]). Alternatively, the high PC1 score of Omo 2 and, to a lesser extent, Omo 1 may indicate high levels of variation and population structure in early *H. sapiens*, as has been argued previously[123,124]. The remaining early African *H. sapiens* or possible *H. sapiens*, including LH18, Eliye Springs and Aduma 3, plot with the African sample; all but Aduma 3 overlap with the Eurasian *H. sapiens* range. In contrast to the African samples, multiple Eurasian *H. sapiens* specimens fall outside the African *H. sapiens* convex hull and confidence ellipse: of those, Qafzeh 6, Pavlov 1 and Mladeč 5 plot within the Neandertal convex hull, while Cro Magnon 1, Cro Magnon 3, Abri Pataud 1, Mladeč 1 and Predmost 3 fall within the Neanderthal 95% confidence ellipse. Several more (Chancelade, Cioclovina, Cro Magnon 2, Dolní Věstonice 16) plot in the region of overlap of the two 'parental' confidence ellipses. Many also show Neanderthal-like shape indices. Some of these individuals have previously been described as possessing occipital 'hemibuns', posterior projections of the occipital bone reminiscent of those shown by Neanderthals, possibly due to Neanderthal ancestry. Unfortunately, no genomic evidence is available to test this possibility further.

Elongated cranial profiles in fossil *H. sapiens* might also result from the retention of ancestral morphology represented here by Omo 1 and possibly Omo 2. However, the Omo specimens greatly predate both the Levantine and the European Upper Palaeolithic samples by ca. 60–90 kyr and >160 kyr, respectively, making recent admixture with Neanderthals a more probable explanation for the observed variation in the Upper Palaeolithic, and perhaps also the Near Eastern sample—a possibility that requires further investigation. On the other hand, Oase 2, which exhibits upper third molar megadontia[83,122] and has also recently been found to have relatively elevated Neanderthal admixture (6.06%, ref. [125]), plots in the centre of the African convex hull in the PCA plot and shows a modern human-like shape index. So does Dolní Věstonice 15, which shows 4.3% Neanderthal ancestry[4] as well as a conical mandibular supernumerary tooth in the region of the left canine root and rotation of the left mandibular premolar[122]; the supernumerary tooth in particular

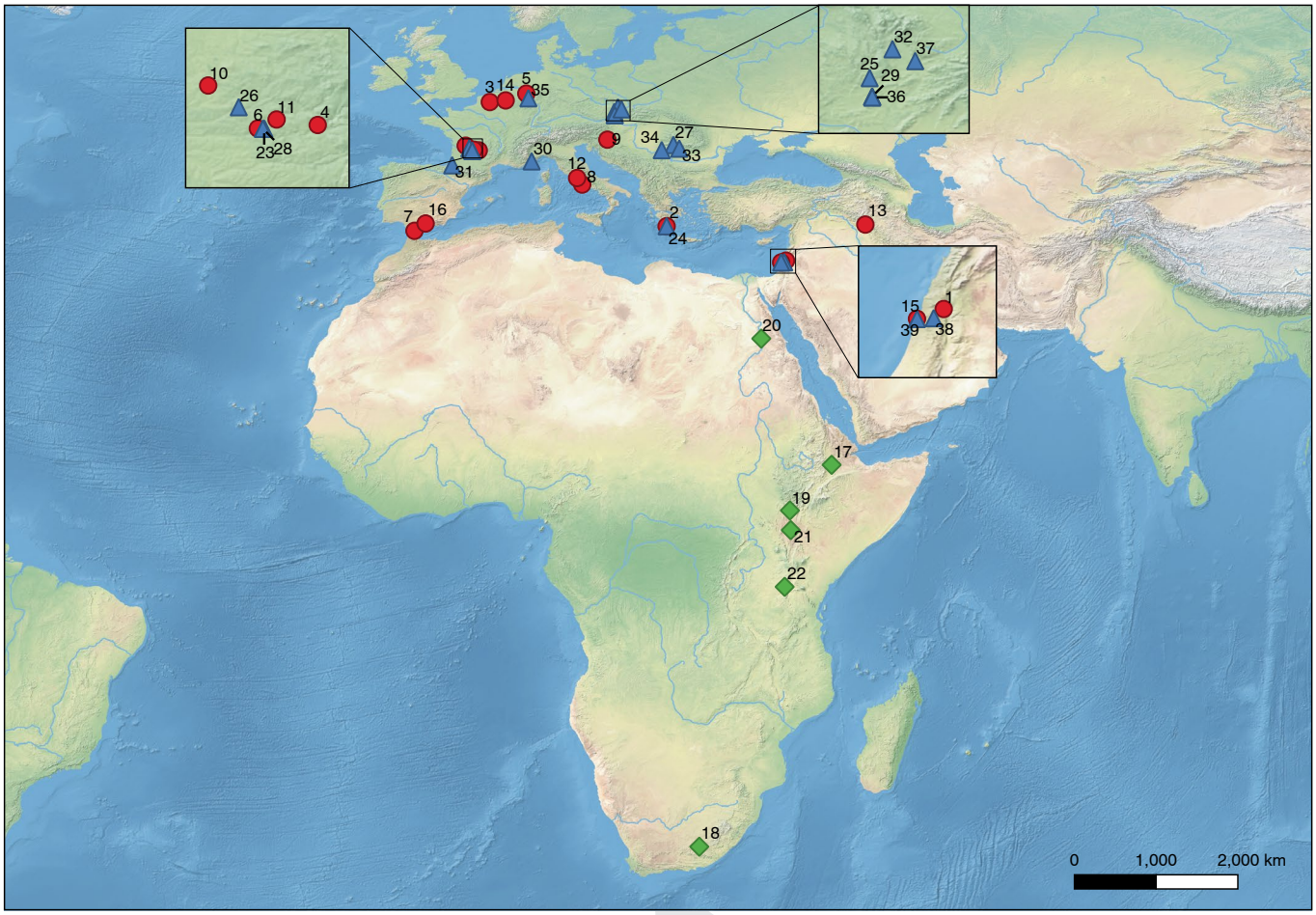

Fig. 2 | Localities for Pleistocene fossil hominin specimens used in the analyses. The Near East and potentially the general Eastern Mediterranean region and Eastern Europe have been proposed as contact areas between Neanderthals and Pleistocene *H. sapiens*. The map was produced using QGIS (https://www.qgis.org) and Natural Earth (http://naturalearthdata.com/).

might be interpreted as possibly resulting from admixture (although being a more common form of supernumerary tooth, it is not strong evidence). Furthermore, several additional specimens with known, relatively low Neanderthal genetic components (Fig. 4) have shape index values within the range of African *H. sapiens*. Finally, the proposed early *H. sapiens* Apidima 1 specimen plots with African *H. sapiens* in both the PCA and shape index plots but is characterized by a smaller centroid size, consistent with retention of ancestral morphology as well as with possible admixture.

The facial dataset shows yet a different pattern in the PCA plot (PC1-2, 39.5% of total variance; Fig. 5). Here the African *H. sapiens* sample (except for the late Pleistocene specimen Hofmeyr) falls within the more dispersed shape space of Eurasian *H. sapiens*, with its 95% confidence ellipse completely nested within that of the Eurasian *H. sapiens* sample. Both plot away from the tightly clustering Neanderthals. The Eurasian *H. sapiens* sample is considerably more variable in shape, as reflected in their more widely diverging PC1 and 2 scores, with most specimens, including all individuals with known Neanderthal genetic components, falling outside of the African *H. sapiens* convex hull or even confidence ellipse (that is, transgressive relative to African *H. sapiens*). The early modern humans from the Near East (Qafzeh 6 and 9) plot in more intermediate positions in the PCA plot and also have intermediate facial shape indices, although still clearly away from the Neanderthal range/confidence ellipse. The greater variability and transgressive/intermediate shape of many individuals in this sample relative to the

