Cioclovina (Romania): affinities of an early modern European

Katerina Harvati a,*, Philipp Gunz a, Dan Grigorescu b

a Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, Leipzig, Germany
b Department of Paleontology, University of Bucharest, Bucharest, Romania

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Abstract

The current modern human origins debate centers on the possibility and degree of admixture between indigenous archaic humans and modern human populations migrating out of Africa into Europe and Asia in the Late Pleistocene. Evidence for such admixture must be sought in the earliest fossil record of modern humans outside Africa, as it is those populations that would have encountered, and possibly interbred with, archaic hominins. In the case of Europe, the recent application of direct dating techniques has eliminated several specimens from the Upper Paleolithic fossil record, while confirming early ages for others. Among these earliest reliably dated specimens is the Cioclovina calvaria from Romania. This individual is of highest importance for the understanding of modern human origins in Europe, and has recently been proposed to represent a Neanderthal-modern human hybrid. We present a short description and a three-dimensional (3D) geometric morphometric analysis of the Cioclovina specimen using a large geographic sample of recent humans, Neanderthals and Middle and Late Pleistocene fossil hominins from Europe, Africa, and the Levant, in order to establish its phenetic affinities and to evaluate its morphology for evidence of admixture between Neanderthals and early modern Europeans. Our results show Cioclovina to be entirely modern in its cranial shape, and do not support the hypothesis that it represents a hybrid.

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Introduction

The last few years have witnessed an evolution of the debate surrounding modern human origins. The two original competing hypotheses, the Multiregional and the Single Origin models, proposed two strikingly different views of the geographic origins and antiquity of modern humanity. The former saw modern humans as evolving from archaic populations around the world into the modern-day geographic groups, all the while maintaining species cohesion through extensive gene flow (e.g., Smith, 1982, 1984; Wolpoff, 1989, 1992; Frayer et al., 1993). In this view the human lineage went back in time unbroken by speciation events to the early Pleistocene (Wolpoff et al., 1994). The Single Origin model, on the other hand, proposed a relatively recent (ca. 200–100 ka) origin of modern humans in one geographic location, most likely Africa, with subsequent modern human migration and replacement of archaic hominins around the world (e.g., Stringer and Andrews, 1988; Stringer, 1989, 1992). In this debate, the place (the Old World vs. Africa) and timing (ancient vs. recent) of the origin of modern humans, as well as the fate of archaic humans (ancestors vs. extinct side branches), were disputed, with some researchers proposing a middle ground [e.g., the Afro-European hypothesis (Brauer, 1984); the Assimilation model (Smith et al., 1989)].

Recently, the focus of the discussion has shifted due to several recent advances in dating methods, modern human and ancient DNA analysis, and the study of the fossil record itself (e.g., Valladas et al., 1987, 1988; Krings et al., 1997, 2000; Ponce de León and Zollikofer, 2001; Harvati, 2003b; Harvati et al., 2004; Serre et al., 2004; Green et al., 2006; Noonan...
et al., 2006; Serre and Pääbo, 2006). The consensus view now agrees on a recent African origin for modern humans (see e.g., Trinkaus, 2005). However, it is the possible degree of admixture between the migrating African modern human populations and the indigenous archaic humans that is now disputed (Wolpoff et al., 2001; Harvati, 2003b; Currat and Excoffier, 2004; Harvati et al., 2004; Pearson, 2004; Serre et al., 2004; Smith et al., 2005; Trinkaus, 2005; Weaver and Roseman, 2005; Evans et al., 2006).

Within this framework, the fossil record of the earliest modern humans outside of Africa is of paramount importance, since it is among these early moderns that evidence of hybridization with penecontemporaneous archaic groups should be observed (Relethford, 2001; Harvati et al., 2004). This fossil sample has recently been pruned on the basis of redating of several important specimens, many of which proved to be substantially younger than initially thought. Nonetheless, early dates have been confirmed for some specimens (see e.g., Trinkaus, 2005, and references therein). Among these earliest reliably dated early modern Europeans is the little-known partial cranium from Cioclovina, Romania (Rainer and Simionescu, 1942; Necrasov and Cristescu, 1965; Pâunescu, 2001; Trinkaus, 2005), recently directly dated by AMS radiocarbon to approximately 28–29 thousand years (Olariu et al., 2005; Soficaru et al., 2007). Despite the specimen’s importance, no detailed morphological description and comparative analysis had been conducted since its original description by Rainer and Simionescu (1942) until recently (Soficaru et al., 2007). The latter study has proposed that this cranium shows evidence of intermixture between Neanderthals and modern humans, thus representing a hybrid.

What do hybrids look like?

