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# Quantifying variation in human dental development sequences: An EVO-DEVO perspective

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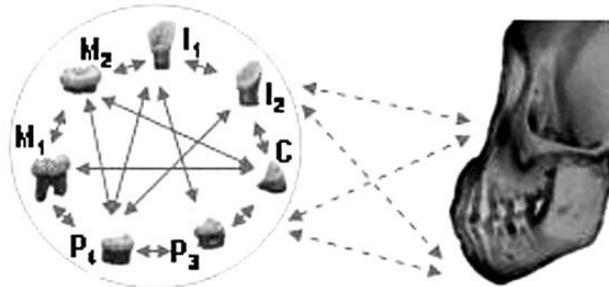
## Abstract

The present paper describes a novel analytical approach to provide a comprehensive description of the complex interactions that exist between the growing permanent mandibular teeth (excluding the third molars), and to quantify variability in sequences of key events during crown and root formation, independent of chronological age. Importantly, our method integrates the fundamental concept of modularity and rejects the old statistical fallacy of analyzing data on the assumption that it contains no information beyond that revealed on a tooth-by-tooth analysis. Indeed, interactions between growing teeth may also contain some information, which enables developmental or evolutionary information to be uncovered. Our training sample is based upon cross-sectional standardized panoramic radiographs of the teeth of a total of 2089 children (1206 girls and 883 boys) of different geographic origins (mainly Western Europe, Southern Iran, and Ivory Coast). We observe that, in extant humans sampled so far, the relative development of the permanent incisors is more plastic and varies more than for other teeth. Therefore, we consider that the quantification of possible variations between onsets, durations and rates of development of different teeth in any given child, within a large sample, is a prerequisite to the analysis of fossil hominids. In particular, we seriously question the assumption that the anterior teeth can serve as a reliable substitute for the other permanent teeth, and in particular for interpretations on somatic maturity and brain size. Our hypothesis of modularity in dental development and our method derived from this concept can serve as a basis for identifying and studying patterns of dental growth and, importantly, for comparisons between extant populations, and/or fossil species. These studies do not need to be hedged with age assessments of unknown accuracy and reliability levels (particularly in fossils), or the assumption of independence between growing teeth.

## Introduction

The aim of our cross-sectional analysis of subadult radiographs is to examine, using an appropriate method and samples from different extant human populations, the variation in the relative sequences (or patterns) of key events during crown and root formation. To date, investigators have focused on the timescale (chronology) of dental development with published median or mean ages of emergence for permanent teeth in different modern human populations (e.g., Kuykendall, 1992; Liversidge, 2003) (in some studies, emergence is often confused with eruption; see Marks and Cahill, 1993 for a definition of the latter term). The debates have focused on the existence of real differences between samples, or on biases due to sampling, rating effects, and statistical procedures (Smith, 1991). However, sequence and timing represent two separate aspects of variation. For example, the first permanent molars of two individuals can mineralize at the same time but in different sequences relative to the other teeth. Compared to variation in timing, variation in patterns is much less documented in the literature. The question of variability in patterns is fundamental to the study of dental developmental processes as a possible foundation for morphological changes during human evolution. For permanent teeth, data are available for variation in sequences of emergence (e.g., Garn et al., 1973; Smith and Garn, 1987; Nonaka et al., 1990) and for differences in sequences of development revealed only between pairs of teeth (e.g., Fanning and Moorrees, 1969; Tompkins, 1996; Liversidge and Speechly, 2001). Variability in sequences of dental development in extant humans is acknowledged by some scholars (Mann et al., 1987; Smith, 1989) but the problem has not properly addressed so far, due to lack of definition, concepts and methods (see below). Little is known of possible shifts in the sequences of formation of teeth that might be due to sex, age, jaw, and geographic origin, among other factors. It is generally assumed that “the pattern of development should be more robust than is age of stage appearance” (Smith, 1989: 77) and that there is little variability in relative sequences of key events during tooth growth. The comparative analyses of the sequences of dental development observed in fossil hominids have led to opposite views regarding their “modern human-like” or “apelike” status (Smith, 1986, 1989, 1994; Mann et al., 1987; Lampl et al., 1993; Conroy and Kuykendall, 1995). Discrepancies are due to the lack of studies of variability in patterns of dental development both within extant humans and between the two chimpanzee species. In addition to the problem of quantifying variability and the controversy over which standard is the most appropriate, other limitations on the study of dental developmental patterns are mainly methodological: (i) first, the understanding of patterns of developmental relationships should not be age related because non-adult dental age assessment is another complex problem with factors influencing its quality (accuracy and reliability) (Ritz-Timme et al., 2000; Braga et al., 2005); (ii) second, developing teeth do not mineralize randomly with respect to one another. We do not expect partial correlations between the rates of tooth formation to approach zero when controlled for age. Indeed, teeth are topographically, developmentally and functionally associated with each other. Teeth essentially grow as a unit (see below) and should be statistically considered as dependent units which grow within a developmental module (Figure 1).

The growing teeth represent a developmental module, with stable or labile interactions (to be quantified) between its hierarchical units (a tooth or a group of teeth)



126 combinations (numbered as follows, in bold) derived from a sequence comprising 7 teeth