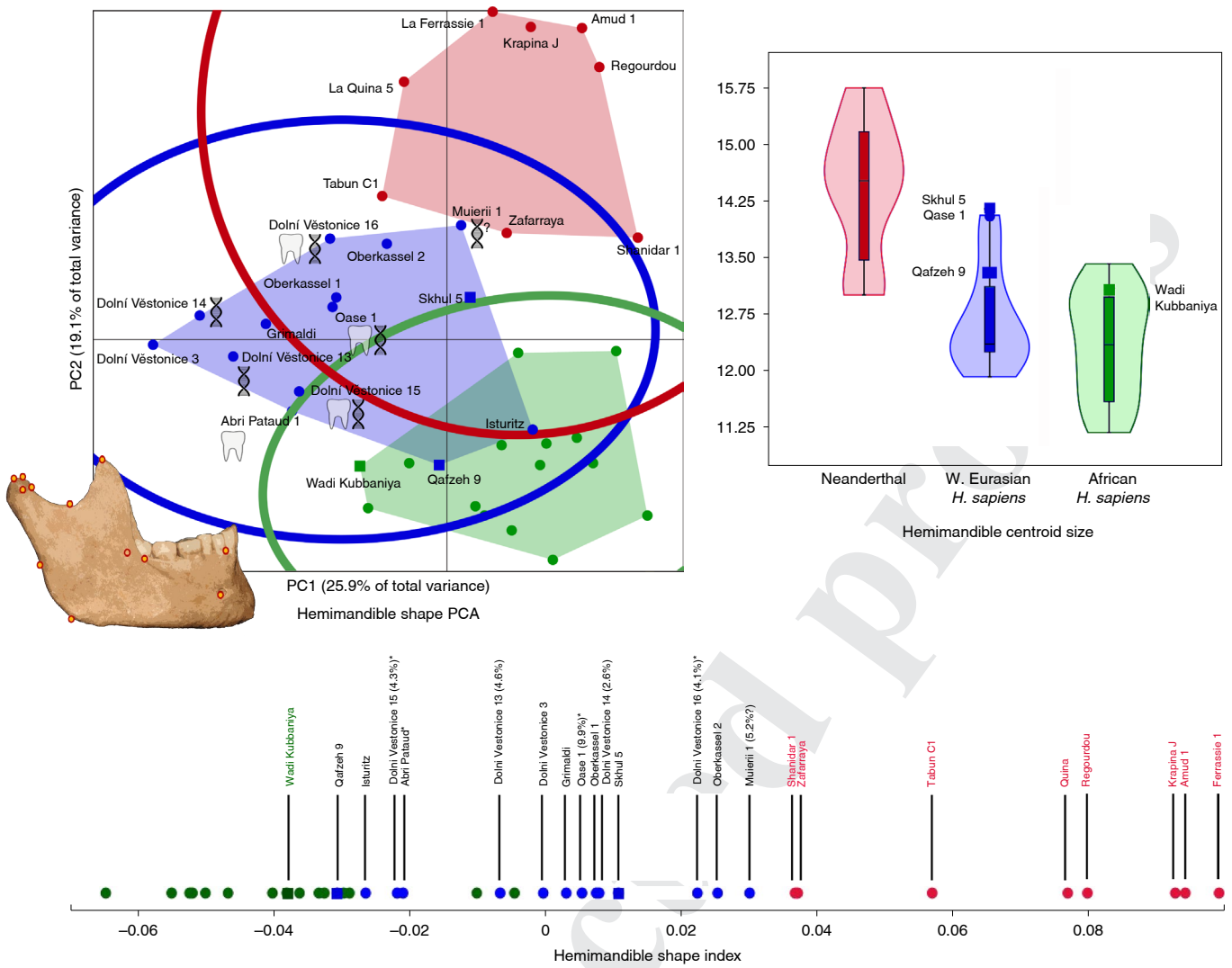

Fig. 3 | Hemimandible analysis. Top left: PCA, landmark dataset shown on modern human mandible model. Convex hulls and 95% confidence ellipses are plotted. Top right: violin plot of centroid size by group, minimum to maximum values; superimposed boxplot shows median and 25–75% quartiles ($n=8$ Neanderthals; $n=12$ Eurasian Upper Palaeolithic *H. sapiens*; $n=14$ recent African *H. sapiens*; earlier specimens also plotted). Bottom: shape index. Red, Neanderthals; green, African recent (filled circles) and Pleistocene *H. sapiens* (filled squares); blue, Eurasian Upper Palaeolithic (filled circles) and early late Pleistocene *H. sapiens* (filled squares). Individuals with genetic evidence for hybridization are marked with a DNA symbol in the PCA plot, with % Neanderthal ancestry given in the shape index. Individuals with atypical dental or sutural variation as reviewed in ref. [122] are marked with a tooth symbol in the PCA plot and with an (*) in the shape index. Tooth and DNA symbols are freely available at https://freesvg.org/pages/about-us. Photo credit for skeletal image: K.H.

'parent' taxa is reflected in its wider confidence ellipse, which overlaps somewhat with the Neanderthal one, even though no *H. sapiens* plot in the region of overlap of the ellipses. Such increased variability is consistent with an admixed sample, but could also result from sampling bias, a greater temporal variability in our Eurasian *H. sapiens* sample, or from within-species geographic variation, although similar temporal and geographic variation did not lead to this pattern in the other analyses.

Similarly, the facial shape index values of the African *H. sapiens* specimens fall within the Eurasian *H. sapiens* range and away from those of Neanderthals. Again, there is no relationship between the facial shape index and the percentage of Neanderthal ancestry in the specimens for which the latter is known (Fig. 5). The two *H. sapiens* samples also show roughly equivalent centroid sizes.

Discussion

We did not approach this study by asking whether hybridization was common in late Pleistocene Europe, although current evidence suggests that it may have been. Instead, we wanted to evaluate how admixture manifests in the skeleton and whether different lines of evidence, morphological as well as genetic, can help reveal the presence of admixture, making it possible to identify hominin hybrids on the basis of either (or both). However, our evaluation of the morphology of late Pleistocene Eurasian *H. sapiens* against predictions based on model organisms is based on small and imperfect samples (that is, poor representation of African early *H. sapiens*, few individuals with both genomic and morphological data available, and representation of primarily later- rather than early-generation hybrids). Recent African individuals are also imperfect models for early *H. sapiens* given that they have gone through their own process of evolution relative to the population for which we are using them as a proxy. Furthermore, the interpretation of the observed patterns is complicated by equifinality, as phenotypic variation consistent with admixture may also result from other processes, especially retention of ancestral features; and by sampling limitations that may underestimate the true variability of the groups included in our analyses. We

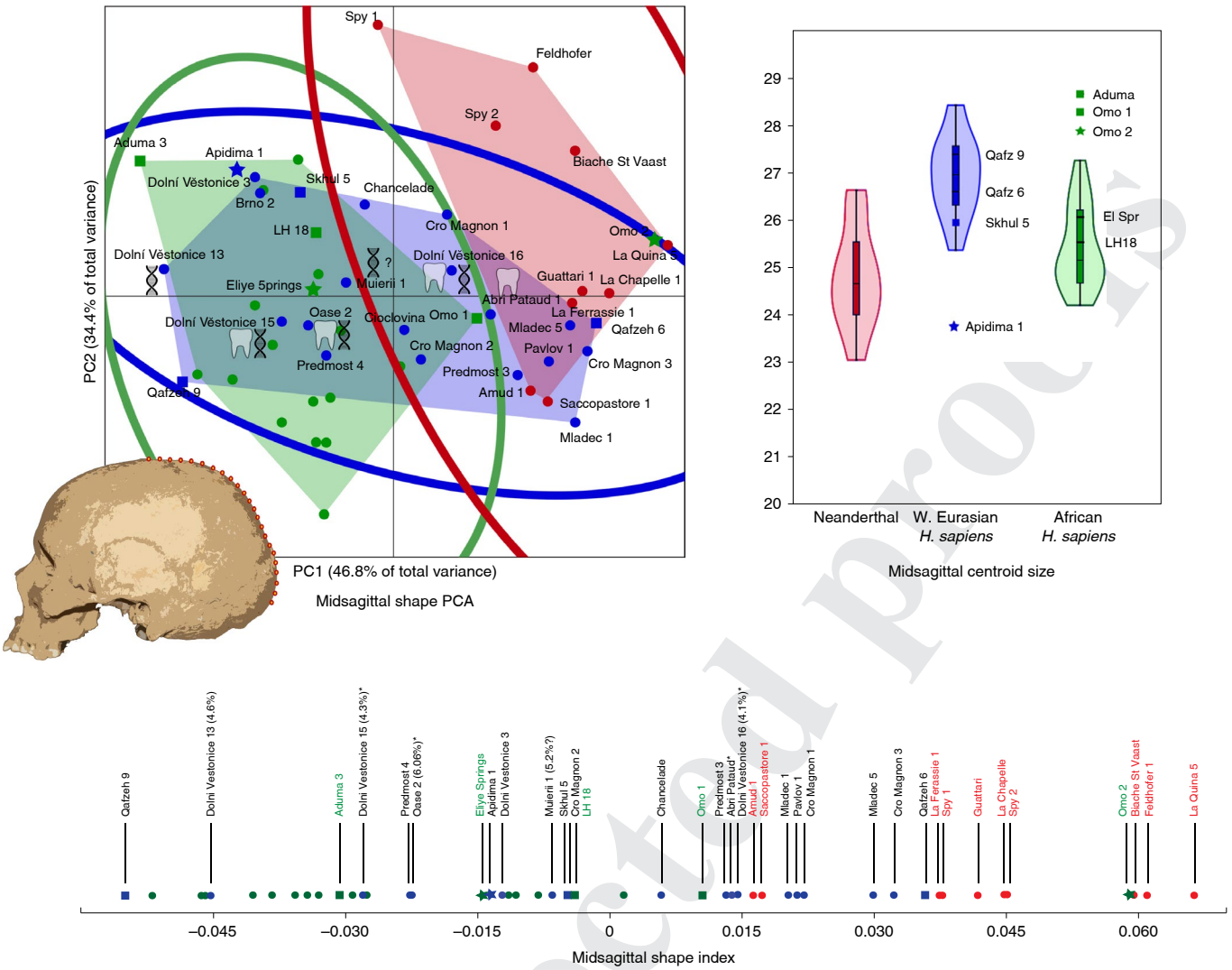

Fig. 4 | Posterior cranial (midsagittal) profile analysis. Top left: PCA, landmark/semilandmark dataset on modern human cranium model. Convex hulls and 95% confidence ellipses are plotted. Top right: violin plot of centroid size by group, minimum to maximum values; superimposed boxplot shows median and 25–75% quartiles ($n = 10$ Neanderthals; $n = 18$ Eurasian Upper Palaeolithic *H. sapiens*; $n = 15$ recent African *H. sapiens*; earlier specimens also plotted). Bottom: shape index. Colours and symbols as in Fig. 3; green/blue stars, specimens of uncertain affinities or incomplete morphology (Omo 2, Eliye Springs, Apidima 1). Individuals with genetic evidence for hybridization are marked with a DNA symbol in the PCA plot, with % Neanderthal ancestry given in the shape index. Individuals with atypical dental or sutural variation as reviewed in ref. [122] are marked with a tooth symbol in the PCA plot and with an (*) in the shape index. Tooth and DNA symbols are freely available at https://freesvg.org/pages/about-us. Photo credit for skeletal image: K.H.

therefore can provide only tentative and preliminary answers to the questions posed. These answers, nevertheless, can form the basis for future work exploring hybridization in the human fossil record.

