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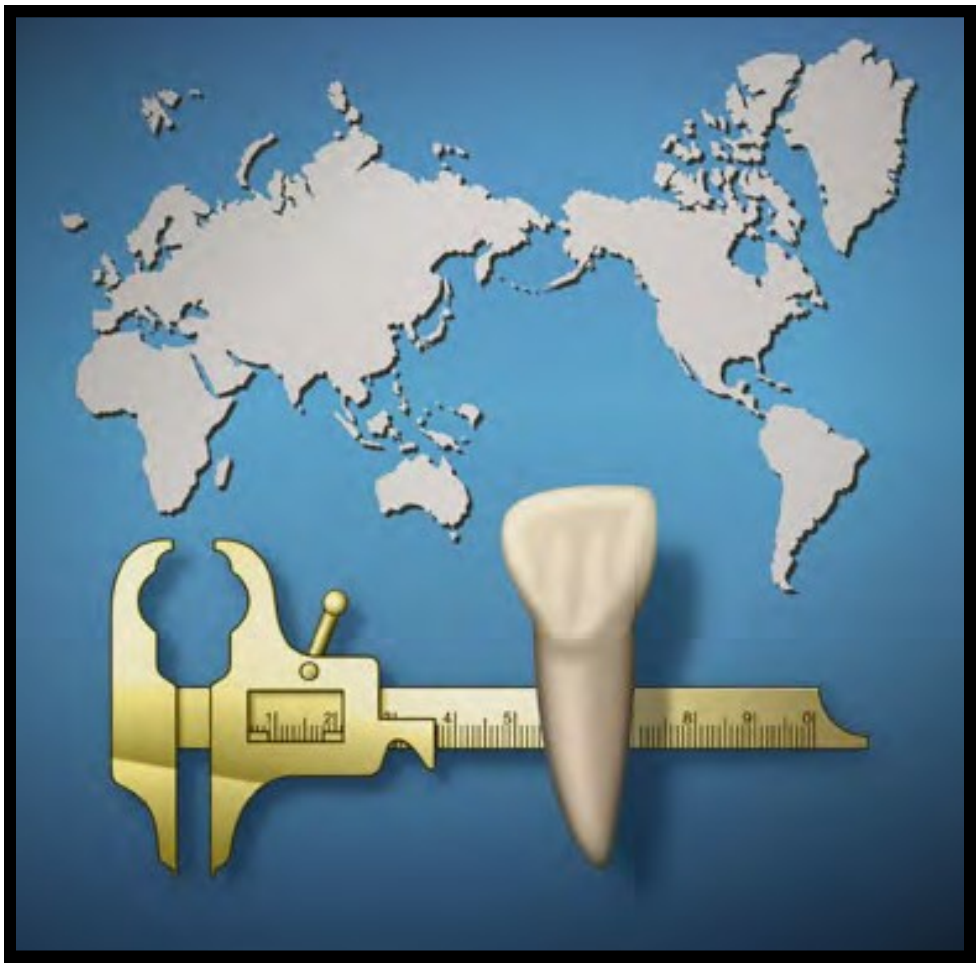

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Childhood Variation in Skeletal and Dental Development

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ABSTRACT The existing research comparing variation in developmental timing of skeletal and dental systems has focused on cross-sectional correlations of group means throughout late childhood. We used a longitudinal sample of 100 White American girls to compare developmental variation from 3-12 years to improve our understanding of developmental variation. The sample was divided into two sets (dental and skeletal) of three subgroups (delayed, average, or advanced) based on development at age three. Repeated measure ANOVA and Tukey's HSD analyses examined the longitudinal maturation of: 1) skeletal development of skeletal subgroups, 2) dental development of skeletal subgroups, 3) dental development of dental subgroups, and 4) skeletal development of dental subgroups.
The four models demonstrated significant differences between subgroup developmental trajectories. Pairwise comparisons of same-system development (analyses 1 and 3) found all comparisons to be significant; this was not the case for pairwise comparisons across systems (analyses 2 and 4). Only the advanced group was consistently different across all combinations.
Results suggest that the pace of development differs among delayed, average, and advanced individuals, and between dental and skeletal systems. Therefore, to fully explore the relationship between the systems, the full range of variation in the timing of development is required.