<b>1 tooth vs 6</b>	<b>6 teeth vs 1</b>	<b>2 teeth vs 5</b>	<b>5 teeth vs 2</b>	<b>3 teeth vs 4</b>	<b>4 teeth vs 3</b>
001. $I_1, I_2, CP, P_1, M_1, M_2$	064. $I_2, CP, P_1, M_1, M_2, I_1$	008. $I_1, I_2, CP, P_1, M_1, M_2$	071. $CP, P_1, M_1, M_2, I_1, I_2$	029. $I_1, I_2, CP, P_1, M_1, M_2$	092. $P_1, M_1, M_2, I_1, I_2, CP$
002. $I_2, CP, P_1, M_1, M_2$	065. $I_1, CP, P_1, M_1, M_2, I_2$	009. $I_1, CP, P_1, M_1, M_2$	072. $I_1, CP, P_1, M_1, M_2, I_2$	030. $I_1, CP, P_1, M_1, M_2$	093. $CP, P_1, M_1, M_2, I_1, I_2$
003. $CP, P_1, M_1, M_2$	066. $I_1, CP, P_1, M_1, M_2, I_2$	010. $I_1, CP, P_1, M_1, M_2$	073. $I_1, CP, P_1, M_1, M_2, I_2$	031. $I_1, CP, P_1, M_1, M_2$	094. $CP, P_1, M_1, M_2, I_1, I_2$
004. $P_1, M_1, M_2$	067. $I_1, CP, P_1, M_1, M_2, I_2$	011. $I_1, CP, P_1, M_1, M_2$	074. $I_1, CP, P_1, M_1, M_2, I_2$	032. $I_1, CP, P_1, M_1, M_2$	095. $CP, P_1, M_1, M_2, I_1, I_2$
005. $P_1, M_1, M_2$	068. $I_1, CP, P_1, M_1, M_2, I_2$	012. $I_1, CP, P_1, M_1, M_2$	075. $I_1, CP, P_1, M_1, M_2, I_2$	033. $I_1, CP, P_1, M_1, M_2$	096. $CP, P_1, M_1, M_2, I_1, I_2$
006. $M_1, M_2$	069. $I_1, CP, P_1, M_1, M_2, I_2$	013. $I_1, CP, P_1, M_1, M_2$	076. $I_1, CP, P_1, M_1, M_2, I_2$	034. $I_1, CP, P_1, M_1, M_2$	097. $CP, P_1, M_1, M_2, I_1, I_2$
007. $M_1, M_2$	070. $I_1, CP, P_1, M_1, M_2, I_2$	014. $I_1, CP, P_1, M_1, M_2$	077. $I_1, CP, P_1, M_1, M_2, I_2$	035. $I_1, CP, P_1, M_1, M_2$	098. $CP, P_1, M_1, M_2, I_1, I_2$
		015. $I_1, CP, P_1, M_1, M_2$	078. $I_1, CP, P_1, M_1, M_2, I_2$	036. $I_1, CP, P_1, M_1, M_2$	099. $CP, P_1, M_1, M_2, I_1, I_2$
		016. $I_1, CP, P_1, M_1, M_2$	079. $I_1, CP, P_1, M_1, M_2, I_2$	037. $I_1, CP, P_1, M_1, M_2$	100. $CP, P_1, M_1, M_2, I_1, I_2$
		017. $I_1, CP, P_1, M_1, M_2$	080. $I_1, CP, P_1, M_1, M_2, I_2$	038. $I_1, CP, P_1, M_1, M_2$	101. $CP, P_1, M_1, M_2, I_1, I_2$
		018. $I_1, CP, P_1, M_1, M_2$	081. $I_1, CP, P_1, M_1, M_2, I_2$	039. $I_1, CP, P_1, M_1, M_2$	102. $CP, P_1, M_1, M_2, I_1, I_2$
		019. $I_1, CP, P_1, M_1, M_2$	082. $I_1, CP, P_1, M_1, M_2, I_2$	040. $I_1, CP, P_1, M_1, M_2$	103. $CP, P_1, M_1, M_2, I_1, I_2$
		020. $I_1, CP, P_1, M_1, M_2$	083. $I_1, CP, P_1, M_1, M_2, I_2$	041. $I_1, CP, P_1, M_1, M_2$	104. $CP, P_1, M_1, M_2, I_1, I_2$
		021. $I_1, CP, P_1, M_1, M_2$	084. $I_1, CP, P_1, M_1, M_2, I_2$	042. $I_1, CP, P_1, M_1, M_2$	105. $CP, P_1, M_1, M_2, I_1, I_2$
		022. $I_1, CP, P_1, M_1, M_2$	085. $I_1, CP, P_1, M_1, M_2, I_2$	043. $I_1, CP, P_1, M_1, M_2$	106. $CP, P_1, M_1, M_2, I_1, I_2$
		023. $I_1, CP, P_1, M_1, M_2$	086. $I_1, CP, P_1, M_1, M_2, I_2$	044. $I_1, CP, P_1, M_1, M_2$	107. $CP, P_1, M_1, M_2, I_1, I_2$
		024. $I_1, CP, P_1, M_1, M_2$	087. $I_1, CP, P_1, M_1, M_2, I_2$	045. $I_1, CP, P_1, M_1, M_2$	108. $CP, P_1, M_1, M_2, I_1, I_2$
		025. $I_1, CP, P_1, M_1, M_2$	088. $I_1, CP, P_1, M_1, M_2, I_2$	046. $I_1, CP, P_1, M_1, M_2$	109. $CP, P_1, M_1, M_2, I_1, I_2$
		026. $I_1, CP, P_1, M_1, M_2$	089. $I_1, CP, P_1, M_1, M_2, I_2$	047. $I_1, CP, P_1, M_1, M_2$	110. $CP, P_1, M_1, M_2, I_1, I_2$
		027. $I_1, CP, P_1, M_1, M_2$	090. $I_1, CP, P_1, M_1, M_2, I_2$	048. $I_1, CP, P_1, M_1, M_2$	111. $CP, P_1, M_1, M_2, I_1, I_2$
		028. $I_1, CP, P_1, M_1, M_2$	091. $I_1, CP, P_1, M_1, M_2, I_2$	049. $I_1, CP, P_1, M_1, M_2$	112. $CP, P_1, M_1, M_2, I_1, I_2$
				050. $I_1, CP, P_1, M_1, M_2$	113. $CP, P_1, M_1, M_2, I_1, I_2$
				051. $I_1, CP, P_1, M_1, M_2$	114. $CP, P_1, M_1, M_2, I_1, I_2$
				052. $I_1, CP, P_1, M_1, M_2$	115. $CP, P_1, M_1, M_2, I_1, I_2$
				053. $I_1, CP, P_1, M_1, M_2$	116. $CP, P_1, M_1, M_2, I_1, I_2$
				054. $I_1, CP, P_1, M_1, M_2$	117. $CP, P_1, M_1, M_2, I_1, I_2$
				055. $I_1, CP, P_1, M_1, M_2$	118. $CP, P_1, M_1, M_2, I_1, I_2$
				056. $I_1, CP, P_1, M_1, M_2$	119. $CP, P_1, M_1, M_2, I_1, I_2$
				057. $I_1, CP, P_1, M_1, M_2$	120. $CP, P_1, M_1, M_2, I_1, I_2$
				058. $I_1, CP, P_1, M_1, M_2$	121. $CP, P_1, M_1, M_2, I_1, I_2$
				059. $I_1, CP, P_1, M_1, M_2$	122. $CP, P_1, M_1, M_2, I_1, I_2$
				060. $I_1, CP, P_1, M_1, M_2$	123. $CP, P_1, M_1, M_2, I_1, I_2$
				061. $I_1, CP, P_1, M_1, M_2$	124. $CP, P_1, M_1, M_2, I_1, I_2$
				062. $I_1, CP, P_1, M_1, M_2$	125. $CP, P_1, M_1, M_2, I_1, I_2$
				063. $I_1, CP, P_1, M_1, M_2$	126. $CP, P_1, M_1, M_2, I_1, I_2$