To summarize, we explored whether our late Pleistocene Eurasian *H. sapiens* sample fits our predictions for a population with a history of hybridization. For our mandibular and posterior cranial datasets, we found that they were intermediate in shape and size between Neanderthals and African *H. sapiens*, with some individuals being transgressive in aspects of shape—patterns consistent with hybridization across the sample as a whole. Facial shape, on the other hand, did not provide a signal that clearly emulates what we see in comparative datasets (for example, baboons, mice), although the large variation in the Eurasian *H. sapiens* sample and high proportion of transgressive individuals outside of the African *H. sapiens* range is suggestive. It is unclear why different anatomical regions would demonstrate different patterns in the presence of hybridization, if indeed that is the signal being detected here. Facial and mandibular shape has been argued to be affected differentially by selection and by adaptive or plastic responses to external environmental factors (for example, ref. [126–128]). Facial morphology is also widely recognized as important in species recognition and social interactions among primates[129], and may therefore be under selective pressure to conform more closely to the backcrossing population. Finally, in all our analyses, late Pleistocene Eurasian *H. sapiens* as a sample was closer in shape to African *H. sapiens* than to Neanderthals, as expected under conditions of asymmetric gene flow (hypothesized for large differences in parental population sizes, as postulated for early European *H. sapiens* relative to late Neanderthals; for example, ref. [130]), or more importantly, for a sample comprising multiple later generations (that is, more backcrossed into modern humans) hybrids.

In terms of individual specimens, no direct relationship was found between estimated levels of Neanderthal ancestry based on genomic evidence where known, and anatomical shape/size, nor

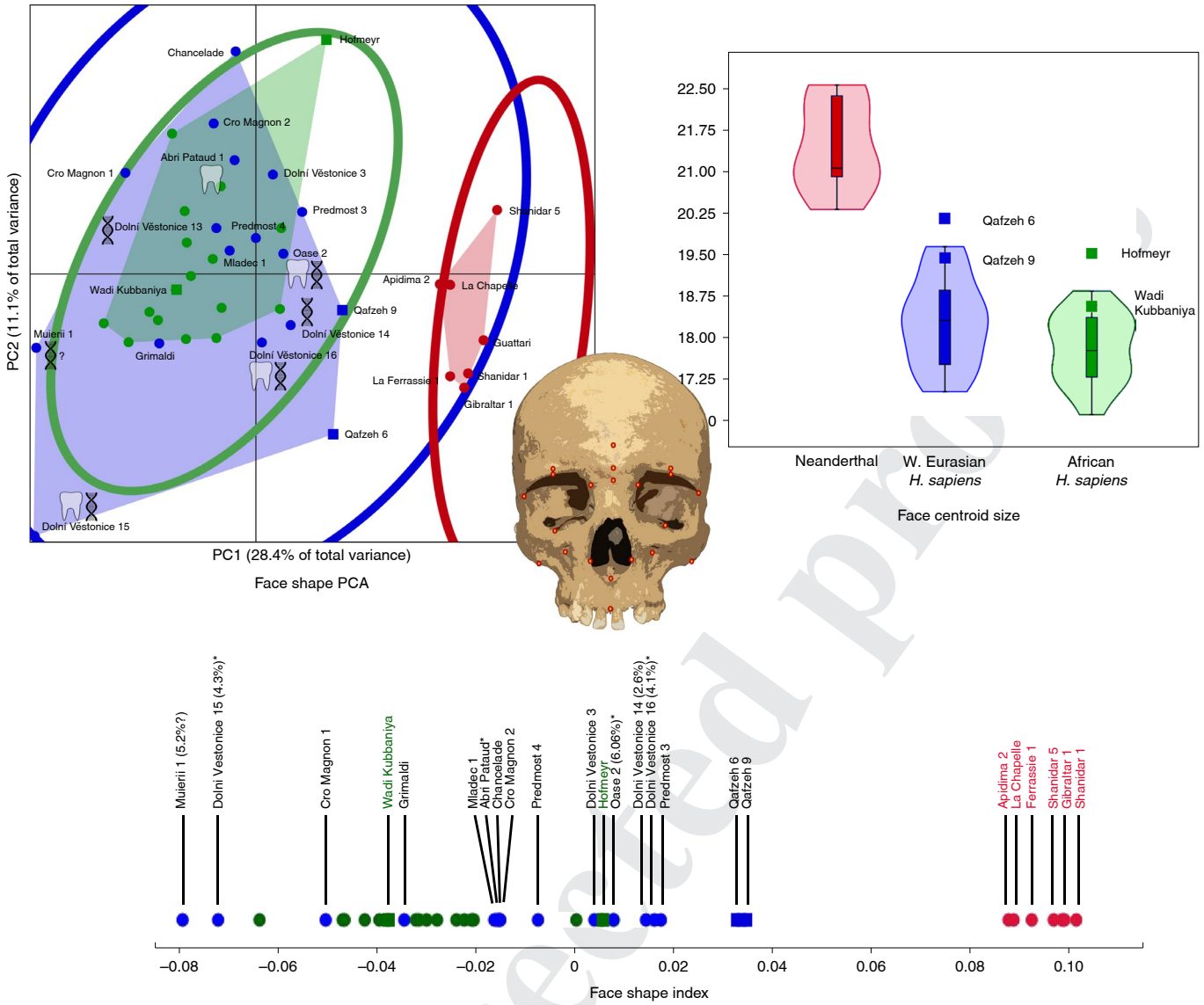

Fig. 5 | Face analysis. Top left: PCA, landmark dataset shown on modern human cranium model. Convex hulls and 95% confidence ellipses are plotted. Top right: violin plot of centroid size by group, minimum to maximum values; superimposed boxplot shows median and 25–75% quartiles ($n = 7$ Neanderthals; $n = 15$ Eurasian Upper Palaeolithic *H. sapiens*; $n = 15$ recent African *H. sapiens*; earlier specimens also plotted). Bottom: shape index. Colours and symbols as in Fig. 3. Individuals with genetic evidence for hybridization are marked with a DNA symbol in the PCA plot, with % Neanderthal ancestry given in the shape index. Individuals with atypical dental or sutural variation as reviewed in ref. [122] are marked with a tooth symbol in the PCA plot and with an (*) in the shape index. Tooth and DNA symbols are freely available at https://freesvg.org/pages/about-us. Photo credit for skeletal image: K.H.

between this genomic evidence and expression of developmentally abnormal dental or sutural features as reported in the literature. This was also the case in the early-generation Neanderthal-modern human hybrid, the mandible Oase 1, whose phenotypic signals of hybridization are limited to its very large overall size and megadontia. This result is perhaps not surprising, as estimated admixture percentages may vary across most specimens due to noise or sequencing depth. Furthermore, the critical factor for the expression of Neanderthal-like features is most probably the presence of particular alleles relevant to the expression of specific phenotypes, rather than overall percentages of Neanderthal ancestry (as has recently been argued for cranial globularity[35]). Assuming that cranio-mandibular morphology is at least in part under genetic control, the comparatively moderately elevated Neanderthal genetic component shown by, for example, Dolní Věstonice 16, may comprise alleles influencing development of the masticatory region and neurocranium, which resulted in shape similarities to Neanderthals reflected by this specimen's mandibular and midsagittal profile shape indices and PC scores (Figs. 3 and 4) and in the known misalignment of the maxillae along the intermaxillary suture[131], but not in marked facial similarities (Fig. 5).

Are these skeletal morphologies useful predictors of admixture in samples where no genetic evidence is available? At the moment, the patterns observed when considering a larger sample/population are the most informative. As regards individual specimens, the signals are often mixed, even across anatomical regions for the same individuals, possibly reflecting differential preservation of the hybridization signal according to anatomical region (see above). The state of preservation and degree of completeness of a fossil, therefore, may influence whether an admixture signal can be detected. This signal will probably further be influenced by the differential expression of Neanderthal-like or developmentally abnormal features

according to the presence of particular Neanderthal alleles or the degree and/or recency of ancestry. Nevertheless, some observations can be made. The individuals Qafzeh 6, Cro Magnon 3, Mladeč 5 and Pavlov 1 are the only ones across all our analyses that plot with Neanderthals in the PCA plot and show Neanderthal-like shape index values in one of our analyses—the posterior midsagittal cranial outline (the only analysis where these three Upper Palaeolithic individuals could be included). On this basis, we may hypothesize that they have a Neanderthal genetic component comprising alleles important for cranial shape. In terms of the Qafzeh specimens, Qafzeh 9 plots completely opposite from Qafzeh 6 in the posterior midsagittal cranial outline analysis, underlining a very high variability in this morphology within a single site. The Qafzeh specimens are also the only ones that show a somewhat intermediate position in the facial analysis. These results, together with the high levels of variation in one site and the geographic origin in the Levant (a postulated contact area between Neanderthals and modern humans[5]), raise the possibility that the Qafzeh individuals may have some Neanderthal ancestry[84]. Even though such indications are intriguing, they cannot be considered conclusive and must be treated as hypotheses, especially since similar phenotypes might be consistent with different underlying causes as mentioned above. Nevertheless, it is possible to evaluate the likelihood of such alternative explanations on a case-by-case basis. For example, because a rounded cranium is a derived *H. sapiens* feature, an alternative hypothesis for a relatively elongated cranial phenotype could be that it results from retention of the ancestral elongated condition. An ancestral retention, however, is more convincing for Qafzeh, which represents an early *H. sapiens* population dating to ca. 100–130 ka (Extended Data Table 1), than for the Upper Palaeolithic Europeans Cro Magnon 3, Mladeč 5 and Pavlov 1, which greatly postdate the establishment of the derived condition[117,132].

Finally, recent suggestions that skeletal anomalies in some Upper Palaeolithic and Neanderthal samples result from inbreeding[122,133] may further complicate the interpretation of developmental abnormalities as indicators of admixture. Indeed, both processes are expected to have taken place in the highly dynamic conditions of cyclical environmental change of Pleistocene Eurasia, which probably resulted in repeated isolation of populations in refugia areas, sometimes leading to local extinctions but also to population expansion and dispersals[134]. Under these conditions, palaeodemes have been proposed to resemble 'tidal islands', often isolated but occasionally flooded with expanding/dispersing populations and their genetic material[134]. However, although empirical evidence from primates for the skeletal expression of inbreeding is limited, the evidence that does exist suggests that it is associated with abnormalities (for example, reduced size, anencephaly, polydactyly, syndactyly, limb malformations[135–139]) that are different from those shown to occur in hybrids (for example, increased size, extremely rare dental and sutural traits with no other associated diseases or syndromes[1,84,96,97]). This indicates that, in the future, it should be possible to distinguish between these causal phenomena and their relative contributions to the morphology we see in the fossil record.