The skeletal and dental systems have long been subjects of study in humans. Existing studies of the two systems focus either on the sample mean expression or on the correlation between the two systems across the entire sample. This paper examines whether the variation between the systems' developmental trajectories varies between individuals who were delayed, average, or advanced in their development at an early age. Individuals within the average subgroup are included as a baseline with which to compare how the developmental trajectories of delayed and advanced individuals differ.

The general order and timing of how a juvenile develops into an adult is consistent among individuals. Here, **development** is used to refer to the change and refinement in shape of objects from their juvenile form to their completed adult appearance (Greulich & Pyle, 1959; Moorrees, Fanning, & Hunt, 1963). This is to differentiate development from **growth,** which refers to changes in size (Ogden et al., 2002; WHO Multicenter Growth Reference Study Group, 2006). The overall order of development, the order at which different bones and epiphyses form and fuse or teeth mineralize, is canalized. **Canalization** refers to the fact that developmental reactions "adjust so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction" (Waddington, 1942:563). The canalization of the skeletal and dental systems has long lent these systems to being used to estimate chronological age (Greulich & Pyle, 1959; Moorrees, Fanning, & Hunt, 1963; Tanner, 1978).

As the development of the skeletal and dental systems roughly correspond to chronological age, it follows that the two systems should be correlated. The correlation is not perfect due to variation between, and even within, individuals. Variation within and between individuals is inherent to canalization (Flatt, 2005; Waddington, 1942). A pletho-

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ra of environmental, genetic, and epigenetic factors contributes to the range of variation. Regardless of the cause or influence, the entire range of skeletal and dental developmental variation between people is the **inter-individual variation in developmental timing (IVDT)**.

The environment can influence IVDT either as developmental stressors (nutritional or pathological) or via developmental plasticity. Developmental plasticity is the phenotypic response to the environment given an individual's genetic and epigenetic profile (Hochberg et al., 2011; Houston & McNamara, 1992; Kuzawa, 2012; Wang et al., 2014). Genetic variation and developmental plastic variation are susceptible to evolutionary forces, referred to at the inter-species level as heterochrony (Bogin, 1997; Love, 2014). An example of genetic and epigenetic differences in IVDT include the known difference between the sexes (Badyaev, 2002; Ogden et al., 2002); males are known to develop more slowly on average than females (Badyaev, 2002; Greulich & Pyle, 1959).

Differences in ancestry also must be considered when using developmental timing standards, as a method developed for one population may not be accurate for another population. This can result in either under or overestimation of an individual's developmental age (Haiter-Neto, Kurita, Menezes, & Casanova, 2006; Zhang, Sayre, Vachon, Liu, & Huang, 2009). Additionally, differences in population histories (e.g. famine or slavery) can delay or slow the development of disadvantaged groups (Jasienska, 2013; Ribot & Roberts, 1996).

Non-evolutionary related variation over time also can occur. This is secular change, which is often associated with variation in environments such as improved nutrition and increased caloric intake (Garn, 1987). While the effects of secular change on the skeletal system and on total body size have been known for well over a century (Boas, 1912), the possibility of secular change affecting the dental system is a more recent field of study (Cardoso, Heuze, & Julio, 2010; Nadler, 1998; Rautman & Edgar, 2013). Regardless of the many causes, origins, and types, the entire range of variation is included in IVDT.

When the two systems are compared to each other, rather than to chronological age, a common finding is that the skeletal system is more susceptible to environmental and developmental stressors than is the dental (Cardoso, 2007b, 2007a; Demirjian, Buschang, Tanguay, & Kingnorth Patterson, 1985; Flores-Mir, Mauicio, Orellena, & Major, 2005; Lewis & Garn, 1960). Large discrepancies between chronological age and either skeletal or dental age may be an indicator of an underlying disease or condition or of some other developmental stressor.

