Dental maturational sequence and dental tissue proportions in the early Upper Paleolithic child from Abrigo do Lagar Velho, Portugal

Priscilla Bayle[a,1], Roberto Macchiarelli[a,b], Erik Trinkaus[c,1], Cidália Duarte[d], Arnaud Mazurier[e], and João Zilhão[f]

[a]Département de Préhistoire, Muséum National d'Histoire Naturelle, 75013 Paris, France; [b]Département Géosciences, Université de Poitiers, 86000 Poitiers, France; [c]Department of Anthropology, Washington University, St. Louis, MO 63130; [d]Departamento Património e Aprovisonamento, Câmara Municipal do Porto, 4049-001 Porto, Portugal; [e]Société Etudes Recherches Matériaux, CRI-Biopôle, 86000 Poitiers, France; and [f]Department of Archaeology and Anthropology, University of Bristol, Bristol BS8 1UU, United Kingdom

Contributed by Erik Trinkaus, December 8, 2009 (sent for review October 13, 2009)

Neandertals differ from recent and terminal Pleistocene human populations in their patterns of dental development, endostructural (internal structure) organization, and relative tissue proportions. Although significant changes in craniofacial and postcranial morphology have been found between the Middle Paleolithic and earlier Upper Paleolithic modern humans of western Eurasia and the terminal Pleistocene and Holocene inhabitants of the same region, most studies of dental maturation and structural morphology have compared Neandertals only to later Holocene humans. To assess whether earlier modern humans contrasted with later modern populations and possibly approached the Neandertal pattern, we used high-resolution microtomography to analyze the remarkably complete mixed dentition of the early Upper Paleolithic (Gravettian) child from Abrigo do Lagar Velho, Portugal, and compared it to a Neandertal sample, the late Upper Paleolithic (Magdalenian) child of La Madeleine, and a worldwide extant human sample. Some aspects of the dental maturational pattern and tooth endostructural organization of Lagar Velho 1 are absent from extant populations and the Magdalenian specimen and are currently documented only among Neandertals. Therefore, a simple Neandertal versus modern human dichotomy is inadequate to accommodate the morphostructural and developmental variation represented by Middle Paleolithic and earlier Upper Paleolithic populations. These data reinforce the complex nature of Neandertal-modern human similarities and differences, and document ongoing human evolution after the global establishment of modern human morphology.

dentition | development | modern humans | Neandertal | Pleistocene

The past two decades have seen a number of studies focused on the possibility and nature of differences between the Neandertals and modern humans in the timing and patterning of dental maturation, as well as in dental tissue proportions (1–11). With few exceptions, these analyses have compared Neandertal dental developmental attributes to the variation among later Holocene human samples. Even though partial mixed (deciduous and permanent) dentitions exist for a number of early modern humans and Middle and Upper Paleolithic associated modern human individuals contemporaneous with or close in time to the Neandertals, many of these mixed dentitions are incomplete and analyses have relied on macroscopic radiographic assessments of relative dental development and structure (1, 12–14). At the same time, it has become apparent that there were significant changes in craniofacial and postcranial morphology between the Middle Paleolithic and earlier Upper Paleolithic modern human populations of western Eurasia and that of the terminal Pleistocene and Holocene inhabitants of the same region (15–18).

It was with the goal of assessing whether the dental developmental patterns and microstructure of these earlier modern humans contrasted with later modern humans, and possibly approached the condition seen in the Neandertals, that we analyzed the remarkably complete dentition of the Lagar Velho 1 child (19). Discovered in Portugal in 1998, this fossil is the >90% complete skeleton of a child (4–5 years old on the basis of dental maturation and limb bone lengths) ritually buried in a recess in the back wall of a rock shelter, at the base of an ~3-m thick Gravettian to Solutrean stratigraphic sequence. The ^{14}C-dated components of the burial context concur in placing the burial ~24.5 ka ^{14}C BP (~30,000 calendar years ago), some five millennia after the replacement/assimilation of the last Neandertal populations of Iberia (20, 21). Preserving 46 of the possible 48 teeth (from calcified germs to complete teeth), all except one upper P4 and one lower P4 (and the four yet-to-calcify M3s) (19), the Lagar Velho 1 dentition provides a unique opportunity to assess these odontological aspects.

This analysis was carried out, not to test previous interpretations of this partial skeleton as reflecting Neandertal assimilation by early modern humans (22, 23), but to provide insight into Late Pleistocene human developmental biology and dental evolution. More specifically, to assess the similarities/differences displayed by the dental maturational status of Lagar Velho 1 (LaVe1) with respect to the developmental patterns reported for Neandertals and extant humans, we calculated the Bayesian probabilities that the mineralization sequences of its deciduous and permanent mandibular dentitions (Tables S1 and S2) are found within the variation represented by the extant human reference sample. These results were then compared with the analogous probabilities from the immature dental sequences of the 2.5- to 3.0-year-old Roc de Marsal 1 Neandertal (RdM1) (10) and the 3- to 4-year-old La Madeleine 4 late Upper Paleolithic child (Mad4) (24). Additionally, as Neandertals and extant humans show quite distinct patterns of tooth internal structural (endostructural) organization (3, 6, 8, 10, 24–28), we assessed the linear, surface, and volumetric proportions of the crown and root components (enamel, dentine, pulp) in five teeth of Lagar Velho 1 (Fig. 1 and Fig. S1), and detailed their global and topographic enamel thickness variation and tooth-specific enamel/dentine ratio.