The hypothesis of admixture between closely related species [or morphologically distinct subspecies—due to the process of evolution the separation between these taxonomic levels is often unclear (see e.g., Jolly, 2001)] is difficult to test. It is often assumed that hybrids in the human fossil record should show morphology that is in some ways intermediate between the two parental populations, or that is a ‘mosaic’ of morphological features from both parental groups (e.g., Bräuer, 1992; Frayer, 1992; Kidder et al., 1992; Frayer et al., 1993; Duarte et al., 1999; Wolpoff et al., 2001; Smith et al., 2005). It has also recently been stated that hybrid specimens may only show one or two nonmetric features from one parental taxon while in all other respects conforming to the norm for just one of the parent groups (Soficaru et al., 2007). Nevertheless, and despite these assumptions about the fossil record, little is known about the skeletal manifestations of admixed ancestry in primate taxa, either at the specific (or even above it) or the subspecific level, at the F1 or later hybrid generations.

The most commonly documented characteristics of hybrids are heterosis and dysgenesis. These can be defined as departures of the hybrids toward either greater (heterosis) or smaller (dysgenesis) size than expected based on the phenotypes of the parental taxa. They have been documented in hybridization between both species and subspecies, and at both the F1 and later hybrid generations. Starting with the species level, Schladl et al. (2005) found heterosis in hybrids (of unknown generation) of two species of Sulawesi macaques in one cranial (cranial vault length) and one postcranial (crown-rump length) dimension, but dysgenesis in body mass. In terms of subspecific hybridization, Cheverud et al. (1993) found strong evidence for heterosis in the crania of hybrids of black-saddled tamarins subspecies, although the magnitude of this effect was variable across cranial dimensions and from one pair of hybridizing subspecies to another. More recently, Ackermann et al. (2006) found a variable expression of heterosis in some cranial dimensions in known-pedigree F1 generation hybrids of yellow and olive baboons (subspecies of Papio, showing mtDNA divergence at as recently as 160 ka).

Some evidence also exists for intermediate morphology in admixed samples. An intergeneric F1 hybrid between a sian mang (Symphalangus syndactylus) and a gibbon (Hylobates moloch) born in the Atlanta Grant Park Zoo showed resemblances to both parental groups in its limb proportions, but also a mixed occurrence of such characteristic traits as webbed toes and throat sac (Myers and Schafer, 1979). Another example of intergeneric F1 hybrids, the naturally occurring hybrids of Papio and Theropithecus described by Jolly et al. (1997), showed largely intermediate cranial, dental, and postcranial morphology to the parental conditions as well as very large body and dental dimensions, but no dental anomalies such as those reported by Ackermann et al. (2006, see below). At the species level, intermediate characteristics in pelage and general morphology were observed by Bernstein (1966) in two presumed naturally occurring Macaca nemestrina and M. irus hybrids, possibly F1. Further, the macaque samples from the western hybrid zone of Macaca maurus and M. tonkeana (South Sulawesi), which presumably encompasses individuals of both F1 and later hybrid generations, exhibit clinal distribution of intermediate phenotypes in cranial and postcranial dimensions, pelage patterns, and dermatoglyphics (Froehlich and Supriatna, 1996; see also Supriatna, 1991). Additional evidence for intermediate morphology in hybrids of well-defined species comes from the canid literature, where hybrids of the C. latrans (coyote) and C. rufus (red wolf) from the early 1900s show intermediate morphology to the parental species (Nowak, 2002). A similar pattern of clinally distributed intermediate craniofacial shape was found for subspecific hybridization in a 3D geometric morphometric analysis by Frost et al. (2003) of a large sample (of unknown pedigree but probably representative of all hybrid generations present in a hybrid zone) of baboons from all six Papio subspecies, some of which are geographically connected by well-documented hybrid zones (e.g., Phillips-Conroy and Jolly, 1986; Phillips-Conroy et al., 1991).

Finally, Ackermann et al. (2006), based on her pedigreed F1 baboon subspecies hybrids, found a high frequency of dental and sutural anomalies in these specimens, such as supernumerary teeth and extra sutures in that sample. They interpreted this as evidence for high levels of developmental instability in admixed individuals even at the subspecific level. Furthermore,
Ackermann et al. (2006) suggested that hybrid samples tend to be characterized by a high level of individual variability, which often exceeds the combined variation of both parental groups.

On the basis of this evidence, it is reasonable to expect that hybrids (whether F1 or later generation) between well-defined fossil human taxa, and, therefore, also between Neanderthals and early modern humans (whether these represent distinct species or subspecies), would exhibit either all or some of the following morphological conditions: 1) heterosis or dysgenesis; 2) intermediate morphology to the parental groups, either in terms of shape or in terms of mixed occurrence of nonmetric traits; 3) possible occurrence of rare anomalies such as supernumerary teeth and extra sutures; and, finally, 4) high levels of individual variability in the hybrid population.