Numerous studies of the skeletal and dental systems have considered the systems individually and together (Cardoso, 2007b; Hunt & Gleiser, 1955; Lauterstein, 1961; Lewis & Garn, 1960). Existing studies comparing the development primarily focus on mean/median/modal developmental phenotype, or else the correlation across the entire sample. The mean (most commonly reported) phenotype is crucial to understanding the development of that phenotype. However, the mean expression is not informative about the range of possible variation. Such studies assume that the approach to development is the same across the range of IVDT, and that the mean expression is sufficient. By reporting or considering standard deviation in addition to the mean, more focus is placed on the range of variation (Al-Juboori, Saloom, & Al-Bustani, 2012; Bagherpour, Pousti, & Adelianfar, 2014; Gupta, Divyashree, Abhilash, Bijle, & Murali, 2013; Sachan, Sharma, & Tandon, 2011). Similarly, studies which utilize correlations do consider the entire range of variation (Anderson, Thompson, & Popovich, 1975; Arora, 2009; Bagherpour et al., 2014; Lauterstein, 1961; Saglam & Gazilerli, 2002). Such studies assume that the skeletal and dental correlation is the same across the entire range of IVDT. They ignore the possibility that the relationship between the systems may vary through the IVDT range.

The current research considers whether the relative relationship between the skeletal and dental systems is the same throughout the range of IVDT by comparing the correlation of skeletal and dental development between subgroups whose skeletal or dental development was delayed, average, or advanced early in life. Subgroups are here defined independently by either the completed skeletal or dental development at age three. The entire sample was divided into subgroups each with 20% of the total IVDT. Of the resulting five quantiles per system (five skeletal and five dental), only three per system were considered in the subsequent analysis. These three were those 20% who were delayed; those who were average, the middle 20%; and those 20% who were advanced, all at age three. The delayed, average, and advanced *skeletal* quantiles were based on percentage of completed skeletal development at age three; while the delayed, average, and advanced dental quantiles were based on percentage of completed *dental* development at age three. The subsequent skeletal and

dental development of each quantile were compared. The null hypothesis was that the relationship would be the same between the three skeletal quantiles and between the three dental quantiles. However, we predicted that the developmental trajectories would vary between those who were delayed, average, or advanced at age three. If the developmental trajectories were to vary between the three quantiles per system's IVDT, this would indicate that the relationship between the skeletal and dental systems is more complicated than is understood from the general assumption based on a consideration only of means or total sample correlations. This analysis of IVDT does not address the cause of the observed variation, nor should the findings be interpreted as being the result of a specific cause of variation.

Materials and Methods

The sample consists of 100 healthy females from the Bolton-Brush Growth Study, who were described by the study designers as White, of seemingly normal development, and who were without known major pathological conditions. Only one sex was considered for this study to avoid potential complication based on known sex differences in rates of development (Greulich & Pyle, 1959; Stinson, 1985). In order to remove sex as a confounding variable females were chosen as they develop more quickly than males (Greulich & Pyle, 1959; Humphrey, 1998).

The Bolton-Brush Growth Study is a combination of two related studies, the Brush Inquiry and Broadbent-Bolton Study, both of which began in the late 1920s in Cleveland, Ohio. The Brush Inquiry began in 1926 (Nelson, Hans, Broadbent Jr., & Dean, 2000) (or 1928 (Behrents, 1984)) in order to study how healthy, normal children grew and developed (Nelson et al., 2000). Included among the data from this study are radiographs of the postcranial skeleton, information on the mental and physical health and growth of the child, and information about the child's family and home environment (Nelson et al., 2000). In 1929, the Broadbent-Bolton Study began with the initial purpose of understanding the dentofacial growth and development of normal, healthy children (Hans, Broadbent Jr., & Nelson, 1994). This study included radiographs of the head and the hand-wrist, dental casts, and information on the health and developmental environment of each child. Although the two studies were independent, many participants were included in both studies. Of those individuals in the Brush Inquiry, 73% also participated in the Broadbent-Bolton Study, while 67% of those in the Broadbent-Bolton Study were also in the Brush Inquiry (Hans et al., 1994; Nelson et al., 2000). Not all participants joined the studies at the same age. However, participants were seen every three months when less than one-year-old, every six months from one to five years old, and once a year after age five.

The selection criteria for the present study were that each girl must have been seen within three months of her third, sixth, ninth, and 12th birthday. In cases in which pairs of sisters were seen at all four ages, only one sister was included. Birth dates ranged from January 1928 to May 1934 and were distributed as evenly as possible during this window. For each visit, the lateral cranial and hand-wrist radiographs were used to measure skeletal and dental development.