For example, the combination numbered 001 and noted " $I_1, I_2, CP, P_1, M_1, M_2$ "



should be read as "the conditional probability of observing, in the training sample, the central incisor ( $I_1$ ) at its rating in the sequence to be tested when the other teeth, considered as a hierarchical unit ( $I_2, CP, P_1, M_1, M_2$ ), are observed at the ratings seen in the same sequence."

Figure 1. Modularity in dental development with 126 combinations (numbered as follows, in bold) derived from any sequence comprising 7 permanent teeth.

Any assumption of independence (in a statistical sense) between developing teeth is a fallacy. Age should be estimated not only from as many available teeth as is possible, but also by considering teeth as dependent units (Braga et al., 2005); this can be done even in the case of fragmentary remains (Heuzé, 2004); and (iii) third, usually more than two teeth are represented in sequences of dental development. The intricate relationships existing in the set of all teeth need to be examined for an accurate, reliable and comprehensive outline of dental developmental patterns.

The dental developmental status of an individual corresponds to the degree of various growth processes: the formation of crypts, the mineralization of crowns and roots, and their eruption status. Any combination of offset, duration and rate between developing teeth results in a sequence of dental development. A sequence of dental development is a series of discrete events, from the first evidence of mineralization to the closure of the root apex in all teeth (i.e., based on ratings of tooth formation made from panoramic radiographs) (Figure 1). It affects the growing teeth during pre-eruptive (movements of the growing tooth germs within the alveolar process before root formation), pre-functional eruptive (pre-emergent and post-emergent) and functional eruptive phases, when teeth move relative to each other and relative to the developing jaws. A dental mineralization sequence then corresponds to growing elementary units (teeth) embedded within the jaws, observed at a given developmental stage of an individual. Teeth are connected by many interactions that channel variation in sequences of dental development in nonrandom ways, and that lead to morphological patterns of co-variation. Moreover, even if minor, teeth also exhibit some degree of interaction with their respective jaws. We still have not clearly identified these interactions; however, experimental and clinical observations have demonstrated that tooth development and alveolar bone growth are interdependent (Marks and Cahill, 1987; Wise et al., 2002). There is no unifying analytical approach that provides a description of the dynamics of dental development. It is, therefore, important to identify within and between species both the more conservative and more plastic patterns of co-variation during dental growth. Indeed, conservative interactions among developing teeth arise primarily by strong, direct connections between their developmental pathways. In this case, developing teeth will be considered to co-vary as a unit during evolution, and represent an integrated module (Olson and Miller, 1958). On the contrary, plastic patterns of interactions arise primarily in parallel but separate developmental pathways. Therefore, analysis of the complex interactions existing between growing teeth may well represent a new field in evolutionary developmental biology (EVO-DEVO). Are developmental changes in a set of teeth accompanied by correlative changes in other teeth in extant humans and chimpanzees? How did morphological patterns of co-variation evolve in humans, and which changes in interactions between growing teeth may have produced evolutionary changes? To contribute to answering this question, we hypothesize that the developing dentition represents a developmental module with its several discernible properties defined by Raff (1996): (i) it has “an autonomous, genetically discrete organization”; (ii) it is composed of “hierarchical units and may in turn be parts of larger hierarchical entities”; (iii) it has a physical location within the developing system; (iv) it exhibits varying degrees of

connectivity to other modules; (v) it undergoes temporal transformations. Indeed, a sequence of dental development in an individual is not necessarily stable through time. For example, in a given individual examined at two successive stages A and B, of his somatic development; teeth A1 and A2 may show, at stage A, the same degree of mineralization whereas, at stage B, the same teeth, B1 and B2, may be shifted and then show two different degrees of mineralization. As a cross-sectional assessment, this study does not examine the temporal variability of a sequence in an individual. This is also the case in studies applied to fossil hominids and those based on radiographic stages.

This paper describes a novel analytical approach: (i) to provide a comprehensive description of the complex interactions existing between the growing permanent teeth (third molars excepted); and (ii) to quantify variability in sequences of dental development among extant humans at the gross anatomical level and independently of chronological age. Importantly, our method integrates the fundamental concept of modularity by rejecting the old fallacy of analyzing data on the assumption that it contains no information beyond that revealed on a tooth-by-tooth analysis. On the contrary, we believe that the phylogenetic and taxonomic significance of dental developmental patterns arises from the interactions between growing teeth, for it is these that may contain the information which enables developmental or evolutionary information to be uncovered.