This study compared genomic and morphological datasets to interrogate the fossil evidence for late Pleistocene hybridization between Neanderthals and early *H. sapiens*, for which we currently have substantial evidence. We urge further studies of the phenotype to expand our ability to detect the ways in which migration, interaction and genetic exchange have shaped the human past, beyond what is currently visible with the lens of ancient DNA. It is particularly important to examine such datasets together to understand the effects of hybridization on the morphology of later-generation hybrids, and whether these effects vary by anatomical region. The results provided here should form the basis for developing hypotheses to be tested against the human fossil record in the future.

Methods

Our sample comprised late middle and late Pleistocene (roughly corresponding to MIS 7-2) fossil human specimens from Europe, Africa and the Middle East assigned to Neanderthals and *H. sapiens* (Extended Data Table 1 and Fig. 2), including but not limited to individuals that are genetically known and morphologically proposed hybrids. We chose an upper age limit of MIS 7 because the suites of diagnostic morphological features of both Neanderthals and modern humans were largely established by this time (see for example, refs. [116,140]). To frame our study in a manner that is consistent with studies from model organisms (see for example, Fig. 1; refs. [96–98,110]), the Neanderthal portion of this sample was considered as representative of one of the 'parental' (unhybridized) populations. We could not rule out *H. sapiens* ancestry in individual Neanderthals, although so far, evidence for introgression of *H. sapiens* genes into Neanderthals is more limited than the reverse. For the second parental population, because of the poor representation of penecontemporaneous African early *H. sapiens* in our dataset and in the fossil record generally, we considered a pooled sample of ancient and recent sub-Saharan Africans, expected to have no or minimal Neanderthal ancestry[9] as a proxy for early *H. sapiens* anatomy. The recent sub-Saharan African portion of the pooled *H. sapiens* sample was represented by three sex-pooled datasets of individuals from eastern and southern Africa (face: $n = 15$; hemimandible: $n = 14$; posterior cranial profile: $n = 15$) from the collections of the American Museum of Natural History, New York, and the University of the Witwatersrand, Johannesburg, which we refer to as African *H. sapiens*. We recognize that the inclusion of these small samples of extant sub-Saharan Africans is not ideal, given the potential effects of recent and ancient demographic processes, as well as the possibility of admixture in deeper time[9,16–21]. We hoped to mitigate such effects to the extent possible by combining the few available ancient individuals with our recent African samples and limiting the extant sample to sub-Saharan Africa, thereby reducing the likelihood of admixture from Neanderthals. We could not rule out the possibility that introgression from other non-Neanderthal 'ghost lineages' might be present in the African samples, or the late survival of archaic lineages not directly ancestral to *H. sapiens* in our ancient African samples. Specimens explicitly proposed as such possible hybrids (for example, the Iwo Eleru calvaria) were excluded from our analyses. The results presented largely position the ancient African samples within the same shape space as the modern ones (and distinct from Neanderthals), thus supporting these choices. All other fossils were considered together as Pleistocene (non-Neanderthal) Western Eurasians, which we refer to as Eurasian *H. sapiens*, and as such are potentially admixed, and the primary focus of this study. A few individuals with incomplete morphology or uncertain attribution (that is, Omo 2, Eliye Springs, Apidima 1) were also included in our analyses. These were not assigned to a group and are discussed separately.

We expect that the morphological datasets investigated would differentiate between Neanderthals and African *H. sapiens*, reflecting their commonly accepted status as distinct lineages. Further, we made a series of predictions aimed at determining whether the Eurasian *H. sapiens* sample shows patterns of variation consistent with hybridization between African *H. sapiens* and Neanderthals. Empirical research on hybridization in primates, mice and a handful of other mammals predicts that an admixed sample should contain: (1) individuals with 'mixed' or intermediate morphologies somewhere between their parental (un-admixed) taxa, (2) individuals with developmentally atypical traits not seen (or seen at extremely low frequency) in the parental taxa (especially dental or sutural anomalies) and/or (3) individuals that are transgressive in shape or size relative to the parental taxa[1]; these characteristics framed our morphological expectations. Taken together, they were expected to result in hybrid populations that are more diverse than parental groups. The consistency of the findings across taxa and generations within taxa[1,97] supported the use of this general pattern for determining hybrid status in the fossil record.

In assessing the question of hybridization between early *H. sapiens* and Neanderthals, some additional dynamics also needed to be taken into account. Introgression from Neanderthals into *H. sapiens* occurred at a low level, probably mediated by differences in population size (with *H. sapiens* being considerably larger[8,130]) as well as directionality of backcrossing and possibly reduced hybrid fitness[141–143]. Moreover, any sample is certain to be composed of multi-generational recombinants rather than first-generation hybrids. As a result, not all specimens in our Eurasian *H. sapiens* sample were expected to be admixed, and those that are admixed would probably represent individuals with substantially more African than Neanderthal ancestry components. Indeed, all Upper Palaeolithic Eurasian specimens for which genetic information is available showed evidence of Neanderthal admixture at least as great as that observed in modern non-Africans, but specimens with recent Neanderthal ancestry were rare[6,125]. This differs from the studies of model organisms which focus primarily on early-generation hybrids. Furthermore, our analyses focused on specific aspects of skull anatomy (mandibular, neurocranial, facial) to maximize samples (see below), and therefore our datasets do not exactly replicate the model organism studies that generally examine size/shape of overall cranial morphology (in addition to key non-metric traits). As a result of these factors, we expected to find substantial overlap between the African *H. sapiens* 'parental' population and a Eurasian admixed sample, with some individuals plotting as expected for hybrids, that is, intermediate, atypical, or transgressive. Alternatively, if no admixture occurred, or if such admixture did not manifest on the aspects of cranial morphology investigated here, the Eurasian

H. sapiens sample would be expected to largely conform to the patterns shown by the African *H. sapiens* 'parental' population. However, it must be stressed again that an important complicating factor in these assessments is the problem of equifinality, that is, that similar morphologies can result from different processes. Some of the predictions outlined above for admixture may also apply to other evolutionary processes, such as the retention of primitive features, or selection for specific phenotypes under particular environmental conditions leading to convergence. The results presented here must therefore be interpreted with caution.

Due to the fragmentary nature of the fossil record, individuals are generally not fully preserved and different individuals are often represented by different parts of the skeleton. To include as many fossils as possible, we evaluated three anatomical regions: the hemimandible, the posterior cranial profile (midsagittal profile) and the face. Data were collected previously by K.H. (the hemimandible dataset was collected jointly by K.H. and E. Lopez; see ref. [144]). They consisted of three-dimensional landmarks and semilandmarks, processed with Procrustes superimposition and semilandmark sliding (in the case of the posterior cranial profile), and analysed using PCA. The datasets were specifically designed to capture salient morphological features that are widely considered Neanderthal or *H. sapiens*-derived traits in the respective anatomical regions and are routinely used for taxonomic identification[144–146]. However, they may be affected differentially by different evolutionary processes. For example, facial and mandibular traits may be influenced by selection resulting from environmental factors, such as climate or diet[126–128], with facial morphology also possibly affected by stabilizing selection due to its importance in species recognition[129]. In contrast, neurocranial shape is proposed to track neutral evolutionary changes and population history more closely[126], and has been linked to Neanderthal genetic ancestry in modern Europeans[35]. Our mandibular and facial datasets, therefore, may be expected to reflect a hybridization signal less clearly than our midsagittal profile dataset. For each dataset, we also developed a shape index by calculating an axis between the mean Neanderthal and mean African *H. sapiens* shapes and projecting all Eurasian *H. sapiens* onto it[35,117]. Finally, data indicating the presence of non-metric skeletal abnormalities and genetic information on % Neanderthal ancestry were compiled from the literature, where available, and integrated in our figures and discussion.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data supporting the findings of this study are available in the Zenodo open source online repository at https://doi.org/10.5281/zenodo.6846628.

Received: 24 April 2019; Accepted: 8 August 2022;