Results

Maturational Sequences. The results of the Bayesian analysis of the series of possible maturational sequences of Lagar Velho 1 mandibular dentition are detailed in Table 1. As a whole, neither the deciduous nor the permanent sequences displayed by Lagar

Author contributions: P.B., R.M., E.T., and J.Z. designed research; P.B., R.M., C.D., and A.M. performed research; P.B., R.M., and A.M. analyzed data; and P.B., R.M., E.T., and J.Z. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. E-mail: bayle@mnhn.fr or trinkaus@artsci.wustl.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0914202107/DCSupplemental.

ANTHROPOLOGY

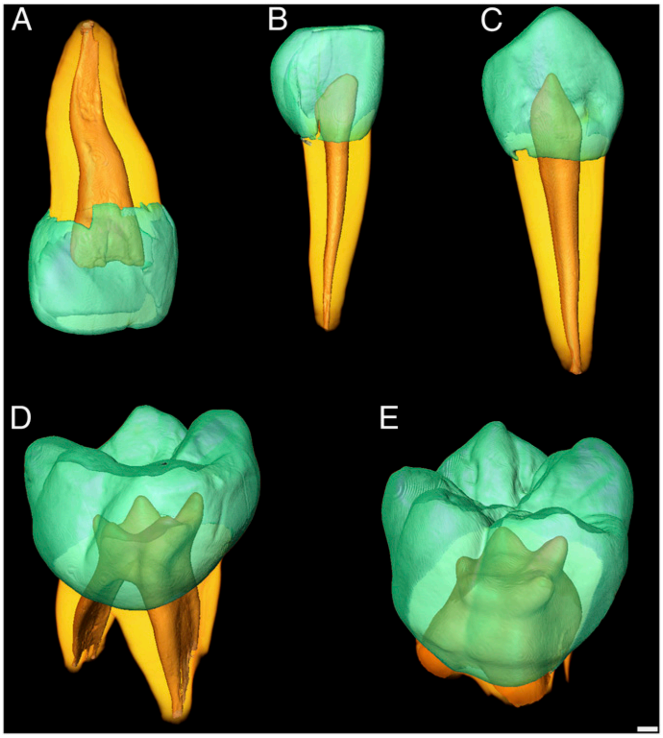

Fig. 1. Lagar Velho 1. Virtual 3D reconstruction of four deciduous and one permanent teeth assessed for linear, surface, and volumetric tissue proportions. (*A*) Deciduous upper right central incisor (Ui1; labial view). (*B*) Deciduous lower right lateral incisor (Li2; labial view). (*C*) Deciduous lower right canine (Lc; labial view). (*D*) Deciduous lower right second molar (Lm2; oblique mesio-buccal view). (*E*) Permanent lower right first molar (LM1; oblique mesio-buccal view). The volumes of the virtually reconstructed teeth are rendered in transparency, and each tooth component (enamel cap, dentine, and pulp chamber) is shown in a different color. (Scale bar, 1 mm.)

Velho 1 are found within our comparative samples. Relative to the extant human sample, its three deciduous combinations are characterized by a relative maturational delay of both incisors associated with a relative advancement of the molars, notably of the lower second deciduous molar (Lm2). In fact, in our extant sample, all of the mineralization sequences with an Lm2 at the same stage as in Lagar Velho 1 have their lower incisors either in resorption or lost. In the permanent dentition, as noted by Hillson (14), a slight discrepancy exists in Lagar Velho 1 between the degree of mineralization of the P4 and of the two molars, which are relatively advanced, and the maturational level reached by both incisors, which are relatively delayed.

For both deciduous and permanent mandibular sequences, a maturational pattern characterized by an advanced first molar stage of mineralization relative to that of the incisors has been reported for the Roc de Marsal 1 Neandertal (10). Conversely, a Bayesian analysis performed on the late Upper Paleolithic La Madeleine 4 showed that its mandibular deciduous sequence is represented in extant European populations (24).

Dental Tissue Proportions. Comparative dental tissue proportions in Lagar Velho 1, La Madeleine 4 (no data are available for Ui1 and LM1), and the Neandertal (Neand) and the extant human (EH) samples are detailed in Table S3 (see also *Methods* and Tables S4 and S5). In Lagar Velho 1, the figures displayed by the four deciduous elements for the absolute values [enamel, crown dentine, crown pulp, and crown dentine+pulp volumes, and enamel-dentine junction (EDJ) surface area] show two tendencies. Whereas the values displayed by the deciduous lower canine (Lc) and lower second molar (Lm2) are close to, or poorly differentiated from, the extant human figures (except for the crown dentine and crown dentine+pulp volumes), those shown by both deciduous incisors, and notably the Ui1 for the crown dentine and crown dentine+pulp volumes, approach the Neandertal estimates or are intermediate. Conversely, limited to the available Li2, this is not seen in La Madeleine 4.