We conduct a comparative morphological analysis of the Cioclovina specimen to test the predictions for hybrid morphology outlined above. We briefly describe the cranium in terms of general morphology and nonmetric traits insofar as these may reflect similarities to Neanderthals, or mixed Neanderthal-early modern human ancestry. A three-dimensional (3D) geometric morphometrics analysis of size and shape is also conducted to more fully explore these hypotheses. These methods were chosen because they present several advantages over traditional morphometrics. In contrast to conventional linear and angular measurements, this coordinate-based approach preserves the geometry of the object studied and allows the intuitive visualization of shape differences between specimens or group averages as landmark displacements (Rohlf and Marcus, 1993; O’Higgins, 2000, Harvati, 2001, 2003a,b). These methods also provide a way of quantifying shape variability of traits which are difficult to measure with traditional measurements and are, therefore, usually described qualitatively (Harvati, 2001, 2003a; Nicholson and Harvati, 2006). In addition to the landmarks we are able to include information from curves (see below), therefore the present analysis quantifies not only the general shape of the cranium, as might be reflected by traditional measurements, but also the detailed shape of particular anatomical regions that have been proposed to show Neanderthal affinities, such as the shape of the supraorbital region and the ‘hemibun’ morphology.

The Cioclovina specimen

The Cioclovina calvaria was discovered in 1941, during phosphate mining of the Cioclovina cave, South Transylvania (Fig. 1). With it were also recovered three lithic artifacts, as well as Aurignacian, as well as three crania and several vertebrae of Ursus spelaeus. These lithic and faunal specimens were at the time interpreted as deriving from the same layer as the cranium on the basis of similarities of the sediment encasing them and because of the similar level of fossilization of both human and Ursus remains (Rainer and Simionescu, 1942). However, since the conditions of discovery are not clear, this association has been questioned (Olariu et al., 2005). Cioclovina was initially described by Rainer and Simionescu (1942) and had not been further studied until very recently (Soficaru et al., 2007). Direct AMS 14C dating of the calvaria at Lund University, Sweden, originally yielded an age of 29,000 ± 700 radiocarbon years (Olariu et al., 2005), indicating that it is among the earliest known modern humans in Europe. A more recent direct dating attempt, using the superior sample preparation technique involving ultrafiltration, has produced a similar age of 28,510 ± 170 radiocarbon years (Soficaru et al., 2007).

Cioclovina preserves the cranial vault and much of its cranial base. The face has been almost entirely destroyed: only the frontal aspect of the orbits and the upper part of the nasal bones are present. Despite some cracks and fractures on the parietals, especially on the right side, the cranium is in good condition and appears to have suffered minimal postmortem distortion. Rainer and Simionescu (1942) tentatively aged and sexed it as a young adult female, though this assignment was later questioned (Smith, 1984; Soficaru et al., 2007). They pointed out Cioclovina’s metric similarities to the Predmosti specimens and attributed it to “Homo sapiens diluvialis” (i.e., Upper Paleolithic Europeans). These authors also saw Neanderthal influences on the specimen’s supraorbital morphology, although at the same time they pointed out that the specimen does not exhibit a true continuous supraorbital torus. The more recent brief description by Soficaru et al. (2007) did not find Cioclovina’s supraorbital morphology to be Neanderthal-like; instead, it suggested that Neanderthal-like features are shown by its nuchal region.

Nonmetric features of the cranium

As both the previous descriptions noted, the overall morphology of the cranium is modern (Fig. 2). The frontal squama is high and vertically rising, though somewhat flattened anteriorly. The glabella is prominent and the glabellar part of the supraorbital region is relatively heavy. Nonetheless, the lateral part of the supraorbital region is differentiated from the middle segment, a modern condition different from the continuous supraorbital morphology observed in Neanderthals (e.g., Smith and Raynard, 1980; Bräuer, 1981; Smith, 1992). In lateral view the vault is high and rounded. The parietals are curved both anteroposteriorly and mediolaterally. In posterior view the vault shape is pentagonal and different from the rounded en bombe shape of the Neanderthals. No unusual sutural anomalies are present.

The temporal bone is characterized by a high, arched squamous portion, a deep glenoid fossa, a relatively short mastoid portion, and a well-marked supramastoid crest. The mastoid process is large, robust, and laterally projecting, with heavy muscle markings and a developed mastoid crest. No anterior mastoid tubercle, a proposed Neanderthal autapomorphic feature by Santa Luca (1978), is present. The juxtamastoid eminence is small and the petrotympanic crest runs from the carotid canal to the anterior edge of the mastoid process. This morphology is typical of modern humans and similar to that shown by other Upper Paleolithic European crania (e.g., Caspari, 1991; Harvati, 2003a, 2004). It contrasts sharply with the small mastoid processes, large juxtamastoid eminences, and coronally oriented petromastoid crests observed in
Neanderthals (e.g., Vallois, 1969; Hublin, 1988; Caspari, 1991; Condemi, 1991; Harvati, 2003a, 2004). The digastric fossa is narrow and almost slit-like, and antero posteriorly elongated, a condition common in modern human crania but different from the wide fossa shown by Neanderthals (Vallois, 1969; Vandermeersch, 1985; Harvati, 2003a, 2004).