Skeletal and Dental Development

The level of skeletal development was determined by visual observation of left hand-wrist radiographs. The stage of development of 15 bones at 11 sites was determined using Greulich and Pyle's atlas of hand and wrist development (Greulich & Pyle, 1959) to quantify the development of carpals, metacarpals, the radius, and ulna (Table 1). When a bone (e.g. trapezium) or epiphysis (e.g. first metacarpal) had not yet begun ossification, it was scored "1" (Greulich & Pyle, 1959). When assignment to Greulich and Pyle's Stage 1 stated that ossification had already begun (e.g. scaphoid: "Stage 1: ossification usually begins from a single center, pg. 201), radiographs that showed no sign of ossification were scored as zero. Radiographs that were too blurry or out of focus to determine the devel-

Table 1. Skeletal development sites (Greulich and Pyle, 1959) with the range of ordinal stages and the total number of stages used to calculate obtained level of development. Stage "0" was added and defined as prior to the beginning of ossification.

Development Site	Range of Stages	Number of Stages
Proximal 1st phalanx	1 to 10	10
Distal 2nd – 4th metacarpals	1 to 9	9
Distal 5th metacarpal	1 to 9	9
Trapezium & 1st metacarpal	1 to 12	12
Trapezoid & 2nd metacarpal	1 to 10	10
Capitate & Hamate	0 to 10	11
Scaphoid	0 to 8	9
Lunate	0 to 8	9
Triquetral & Pisiform	0 to 8	9
Radius	1 to 11	11
Ulna	1 to 11	11

opment at a site were scored as "non-observable" and excluded from further analysis.

One author (ALMR) determined the level of attained dental development by visual examination of the permanent dentition as observed from lateral radiographs. The presence or absence of each tooth was noted, as was the stage of development. Stages were determined using Moorrees et al. (1963) stages from AlQahtani et al.'s (2010) dental age estimation chart (Table 2). Due to the nature of lateral radiographs, differentiating the central versus lateral incisor was complicated and was solved by scoring only one, presumably the first central incisor in both the maxilla and mandible. Although orthopantomograms are better suited for observing individual tooth development, the Moorrees et al. (1963) method was developed based on lateral radiographs. Furthermore, orthopantomograms are a more recent technological image and not commonly available in longitudinal studies such as the Bolton-Brush Growth Study. Additional teeth scored included maxillary and mandibular canines, third and fourth premolars, as well as first, second, and third molars. Siding was not possible, but only one tooth at each position was scored. When the quality of the radiograph or the angle prevented positively identifying a specific tooth, the tooth was scored as "non-observable."

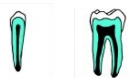
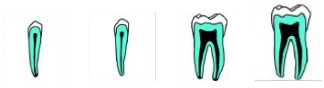
Intra-Observer Error

To test for consistent scoring, a subset of 20% of the radiographs were randomly selected to form an intra-observer data subset. This subset of 78 hand-wrist radiographs and 80 lateral cephalograms were then scored a second time. The numeric and "non-observable" scores per hand-wrist location and tooth were included. All scores within the intra-observer subset were compared between rounds of observations using a weighted Cohen's Kappa test (Viera & Garrett, 2005) using the statistical package R x64 3.2.3. Data from repeat observations were used only for the intra-observer test and were not included in further analyses.

Developmental Level Scoring

At each age, a composite score of percentage of attained skeletal and dental development was calculated for each individual. Hand-wrist radiographs with fewer than seven scored sites were excluded from analysis. Skeletal ordinal stages were converted into numbered levels (see Table 1). Ratios per site of percent development obtained were calculated based on the sites' number of stages and then a composite score of average skeletal

Table 2. Dental development stages (Moorrees et al., 1963; AlQahtani et al., 2010) and their description. The number in the Stage column is the number used to calculate level of obtained development.

Stage	Description	Stage	Description
A-NP 0	Tooth absent, formation not yet begun. Comparison between ages was used to distinguish from congenitally absent teeth.	Crc 6	Crown complete with defined pulp roof 
Ci 1	Initial cusp formation 	Ri 7	Initial root formation 
Cco 2	Coalescence of cusps 	R ¼ 8	Root length less than crown length. Posterior teeth have visible bifurcation area. 
Coc 3	Cusp outline complete 	R ½ 9	Root length equals crown length 
Cr ½ 4	Crown half complete with dentine formation 	R ¾ 10	Three quarters of root length developed with diverge ends 
Cr ¾ 5	Crown three quarters complete 	Rc+ 12	Root length complete. With parallel ends or closed apex. 

development was calculated. Similarly, lateral radiographs with fewer than eight scored teeth were excluded from analysis. The ordinal stages of dental development were converted into numbered levels (see Table 2), ranging from zero for teeth whose formation had yet to begin, to 12 for completely formed teeth. Numeric levels were then converted to percentages of completed development and a composite score of average dental development was calculated. The final sample sizes of usable radiographs per age varied from 92 to 97 for skeletal development and 90 to 97 for dental development (Table 3a).