Moreover, whereas three of these five absolute variables (crown dentine and crown dentine+pulp volumes, and EDJ surface area) show a time-related reduction trend, from Neandertals to extant humans, through the earlier and later Upper Paleolithic specimens, the same tendency is not shown by the enamel and crown pulp volumes.

The deciduous upper central incisor (Ui1) of Lagar Velho 1 fits the Neandertal figures also for the percent of the crown volume that is dentine and pulp (Fig. 2). For the Li2, the estimates for both Upper Paleolithic immature individuals are intermediate between the Neandertals and the extant humans. For the two other deciduous teeth, the differences for this relative parameter recorded among the individual specimens (LaVe1 and Mad4) and samples (Neand and EH) are negligible.

Table 1. Results of the Bayesian analysis of the Lagar Velho 1 dental maturational sequence.

		Deciduous maturational sequences			Permanent maturational sequences			
		1	2	3	I	II	III	IV
N individual comparative extant sample		45	45	45	351	351	351	351
N theoretical combinations		30	30	30	126	126	126	126
N calculated combinations		0	0	0	0	0	4	2
N sequences cf LaVe1		0	0	0	0	0	0	0
Probability balance (%)	$P < 0.25$	—	—	—	—	—	100	100
	$0.25 \leq P \leq 0.75$	—	—	—	—	—	0	0
	$P > 0.75$	—	—	—	—	—	0	0

Results of the Bayesian probabilistic analysis run for three deciduous (1–3) and four permanent (I–IV) possible maturational sequences assessed for the Lagar Velho 1 (LaVe1) mandibular dentition (Tables S1 and S2). For each deciduous and permanent sequence, a probability (*P*) that, given one Lagar Velho 1 specific maturational unit (a selected part of the sequence), its other developmental subset (the remaining part of the sequence) is found within a comparative reference sample representing, respectively, 45 (deciduous) and 351 (permanent) extant human sequences is theoretically calculable for 30 and 126 combinations (N theoretical combinations). For the probabilities associated with the effectively calculated combinations (N calculated combinations), the balance (in %) is given for both series of sequences. For all deciduous sequences and the first two permanent ones, no probability is calculable and, for the last two permanent sequences, all calculable probabilities are lower than 0.25. As a whole, the Lagar Velho 1 maturational pattern is not represented in the extant comparative sample.

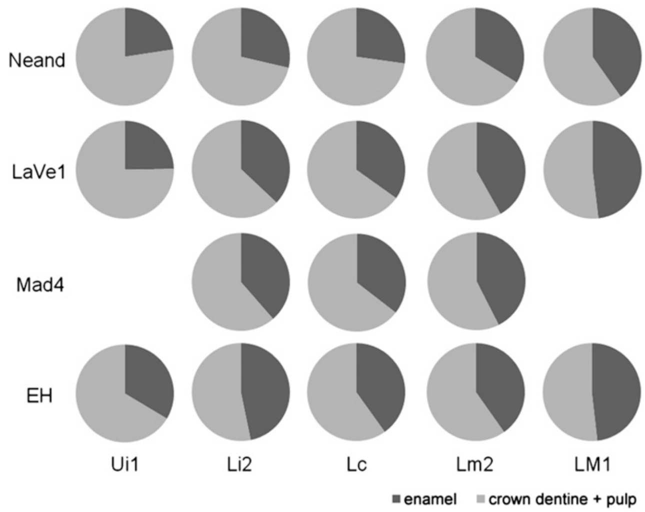

Fig. 2. Comparative estimates of the percent of the crown volume that is dentine and pulp of four deciduous and one permanent teeth for Lagar Velho 1 (LaVe1), La Madeleine 4 (Mad4; no data are available for Ui1 and LM1), Neandertal mean (Neand), and extant human mean (EH). Data are in Table S3.

The evidence of an endostructural organization of the two Lagar Velho 1 incisors, absolutely and relatively closer to the Neandertal pattern than to the extant human one, also persists when the Neandertal dental sample is reduced to include only the teeth affected by occlusal wear to the same degree as that displayed by Lagar Velho 1 (Engis 2 for the Ui1; La Chaise S14-3 and Roc de Marsal 1 for the Li2; Table S5). In this case, the Neandertal percent of the crown volume that is dentine+pulp increases from 77.4% to 78.9% for the Ui1 (vs. 75.3% in LaVe1 and a mean of 66.4% in EH) and from 71.4% to 73.3% for the Li2 (vs. 63.0% and 61.4% for LaVe1 and Mad4, respectively, and a mean of 53.2% in EH).