The occipital bone is rounded in lateral view, with a supero-inferiorly high upper scale. There is a slight posterior bulging on the upper scale of the occipital and a small depression above lambda on the parietals. There is a slight posterior bulging on the upper scale of the occipital and a small depression above lambda on the parietals. This morphology, noted also by Soficaru et al. (2007), is reminiscent of the ‘hemibuns’ exhibited by some Upper Paleolithic crania and common among recent humans (Smith et al., 2005; Gunz and Harvati, 2007). This ‘hemibun’, however, does not show the typical lateral expansion and is not associated with the flat parietals and an elevated nuchal plane of Neanderthal ‘chignons’ (Ducros, 1967; Caspari, 1991; Gunz and Harvati, 2007). The superior nuchal line is well-developed and courses the entire width of the occipital bone to the occipitomastoid sutures on either side (see also Soficaru et al., 2007), where it forms a thickening near asterion. In this respect Cioclovina is similar to the morphology described for Upper Paleolithic specimens, even some considered female (Caspari, 1991). It is quite different from the Neanderthal condition, characterized by very weak superior nuchal lines, and by a high, thick, horizontal occipital torus, well-defined inferiorly but without distinct superior border, and with its greatest expression to the sides of the midline (Hublin, 1978a,b; Caspari, 1991). Unlike many Upper Paleolithic crania, however, Cioclovina shows no external occipital protuberance; instead, a tuberculum linearium, derived from the superior nuchal lines, connects to the external occipital crest inferiorly. Although Neanderthals lack an external occipital protuberance and show a tuberculum linearium instead, this condition is also common in modern humans (Caspari, 1991).

One of the frequently cited Neanderthal autapomorphic traits is the supraoiniac fossa. This is a pitted, large oblong or rectangular depression above the center of the Neanderthal torus and running parallel to the torus’ long axis (Hublin, 1978a,b; Santa Luca, 1978; Bräuer and Broeg, 1998). Despite variability in the expression of this trait among Neanderthals, it differs from the simple occurrence of a resorptive area in the general region above inion (a feature common in many modern humans and some Upper Paleolithic specimens) in its specific shape, strong expression, and associated structures (Hublin, 1978a,b; see also Caspari, 1991). As observed by Soficaru et al. (2007), a slightly rough area, possibly a resorptive region, is found above the superior nuchal lines in the midline in Cioclovina. However, contra Soficaru et al. (2007), this feature is neither a fossa nor does it present the features associated with the Neanderthal condition (see Fig. 2c,d). We consider that such morphology is common in modern human crania, and, therefore, cannot be used to deduce Neanderthal affinities, contrary to the claims of Soficaru et al. (2007) to this effect.

Shape analysis

Materials and methods

Comparative samples. Our samples included several Middle and Late Pleistocene fossil specimens from Europe, Africa, and the Near East, and 263 recent human crania...
representing six broad geographic groups (Tables 1 and 2). With the exception of the Andamanese and the Khoisan, these samples do not represent biological populations in the sense of demes, but instead group subsamples from a larger geographic region. Only adults were included, as determined by fully erupted permanent dentition. The sex of the recent human crania was often unknown and was assigned based on cranial morphology and size. Since such sex assignment is imperfect for recent humans, and even more problematic for fossil specimens, sexes were pooled in the analysis. Where original fossil specimens were not available, we measured high quality casts or stereolithographs. In some cases, measurements on the original fossils were supplemented with measurements on casts.

For the purposes of the canonical variates and Mahalanobis D^2 analyses (see below), the fossil samples were partitioned in the following groups: Neanderthals (NEA); Middle Pleistocene Europeans (MPE); late Middle and Late Pleistocene humans from Africa and the Levant (MLP); and Upper Paleolithic Europeans (UPE).

**Data.** Three-dimensional coordinates of 19 osteometric landmarks and several semilandmarks were collected by one of us (K.H.) on the cranium, defined as homologous points that can be reliably and repeatedly located, using the Microscribe 3D-X (Immersion Corporation, 1998) portable digitizer (Table 3). Landmarks along the midsagittal profile from glabella to inion, along the coronal and lambdoid sutures, and along the upper margin of the supraorbital torus were also registered (Table 3). The points along sutures were later automatically resampled to yield the same semilandmark count on every specimen. Despite some questions on the reliability of cranial vault morphology in reconstructing phylogeny and population history due to its presumed plasticity (see e.g., Wood and Lieberman, 2001), 3D landmark data from this cranial region have been shown to track population history (as reflected by genetic distances) well among recent human groups (Harvati and Weaver, 2006a, b). Our morphometric analysis of cranial vault shape, therefore, is likely to be informative on the phylogenetic relationships of the Cioclovina specimen.