References

1. Ackermann, R. et al. Hybridization in human evolution: insights from other organisms. *Evol. Anthropol.* **28**, 189–209 (2019).
2. Taylor, S. A. & Larson, E. L. Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. *Nat. Ecol. Evol.* **3**, 170–177 (2019).
3. Fu, Q. et al. Genome sequence of a 45,000-year-old modern human from western Siberia. *Nature* **514**, 445–449 (2014).
4. Fu, Q. et al. The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).
5. Green, R. E. et al. A draft sequence of the Neandertal genome. *Science* **328**, 710–722 (2010).
6. Hajdinjak, M. et al. Initial Upper Palaeolithic humans in Europe had recent Neanderthal ancestry. *Nature* **592**, 253–257 (2021).
7. Prüfer, K. et al. A genome sequence from a modern human skull over 45,000 years old from Zlatý kůň in Czechia. *Nat. Ecol. Evol.* **5**, 820–825 (2021).
8. Villanea, F. A. & Schraiber, J. G. Multiple episodes of interbreeding between Neanderthal and modern humans. *Nat. Ecol. Evol.* **3**, 39–44 (2019).
9. Chen, L., Wolf, A. B., Fu, W., Li, L. & Akey, J. M. Identifying and interpreting apparent Neanderthal ancestry in African individuals. *Cell* **180**, 677–687.e16 (2020).
10. Posth, C. et al. Deeply divergent archaic mitochondrial genome provides lower time boundary for African gene flow into Neanderthals. *Nat. Commun.* **8**, 16046 (2017).
11. Meyer, M. et al. A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222–226 (2012).
12. Reich, D. et al. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* **468**, 1053–1060 (2010).
13. Prufer, K. et al. The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43–49 (2014).
14. Slon, V. et al. Neandertal and Denisovan DNA from Pleistocene sediments. *Science* https://doi.org/10.1126/science.aam9695 (2017).
15. Slon, V. et al. The genome of the offspring of a Neanderthal mother and a Denisovan father. *Nature* **561**, 113–116 (2018).
16. Durvasula, A. & Sankararaman, S. Recovering signals of ghost archaic introgression in African populations. *Sci. Adv.* **6**, eaax5097 (2020).
17. Hammer, M. F., Woerner, A. E., Mendez, F. L., Watkins, J. C. & Wall, J. D. Genetic evidence for archaic admixture in Africa. *Proc. Natl Acad. Sci. USA* **108**, 15123–15128 (2011).
18. Harvati, K. et al. The later stone age Calvaria from Iwo Eleru, Nigeria: morphology and chronology. *PLoS ONE* **6**, e24024 (2011).
19. Lachance, J. et al. Evolutionary history and adaptation from high-coverage whole-genome sequences of diverse African hunter-gatherers. *Cell* **150**, 457–469 (2012).
20. Lipson, M. et al. Ancient West African foragers in the context of African population history. *Nature* **577**, 665–670 (2020).
21. Wang, K., Mathieson, I., O'Connell, J. & Schiffels, S. Tracking human population structure through time from whole genome sequences. *PLoS Genet.* **16**, e1008552 (2020).
22. Smith, F. H., Ahern, J. C. M., Janković, I. & Karavanić, I. The Assimilation Model of modern human origins in light of current genetic and genomic knowledge. *Quat. Int.* **450**, 126–136 (2017).
23. Dannemann, M., Andrés, A. M. & Kelso, J. Adaptive variation in human toll-like receptors is contributed by introgression from both Neandertals and Denisovans. (2015). Q11
24. Hu, Y., Ding, Q., He, Y., Xu, S. & Jin, L. Reintroduction of a homocysteine level-associated allele into East asians by Neanderthal introgression. *Mol. Biol. Evol.* **32**, 3108–3113 (2015).
25. Huerta-Sánchez, E. et al. Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature* **512**, 194–197 (2014).
26. Lin, Y. L., Pavlidis, P., Karakoc, E., Ajay, J. & Gokcumen, O. The evolution and functional impact of human deletion variants shared with archaic hominin genomes. *Mol. Biol. Evol.* **32**, 1008–1019 (2015).
27. Sankararaman, S. et al. The genomic landscape of Neanderthal ancestry in present-day humans. *Nature* **507**, 354–357 (2014).
28. Schlebusch, C. M. et al. Genomic variation in seven Khoe-San groups reveals adaptation and complex African history. *Science* **338**, 374–379 (2012).
29. Simonti, C. N. et al. The phenotypic legacy of admixture between modern humans and Neandertals. *Science* **351**, 737–741 (2016).
30. Sudmant, P. H. et al. Global diversity, population stratification, and selection of human copy-number variation. *Science* **349**, aab3761 (2015).
31. Vernot, B. & Akey, J. M. Resurrecting surviving Neandertal lineages from modern human genomes. *Science* **343**, 1017–1021 (2014).
32. Skov, L. et al. The nature of Neanderthal introgression revealed by 27,566 Icelandic genomes. *Nature* https://doi.org/10.1038/s41586-020-2225-9 (2020).
33. Mozzi, A. et al. Distinct selective forces and Neanderthal introgression shaped genetic diversity at genes involved in neurodevelopmental disorders. *Sci. Rep.* **7**, 6116 (2017).
34. Gregory, M. D. et al. Neanderthal-derived genetic variation shapes modern human cranium and brain. *Sci. Rep.* **7**, 6308 (2017).
35. Gunz, P. et al. Neandertal introgression sheds light on modern human endocranial globularity. *Curr. Biol.* **29**, 120–127.e5 (2019).
36. Zeberg, H. & Pääbo, S. The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. *Nature* **587**, 610–612 (2020).
37. Zeberg, H. & Pääbo, S. A genomic region associated with protection against severe COVID-19 is inherited from Neandertals. *Proc. Natl Acad. Sci. USA* **118**, e2026309118 (2021).
38. Gokcumen, O. Archaic hominin introgression into modern human genomes. *Am. J. Phys. Anthropol.* https://doi.org/10.1002/ajpa.23951 (2019).
39. Arnold, M. L. *Evolution Through Genetic Exchange* (Oxford Univ. Press, 2006).
40. Jolly, C. J. A proper study for mankind: analogies from the Papionin monkeys and their implications for human evolution. *Am. J. Phys. Anthropol.* **116**, 177–204 (2001).
41. Mallet, J. Hybridization as an invasion of the genome. *Trends Ecol. Evol.* **20**, 229–237 (2005).
42. Stelkens, R. & Seehausen, O. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution* **63**, 884–897 (2009).
43. Ackermann, R., Brink, J., Vrahimis, S. & de Klerk, B. Hybrid wildebeest (Artiodactyla: Bovidae) provide further evidence for shared signatures of admixture in mammalian crania. *S. Afr. J. Sci.* **106**, 1–4 (2010).
44. Baranov, A. S. & Zakharov, V. M. Developmental stability in hybrids of European bison, *Bison bonasus*, and domestic cattle. *Acta Theriol.* **42**, 87–90 (1997).
45. Brink, J. S. *The Evolution of the Black Wildebeest, Connochaetes Gnou, and Modern Large Mammal Faunas in Central Southern Africa*. PhD thesis, Univ. Stellenbosch (2005).
46. Cahill, J. A. et al. Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Mol. Ecol.* **24**, 1205–1217 (2015).