With the exception again of the Ui1 limited to the relative enamel thickness, both the average (AET) and relative (RET) enamel thickness values (*Methods*) recorded for all of Lagar Vehlo 1's teeth follow the EH estimates, systematically setting it apart from the Neandertals because of their lower values (Figs. S2 and S3). In this respect, notably for the two deciduous and permanent molars, the extant human-like pattern of enamel distribution displayed by this child's dentition is also evinced by the comparative thickness cartographies (Fig. S4).

For all of the absolute and relative variables describing dental tissue proportions, except the EDJ surface area, the results shown by the Lagar Velho 1's lower first permanent molar (LM1) conform to those from its deciduous Lc and Lm2; they closely resemble the extant human figures. In all three cases, the potential influence of dental wear is negligible or null (Table S5). It is noteworthy that the lower canine of Lagar Velho 1 is affected by localized enamel hypoplasia (29) (Fig. 1*C* and Fig. S1*C*). Accordingly, the values displayed by both Upper Paleolithic children for the percent of the crown volume that is dentine and pulp and for the relative enamel thickness (Table S3) should be further shifted closer to the extant human values.

Discussion

With respect to a "Neandertal versus extant" reference model, the comparative analysis of the developmental pattern and endostructural organization of the mixed dentition of the Gravettian child from the Abrigo do Lagar Velho provides contrasting results (Table 2). To some extent this is not surprising, because it reflects the modest level of knowledge that still characterizes the Middle and Upper Paleolithic odontological record. Deciduous teeth are rare for Late Pleistocene humans, and few entire sequences of the primary dentition have been detailed for their developmental status (1, 14, 24, 30–32). Also, the only two immature dentitions reported so far for their inner structural morphology and volumetric proportions are those of La Madeleine 4 (24, 27, 28) and, now, of Lagar Velho 1.

Table 2. Comparative analysis of the dental tissue proportions of Lagar Velho 1.

Lagar Velho		Neandertals		Extant humans
Ui1	Enamel*		∼	
	Dentine†	++		
	Enamel/dentine‡	++		
Li2	Enamel			++
	Dentine		∼	
	Enamel/dentine		∼	
Lc	Enamel			++
	Dentine		∼	
	Enamel/dentine			+
Lm2	Enamel			++
	Dentine	∼/+		
	Enamel/dentine			++
LM1	Enamel			++
	Dentine			+
	Enamel/dentine			++

Schematic assessment of the degree of similarity for the endostructural organization (tissue proportions) of five investigated teeth from the Lagar Velho's mixed dentition with respect to the Neandertal and the extant human conditions (based on Table S3). ∼, intermediate condition; +, condition more similar to; ++, strong similarity.
*Enamel volume, AET and RET (see Methods and Table S3).
†Crown dentine volume and crown dentine+pulp volume.
‡The percentage of the crown volume that is dentine+pulp.

The deciduous and permanent dental maturational sequences of Lagar Velho 1 are absent from the currently available Upper Paleolithic and extant human reference samples. Based on the same Bayesian statistical approach, an analogous result applies to the Roc de Marsal 1 Neandertal child (10), but not to the La Madeleine 4 late Upper Paleolithic child (24). In this respect, the mixed dentitions of the Neandertal and Gravettian individuals share a relatively delayed incisor calcification and/or a relative developmental advancement of the molars, a condition rarely if ever found in recent human populations (33, 34).

The process of relative dental mineralization is quite variable in Late Pleistocene and post-Pleistocene populations (1, 6, 30, 33, 35–39). An extensive radiographic study of immature fossil dentitions has recorded numerous similarities in relative maturational patterns of the permanent teeth between Neandertals (∼130–35 ka BP) and Upper Paleolithic specimens (∼34–11 ka BP) from Europe and southwest Asia (1, 39). Nonetheless, although both groups vary and show only minor differences with respect to the Holocene samples, a slight evolution in dental developmental patterning has been recognized through the Late Pleistocene. Also, compared to the Upper Paleolithic record, in their dental developmental patterns, Neandertals more typically evince a development of the molars relatively earlier than that of the incisors and the third premolar (1, 6, 35).

In terms of dental tissue proportions, although affected minimally by occlusal dental wear, the endostructural organization of the relatively voluminous Lagar Velho 1 deciduous upper central incisor closely approaches the Neandertal condition, characterized by absolutely and relatively larger dentine volumes and a larger EDJ surface (3, 8, 10, 24–28, 40). Conversely, whereas its deciduous lower lateral incisor has an intermediate position between Neandertals and extant humans, the remaining teeth, and notably the permanent lower first molar, fit the extant human pattern and are distinct from the Neandertals in endo-

ANTHROPOLOGY

structural tissue organization. In the case of these latter teeth, the signal is not affected by dental wear, as noted for the incisors, or by gross enamel developmental disturbances, as seen in the deciduous lower canine.

Finally, the tooth enamel topographic distribution displayed by the Lagar Velho child unambiguously gets away from the Neandertal pattern, which is characterized by an absolutely and relatively thinner enamel deposited over a larger volume of coronal dentine (3, 8, 25, 40).