Since this type of morphometric analysis does not accommodate missing data, some level of data reconstruction was necessary. Landmarks on specimens with minimal damage were estimated during data collection, using anatomical clues from the preserved surrounding areas. Bilateral landmarks missing on one side were estimated by mirroring-imaging, using reflected relabelling (Mardia and Bookstein, 2000), which exploits the Procrustes geometry to reflect the paired
landmarks without having to specify a mirroring plane. During this procedure, specimens with a missing or deformed curve on one side were least-squares superimposed with their reflections. The coordinates for the missing landmarks and semilandmarks were then substituted from the fitted homologous landmarks without having to specify a mirroring plane. During this procedure, specimens with a missing or deformed curve on one side were least-squares superimposed with their reflections. The coordinates for the missing landmarks and semilandmarks were then substituted from the fitted homologous

<table>
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<th>Table 1</th>
<th>Fossil comparative samples used in the analysis. In parentheses are indicated the symbols for each specimen in Fig. 5 and 8</th>
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<tbody>
<tr>
<td>Neanderthals (n = 10)</td>
<td>Amud 1 (Am)</td>
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<tr>
<td>Middle Pleistocene Europeans (n = 2)</td>
<td>Petralona (Pet)</td>
</tr>
<tr>
<td>Late Middle-Late Pleistocene fossils from Africa and the Levant (n = 6)</td>
<td>Ngaloba (LH18)</td>
</tr>
<tr>
<td>Upper Paleolithic European modern humans (n = 9)</td>
<td>Abri Pataud (AbP)</td>
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</tbody>
</table>

* indicates specimens for which high-quality casts or stereolithographs were measured.

Table 2 | Recent human comparative samples employed |
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<tr>
<td>Recent human samples</td>
<td>African Bantu (S. Africa, Kenya; WITS*, NHM)</td>
</tr>
<tr>
<td></td>
<td>Khoisan (S.Africa: SAM, UCT)</td>
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<td></td>
<td>Andamanese (Andaman Islands; NHM)</td>
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<td></td>
<td>Asian (China, Thailand; MH)</td>
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<td></td>
<td>Australasian (South Australia, Tasmania, Torres Islands; NHM)</td>
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<tr>
<td></td>
<td>New World (Utah, Alaska, Greenland; AMNH)</td>
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<tr>
<td></td>
<td>Western Eurasian (Northern Europe, Near East; LIA, MH)</td>
</tr>
<tr>
<td>Total</td>
<td>n = 263</td>
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* Abbreviations as follows. AMNH: American Museum of Natural History; LIA: Leipzig Anatomical Institute; MH: Musée de l’Homme; NHM: Natural History Museum; SAM: South African Museum; UCT: University of Cape Town; WITS: University of the Witswaterand.

Table 3 | Landmarks and semi landmarks used in the analysis |
<table>
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<tr>
<td>LANDMARKS</td>
<td>1. Glabella (GLA)</td>
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<tr>
<td></td>
<td>2. Post-toral Sulcus</td>
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<tr>
<td></td>
<td>3. Bregma</td>
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<tr>
<td></td>
<td>5. Inion (IN)</td>
</tr>
<tr>
<td></td>
<td>Superior Right and Left</td>
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<tr>
<td></td>
<td>8. 9. Mid-orbit Torus</td>
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<td></td>
<td>Inferior Right and Left</td>
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<td></td>
<td>10, 11. Frontomalar Orbitale (FMO) Right and Left</td>
</tr>
<tr>
<td></td>
<td>12. 13. Frontomalar Temporale (FMT) Right and Left &amp; 14. 15. Anterior Pterion Right and Left (PTE)</td>
</tr>
<tr>
<td></td>
<td>16. 17. Parietal Notch Right and Left</td>
</tr>
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RIDGE CURVES | 1. Supraorbital torus | 21 semilandmarks from FMT Right to FMT Left |
| 2. Midsagittal profile | 26 semilandmarks from GLA to IN |
| 3. Coronal suture | 20 semilandmarks from PTE Right to PTE Left |
| 4. Lambdoid suture | 14 semilandmarks from AST right to LBD to AST Left |

The landmarks and slid semilandmarks were superimposed with Generalized Procrustes Analysis (GPA) using the Morpheus software package (Slice, 1998). This procedure translates the landmark configurations to common origin, scales them to unit centroid size (the square root of the sum of squared distances of all landmarks to the centroid of the object, the measure of size used here), and rotates them according to minimal missing data. Such were estimated by minimizing the bending energy of the thin-plate spline between the incomplete specimen and the sample Procrustes average, following (Gunz, 2005; Gunz and Harvati, 2007).

Geometric morphometrics. All semilandmarks were iteratively allowed to slide along their curve to minimize the bending energy of the thin-plate spline interpolation function computed between each specimen and the sample Procrustes average, using the algorithm of Bookstein (1997) and Gunz et al. (2005). This allows points to slide along tangents to the curve, approximated for each semilandmark by the vector between the two neighboring points. Missing points were allowed to slide without constraining them to a curve. Spline-relaxation removes the effects of ‘digitizing error’ in the tangent direction that results from the practical necessity of having to place the semilandmarks somewhere along the curves. By sliding, they acquire geometric correspondence within the sample (Bookstein, 1997; Bookstein et al., 1999; Gunz et al., 2005; Gunz and Harvati, 2007). Semilandmark processing was performed with the software Mathematica (Wolfram Research, Inc., 2007) using routines developed by PG and Philipp Mitteroecker.