Determining Quantile Subgroups: Delayed, Average, and Advanced

Quantiles used for analysis included individuals who were delayed, average, or advanced in their skeletal or dental development at age three, representing the range of normal variation. To define these quantiles, we divided the entire sample into two matched sets of five subgroups. Each set of quantiles was defined either by the percentage of completed skeletal development (skeletal quantiles) or the percentage of completed dental development (dental quantiles) at age three. When defining each set of quantiles, the systems were considered independently. Therefore, an individual's ranking of skeletal development influenced only their classification in the skeletal quantiles and did not influence the placement in the dental quantiles, and vice versa. The delayed quantile includes those individuals who had achieved the least amount of development, those in the lowest 20th percentile. The average quantile included individuals in the middle quantile, those whose development was between the 40th and 60th percentiles. The advanced quantile contained the most developmentally advanced individuals, those in the highest 20th percentile.

Subsequent analyses comparing developmental trajectories were based on two sets of three quantiles: delayed, average, and advanced *skeletal* development quantiles; and delayed, average, and advanced *dental* quantiles. All six quantiles were of similar size (Table 3b). Although quantiles were defined based on one system at a time, some individuals fell in quantiles of interest for both systems (Table 3c). By using these six quantiles, four questions could be examined:

For quantiles based on *skeletal* development:
1) How do the *skeletal* developmental trajectories compare between *skeletal* quantiles?
2) How do the *dental* developmental trajectories compare between *skeletal* quantiles?

For quantiles based on *dental* development:
1) How do the *dental* developmental trajectories compare between *dental* quantiles?
2) How do the *skeletal* developmental trajectories compare between *dental* quantiles?

Table 3. Sample sizes after percent of obtained development was calculated. (a) Total sample size of usable radiographs prior to quantile assignment. (b) Sample size per skeletal and dental quantiles of interest. (c) Sample size of individuals who were in the quantiles of interest in both systems.

(a) Sample pre-quantiles assigned

Age	Dental	Skeletal	Both
3	90	92	84
6	93	97	90
9	97	97	95
12	97	95	92
all ages	83	84	72

(b) Per quantile of interest by system

	Delayed	Mean	Advanced
Skeletal development based quantiles (skeletal quantiles)	18	18	18
Dental development based quantiles (dental quantiles)	19	18	19

(c) Per quantiles of interest for both systems

		Skeletal quantiles		
		Delayed	Mean	Advanced
Dental quantiles	Advanced	3	6	4
	Mean	3	1	4
	Delayed	5	1	5

Statistical Analysis

Descriptive statistics were calculated for the total original sample and for each quantile of interest at all four ages. Additionally, attained development composite scores were plotted against exact chronological age. For each plot, logistic growth curves (Fox & Weisberg, 2010) were calculated and added to the plots. Repeated measure ANOVA was used to test the significance of statistical models based on each question. These statistical models incorporated the composite score of obtained development

for a given system (*Development*) as the dependent variable and individual (*Pt.ID*), chronological age (*Age*), baseline quantile group (*Q.subgroup*), and the interaction of age and baseline quantile group as the independent variables, with repeated measures by individual (*Pt. ID*).

Development ~ Pt.ID +Age+ Q.subgroup

Model 1 corresponds with the first question: How do the *skeletal* developmental trajectories compare between *skeletal* quantiles? Therefore, in Model 1 the dependent developmental variable is skeletal development, and the *Q.subgroup* are the three skeletal baseline quantile groups. Model 2 corresponds to the second question and uses dental development for the dependent variable, and uses the same three skeletal baseline quantile groups for the *Q.subgroup*. This pattern continues through the remaining two questions.

When the repeated measure ANOVA showed the statistical model to be significant, a Tukey's HSD (honest significant difference) test was run for pairwise comparison of quantile groups. Analyses were completed using R x64 3.2.3 and STATA/IC 11.2 statistical programs.