47. Kumar, V. et al. The evolutionary history of bears is characterized by gene flow across species. *Sci. Rep.* **7**, 46487 (2017).
48. Figueiró, H. V. et al. Genome-wide signatures of complex introgression and adaptive evolution in the big cats. *Sci. Adv.* https://doi.org/10.1126/sciadv.1700299 (2017).
49. Li, G., Davis, B. W., Eizirik, E. & Murphy, W. J. Phylogenomic evidence for ancient hybridization in the genomes of living cats (Felidae). *Genome Res.* **26**, 1–11 (2016).
50. Benson, J. F., Patterson, B. R. & Wheeldon, T. J. Spatial genetic and morphologic structure of wolves and coyotes in relation to environmental heterogeneity in a Canis hybrid zone. *Mol. Ecol.* **21**, 5934–5954 (2012).
51. Khosravi, R., Rezaei, H. R. & Kaboli, M. Detecting hybridization between Iranian wild wolf (*Canis lupus pallipes*) and free-ranging domestic dog (*Canis familiaris*) by analysis of microsatellite markers. *Zool. Sci.* **30**, 27–34 (2013).
52. Mahan, B. R., Gipson, P. S. & Case, R. M. Characteristics and distribution of coyote X dog hybrids collected in Nebraska. *Am. Midl. Nat.* **100**, 408–415 (1978).
53. Monzon, J., Kays, R. & Dykhuizen, D. E. Assessment of coyote-wolf-dog admixture using ancestry-informative diagnostic SNPs. *Mol. Ecol.* **23**, 182–197 (2014).
54. Vila, C. et al. Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf-dog hybrids. *Heredity* **90**, 17–24 (2003).
55. Pastorini, J., Zaramody, A., Curtis, D. J., Nievergelt, C. M. & Mundy, N. I. Genetic analysis of hybridization and introgression between wild mongoose and brown lemurs. *BMC Evol. Biol.* **9**, 32 (2009).
56. Wyner, Y. M., Johnson, S. E., Stumpf, R. M. & Desalle, R. Genetic assessment of a white-collared×red-fronted lemur hybrid zone at Andringitra, Madagascar. *Am. J. Primatol.* **57**, 51–66 (2002).
57. Aguiar, L. M. et al. Sympatry between *Alouatta caraya* and *Alouatta clamitans* and the rediscovery of free-ranging potential hybrids in Southern Brazil. *Primates* **48**, 245–248 (2007).
58. Cortés-Ortiz, L. et al. 107–131 (Springer-Verlag, 2015).
59. Malukiewicz, J. et al. Hybridization effects and genetic diversity of the common and black-tufted marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. *Am. J. Phys. Anthropol.* **155**, 522–536 (2014).
60. Peres, C. A., Patton, J. L., Nazareth, F. & da Silva, M. Riverine barriers and gene flow in Amazonian saddle-back tamarins. *Folia Primatol.* **67**, 113–124 (1996).
61. Rossan, R. N. & Baerg, D. C. Laboratory and feral hybridization of *Ateles geoffroyi panamensis* Kellogg and Goldman 1944 and *A. fusciceps robustus* Allen 1914 in Panama. *Primates* **18**, 235–237 (1977).
62. Detwiler, K. M., Burrell, A. S. & Jolly, C. J. Conservation implications of hybridization in African cercopithecine monkeys. *Int. J. Primatol.* **26**, 661–684 (2005).
63. Fooden, J. Rhesus and crab-eating macaques: intergradation in Thailand. *Science* **143**, 363–364 (1964).
64. Schillaci, M. A., Froehlich, J. W., Supriatna, J. & Jones-Engel, L. The effects of hybridization on growth allometry and craniofacial form in Sulawesi macaques. *J. Human Evol.* **49**, 335–369 (2005).
65. Wildman, D. E. et al. Mitochondrial evidence for the origin of hamadryas baboons. *Mol. Phylogenet. Evol.* **32**, 287–296 (2004).
66. Zinner, D., Groeneveld, L. F., Keller, C. & Roos, C. Mitochondrial phylogeography of baboons (*Papio* spp.): indication for introgressive hybridization? *BMC Evol. Biol.* **9**, 83 (2009).
67. Harvati, K., Frost, S. R. & McNulty, K. P. Neanderthal taxonomy reconsidered: implications of 3D primate models of intra- and interspecific differences. *Proc. Natl Acad. Sci. USA* **101**, 1147–1152 (2004).
68. Jolly, C. J. in *Species, Species Concepts and Primate Evolution* (eds Kimbel, W. H. & Martin, L. B.) 67–107 (Springer, 1993).
69. Brockelman, W. Y. in *The Lesser Apes: Evolutionary and Behavioural Biology* (1984).
70. Marshall, J. & Sugardjito, J. in *Comparative Primate Biology, 1. Systematics, Evolution and Anatomy* (eds Swindler, D. R. & Erwin, J.) 137–185 (Liss, 1986).
71. Prado-Martinez, J. et al. Great ape genetic diversity and population history. *Nature* https://doi.org/10.1038/nature12228 (2013).
72. de Manuel, M. et al. Chimpanzee genomic diversity reveals ancient admixture with bonobos. *Science* **354**, 477–481 (2016).
73. Arnold, M. L. & Kunte, K. Adaptive genetic exchange: a tangled history of admixture and evolutionary innovation. *Trends Ecol. Evol.* **32**, 601–611 (2017).
74. Nye, J. et al. Selection in the introgressed regions of the chimpanzee genome. *Genome Biol. Evol.* **10**, 1132–1138 (2018).
75. Sankararaman, S., Mallick, S., Patterson, N. & Reich, D. The combined landscape of Denisovan and Neanderthal ancestry in present-day humans. *Curr. Biol.* **26**, 1241–1247 (2016).
76. Vernot, B. et al. Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals. *Science* **352**, 235–239 (2016).
77. Kuhlwilm, M. The evolution of FOXP2 in the light of admixture. *Curr. Opin. Behav. Sci.* **21**, 120–126 (2018).
78. Kuhlwilm, M., Han, S., Sousa, V. C., Excoffier, L. & Marques-Bonet, T. Ancient admixture from an extinct ape lineage into bonobos. *Nat. Ecol. Evol.* **3**, 957–965 (2019).
79. Duarte, C. et al. The early Upper Paleolithic human skeleton from the Abrigo do Lagar Velho (Portugal) and modern human emergence in Iberia. *Proc. Nat Acad. Sci. USA* **96**, 7604–7609 (1999).
80. Wolpoff, M., Hawks, J., Frayer, D. & Hunley, K. Modern human ancestry at the peripheries: a test of the replacement theory. *Science* **291**, 293–297 (2001).
81. Soficaru, A., Petrea, C., Dobos, A. & Trinkaus, E. Early modern humans from the Pestera Muierii, Baia de Fier, Romania. *Proc. Natl Acad. Sci. USA* **103**, 17196–17201 (2006).
82. Rougier, H. et al. Peştera cu Oase 2 and the cranial morphology of early modern Europeans. *Proc. Natl Acad. Sci. USA* **104**, 1165–1170 (2007).
83. Trinkaus, E., Constantin, S. & Zilhão, J. *Life and Death at the Pestera cu Oase. A Setting for Modern Human Emergence in Europe* (Oxford Univ. Press, 2013).
84. Ackermann, R. R. Phenotypic traits of primate hybrids: recognizing admixture in the fossil record. *Evol. Anthropol.* **19**, 258–270 (2010).
85. Smith, F., Lacy, K. & Caldwell, S. Morphological evidence for modern human influences in late central European Neandertals. *Anthropologie* **53**, 61–76 (2015).
86. Smith, F. H., Hutchinson, V. T. & Janković, I. in *African Genesis: Perspectives on Hominin Evolution* (eds Reynolds, S. C. & Gallagher, A.) 365-–393 (Cambridge Univ. Press, 2012).
87. Smith, F. H., Falsetti, A. B., & Simmons, T. in *Man and Environment in the Paleolithic* (ed. Ullrich, H.) 167–179 (ERAUL, 1995).
88. Ahern, J. C., Janković, I., Voisin, J. & Smith, F. H. in *Origins of Modern Humans: Biology Reconsidered* (eds Smith, F. H. & Ahern, J. C.) 151–222 (Wiley-Blackwell, 2013).
89. Cartmill, M. & Smith, F. H. *The Human Lineage* (John Wiley & Sons, 2009).
90. Condemi, S. et al. Possible interbreeding in late Italian Neanderthals? New data from the Mezzena Jaw (Monti Lessini, Verona, Italy). *PLoS ONE* **8**, 1–9 (2013).
91. Harvati, K., Gunz, P. & Grigorescu, D. Cioclovina (Romania): affinities of an early modern European. *J. Hum. Evol.* **53**, 732–746 (2007).
92. Stringer, C. What makes a modern human. *Nature* **485**, 33 (2012).
93. Tattersall, I. & Schwartz, J. H. Hominids and hybrids: the place of Neanderthals in human evolution. *Proc. Natl Acad. Sci. USA* **96**, 7117–7119 (1999).
94. Klein, R. *The Human Career* 3rd edn (Univ. Chicago Press, 2009).
95. Klein, R. G. Paleoanthropology. Whither the Neanderthals? *Science* **299**, 1525–1527 (2003).
96. Ackermann, R. R., Rogers, J. & Cheverud, J. Identifying the morphological signatures of hybridization in primate and human evolution. *J. Hum. Evol.* **51**, 632–645 (2006).
97. Ackermann, R. R., Schroeder, L., Rogers, J. & Cheverud, J. Further evidence for phenotypic signatures of hybridization in descendant baboon populations. *J. Hum. Evol.* **76**, (2014).
98. Warren, K. A. et al. Craniomandibular form and body size variation of first generation mouse hybrids: a model for hominin hybridization. *J. Hum. Evol.* **116**, 57–74 (2018).
99. Harvati, K. & Roksandic, M. in *Paleoanthropology of the Balkans and Anatolia: Human Evolution and its Context* (eds Harvati, K. & Roksandic, M.) 51–68 (Springer, 2016).
100. Ackermann, R. R. in *Tinkering: the Microevolution of Development* Symposium 284 (ed. Novartis Foundation) 262–279 (Wiley, 2007).
101. Goodwin, T. Supernumerary teeth in Pleistocene, recent, and hybrid individuals of the *Spermophilus richardsonii* Complex (Sciuridae) **79**, (1998).
102. Zdjelar, N., Nagendran, L., Kendall, C., Ackermann, R. R. & Schroeder, L. The hybrid skull of the eastern coyote (*Canis latrans* var.): nonmetric traits and craniomandibular shape. *J. Morphol.* **282**, 1745–1764 (2021).
103. Eichel, K. & Ackermann, R. R. Variation in the nasal cavity of baboon hybrids with implications for late Pleistocene hominins. *J. Hum. Evol.* **94**, 134–145 (2016).
104. Rieseberg, L., Archer, M. & Wayne, R. Transgressive segregation, adaptation and speciation. *Heredity* **83**, 363–372 (1999).
105. Leamy, L. Morphometric studies in inbred and hybrid house mice. I. Patterns in the mean values. *J. Hered.* **73**, 171–176 (1982).
106. Leamy, L. Morphometric studies in inbred and hybrid house mice. VII. Heterosis in fluctuating asymmetry at different ages. **191**, (1992).
107. Leamy, L. & Thorpe, R. *Morphometric studies in inbred and hybrid house mice. Heterosis, homeostasis and heritability of size and shape.* **22**, (1984).
108. Percival, C. J. et al. Genetics of murine craniofacial morphology: diallel analysis of the eight founders of the Collaborative Cross. *J. Anat.* **228**, 96–112 (2016).