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analyses. Centroid size was analyzed separately from shape. The expected value for our evaluation of heterosis/dysgenesis was the midparental mean centroid size, with the NEA and MLP samples as the parental samples. The significance of possible heterosis or dysgenesis was assessed using a t-test with the half of the squared root of the sum of squares of the sample variance of the parental populations as standard error for the expected values, after Ackermann et al. (2006).

The landmark configuration of the Cioclovina cranium was compared with mean configurations of the MLP, UPE, Neanderthals, and recent human populations superimposed, so as to evaluate the specimen’s morphological shape-similarities after scaling. In order to further assess the shape of the Cioclovina cranium and its possible intermediate position, the Procrustes coordinates were analyzed using principal components analysis (PCA) and Mahalanobis D2 analyses. Procrustes distances among specimens were also calculated. All statistics were calculated with the statistical software packages SAS (SAS Institute, 1999—2001), NTSys (Applied Biostatistics Inc., 1986—2000), and R (R Development Core Team, 2007).

PCA visualizes the main axes of variation as scores, which are projections of the data onto the principal component axes, and as deformations. These deformations were visualized by subtracting and adding a multiple of the principal component vector to the Procrustes mean shape. Thus, a negative and a positive “extreme” shape were created, three standard deviations from the mean in each direction. We then used the thin-plate spline interpolation function calculated from these extremes to morph the surface of a single specimen, thus visualizing the shape change associated with this PC. Because this interpolation is based on minimal bending energy, it is perfectly smooth in areas not covered by semilandmarks or landmarks.

Morphological similarity among recent and fossil samples was evaluated by the Mahalanobis D2 statistic. The larger the values of the D2 distance, the farther the group centroids are from each other. The first 15 principal components were used as variables (accounting for approximately 80% of the total variance). Because sample sizes were not equal, a correction was used following (Marcus, 1993; see also Harvati, 2003b). In order to better visualize the Mahalanobis distance results a minimum spanning tree (MST) was calculated in the program NTSys (Applied Biostatistics Inc., 1986—2000). A MST is the graph that links up all specimens of the dataset with the smallest sum of distances. A MST was also calculated from the Procrustes distances among individual specimens and plotted in principal components space. This distance metric is the squared root of the sum of squared distances between two superimposed landmark configurations. In contrast to Mahalanobis D2, this distance does not take into account the group-covariance matrix, but allows the assessment of the degree of shape similarity among individual specimens in all dimensions of shape space. It also requires no prior assumptions about group membership of specimens.

Because the MST is calculated based on all dimensions of shape space, two specimens linked in the Procrustes-distance MST are close to each other not only in the projection plotted, but also in full shape space.

Results

Centroid size and heterosis/dysgenesis

The ranges of the different samples in centroid size are reported in Table 4 and Fig. 4. The means of all fossil samples, except the MPE, are significantly larger than the recent human mean. The Neanderthal mean centroid size is not significantly different from that of the MPE, UPE, or MLP, and neither are the UPE and MLP means from each other. Cioclovina is smaller than either the MLP or the Neanderthal mean, considered here as the two parental populations, and is also smaller than the midparental mean. This difference, however, is not significant (t = −1.33, p = 0.2).

Comparison of landmark and semilandmark configurations

When Cioclovina is superimposed with the Neanderthal and modern human mean configurations, it is clear that it differs dramatically from the former but is very similar to the latter (Fig. 3). It differs from Neanderthals in all aspects that differentiate the modern human from the Neanderthal mean configuration: in the vault height and curvature of the frontal and parietal bones, the shape and relative size of the occipital bone, and the shape and projection of the supraorbital region. In all these respects Cioclovina is almost identical to the modern human mean configuration, differing from it only slightly in its prominent glabella, somewhat flat frontal squama, and posteriorly rounded occipital. Its supraorbital region shows the typical modern human shape, albeit heavier than average. With respect to its somewhat flatter frontal bone and heavy browridge the Cioclovina configuration is most similar to the Australasian mean configuration among recent human samples.

Principal components analysis (PCA)

The fossil samples were separated from recent humans along the first principal component (PC 1, 27.08% of the total variance).

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<th>Mean</th>
<th>Min.</th>
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<tr>
<td>Recent humans (n = 263)</td>
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<tr>
<td>NEA (n = 10)</td>
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<td>UPE (n = 9)</td>
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<tr>
<td>Cioclovina</td>
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PC 1 completely separated Neanderthals and MPE from all modern humans (Fig. 5a). The three LMP African specimens and Qafzeh 6 spanned the gap between these two groupings, while Skhul 5 and Qafzeh 9 fell within the recent human range along this axis. Cioclovina and all the UPE specimens clustered within the recent human range of variation, with only Mladeč 5 falling just outside the recent human cloud.