A possible link between larger tooth size, which may require more time for development and completion, and delayed relative dental calcification rates has been hypothesized for the Neandertal anterior dentition (1, 10, 24, 41), even if there is no agreement about the crown formation times of their incisors (2, 4, 6). In particular, tooth structural evidence from traditional radiology (1, 30, 42–45) and microtomography and histology (3, 6, 10, 27, 28) suggests that the Neandertal relative anterior versus posterior formation stages may result from volumetric and tissue proportional differences between the anterior and posterior teeth.

Some support for this hypothesis comes from the comparison, in terms of total tooth volumes, between the four Lagar Velho 1 deciduous elements and those available in our extant reference sample displaying a comparable mineralization stage. Whereas at each tooth position Lagar Velho 1 exceeds the extant human estimates (Σ tooth volumes = 1118.4 mm^3 in LaVe1 vs. 945.2 mm^3 in EH; LaVe1/EH = 1.18), the proportions decrease mesiodistally from the incisors (LaVe1/EH = 1.27 for Ui1 and 1.33 for Li2), to the Lm2 (1.13), through the canine (1.15). Taking into account the degree of wear exhibited by the incisors in Lagar Velho 1 (29) makes this pattern appear even stronger. Conversely, with respect to the EH estimates, the volumetric differences for the three available deciduous teeth from La Madeleine 4 are absolutely and relatively lower (Σ tooth volumes = 822.2 mm^3 in Mad4 vs. 767.1 mm^3 in EH; Mad4/EH = 1.07) and especially more homogeneously distributed (Mad4/EH = 1.02 for Li2; 1.11 for Lc; 1.07 for Lm2; Table S3).

This study confirms that aspects of the Lagar Velho 1 anatomy, namely in the dentition, are unknown in extant and more recent Upper Paleolithic modern humans, and currently documented only among the Neandertals. Our results thus concur with those based on the analysis of discrete dental morphological trait variation in early Upper Paleolithic dentitions, and other morphological aspects, that they are not resolvable into a simple Neandertal versus modern human dichotomy (15–17, 46, 47). These earlier Upper Paleolithic modern humans are not simply older versions of extant humanity.

Assessment of the full paleobiological (and by extension phylogenetic) implications of these findings requires further analysis. Yet, as highlighted here, it is likely that a significant contribution will arise from the integration of detailed dental quantitative information on mineralization stages with the three-dimensional characterization of inner structural morphology and volumetric proportions, comparatively assessed on a larger sample of immature dentitions from individuals covering the last 100–200 thousand years.

Methods

To assess comparatively the Lagar Velho 1 dental maturational sequences, we considered the mandibular dentition, represented by five deciduous (Li1–Lm2) and seven permanent (LI1–LM2) elements. The degree of maturation of each tooth is based on the original descriptions (14, 29), in combination with new estimates performed on the original specimen using the Demirjian et al. (48) scoring system for the permanent teeth, revised by Liversidge and Molleson (49) for the deciduous teeth, and modified by Bayle et al. (10) (Table S1). By considering all possible combinations among the series of stage attributions performed by the different observers, sets of three (1–3) and four (I–IV) maturational sequences have been obtained for the deciduous and the permanent dentition, respectively (Table S2).

The three possible deciduous sequences displayed by Lagar Velho 1 have been compared to those assessed following the same scoring methods on a panoramic radiographic and computed tomographic (CT) -based extant human reference sample of 45 individuals (20 females and 25 males), mainly of western European origin (10, 24, and original data). Similarly, each Lagar Velho 1 possible permanent sequence has been compared to the set of maturational combinations represented in a sample of 351 living individuals (162 females and 189 males) of African, European, and southwest Asian origins, aged 3–6 years (10, 37, 50, and original data).

The statistical analysis of each dental mineralization sequence applied the approach of Braga and Heuzé (37) using Bayes' rule of conditional probability (51, 52), with teeth being considered as statistically dependent units and prior probabilities as uniform (10, 24).

Dental tissue proportions in Lagar Velho 1 have been assessed on four well-preserved deciduous teeth [the upper right central incisor (Ui1), the lower right lateral incisor (Li2), the lower right canine (Lc), and the lower right second molar (Lm2)] and one permanent tooth [the lower right first molar (LM1)] (Fig. 1). The specimens, all isolated, have been imaged using high-resolution microtomography (μCT) at the Centre de Microtomographie of the Université de Poitiers (equipment X8050-16 Viscom AG; camera 1004 × 1004). The scans were: 105 kV, 0.5 mA current, 32 integrations/projection, and a projection each 0.2°. In the case of the two lower molars, the energy was set at 110 kV and the current at 0.52 mA. The final volumes were reconstructed using DigiCT v.2.3.3 (DIGISENS) with an isotropic voxel size of 22.6 μm^3 for the three anterior teeth and of 24.2 μm^3 for the two molars.