Results

Weighted Cohen's Kappa test (Viera & Garrett, 2005) of intra-observer error showed consistent agreement in development scores. For dental development, the weighted Cohen's Kappa was 0.811, demonstrating almost perfect agreement, while the weighted Cohen's Kappa for skeletal development was 0.792, demonstrating substantial agreement and falling just below the 0.81 cutoff for almost perfect agreement (Landis & Koch, 1977).

In agreement with previous studies (Cardoso, 2007b; Flores-Mir et al., 2005; Lewis & Garn, 1960), descriptive statistics show that, overall (Table 4a), skeletal development is more variable than dental development at all ages (Table 5a). However, this difference did not hold up for all quantiles of interest, as for some quantiles, the variation in dental development was greater than for skeletal.

Results for Quantiles Based on Skeletal Development

For skeletal based quantiles, the descriptive statistics (Table 4b) fail to demonstrate consistently greater skeletal development variation than dental (Table 5b). At age three, the reverse is true for all three quantiles. The dental development continues to be more variable at age six for the average and advanced quantiles. At age nine, the dental variation is greater only for the advanced quantile, but at age 12, only the average quantile demonstrates higher dental variation than skeletal. These differences suggest that there are differences in skeletal and dental development between those who were delayed, average, or advanced in their skeletal development at age three.

Model 1: Skeletal Developmental Trajectories of Skeletal Quantiles

Figure 1a depicts skeletal developmental trajectories of the three skeletal quantiles versus exact chronological age. The three lines represent the logistic growth curves per skeletal quantile. The difference in mean age per quantile decreases continuously between the delayed and advanced quantiles as the individuals age. Despite the narrowing differences, the three quantiles continue to follow their own trajectories. A repeated measure ANOVA was run on Model 1, comparing the skeletal developmental trajectories of the three skeletal quantiles (Table 6a). The model was found to be significant ($F=179.43$; $p<0.0001$). Age, as well as the interaction of age and skeletal quantile, was also significant. The R-squared value for the model was 0.9869. Based on the model's significance, a Tukey's HSD pairwise comparison was run to test the effect each quantile's pairing had on the complete model (Table 6b). This test demonstrated that all three comparisons between the skeletal quantiles were significantly different in their mean scores.

Model 2: Dental Developmental Trajectories of Skeletal Quantiles

Figure 1a and 1b depict the developmental trajectories versus exact chronological age of the same individuals within the skeletal quantiles of interest. However, while Figure 1a compares the skeletal development, Figure 1b compares the dental development. The trajectories of the delayed and average quantiles are similar and, in fact, cross over each other. A repeated measure ANOVA was run on Model Q2, comparing the dental development of the three skeletal quantiles (Table 7a). The model was significant ($F=128.47$; $p<0.0001$). The influence of age was significant in Model 2, as it was in Model 1. However, unlike Model 1, the interaction between age and the skeletal quantiles was not significant ($p=0.4578$). The R-squared was 0.9822, which is slightly lower than that for Model 1 yet still a high value. Because the model was significant, a Tukey's HSD pairwise comparison was run (Table 7b). The results of this test differ from those of Model 1 in that not all the pairwise comparisons

Table 4. Descriptive statistics of the skeletal and dental development for the entire sample, (b) three skeletal quantiles of interest, and (c) the three dental quantiles of interest.

(a) Total sample's development				
	Skeletal		**Dental**	
	mean	SD	mean	SD
age 3	0.3226	0.0630	0.2762	0.0364
age 6	0.5372	0.0628	0.5199	0.0561
age 9	0.7083	0.0658	0.7326	0.0503
age 12	0.8906	0.0483	0.9107	0.0405

(b) Skeletal based Quantiles								
			Delayed-Skeletal		Average-Skeletal		Advanced-Skeletal	
			mean	SD	mean	SD	mean	SD
Development	Skeletal	age 3	0.2424	0.0250	0.3146	0.0058	0.4170	0.0401
		age 6	0.4654	0.0532	0.5521	0.0458	0.6059	0.0337
		age 9	0.6585	0.0522	0.7174	0.0554	0.7815	0.0499
		age 12	0.8890	0.0512	0.8976	0.0377	0.9081	0.0460
			Delayed-Skeletal		Average-Skeletal		Advanced-Skeletal	
			mean	SD	mean	SD	mean	SD
	Dental	age 3	0.2758	0.0303	0.2613	0.0320	0.2819	0.0410
		age 6	0.4987	0.0498	0.5154	0.0601	0.5486	0.0639
		age 9	0.7265	0.0503	0.7145	0.0443	0.7527	0.0632
		age 12	0.9022	0.0324	0.8995	0.0625	0.9281	0.0355