PC 1 was significantly correlated with centroid size but with a very low correlation coefficient ($r^2 = 0.06; p < 0.0001$). This correlation was driven in large part by the differences in centroid size between the fossils and the recent specimens (Fig. 5b). When the samples were separated by species (Neanderthals, $H. sapiens$) there remained no significant correlation between PC 1 and centroid size. The shape differences along PC 1 are shown in Fig. 6, by superimposing the ‘left’ extreme shape in blue and the ‘right’ extreme shape (red). Neanderthal-like high PC 1 scores reflect a low and elongated cranial vault, with a continuous, superoinferiorly thick and anteriorly projecting supraorbital torus, and a wide cranial base. Modern human-like scores, on the other hand, reflect a high, rounded, and anteroposteriorly short cranial vault, supraorbital ridges whose lateral parts are neither superoinferiorly thick nor anteriorly projecting (though the glabellar part may be more pronounced), and narrow cranial bases.

PC 2 accounted for 11.48% of the total variance reflected aspects of recent human variation. Along this component, Cioclovina and the other UPE clustered approximately in the center of the modern human range.

Procrustes and Mahalanobis distances


MST demonstrates that these specimens cluster in the higher principal components, and that their position within modern human variation is not a projection artifact. Furthermore, Mladeč 5 was linked to Saldahna, suggesting morphological affinities with the earlier African/Levantine specimens. None of the UPE specimens showed links to Neanderthals or to the EMP specimens. Skhul 5 and Qafzeh 9 were not linked to each other or to other early modern fossils by smallest Procrustes distance, but were both linked to recent human specimens (see Fig. 5a, links in orange). Finally, all Neanderthal and MPE fossils were linked as a group distinct from that of modern humans, with the ‘bridge’ between these two clusters between Spy 2 and Qafzeh 6.

Mahalanobis squared distances among recent and fossil human samples were calculated from the first 15 principal components (81.5% of the total variance) and corrected for unequal sample sizes. Both corrected and uncorrected values are reported (Table 5). Cioclovina showed very large distances to both the MPE and Neanderthals, and was quite close to both the UPE and the MLP samples. It showed very small distances to most modern human groups, being closest to the sub-Saharan African Bantu, the Australasian, Asian, and W. Eurasian samples, while farthest from the New World group. The UPE also showed very large distances to Neanderthals and the MPE, and much smaller D² to modern human groups. The smallest squared distances shown by this group were to Cioclovina and, among recent groups, to the Khoisan and Australasian samples. The MLP was closest to Cioclovina and to the UPE, but also relatively close to the Neanderthals in D². The minimum spanning tree calculated from the Mahalanobis D² (Fig. 7) shows Cioclovina in a central position linking to all recent human groups as well as to the UPE and MLP sample. The MLP group in turn is joined to the Neanderthal and MPE fossils.

Discussion

This study aimed to evaluate the hypothesis that Cioclovina represents a hybrid individual between early modern humans and Neanderthals. Four predictions of this hypothesis were laid out in the introduction: 1) presence of heterosis/dysgenesis; 2) intermediate shape and/or mixed nonmetric Neanderthal and modern human features; 3) rare sutural anomalies; and 4) great individual variability among UPE specimens.

None of these predictions are met. Our analysis shows that Cioclovina is smaller than the MLP and the NEA mean in centroid size. However, it is not significantly smaller than the midpoint of the MLP-NEA mean centroid size value, and, therefore, cannot be said to be dysgenic. The specimen also does not exhibit any rare sutural abnormalities which would support developmental instability of the sort observed by Ackermann et al. (2006). Cioclovina shows no intermediate morphology between Neanderthals and early modern humans, or any affinity with Neanderthals in either the details of its anatomy or the three-dimensional shape of its vault (as reflected by the PCA, Mahalanobis D², and Procrustes distance, and including the form of its supraorbital region). Finally, the UPE sample in general shows no evidence of high shape or size variability in our analysis, despite the great extent of geography and time that these specimens span. The morphological cohesion of this sample is highlighted in the Procrustes distance MST, where eight of the nine UPE specimens, including Cioclovina, are linked.

Our analysis is by necessity limited by the fossil samples available. Several caveats can be pointed out, not least of which is the small sample size and the wide temporal and geographic dispersion of all the fossil groups used (especially of the MLP fossils). These groups cannot be said to represent...
biological populations and, therefore, ultimately perhaps cannot provide a conclusive test of the hypotheses outlined above. Nonetheless, since these are the specimens from which scenarios about the late stages of human evolution are commonly developed, we feel justified in using them to test the predictions of hybridization in a rigorous manner. While none of the individual tests are conclusive with regard to the possible hybrid status of Cioclovina, taken in combination they constitute a strong refutation of that hypothesis. The analysis presented here indicates that there is nothing other than a typical modern human.