A semiautomatic segmentation with manual corrections was carried out using AMIRA v.5.2 (Mercury Computer Systems) and Artecore v.1.0 (NESPOS Society). Threshold values between segmented components were found according to the methodology of Spoor et al. (53). Because of their good preservation and modest degree of mineralization and diagenetic change, no particular technical difficulties were encountered in segmenting the five teeth, all characterized by a rather distinct endostructural signal. Crowns were thus digitally isolated from roots (8), and surface rendering was performed using triangulation and constrained smoothing from the volumetric data [marching cube algorithm (54)].

Eight linear, surface, and volumetric variables describing tooth tissue proportions were digitally measured or calculated: the volume of the enamel cap (mm^3); the volume of the crown dentine (mm^3); the volume of the crown pulp (mm^3); the volume of the crown dentine+pulp (mm^3); the surface area of the enamel-dentine junction (EDJ; mm^2); the percent of the crown volume that is dentine and pulp; the average enamel thickness (AET; mm); and the scale-free relative enamel thickness (RET) (methodological details are in ref. 8). Notably, AET is the average straight-line distance between the EDJ and the outer enamel surface, calculated as the quotient of the enamel volume and EDJ surface area; RET is the AET scaled by the cube root of the crown dentine+pulp volume and multiplied by 100 (8, 55). Intra- and interobserver tests for accuracy of the measures run by two observers provided differences of <5%.

The 3D topographic mapping of the site-specific enamel thickness variation (Fig. S4) was realized from the segmented enamel and crown dentine components and rendered using a chromatic scale (thickness increasing from dark blue up to red).

The results from the virtual analysis of the Lagar Velho 1 dentition are compared to the microtomographic-based evidence from a sample of 26 Neandertal teeth from the sites of Abri Suard (8, 25, 27, and original data), Abri Bourgeois-Delaunay (3, 27), and Roc de Marsal (10, 27, 28) in France; El Sidrón in Spain (8); Engis (8, and original data) and Spy (40) in Belgium; the late Upper Paleolithic child of La Madeleine in France (24, 27, 28); and to a worldwide extant human dental sample of 35 teeth (3, 8, 10, 24, 27, 28, 56, and original data) (Tables S3–S5).

ACKNOWLEDGMENTS. The Portuguese Ministério da Cultura, the French Musée National de Préhistoire, the Musée d'Angoulême, the Institut Royal des Sciences Naturelles de Belgique, and the Université de Liège kindly granted access to the comparative fossil record. The Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 5199 De la Préhistoire à l'Actuel : Culture, Environnement et Anthropologie", équipe du "Laboratoire d'Anthropologie des Populations du Passé (PACEA-LAPP) of the Université Bordeaux 1 and the Institut d'Anatomie of Strasbourg provided extant human comparative materials. We acknowledge the Centre de Microtomographie at the Université de Poitiers, European Synchrotron Radiation Facility (ESRF) beamline ID17, Grenoble, and the Neanderthal Studies Professional Online Service (NESPOS) Society. Thanks go to Radmedica, Rome, the CHU Pellegrin, Bordeaux, the APHP Necker, Paris, and the Unité de Neuroradiologie of the Clinique Pasteur, Toulouse, for access to their radiographic and CT datasets. Research was supported by the EU FP6 Marie Curie Actions MRTN-CT-2005-019564 (EVAN), and the TNT Project, the NESPOS Society (http://www.nespos.org).