(c) Dental based Quantiles								
			Delayed-Dental		Average-Dental		Advanced-Dental	
			mean	SD	mean	SD	mean	SD
Development	Dental	age 3	0.2256	0.0182	0.2783	0.0053	0.3265	0.0219
		age 6	0.4974	0.0270	0.5230	0.0568	0.5298	0.0648
		age 9	0.7033	0.0424	0.7358	0.0525	0.7647	0.0517
		age 12	0.9026	0.0529	0.9160	0.0362	0.9199	0.0469
			Delayed-Dental		Average-Dental		Advanced-Dental	
			mean	SD	mean	SD	mean	SD
	Skeletal	age 3	0.3153	0.0607	0.3181	0.0592	0.3274	0.0852
		age 6	0.5348	0.0820	0.5237	0.0603	0.5335	0.0616
		age 9	0.7100	0.0814	0.7006	0.0720	0.7135	0.0706
		age 12	0.8716	0.0613	0.8912	0.0409	0.9128	0.0300

Table 5. Calculated difference of the variation (as measured by standard deviation) between skeletal and dental development.

a) SD: TS – TS

Total sample: skeletal develop. – dental develop.	
Age 3	0.0266
Age 6	0.0066
Age 9	0.0155
Age 12	0.0078

(b) SD: Q2 – Q3

Skeletal quantiles: skeletal develop. – dental develop.			
	Delayed	**Average**	**Advanced**
Age 3	-0.0053	-0.0261	-0.0009
Age 6	0.0034	-0.0144	-0.0303
Age 9	0.0018	0.0111	-0.0132
Age 12	0.0188	-0.0247	0.0104

(c) SD: Q4 – Q1

Dental quantiles: skeletal develop. – dental develop.			
	Delayed	**Average**	**Advanced**
Age 3	0.0426	0.0539	0.0632
Age 6	0.0549	0.0035	-0.0032
Age 9	0.0390	0.0196	0.0189
Age 12	0.0084	0.0048	-0.0169

Table 6. Model 1: Skeletal development between skeletal quantiles. Pt.ID: individual; Age: patient's chronological age; Skeletal.Q: patient's skeletal quantile.

(a) Repeat Measure ANOVA

Number of observations =218				R-squared =	0.9869
	Root MSE =	0.0305		Adj R-squared =	0.9814
Source	Partial SS	df	MS	F	Prob > F
Model	10.7138	64	0.1674	179.43	0
Pt.ID	0.5387	55	0.0098	10.5	0
Age	9.7514	3	3.2505	3483.98	0
Skeletal.Q	0	0			
Age # Skeletal.Q	0.1280	6	0.0213	22.87	0
Residual	0.1427	153	0.0009		
Total	10.8565	217	0.0500		

Between-subjects error term:	Age # Skeletal.Q
Levels:	12 (6df)
Lowest b.s.e. variable:	Age
Covariance pooled over:	Skeletal.Q (for repeated variable)
Repeated variable:	Pt.ID

(b) Tukey's HSD

studentized range critical value (.05, 3, 153) =				3.3472
uses harmonic mean sample size =				72.608
quantile vs quantile	quantile means		mean dif	HSD-test
delayed vs average	0.5563	0.6174	0.0612	17.0724*
delayed vs advanced	0.5563	0.6767	0.1205	33.6101*
average vs advanced	0.6174	0.6767	0.0593	16.5377*