## Materials and Methods

### Materials

The training sample is based on cross-sectional standardized panoramic radiographs of the teeth of 2089 children (1206 girls and 883 boys) aged 36 to 192 months. The geographic composition of the sample is presented in Table 1. These radiographs were taken when indicated by treatment need, and later digitally photographed for subsequent studies in our department (University of Bordeaux 1). Importantly, because magnification may vary and distortions are not uncommon, and also because of the absence of growth standards for tooth length in children from various geographic populations, we decided to assess mineralization radiographically by using fractions of crown and root formed (see above). We selected the children who were clinically free of anomalies in tooth numbers, size or shape. None of the children had undergone orthodontic treatment at the time of the selected radiograph. Our study was ethically approved by a French institutional board (Commission Nationale de l'Informatique et des Libertés); no regulation existed in Iran or in Ivory Coast. Our study includes the left mandibular permanent teeth except third molars, owing to the well-known practical difficulties in the radiographic visualization of the maxillary teeth. We exclude the third molar from our study for several reasons. First, this tooth is often not observed in our sample, and, second, the absence of this developing tooth may or may not correspond to its agenesis. Owing to the high variability (still to be measured across extant human populations) in the timing of the third molar development (Mincer et al., 1993), we cannot determine whether an absence of the germ corresponds to an agenesis or to a "delay" in the mineralization of this tooth.

Radiology has the disadvantage of detecting mineralization with some delay as compared to histology. However, the principal strength of radiology is that it allows a much better assessment

of developmental variability: it allows large samples of individuals of varying geographic origin to be observed and any given child will be represented by each tooth category (i.e., incisors, canine, premolars, and molars). Indeed, when microstructural studies have been conducted on relatively large samples (e.g., Yuan, 2000) (still smaller samples than in radiographic studies), all the teeth of a dentition have never been analysed.

Table 1. Geographic composition of the sample. (\*some of them being, at least in part, of European origin)

Number of individuals		Geographic origin	Age range (in months)		Parental Origin
Girls	Boys		Girls	Boys	
242	214	South of France	54 to 192	47 to 189	Four grand parents from Western Europe
392	291	South of France	42 to 192	43 to 192	Unknown, or mixed, geographic origin
249	135	Southern Iran	69 to 191	77 to 190	Iran
323	244	Ivory Coast	36 to 181	45 to 190	Ivory Coast *

### Rating Dental Development

Each tooth was assigned a developmental status from the first evidence of crown mineralization to the closure of the root apex, by using the height-stages (from A to H) system described by Demirjian et al. (1973) with its precise descriptive points, radiographs and line drawings. We believe that this rating method, with its relatively small number of formation stages, is more accurate than the 14- stage system developed by Moorrees et al. (1963a) for the permanent teeth. Reducing the number of stages decreases the possibilities of inter- and intra-observer errors. These errors also decrease with increased training in scoring. Some scholars argue that rating dental development by using fractions of crown and root formed is largely subjective, because we do not know the total length of the future completed crown or root. However, when quantifying mineralization from linear measurements taken from radiographs (even if not considering the problem of magnification and distortion; see above), we also do not know the total length of the future completed crown and root. So how can we handle the problem of variability in crown and root length in extant human populations, and how do we take it into account to assess dental development? Moreover, inter- and intra-observer errors also exist when taking measurements (only automatic, operator independent, methods are purely non-subjective). One of us (YH) rated the crown and root mineralization in the total training sample. The other author rated dental development in a fraction of our sample. Disagreement occurred in less than 5% and, at the most, by one stage (usually, at the end of the incisor root formation) (4.2%).

### Decomposing a Sequence Into Combinations

Our method aims to quantify patterns of dental development and is based on the hypothesis that any sequence represents a developmental module composed of its hierarchical units (i.e., isolated teeth or groups of teeth) (Figure 1), which exhibit varying degrees of interactions. Indeed, any method aiming to measure patterns yet ignoring this aspect in statistical analyses

by assuming independence between teeth is rooted on a circular fallacy. For example, in Smith's (1989) method, a dental mean age is assigned to each developing tooth of an individual from average standards of human and great ape development. This tooth-by-tooth age assessment method obviously assumes independence between teeth because average standards for each tooth are derived from different subsamples of individuals with differing age distributions (e.g., the average standard for the stage observed on the canine is not derived from the same individuals than the average standard observed on the first molar). Indeed, Moorrees et al. (1963a) developed the human standards used by Smith (1989) and mentioned this important limitation on the assessment of dental maturation by acknowledging "possible variation between rates of development of different teeth in a given child". As previously stated by Braga et al. (2005), the variability across age classes is more likely higher for a given radiographic stage observed on an isolated tooth than for a given mineralization sequence from which this isolated tooth is derived. In other words, individuals will be distributed in a larger age range when considering a single tooth with its radiographic stage than when considering the same tooth within a given sequence. As a consequence, we simply argue that Smith's (1989) age based method produces results which largely depend on the independence assumption. However, this method has the advantage that it is easy to use with a simple program and with only stages of dental development as input. Our aim is also to devise an easily used program, but one that is not based on age assessments and an assumption of independence between growing teeth. Inversely, we use the concept of modularity.