1. Tompkins RL (1996) Relative dental development of Upper Pleistocene hominids compared to human population variation. *Am J Phys Anthropol* 99:103–118.
2. Ramirez Rozzi FV, Bermúdez De Castro JM (2004) Surprisingly rapid growth in Neanderthals. *Nature* 428:936–939.
3. Macchiarelli R, et al. (2006) How Neanderthal molar teeth grew. *Nature* 444:748–751.
4. Guatelli-Steinberg D, Reid DJ, Bishop TA (2007) Did the lateral enamel of Neandertal anterior teeth grow differently from that of modern humans? *J Hum Evol* 52:72–84.
5. Smith TM, et al. (2007) Earliest evidence of modern human life history in North African early *Homo sapiens*. *Proc Natl Acad Sci USA* 104:6128–6133.
6. Smith TM, Toussaint M, Reid DJ, Olejniczak AJ, Hublin JJ (2007) Rapid dental development in a Middle Paleolithic Belgian Neanderthal. *Proc Natl Acad Sci USA* 104: 20220–20225.
7. Guatelli-Steinberg D, Reid DJ (2008) What molars contribute to an emerging understanding of lateral enamel formation in Neandertals vs. modern humans. *J Hum Evol* 54:236–250.
8. Olejniczak AJ, et al. (2008) Dental tissue proportions and enamel thickness in Neandertal and modern human molars. *J Hum Evol* 55:12–23.
9. Reid DJ, Guatelli-Steinberg D, Walton P (2008) Variation in modern human premolar enamel formation times: Implications for Neandertals. *J Hum Evol* 54:225–235.
10. Bayle P, Braga J, Mazurier A, Macchiarelli R (2009) Dental developmental pattern of the Neanderthal child from Roc de Marsal: A high-resolution 3D analysis. *J Hum Evol* 56:66–75.
11. Guatelli-Steinberg D (2009) Recent studies of dental development in Neandertals: Implications for Neandertal life histories. *Evol Anthropol* 18:9–20.
12. Skinner MF, Sperber GH (1982) *Atlas of Radiographs of Early Man* (Alan R. Liss, New York).
13. Tillier AM (1999) *Les Enfants Moustériens de Qafzeh. Interprétation Phylogénétique et Paléoauxologique* (CNRS, Paris).
14. Hillson SW (2002) The dental age-at-death. *Portrait of the Artist as a Child—The Gravettian Human Skeleton from the Abrigo do Lagar Velho and Its Archeological Context*, eds Zilhão J, Trinkaus E (Instituto Português de Arqueologia, Lisbon), pp 242–245.
15. Trinkaus E (2007) European early modern humans and the fate of the Neandertals. *Proc Natl Acad Sci USA* 104:7367–7372.
16. Frayer DW, Jelínek J, Oliva M, Wolpoff MH (2006) Aurignacian male crania, jaws and teeth from the Mladeč caves, Moravia, Czech Republic. *Early Modern Humans at the Moravian Gate*, ed Teschler-Nicola M (Springer, Vienna), pp 185–272.
17. Soficaru A, Doboș A, Trinkaus E (2006) Early modern humans from the Pestera Muierii, Baia de Fier, Romania. *Proc Natl Acad Sci USA* 103:17196–17201.
18. Rougier H, et al. (2007) Peștera cu Oase 2 and the cranial morphology of early modern Europeans. *Proc Natl Acad Sci USA* 104:1165–1170.
19. Duarte C, Hillson SW, Holliday TW, Trinkaus E (2002) The Lagar Velho 1 human skeletal inventory. *Portrait of the Artist as a Child—The Gravettian Human Skeleton from the Abrigo do Lagar Velho and Its Archeological Context*, eds Zilhão J, Trinkaus E (Instituto Português de Arqueologia, Lisbon), pp 221–241.
20. Zilhão J, Trinkaus E (2002) *Portrait of the Artist as a Child—The Gravettian Human Skeleton from the Abrigo do Lagar Velho and Its Archeological Context* (Instituto Português de Arqueologia, Lisbon).
21. Zilhão J (2006) Chronostratigraphy of the Middle-to-Upper Paleolithic transition in the Iberian peninsula. *Pyrenae* 37:7–84.
22. Duarte C, et al. (1999) The early Upper Paleolithic human skeleton from the Abrigo do Lagar Velho (Portugal) and modern human emergence in Iberia. *Proc Natl Acad Sci USA* 96:7604–7609.
23. Trinkaus E, Zilhão J (2002) Phylogenetic implications. *Portrait of the Artist as a Child—The Gravettian Human Skeleton from the Abrigo do Lagar Velho and Its Archeological Context*, eds Zilhão J, Trinkaus E (Instituto Português de Arqueologia, Lisbon), pp 497–518.
24. Bayle P, Braga J, Mazurier A, Macchiarelli R (2009) High-resolution comparative analysis of the dental development in the late Paleolithic child from La Madeleine. *Am J Phys Anthropol* 138:493–498.
25. Macchiarelli R, Mazurier A, Volpato V (2007) L'apport des nouvelles technologies à l'étude des Néandertaliens. *Les Néandertaliens. Biologie et Cultures*, eds Vandermeersch B, Maureille B (CTHS, Paris), pp 169–179.
26. Macchiarelli R, Bondioli L, Mazurier A (2008) Virtual dentitions: touching the hidden evidence. *Technique and Application in Dental Anthropology*, eds Irish JD, Nelson GC (Cambridge Univ Press, Cambridge, UK), pp 426–448.
27. Bayle P (2008) Analyses quantitatives par imagerie à haute résolution des séquences de maturation dentaire et des proportions des tissus des dents déciduales chez les Néanderthaliens et les Hommes modernes. *PhD thesis* (Université Toulouse III–Paul Sabatier, Toulouse).
28. Bayle P (2008) Proportions des tissus des dents déciduales chez deux individus de Dordogne (France): L'enfant Néanderthalien du Roc de Marsal et le spécimen du Paléolithique supérieur final de La Madeleine. *Bull Mem Soc Anthropol Paris* 20: 151–163.