109. Thorpe, R. & Leamy, L. Morphometric studies in inbred and hybrid house mice (*Mus* sp.): multivariate analysis of size and shape. **199**, (1983).
110. Warren, K. A. *Using the Craniomandibular Morphology of Hybrid Mice to Better Understand Hybrid Morphologies in the Hominin Fossil Record*. PhD thesis, Univ. Cape Town (2017).
111. Cheverud, J. M., Jacobs, S. C. & Moore, A. J. Genetic differences among subspecies of the saddle-back tamarin (*Saguinus fuscicollis*):evidence from hybrids. *A. J. Primatol.* **31**, 23–39 (1993).
112. Carmon, J. L. Heterosis, combining ability, and maternal effects in mice. *J. Genet.* **58**, 225–231 (1963).
113. Kohn, L. A. P., Langton, L. B. & Cheverud, J. M. Subspecific genetic differences in the saddle-back tamarin (*Saguinus fuscicollis*) postcranial skeleton. *Am. J. Primatol.* **54**, 41–56 (2001).
114. Kurnianto, E., Shinjo, A., Suga, D. & Uema, N. Diallel cross analysis of body weight in subspecies of mice. *Exp. Anim.* **48**, 277–283 (1999).
115. Buck, L. T. et al. Effects of admixture on pelvic morphology: a macaque model. *J. Hum. Evol.* **159**, 1030–1049 (2021).
116. Bergström, A., Stringer, C., Hajdinjak, M., Scerri, E. M. L. & Skoglund, P. Origins of modern human ancestry. *Nature* **590**, 229–237 (2021).
117. Harvati, K. et al. Apidima Cave fossils provide earliest evidence of *Homo sapiens* in Eurasia. *Nature* **571**, 500–504 (2019).
118. Tryon, C. A. et al. Late Pleistocene age and archaeological context for the hominin calvaria from GvJm-22 (Lukenya Hill, Kenya. *Proc. Natl Acad. Sci. USA* **112**, 2682–2687 (2015).
Q18 119. (!!! INVALID CITATION!!! 125-127).
120. (!!! INVALID CITATION!!! 35,117).
121. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
122. Trinkaus, E. An abundance of developmental anomalies and abnormalities in Pleistocene people. *Proc. Natl Acad. Sci. USA* **115**, 11941–11946 (2018).
123. Gunz, P. et al. Early modern human diversity suggests subdivided population structure and a complex out-of-Africa scenario. *Proc. Natl Acad. Sci. USA* **106**, 6094–6098 (2009).
124. Scerri, E. M. L. et al. Did our species evolve in subdivided populations across Africa, and why does it matter? *Trends Ecol. Evol.* **33**, 582–594 (2018).
125. Siska, V. *Human Population History and its Interplay with Natural Selection*. PhD thesis, Univ. Cambridge (2018).
126. Harvati, K. & Weaver, T. D. Human cranial anatomy and the differential preservation of population history and climate signatures. *Anat. Rec. A* **288**, 1225–1233 (2006).
127. Hubbe, M., Hanihara, T. & Harvati, K. Climate signatures in the morphological differentiation of worldwide modern human populations. *Anat. Rec.* **292**, 1720–1733 (2009).
128. Noback, M. L. & Harvati, K. The contribution of subsistence to global human cranial variation. *J. Hum. Evol.* **80**, 34–50 (2015).
129. Schmidt, K. L. & Cohn, J. F. Human facial expressions as adaptations: evolutionary questions in facial expression research. *Am. J. Phys. Anthropol.* **116**, 3–24 (2001).
130. Mellars, P. & French, J. C. Tenfold population increase in Western Europe at the Neandertal-to-modern human transition. *Science* **333**, 623–627 (2011).
131. Franciscus, R. & Vlček, E. in *Early Modern Human Evolution in Central Europe: the People of Dolní Věstonice and Pavlov* (eds Trinkaus, E. & Svoboda, J. A.) 63–152 (Oxford Univ. Press, 2006).
132. Galway-Witham, J. & Stringer, C. How did *Homo sapiens* evolve? *Science* **360**, 1296–1298 (2018).
133. Ríos, L. et al. Skeletal anomalies in the Neandertal family of El Sidrón (Spain) support a role of inbreeding in Neandertal extinction. *Sci. Rep.* **9**, 1697 (2019).
134. Dennell, R. W., Martinón-Torres, M. & Bermúdez de Castro, J. M. Hominin variability, climatic instability and population demography in Middle Pleistocene Europe. *Quat. Sci. Rev.* **30**, 1511–1524 (2011).
135. Charpentier, M. J. E., Widdig, A. & Alberts, S. C. Inbreeding depression in non-human primates: a historical review of methods used and empirical data. *Am. J. Primatol.* **69**, 1370–1386 (2007).
136. G. Rawlins, R. & J. Kessler, M. Congenital and hereditary anomalies in the rhesus monkeys (*Macaca mulatta*) of Cayo Santiago. **28**, (1983).
137. Nakamichi, M., Nobuhara, H., Nobuhara, T., Nakahashi, M. & Nigi, H. Birth rate and mortality rate of infants with congenital malformations of the limbs in the Awajishima free-ranging group of Japanese monkeys (*Macaca fuscata*). **42**, (1997).
138. Chalifoux, L. V. & Elliott, M. W. Congenital anomalies in two neonatal tamarins *(Saguinus oedipus* and *Saguinus fuscicollis)*. **15**, (1986).
139. van der Valk, T., Díez-del-Molino, D., Marques-Bonet, T., Guschanski, K. & Dalén, L. Historical genomes reveal the genomic consequences of recent population decline in eastern Gorillas. *Curr. Biol.* **29**, 165–170.e6 (2019).
140. Hublin, J. J. The origin of Neandertals. *Proc. Natl Acad. Sci. USA* **106**, 16022–16027 (2009).
141. Currat, M. & Excoffier, L. Strong reproductive isolation between humans and Neanderthals inferred from observed patterns of introgression. *Proc. Natl Acad. Sci. USA* **108**, 15129–15134 (2011).
142. Juric, I., Aeschbacher, S. & Coop, G. The strength of selection against Neanderthal introgression. *PLoS Genet.* **12**, e1006340 (2016).
143. McCoy, R. C., Wakefield, J. & Akey, J. M. Impacts of Neanderthal-introgressed sequences on the landscape of human gene expression. *Cell* **168**, 916–927.e2 (2017).
144. Nicholson, E. & Harvati, K. Quantitative analysis of human mandibular shape using three-dimensional geometric morphometrics. *Am. J. Phys. Anthropol.* **131**, 368–383 (2006).
145. Gunz, P. & Harvati, K. The Neanderthal "chignon": variation, integration, and homology. *J. Hum. Evol.* **52**, 262–274 (2007).
146. Harvati, K., Hublin, J.-J. & Gunz, P. Evolution of middle-late Pleistocene human cranio-facial form: a 3-D approach. *J. Hum. Evol.* **59**, 445–464 (2010).
147. Valladas, H. et al. TL dates for the Neanderthal site of the Amud Cave, Israel. *J. Archaeol. Sci.* **26**, 259–268 (1999).
148. Bahain, J. J., Sarcia, M. N., Falguères, C. & Yokoyama, Y. Attempt at ESR dating of tooth enamel of French middle Pleistocene sites. *Appl. Radiat. Isot.* **44**, 267–272 (1993).
149. Grün, R. & Stringer, C. ESR dating and the evolution of modern humans. **33**, (2007).
150. Schmitz, R. W. et al. The Neandertal type site revisited: interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. *Proc. Natl Acad. Sci. USA* **99**, 13342–13347 (2002).
151. Guérin, G. et al. A multi-method luminescence dating of the palaeolithic sequence of La Ferrassie based on new excavations adjacent to the La Ferrassie 1 and 2 skeletons. **58**, (2015).
152. Oakley, K., Campbell, B. & Molleson, T. (Mus. Nat. Hist., London, 1971).
153. Grün, R. & Stringer, C. B. Electron spin resonance dating and the evolution of modern humans. *Archaeometry* **33**, 153–199 (1991).
154. Schwarcz, H. P. et al. On the reexamination of Grotta Guattari: uranium-series and electron-spin-resonance dates. *Curr. Anthropol.* **32**, 313–316 (1991).
155. Rink, W. J., Schwarcz, H. P., Smith, F. H. & Radovčić, J. ESR ages for Krapina hominids. **378**, (1995).
156. Debénath, A. & Jelinek, A. J. Nouvelles fouilles à La Quina (Charente). Résultats préliminaires. **40**, (1998).
157. Vandermeersch, B. & Trinkaus, E. The postcranial remains of the Régourdou 1 Neandertal: the shoulder and arm remains. *J. Hum. Evol.* **28**, 439–476 (1995).
158. Marra, F. et al. The aggradational successions of the Aniene River Valley in Rome: age constraints to early Neanderthal presence in Europe. *PLoS ONE* **12**, e0170434 (2017).
159. Solecki, R. S. *Shanidar, the First Flower People* (Knopf, 1971).
160. Devièse, T. et al. Reevaluating the timing of Neanderthal disappearance in Northwest Europe. *Proc. Natl Acad. Sci. USA* **118**, e2022466118 (2021).
161. Grün, R. & Stringer, C. Tabun revisited: revised ESR chronology and new ESR and U-series analyses of dental material from Tabun C1. **39**, (2001).
162. Grün, R. et al. U-series and ESR analyses of bones and teeth relating to the human burials from Skhul. *J. Hum. Evol.* **49**, 316–334 (2005).
163. Michel, V., Delanghe-Sabatier, D., Bard, E. & Barroso Ruiz, C. U-series, ESR and 14C studies of the fossil remains from the Mousterian levels of Zafarraya Cave (Spain): a revised chronology of Neandertal presence. *Quat. Geochronol.* **15**, 20–33 (2013).
164. Wood, R. E. et al. Radiocarbon dating casts doubt on the late chronology of the Middle to Upper Palaeolithic transition in southern Iberia. *Proc. Natl Acad. Sci. USA* **110**, 2781–2786 (2013).
165. Haile-Selassie, Y., Asfaw, B. & White, T. D. Hominid cranial remains from upper Pleistocene deposits at Aduma, Middle Awash, Ethiopia. *Am. J. Phys. Anthropol.* **123**, 1–10 (2004).
166. Grine, F. et al. Late Pleistocene human skull from Hofmeyr. **315**, (2007).
167. Schwartz, J. H. & Tattersall, I. *The Human Fossil Record, Craniodental Morphology of Genus Homo (Africa and Asia)* Vol. 2. (Wiley-Liss, 2003).
168. Wood, B. *Wiley-Blackwell Encyclopedia of Human Evolution* 1st edn (Wiley-Blackwell, 2011).
169. Day, M. H., Leakey, M. D. & Magori, C. A new hominid fossil skull (L.H. 18) from the Ngaloba Beds, Laetoli, northern Tanzania. *Nature* **284**, 55–56 (1980).
170. Leakey, M. D. & Harris, J. M. (eds) *Laetoli: A Pliocene Site in Northern Tanzania* (Oxford Univ. Press, 1987). Q19
171. Vidal, C. M. et al. Age of the oldest known *Homo sapiens* from eastern Africa. *Nature* **601**, 579–583 (2022).
172. McDougall, I., Brown, F. H. & Fleagle, J. G. Stratigraphic placement and age of modern humans from Kibish, Ethiopia. *Nature* **433**, 733–736 (2005).
173. Schild, R. & Wendorf, F. Palaeolithic living sites in upper and middle Egypt: a review article. *J. Field Archaeol.* **29**, 447–461 (2002).

174. Mellars, P. A., Bricker, H. M., Gowlett, J. A. J. & Hedges, R. E. M. Radiocarbon accelerator dating of French Upper Palaeolithic sites. *Curr. Anthropol.* **28**, 128–133 (1987).
175. Holt, B. M. & Formicola, V. Hunters of the Ice Age: the biology of Upper Paleolithic people. *Am. J. Phys. Anthropol.* **137**, 70–99 (2008).
176. Barshay-Szmidt, C. et al. New extensive focused AMS ^{14}C dating of the Middle and Upper Magdalenian of the western Aquitaine/Pyrenean region of France (ca. 19–14 ka cal BP): proposing a new model for its chronological phases and for the timing of occupation. *Quat. Int.* **414**, 62–91 (2016).
177. Soficaru, A., Petrea, C., Doboș, A. & Trinkaus, E. The human cranium from the Peștera Cioclovina Uscată, Romania: context, age, taphonomy, morphology, and paleopathology. *Curr. Anthropol.* **48**, 611–619 (2007).
178. Henry-Gambier, D. Les fossiles de Cro-Magnon (Les Eyzies-de-Tayac, Dordogne): nouvelles données sur leur position chronologique et leur attribution culturelle. **14**, (2002).
179. Trinkaus, E. & Svoboda, J. *Early Modern Human Evolution in Central Europe: The People of Dolní Věstonice and Pavlov* (2006).
180. Formicola, V., Pettitt, P. B. & Del Lucchese, A. A direct AMS radiocarbon date on the Barma Grande 6 Upper Paleolithic skeleton. *Curr. Anthropol.* **45**, 114–118 (2004).
181. Schwartz, J. H. & Tattersall, I. *The Human Fossil Record, Terminology and Craniodental Morphology of Genus I Homo/I (Europe)* Vol. 1 (Wiley-Liss, 2002).
182. Wild, E. M. et al. Direct dating of Early Upper Palaeolithic human remains from Mladeč. *Nature* **435**, 332–335 (2005).
183. Trinkaus, E. et al. An early modern human from the Peștera cu Oase, Romania. *Proc. Natl Acad. Sci. USA* **100**, 11231–11236 (2003).
184. Street, M., Terberger, T. & Orschiedt, J. A critical review of the German Paleolithic hominin record. *J. Hum. Evol.* **51**, 551–579 (2006).
185. Svoboda, J. A. The Upper Paleolithic burial area at Předmostí: ritual and taphonomy. *J. Hum. Evol.* **54**, 15–33 (2008).