Degrees of interactions between isolated teeth, or groups of teeth, can be measured by decomposing any sequence into two subsets with no element in common (Figure 1). Decomposition begins because any sequence comprising 7 developing permanent teeth can be represented by a rearrangement of its seven elements, where none are lost, added, or changed, but distributed into two subsets (each comprising one tooth or more, and noted "sub.k" and "sub.q"; where  $k + q = n$  and  $n = 7$ ), which are combined by a conditional probability. Any sequence of dental development with seven teeth can then be decomposed in a finite number of combinations (as opposed to permutations because in combinations the order of the teeth in each subset is not important, e.g., a subset comprising the canine and the lateral incisor will be identical to the subset comprising the lateral incisor and the canine). Such combinations correspond to conditional probabilities (Figure 1) of observing one subset at radiographic stages (a prior event) as we condition on the attained developmental status of the other subset (a posterior event). The total number of combinations derived from 7 teeth by grouping into two subsets, is 126 (Figure 1). This number is given by the so-called binomial coefficient. Importantly, this decomposition approach is suitable for use with fragmented remains (e.g., fossil hominids). In this case, the total number of combinations will decrease according to the number of preserved teeth.

#### Calculating Conditional Probabilities

The probability of observing, in our training sample, each of the 126 combinations derived from any complete (7 teeth) sequence of dental development, is calculated using Bayes's rule of conditional probability, with teeth being considered as statistically dependent units (as opposed to "Naive Bayes", with units are considered as independent). The Bayesian approach has recently been used in non-adult dental age assessment from ratings of crown and root formation,

considered as statistically dependent units (Braga et al., 2005). The conditional probability of observing the development status of “subset q” (noted “sub.q”), as we condition on the developmental status of “subset k” (noted “sub.k”), is written as follows:

$P(\text{sub}q/\text{sub}k) =$

$$\frac{P(\text{sub.k} / \text{sub.q}) \times P_{\text{prior}}(\text{sub.q})}{[P(\text{sub.k} / \text{sub.q}) \times P_{\text{prior}}(\text{sub.q})] + [P(\text{sub.k} / \text{sub.q}) \times P_{\text{prior}}(\text{sub.q})]}$$

The term  $P(\text{sub.k}/\text{sub.q})$  represents, for subset q, the observed proportion of individuals evincing, in the training sample, subset k. The term  $P(\text{sub.k}/\text{sub.q})$  represents, for all subsets differing from subset q, the observed proportion of individuals evincing subset k (see Braga et al., 2005; for more details about the computations). Crucial to this approach is the selection of appropriate prior probabilities (“priors”). A comparison of conditional probabilities from different trials requires the assumption that individuals are derived from samples of balanced distributions of radiographic stages. Training samples never exhibit unbiased distributions of radiographic stages, because they are constructed by “availability” sampling. Consequently, in absence of any specific prior knowledge, we assume that each mineralization stage has the same prior probability to be observed (a so-called “uninformative prior”). The Bayesian procedure is then effective in removing part of the factors of confusion due to sampling bias. As the uniform priors appear in both numerator and denominator, by factorization, they have no influence on the calculation of the conditional probabilities.

### Bootstrapping and Mean Conditional Probabilities

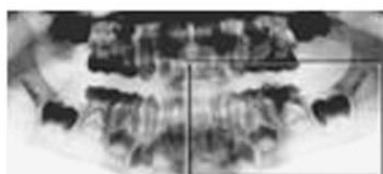
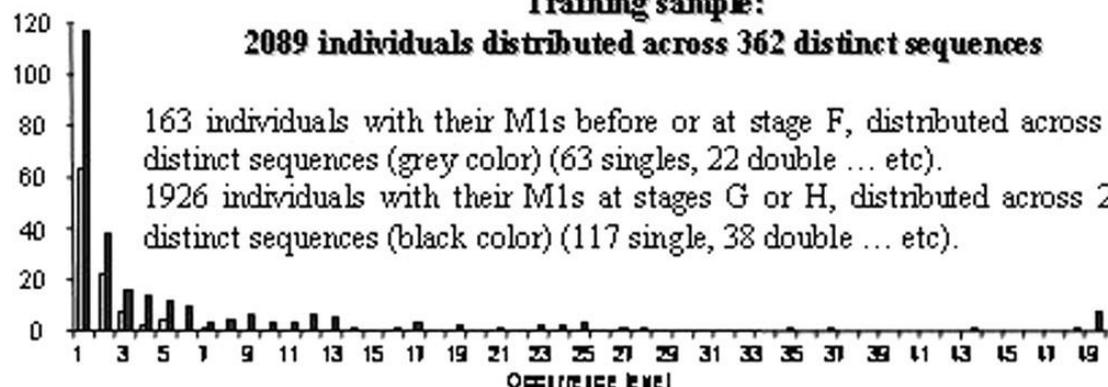
Because of finite training sample size, it is possible that some dental mineralization sequences in extant human populations will not be sampled. Bootstrapping is a way of testing the reliability of our training sample, i.e., the degree to which adding or removing individuals in the training sample changes the estimate of variability in sequences of dental development. Therefore, we generated 1000 pseudoreplicate datasets by randomly resampling with replacement 1000 individuals from our training sample (bootstrapping). Consequently, instead of deriving one conditional probability from our total training sample, we obtained distribution of 1000 randomized conditional probabilities for each of the 126 combinations. The mean value of each distribution is then used as a measure of the likelihood of each of the 126 combinations (Figure 2). If this mean probability is higher or equal to 0.75 it is considered to be likely. Inversely, if the mean probability is lower or equal to 0.25 the combination is considered to be unlikely

(Figure 2). A mean probability ranging between 0.25 and 0.75 is considered to be not informative (Figure 2).