With respect to the specimen’s sex, Soficaru et al. (2007) agreed with Smith (1984) that the specimen is male rather than female (Rainer and Simionescu, 1942), based on its large mastoid processes and other robust features. In our analysis, Cioclovina’s nearest neighbor in total shape is Abri Pataud, itself further linked to Mladeč 1 in the Procrustes distance MST. These three specimens have all been described as female. A second cluster joins the males Mladeč 5, Cro-Magnon 1, and Předmostí 3, while Předmostí 4 is linked to Cro-Magnon 3. Assuming that the sex assignment of Abri Pataud and Mladeč 1 is correct, their striking similarity in shape to Cioclovina may be interpreted as supporting the latter specimen’s assignment as female by Rainer and Simionescu (1942), contra Smith (1984) and Soficaru et al. (2007).

Finally, among recent humans, Cioclovina is closest in Mahalanobis D^2 uncorrected for unequal sample sizes (above diagonal in Table 5) to our Australasian sample, as is the UPE. Nonetheless, when the D^2 values are corrected for unequal sample sizes (Table 5, below diagonal), Cioclovina shows very small distances to several recent human groups and to the UPE sample, suggesting no close affinities with one particular group or the other. In this corrected distance matrix, the

Fig. 5. Principal component analysis. a) Scores of PC 1 vs. PC 2. Superimposed is a minimum spanning tree (MST), calculated from Procrustes distances; for the purposes of clarity, only the connections that involve fossil specimens are shown. Line-segments between two specimens of the same group are colored in their respective color; connections between members of different groups are plotted as orange lines. This MST is based on all dimensions of shape space, not only the two shown here, and thus allows one to check for projection artifacts. It is evident that archaic and modern humans are well-separated in all dimensions of shape space. b) Scores of PC 1 vs. log centroid size. Color scheme as in Fig. 4; specimen abbreviations as shown in Table 1.
UPE appear closest to Cioclovina, and among recent samples to the Khoisan and the Australasians, though they show distances of similar magnitude to all recent human groups. In both the PCA and the Mahalanobis D², Cioclovina and the UPE occupy a central position, and Cioclovina links to all recent human groups, to the MLP, and to the UPE in the Mahalanobis MST. This central position may be interpreted as reflecting an early modern human morphology that appears more or less equally dissimilar to that of all recent human groups. This morphology may be close to the ancestral modern human shape, from which recent human groups later became differentiated (see also Stringer, 1989, 2002).

The results of our geometric morphometric analysis are summarized in Fig. 8, which shows the Procrustes distance MST superimposed on a plot of the PC 1 scores of all specimens against rough divisions of time. This figure shows the

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Fig. 6. Shape differences along Principal Component 1 (PC 1). Frontal, posterior, superior and lateral views. We calculated a negative (modern human-like, blue) and a positive (Neanderthal-like, red) “extreme” shape, three standard deviations from the mean in each direction. We then used the thin-plate spline interpolation function calculated from these extremes to morph the surface of a single specimen. Because this interpolation is based on minimal bending energy, it is perfectly smooth in areas not covered by semilandmarks or landmarks.
connections of all UPE and several of the more archaic MLP specimens to recent humans, and the isolation both in PC 1 scores and in total shape of the Neanderthal (and the very limited European MPE) sample. As noted earlier, although this plot shows only one axis (PC 1), the connections between pairs of specimens, as they depend on Procrustes distances, reflect 100% of variance. Strong morphological similarity, illustrated by links in the Procrustes MST, can be interpreted as reflecting phylogenetic relationships. The most striking of our findings are the connections between the MLP specimens and modern humans, both Upper Paleolithic Europeans and recent, which imply a phylogenetic link between them. We interpret the bridge between the Neanderthal cluster, shown forming a branch with the MPE, and the MLP sample (between Spy 2 and Qafzeh 6) as reflecting greater similarity between these two samples due to more recent common ancestry (i.e., shared ancestral morphology). Although the ancestry of the MLP appears to exclude the European Middle Paleolithic specimens based on this plot, a future addition of older African specimens might change this picture.

Fig. 7. Minimum spanning tree of Mahalanobis squared distances, corrected for unequal sample size, among groups. The MST is plotted in the space of the first three canonical variates—only the groups’ centroids are plotted. The CVA used ‘population’ (Tables 1 and 2) as a grouping variable; fossil groups were treated as if they were a population.

Fig. 8. PC 1 plotted against time (in broad subdivisions), with Procrustes distance MST superimposed. Colors and abbreviations as in Fig. 4 and 5.
Conclusions

Our description and analysis rejected all four tests of the hypothesis that Cioclovina represents a Neanderthal-early modern human hybrid. The specimen does not exhibit any of the morphological patterns predicted for a hybrid. It does not show heterosis or dysgenesis, it is not intermediate either in its shape or in the details of its anatomy, and does not have a mosaic of parental features; it does not show rare sratural anomalies; and, finally, it can be accommodated quite plainly within the variability of the Upper Paleolithic sample. Cioclovina is a fully modern human in all respects examined and analyzed and there is no hint in its anatomy that it may be a Neanderthal-early modern human hybrid.

Acknowledgments

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References