Acknowledgements

This research was supported by the European Research Council (ERC AdG 101019659 (KH)), the German Research Foundation (DFG FOR 2237 'Words, Bones, Genes, Tools' (KH)) and the National Research Foundation of South Africa (Grant No. 117670 (RRA)). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. We thank all curators and institutions that allowed us access to the fossil specimens used in our analyses, E. Lopez for collecting part of the mandibular data used, K. Warren for collecting and analysing the data reproduced in Fig. 1, A. M. Bosman and C. Röding for help with processing the datasets used in Figs. 3–4, H. Rathmann and J. Kunze for help with the figures, and C. Posth for important feedback.

Author contributions

Both K.H. and R.R.A. conceived and designed the study; KH collected and analysed the coordinate data; RRA compiled data from the literature; both K.H. and R.R.A. wrote the manuscript.

Competing interests

K.H. has an additional affiliation with the Centre for Early Sapiens Behavior (SapienCE) Department of Archaeology, History, Cultural Studies and Religion, University of Bergen, Norway, which was not involved in this project and is therefore not listed in this manuscript. R.R.A. declares no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-022-01875-z.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41559-022-01875-z.

Correspondence and requests for materials should be addressed to K. Harvati or R. R. Ackermann.

Peer review information *Nature Ecology & Evolution* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Extended Data Table 1 | Hominin samples used in analyses

Neanderthals	Face	Mandible	Posterior Profile	Apx. geological age (ka)	Reference
Amud 1		x	x	55–60	147
Apidima 2	x			ca. 170	117
Biache St Vaast			x	ca. 250	148
La Chapelle-aux-Saints	x		x	47 / 56	149
Feldhofer			x	40	150
Ferrassie 1	x	x	x	43–45	151
Gibraltar 1	x			ca. 50	152
Guattari 1	x		x	50–60	153,154
Krapina J		x		140-120	155
La Quina 5		x	x	>48 (OIS 3-4)	156
Regourdou		x		MIS 4-5	157
Saccopastore 1			x	295-220	158
Shanidar 1	x	x		46–50 Uncalibrated	159
Shanidar 5	x			46–50 Uncalibrated	159
Spy 1			x	40.6–44.2 cal BP	160
Spy 2			x	40.6–44.2 cal BP	160
Tabun C1		x		130-100	161,162
Zafarraya		x		ca 30–46, >46	163,164
(*possible) Homo sapiens Late Middle – Late Pleistocene Africa					
Aduma 3			x	79–105	165
Hofmeyr	x			36	166
*Eliye Springs			x	Middle/Late Pleistocene	167,168
LH 18			x	120 ± 30	169,170
*Omo 1			x	233 ± 22	171,172
Omo 2			x	195 ± 5	172
Wadi Kubbaniya	x	x		ca. 20	173
(*possible) Homo sapiens Late Middle – Late Pleistocene Eurasia					
Abri Pataud 1	x	x	x	28-26 (22 uncalibrated)	174
*Apidima 1			x	ca. 210	117
Brno 2			x	23.7 uncal (ca. 28.5 cal BP)	175
Chancelade	x		x	18	176
Cioclovina			x	ca. 33	177
Cro Magnon 1	x		x	ca 30	178
Cro Magnon 2	x		x	ca 30	178
Cro Magnon 3			x	ca 30	178
Dolní Věstonice 13	x	x	x	ca. 31	179
Dolní Věstonice 14	x	x		ca. 31	179
Dolní Věstonice 15	x	x	x	ca. 31	179
Dolní Věstonice 16	x	x	x	ca. 30	179
Dolní Věstonice 3	x	x	x	undated	179
Grimaldi	x	x		25 uncal (ca. 29.5 cal BP)	180
Isturitz III		x		Upper Paleolithic	181
Mladec 1	x		x	35–36.5	182
Mladec 5			x	35–36.5	182
Muierii 1	x	x	x	ca. 35	81
Oase 1		x		ca. 40.5	183
Oase 2	x		x	ca. 40.5	82
Oberkassel 1		x		12 uncalb (ca. 14.2 cal BP)	184
Oberkassel 2		x		12 uncalb (ca. 14.2 cal BP)	184
Pavlov 1			x	ca. 30	179
Predmost 3	x		x	27–29	185
Predmost 4	x		x	27–29	185
Qafzeh6	x		x	100–130	162
Qafzeh9	x	x	x	100–130	162
Skhul 5		x	x	100–130	162

Dates are reported in calendar years unless otherwise stated

QUERY FORM

Nature Ecology & Evolution	
Manuscript ID	**[Art. Id: 1875]**
Author	**K. Harvati**

AUTHOR:

The following queries have arisen during the editing of your manuscript. Please answer by making the requisite corrections directly in the e.proofing tool rather than marking them up on the PDF. This will ensure that your corrections are incorporated accurately and that your paper is published as quickly as possible.

Query No.	*Nature of Query*
Q1:	Please confirm or correct the city name inserted in affiliation 1,2
Q2:	Please check your article carefully, coordinate with any co-authors and enter all final edits clearly in the eproof, remembering to save frequently. Once corrections are submitted, we cannot routinely make further changes to the article.
Q3:	Note that the eproof should be amended in only one browser window at any one time; otherwise changes will be overwritten.
Q4:	Author surnames have been highlighted. Please check these carefully and adjust if the first name or surname is marked up incorrectly. Note that changes here will affect indexing of your article in public repositories such as PubMed. Also, carefully check the spelling and numbering of all author names and affiliations, and the corresponding email address(es).
Q5:	You cannot alter accepted Supplementary Information files except for critical changes to scientific content. If you do resupply any files, please also provide a brief (but complete) list of changes. If these are not considered scientific changes, any altered Supplementary files will not be used, only the originally accepted version will be published.
Q6:	If applicable, please ensure that any accession codes and datasets whose DOIs or other identifiers are mentioned in the paper are scheduled for public release as soon as possible, we recommend within a few days of submitting your proof, and update the database record with publication details from this article once available.
Q7:	Per style, figures should have a brief title that should ideally not include references to panels a,b, etc. Please provide a brief title for Fig. 1.
Q8:	Please define MIS at its first mention, in the sentence 'Our analyses include late...'.
Q9:	In Figs. 3-5(top left): please indicate what the red circles (yellow-filled) on the models represent.
Q10:	In Figs. 3-5: please correct or confirm edit to the phrase 'earlier specimens also plotted'. Also please indicate marker for earlier specimens, if appropriate.
Q11:	Please provide the journal title, volume number and page range or article number for reference 23.
Q12:	Please provide the article/book title, edition/volume number and name(s) of editor(s) for reference 58, as appropriate.
Q13:	Please provide name(s) of editor(s), page range or chapter number and publisher for reference 69.

QUERY FORM

Nature Ecology & Evolution	
Manuscript ID	**[Art. Id: 1875]**
Author	**K. Harvati**

AUTHOR:

The following queries have arisen during the editing of your manuscript. Please answer by making the requisite corrections directly in the e.proofing tool rather than marking them up on the PDF. This will ensure that your corrections are incorporated accurately and that your paper is published as quickly as possible.

Query No.	*Nature of Query*
Q14:	Please provide the article number for reference 90.
Q15:	Please provide the final page number for reference 92.
Q16:	Please provide the page range for reference 97.
Q17:	Please provide the journal title and page range or article number for references 101, 106, 107, 109, 136-138, 149, 151, 155, 156, 161, 166, 178.
Q18:	Please provide appropriate publication details for references 119, 120, 152.
Q19:	Please check and confirm edits to ref. 170.
Q20:	Please provide name of publisher for reference 179.

nature portfolio

Corresponding author(s): Katerina Harvati
Rebecca R. Ackermann

Last updated by author(s): Katerina Harvati

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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ 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 *Give P values as exact values whenever suitable.*
☒	☐ 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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	*Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.*

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

Data availability statement included. Data will be made freely available on the zenodo platform

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

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

Sample size	*Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data exclusions	*Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*

Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	yes
Research sample	yes
Sampling strategy	yes
Data collection	yes
Timing and spatial scale	n/a
Data exclusions	n/a
Reproducibility	n/a
Randomization	n/a
Blinding	n/a

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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

Methods

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

Antibodies

Antibodies used: *Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Validation: *Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): *State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*

Authentication: *Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination: *Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*

Commonly misidentified lines (See ICLAC register): *Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance: yes

Specimen deposition: yes

Dating methods: n/a

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight: All samples were accessed with permission from the curating institutions and following all relevant regulations

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: *For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*

Wild animals: *Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*

Reporting on sex: *Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*

Field-collected samples: *For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s) — *Specify: functional, structural, diffusion, perfusion.*

Field strength — *Specify in Tesla*

Sequence & imaging parameters — *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition — *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software — *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization — *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template — *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal — *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring — *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings — *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested — *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016) — *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction — *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity — *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis — *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis — *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*