Let us now consider the example given in Figure 2. Using Demirjian et al.'s (1973) rating system for all permanent teeth (except the third molar), from mesial to distal, the sequence tested is written as follows: G, F, F, E, E, G, E. In this example, 4 combinations (numbered 22, 58, 85 and 121) out of 126, are unlikely, with their mean probabilities falling under 0.25. As shown in Figure 1, the combinations numbered 22, 58, 85 and 121 correspond, respectively, to the following conditional probabilities:  $CM_2$  if  $I_1I_2P_3P_4M_1$ ,  $CP_4M_2$  if  $I_1I_2P_3M_1$ ,  $I_1I_2P_3P_4M_1$  if  $CM_2$ ,  $I_1I_2P_3M_1$  if  $CP_4M_2$ . Therefore, from these combinations, we can conclude that, in this sequence, the developmental rating(s) of the canine and/or the second premolar and/or the second molar deviate significantly from what we observe in our training sample. When we now examine the combinations corresponding to the developmental status of each of these three teeth taken separately, versus of all other permanent teeth (i.e., C if  $I_1I_2P_3P_4M_1M_2$ ,  $P_4$  if  $I_1I_2CP_3M_1M_2$ ,  $M_2$  if  $I_1I_2CP_3P_4M_1$ ,  $I_1I_2P_3P_4M_1M_2$  if C,  $I_1I_2CP_3M_1M_2$  if  $P_4$ , and  $I_1I_2CP_3P_4M_1$  if  $M_2$ ; corresponding, respectively, to numbers 3, 5, 7, 66, 68 and 70), we observe that the lowest probabilities are for the relative dental development of the second molar. Therefore, we conclude that this tooth shows an abnormal developmental status in this sequence.

### Training sample:

#### 2089 individuals distributed across 362 distinct sequences



For each individual to be tested with this training sample



#### Independence assumption

#### Dependent approach

The sequence is decomposed into 126 combinations; bold numbers, see Figure 1).  
For each of the 126 combinations (represented below, from right to left), we calculate the mean of 1000 probabilities obtained after 1000 resamplings with replacement.

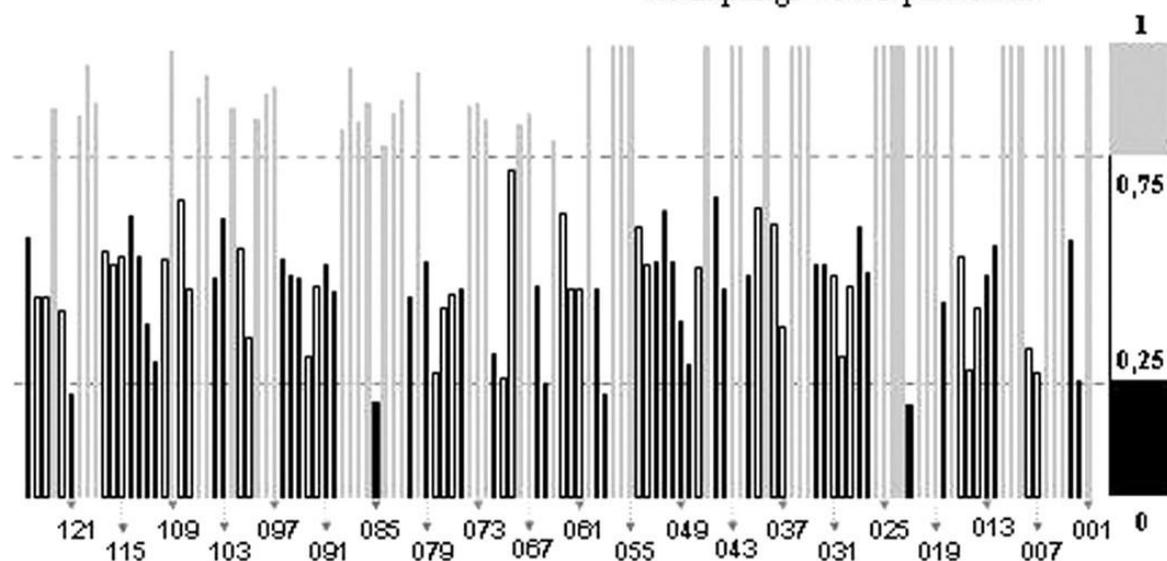


Figure 2. Example of a sequence of dental development with each of its 126 combinations corresponding to a conditional probability. For each combination, we calculate the mean of 1000 probabilities obtained after 1000 resamplings with replacement.

In order to automate the calculations, Dr. F. Houët (from our department) created a Microsoft Excel 2000® Visual Basic macro to determine the relative frequencies of subsets  $q$  and  $k$ , and to apply Bayes's rule of conditional probability. This program is easy to use, with only stages of dental development as input. We will make it accessible through a web-site in the future.

### Testing Sequences and Cross-Validation

After devising our method, which decomposes any sequence into 126 combinations (Figure 1), and which estimates the generalization error (using bootstrapping) for each of the corresponding 126 conditional probabilities (Figure 2), we need to test each sequence of dental development by using a leave-one-out cross-validation. We test sequences of dental development rather than individuals because most individuals share a common sequence, as illustrated in Figure 2 (see above). After cross-validation, the generalization error of our model can be assessed with a representation of the likelihood and the variability of each of the 126 combinations. For each combination, the likelihood and its variability are represented, respectively, by the mean conditional probability and its coefficient of variation, calculated across all sequences that have been tested (Figure 3). This method can then help us to identify the more conservative and the more plastic combinations, or morphological patterns of covariation during dental growth, in our training sample.

### Results

In our training sample, 1926 individuals out of 2089 had their first permanent molars at stages G or H (roots completed or almost completed; see Demirjian et al., 1973). These 1926 individuals do not share the same developmental sequence. Instead, they are distributed across 263 different sequences that need to be tested. As shown in Figure 2, some of these 263 sequences with the M1 at stages G or H (117), are represented only once in our training sample. This is the lowest occurrence level for a sequence. These sequences are called "single" as opposed to "multiple". Each single sequence corresponds only to one individual. Each multiple sequence corresponds to two or more individuals. Multiple sequences demonstrate different occurrence levels. Nevertheless, as the occurrence level increases, the number of distinct sequences showing this occurrence level, decreases rapidly in the training sample (Figure 2). In our training sample, when we consider only the 1926 individuals with their first permanent molars at stages G or H, there are 117 distinct single sequences (sequences occurring once, each representing only one individual), 38 distinct double sequences (sequences occurring twice, each representing two individuals), 16 distinct triple sequences, and so on (see Figure 2). When we now consider, in our training sample, the 163 individuals with their first permanent molars before or at stage F, we observe the same trend: as the occurrence level increases, the number of distinct sequences showing this occurrence level decreases rapidly.