29. Hillson SW, Santos Coelho JM (2002) The dental remains. *Portrait of the Artist as a Child—The Gravettian Human Skeleton from the Abrigo do Lagar Velho and Its Archeological Context*, eds Zilhão J, Trinkaus E (Instituto Português de Arqueologia, Lisbon), pp 342–355.
30. Legoux P (1966) *Détermination de L'âge Dentaire de Fossiles de la Lignée Humaine* (Maloine, Paris).
31. Tompkins RL (1991) Relative dental development in Upper Pleistocene hominids and recent humans. PhD thesis (University of New Mexico).
32. Henry-Gambier D (2001) *La Sépulture des Enfants de Grimaldi (Baoussé-Roussé, Italie). Anthropologie et Palethnologie Funéraire des Populations de la fin du Paléolithique Supérieur* (CTHS, Réunion des Musées Nationaux, Paris).
33. Mann A, Lampl M, Monge J (1990) Patterns of ontogeny in human evolution: Evidence from dental development. *Yearb Phys Anthropol* 33:111–150.
34. Dean MC, Beynon AD, Reid DJ, Whittaker DK (1993) A longitudinal study of tooth growth in a single individual based on long- and short-period incremental markings in dentine and enamel. *Int J Osteoarchaeol* 3:249–264.
35. Wolpoff MH (1979) Variation in modern human dental development. The Krapina dental remains. *Am J Phys Anthropol* 50:67–114.
36. Liversidge HM (2003) *Patterns of Growth and Development in the Genus Homo*, eds Thompson JL, Krovitz GE, Nelson AJ (Cambridge Univ Press, Cambridge, UK), pp 73–113.
37. Braga J, Heuzé Y (2007) Quantifying variation in human dental development sequences. An EVO-DEVO perspective. *Dental Perspectives on Human Evolution: State of the Art Research in Dental Anthropology*, eds Bailey SE, Hublin JJ (Springer, Berlin), pp 247–261.
38. Monge J, Mann A, Stout A, Rogér J, Wadenya R (2007) Dental calcification stages of the permanent M1 and M2 in U.S. children of African-American and European-American ancestry born in the 1990s. *Dental Perspectives on Human Evolution: State of the Art Research in Dental Anthropology*, eds Bailey SE, Hublin JJ (Springer, Berlin), pp 263–274.
39. Tompkins RL (1996) Human population variability in relative dental development. *Am J Phys Anthropol* 99:79–102.
40. Bayle P, Macchiarelli R (2009) The "virtual dentition" of the Spy VI child. *Spy Cave. State of 120 Years of Pluridisciplinary Research on the Betche-aux-Rotches from Spy (Jemeppe-sur-Sambre, Province of Namur, Belgium)*, eds Semal P, Hauzeur A (Royal Belgian Institute of Natural Sciences, Brussels).
41. Wolpoff MH (1971) *Metric Trends in Hominid Dental Evolution* (Case Western Reserve Univ Press, Cleveland).
42. Zilberman U, Smith P (1992) A comparison of tooth structure in Neanderthals and early *Homo sapiens sapiens*: A radiographic study. *J Anat* 180:387–393.
43. Zilberman U, Skinner M, Smith P (1992) Tooth components of mandibular deciduous molars of *Homo sapiens sapiens* and *Homo sapiens neanderthalensis*: A radiographic study. *Am J Phys Anthropol* 87:255–262.
44. Molnar S, Hildebolt C, Molnar IM, Radovčić J, Gravier M (1993) Hominid enamel thickness: I. The Krapina Neandertals. *Am J Phys Anthropol* 92:131–138.
45. Tillier AM (1996) The Pech de l'Azé and Roc de Marsal children (Middle Paleolithic, France): Skeletal evidence for variation in Neanderthal ontogeny. *Hum Evol* 11: 113–119.
46. Bailey SE, Weaver TD, Hublin JJ (2009) Who made the Aurignacian and other early Upper Paleolithic industries? *J Hum Evol* 57:11–26.
47. Ramirez-Rossi FV, et al. (2009) Cutmarked human remains bearing Neandertal features and modern human remains associated with the Aurignacian at Les Rois. *J Anthropol Sci* 87:153–185.
48. Demirjian A, Goldstein H, Tanner JM (1973) A new system of dental age assessment. *Hum Biol* 45:211–227.
49. Liversidge HM, Molleson T (2004) Variation in crown and root formation and eruption of human deciduous teeth. *Am J Phys Anthropol* 123:172–180.
50. Braga J, Treil J (2007) Estimation of pediatric skeletal age using geometric morphometrics and three-dimensional cranial size changes. *Int J Legal Med* 121: 439–443.
51. Vieland VJ (1998) Bayesian linkage analysis, or: How I learned to stop worrying and love the posterior probability of linkage. *Am J Hum Genet* 63:947–954.
52. Aitken C, Taroni F (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists* (Wiley, Chichester), 2nd Ed.
53. Spoor CF, Zonneveld FW, Macho GA (1993) Linear measurements of cortical bone and dental enamel by computed tomography: Applications and problems. *Am J Phys Anthropol* 91:469–484.
54. Lorensen WE, Cline HE (1987) Marching cubes: A high resolution 3D surface construction algorithm. *Comput Graph (ACM)* 21:163–169.
55. Martin L (1985) Significance of enamel thickness in hominoid evolution. *Nature* 314: 260–263.
56. Kono R (2004) Molar enamel thickness and distribution patterns in extant great apes and humans: New insights based on a 3-dimensional whole crown perspective. *Anthropol Sci* 112:121–146.