For each combination (bold numbers, see Figure 1),  
 across all sequences that have been tested,  
 the likelihood (mean probability across sequences)  
 and variability (coefficient of variation)  
 have been calculated

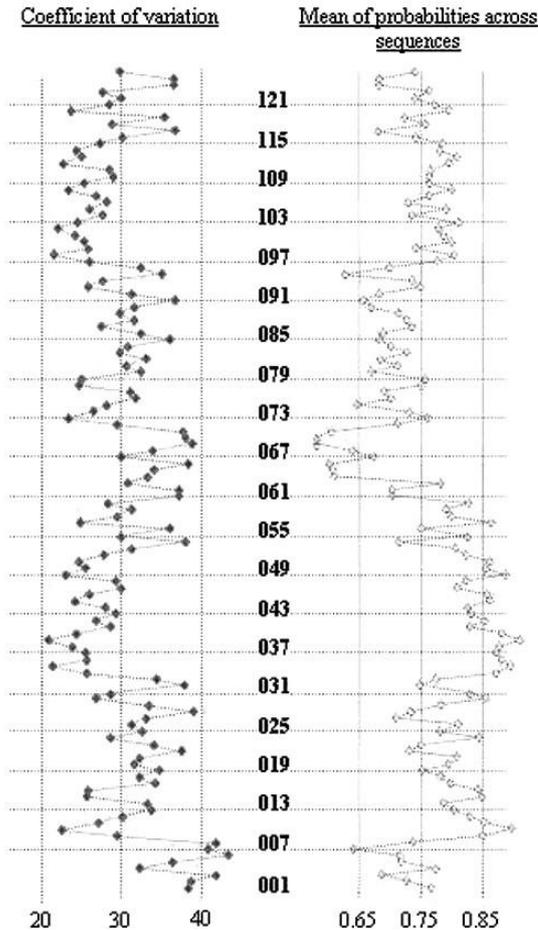


Figure 3. Leave-one-out cross validation in our training sample: 182 multiple sequences (see Figure 2) have been tested.

We now examine which of the 126 combinations are plastic or conserved across the 182 multiple sequences that were tested. In Figure 3, the combinations are numbered and ordered as in Figures 1 and 2, with their corresponding mean probabilities (ranging from 0.58 to 0.91) and coefficients of variation (ranging from 20.8 to 43.9). Assuming a normal distribution of all 126 mean probabilities, 95% are within two standard deviations (within 0.62 and 0.90). The following six combinations fall outside this range:  $I_2CP_3P_4M_1M_2$  if  $I_1$ ,  $I_1CP_3P_4M_1M_2$  if  $I_2$ ,  $I_1I_2P_3P_4M_1M_2$  if  $C$ ,  $I_1I_2CP_3P_4M_2$  if  $M_1$ ,  $I_1I_2CP_3P_4M_1$  if  $M_2$ ,  $CP_3P_4M_1M_2$  if  $I_1I_2$  (respectively, numbers 64 to 66 and 69 to 71) (Figure 3). Five out of six of these combinations represent the developmental status of six permanent teeth given the developmental rating of one isolated tooth. From these results, the premolars do not seem to deviate significantly from the other teeth during dental growth. Interestingly, the sixth combination falling outside the 95% confidence

interval of the distribution of all mean probability values corresponds to the combination numbered 71: CP<sub>3</sub>P<sub>4</sub>M<sub>1</sub>M<sub>2</sub> if I<sub>1</sub>I<sub>2</sub> (Figure 1). This means that, in any sequence that was tested, the probability of observing the permanent incisors at their relative developmental status, in the training sample, is low. The corresponding low mean probability is associated with the second higher coefficient of variation (41.9) (the highest – 43.9 – is for combination number 6 which is associated with a mean probability falling inside the 95% confidence interval). In other words, these results mean that the mandibular incisors are generally developmentally more plastic compared to other permanent mandibular teeth.

## Discussion

Robert (2004: 129) proposes “...a broad interpretation of evo-devo, one according to which the development of whole organisms and the evolution of their modular parts are deemed the primary analysands, rather than as secondary to the epigenetic expression of purely or primarily genetic potential.” Robert (2004: 129) goes on to write: “Such is the force of taking development seriously. A developing system is clearly organized, but in a systemic way; that is, the interrelations between its parts are structured into causal, generative systems with ontogenetic control dispersed throughout these systems”. In this evo-devoist perspective, we believe that our results are important. Using radiographic studies (Smith, 1989; Tompkins, 1996), scholars have asserted that there is “a modern human-like sequence of dental development” that was already present in *Homo erectus* and Neanderthals (Dean et al., 2001; RamirezRozziandBermúdezdeCastro,2004). It is assumed that “a modern human-like sequence of dental development”, as opposed to various human-like sequences, characterizes extant humans and their Upper and Middle Pleistocene fossil relatives. This assumed ‘modern human-like sequence’ is then used to compare fossil hominids represented by single, or few, isolated teeth and to understand tooth (especially enamel) growth processes (Dean et al., 2001; Ramirez Rozzi and Bermúdez de Castro, 2004). In particular, this assumption has been used in microanatomical (or histological) analyses to argue that, the anterior teeth can serve as a reliable substitute for the other permanent teeth (e.g., the molars). It has also been used for interpretations of somatic maturation and brain size. However, even if microanatomical studies now allow the study of small samples of children and the quantification of variation in rates of enamel formation of a given tooth (usually, the permanent incisors), we still have not qualified possible variability between rates of development of different teeth in a given child.

The results of our basic analytical approach (Figure 3) suggest that the developmental relationships between, on the one hand, the permanent incisors (one subset), and on the other hand, the canine, premolars and molars (another subset), are the most statistically plastic in extant humans. Therefore, these two subsets of teeth may well represent two hierarchical units within the same developmental module. Obviously, before applying our method to fossil hominids (preferably in single fossil hominids rather than in fossil samples mixing different periods, populations, or even species), our results need to be confirmed by further testing in extant human populations. We should add that the incisor developmental instability and variation demonstrated in this study may arise primarily by parallel variation of separate developmental pathways and simultaneous effects by an extrinsic source of variation. This source could be the developmental constraints due to the growing premaxilla or incisive bone, which may represent another modular unit. In this case, the developing canine, premolars and

molars could be considered to co-vary as a hierarchical unit during evolution, arising primarily by strong connections between developmental pathways.

This study aimed to assess variation in the relative sequences (or patterns) of key events during formation of crowns and roots from samples representing different extant human populations, by using a method that does not assume tooth independence. Our training sample is biased because early radiographic stages (from A to F), especially for early developing permanent teeth (incisors and first molar), are underrepresented (Figure 2). Indeed, a high proportion of individuals with their first permanent molars before or at stage F, is represented by single dental mineralization sequence (63 individuals out of 163; i.e., 38.7%) (Figure 2). For individuals with their first permanent molars at stages G or H, this proportion decreases considerably (117 individuals out of 1926; i.e., 6%) (Figure 2). A temporary limitation of our method, which does not assume independence, is that no computations can be made for those individuals represented by unique dental development sequences. For these unique sequences, a “Naïve Bayes” approach can easily be developed but, again, will not be biologically realistic (because of the independence assumption) (Heuzé, 2004; Braga et al., 2005). Therefore, in future studies, we need to sample more individuals with their first molars before or at stage F. More sampling will also allow us to quantify possible sexual dimorphism and/or geographic variation of sequences of dental development across extant human populations.

So far, our results are limited to extant humans with their very limited lower facial prognathism coupled with reduced jaw elevator muscles (when compared with chimpanzees). An interpretation of our results in a totally different context of facial morphology and growth would be misleading. Importantly, we still need data on dental development in the pygmy chimpanzee and close attention to the interrelationships between jaw and lower/upper tooth growth in both chimpanzee species in which the patterns of closure of the premaxillary (or incisive) suture are totally different (Braga, 1998) but also in extant humans. In this context, we suggest that it would be premature to make direct comparisons, using our method, between extant humans and chimpanzees, or even, between fossil hominids and chimpanzees, in which the premaxilla (containing the alveolar ridge of the four upper incisors), acting as a stabilizing element within the facial skeleton, plays a totally different role in both craniofacial growth and morphology.

Finally, more testing is needed to determine how stable a crown and root-formation sequence is through ontogeny. In other words, would our results be the same using a different sample with a much younger age distribution? Indeed, Moorrees et al. (1963a, b) have demonstrated that later formation stages vary more in their timing than early stages and later-forming teeth vary more in their timing than early forming teeth. However, timing and pattern are two separate things and this trend for timing and its implications for developmental patterns need to be confirmed. This question cannot be addressed simply by examining pairs of developing teeth and testing whether the stage of development of one given tooth increases when another given tooth may increase its range of calcification. This kind of simplistic observation may represent a false correlation caused by the influence of a third single tooth or group of teeth. Therefore, as previously stated, all possible combinations between teeth need to be examined. Nevertheless, our results suggest a developmental instability of the incisors and integration of the developing canine, premolars and molars. We regard this result as consistent with findings

suggesting that the first permanent molar provides indications of overall somatic maturity and brain size in living primates (Smith, 1989).

## Conclusions

Dental development studies fall mainly into two methods of observation, which now co-exist but which succeeded each other historically, the first method leading to the hypothesis of an evolutionarily early appearance of a human-like dental development and the later method giving birth its negation. Indeed, scholars first used radiographs and attempted to focus on dental macrostructural development. Pioneering comparative studies (Mann, 1975; Skinner, 1978; Skinner and Sperber, 1982) suggested that early hominids followed a human-like schedule of dental growth. This hypothesis of an evolutionarily early appearance of a human-like dental development was established on the principal strength of developmental variability in both extant humans and the great apes (Mann et al., 1987). The opposing hypothesis was built up with a different method of observation, which was based on the study of incremental growth features in enamel to estimate age at death of fossil hominids (Bromage and Dean, 1985). These microanatomical observations are based on the record of a precise timescale for enamel developmental events on growth increments. A large number of studies (e.g., Dean, 1987; Beynon et al., 1998; Dean et al., 2001) now consider this latter approach as a more secure model on which to base studies of the paleodemography, growth and maturation of early hominids. This led to the more general consensus that truly human-like dental development emerged relatively late in human evolution because all early hominids documented thus far, including some earliest fossils attributed to *Homo*, did not resemble modern humans in their development. These two views can now be reconciled because the conceptual framework of modularity enables us to build new methods to achieve an agreement. More precisely, modularity and EVO-DEVO (Raff, 1996; Bolker, 2000) offer a new conceptual framework and new perspectives to provide a picture of the variability in sequences of dental development, as well as in enamel and dentine growth processes, as possible foundations for morphological changes during human evolution. Indeed, our hypothesis of modularity in dental development and our method derived from this concept can serve as a basis for identifying and studying patterns of dental growth and, importantly, for comparisons populations or species that do not need to be hedged with age assessments based on a tooth-by-tooth analysis and its fallacies. At this stage, we suggest that our first comprehensive quantification of variability in patterns of development of permanent teeth in three extant human populations opens an EVO-DEVO perspective. Therefore, we aim to contribute to EVO-DEVO in the future by examining modularity/patterning in fossil hominid samples and by further testing (e.g., influence of sex, age and geographic origin) of the conclusions presented here.

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