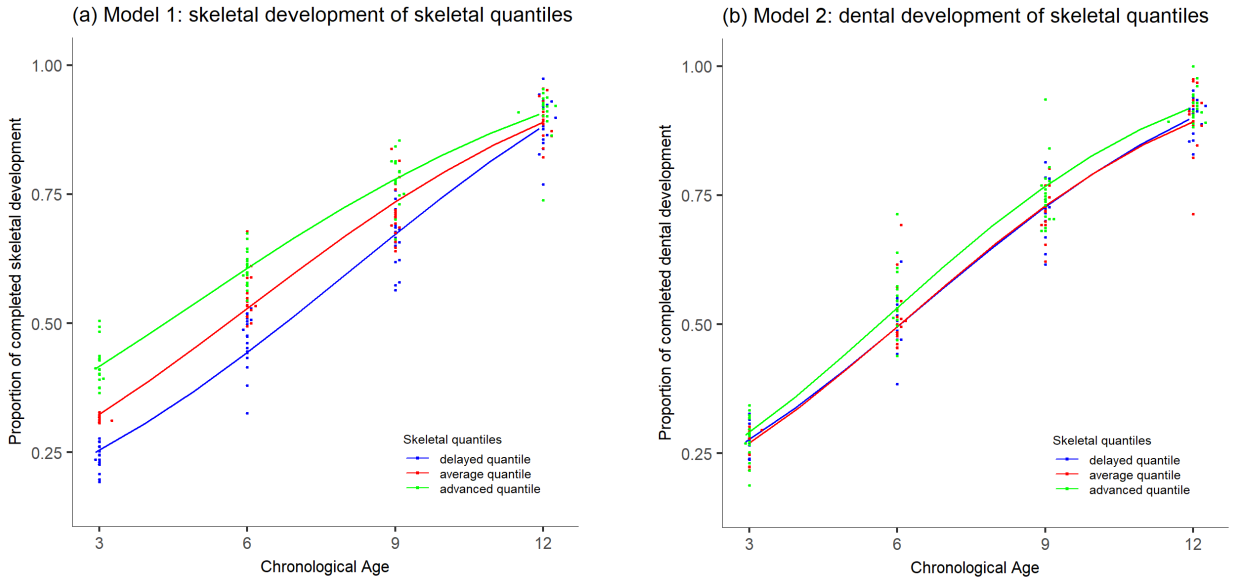


Figure 1. Logistic growth curves of skeletal quantiles: chronological age versus development. Plots of chronological age by proportion of completed skeletal (a) or dental (b) development based on skeletal development at age three, the delayed, average, and advanced skeletal quantiles. Logistic growth curves depict the developmental trajectories taken by those within each quantile.

are significant. Only those comparisons that include the advanced quantile are significant, while the interaction between the delayed and average quantiles is not.

Table 7. Model 2: Dental development between skeletal quantiles. Pt.ID: individual; Age: patient's chronological age; Skeletal.Q: patient's skeletal quantile.

(a) Repeat Measure ANOVA

Number of observations =214			R-squared = 0.9822		
Root MSE = 0.0388			Adj R-squared = 0.9746		
Source	Partial SS	df	MS	F	Prob > F
Model	12.3599	64	0.1931	128.47	0
Pt.ID	0.2582	55	0.0047	3.12	0
Age	11.9463	3	3.9821	2648.89	0
Skeletal.Q	0	0			
Age # Skeletal.Q	0.0086	6	0.0014	0.96	0.4578
Residual	0.2240	149	0.0015		
Total	12.5839	213	0.0591		
Between-subjects error term:		Age # Skeletal.Q			
Levels:		12 (6df)			
Lowest b.s.e. variable:		Age			
Covariance pooled over:		Skeletal.Q (for repeated variable)			
Repeated variable:		Pt.ID			

(b) Tukey's HSD

studentized range critical value (.05, 3, 149) = 3.3480
uses harmonic mean sample size = 71.292

quantile vs quantile	quantile means		mean dif	HSD-test
delayed vs average	0.6064	0.6030	0.0034	0.7330
delayed vs advanced	0.6064	0.6319	0.0255	5.5630*
average vs advanced	0.6030	0.6319	0.0289	6.2960*

Results for Quantiles Based on Dental Development
The descriptive statistics of the dental based quantiles (see Table 4c) demonstrate that, for the delayed and average quantiles, dental development is consistently less varied than skeletal development (see Table 5c), as is predicted. However, the advanced quantile varies by age in terms of which systems' development has greater variation. At ages three and nine the skeletal development is more varied, while ages six and 12 have greater variation in the dental development.

Model 1: Dental Developmental Trajectories of Dental Quantiles
In Figure 2a, the dental developmental trajectories of the three dental quantiles versus the exact age is shown. Model 3 is similar to Model 1 in that the system's development being measured (dental for Model 3, skeletal for Model 1) is the same as the system upon which the quantiles were defined. From age three to age 12, the difference in dental development between the delayed-dental quantile and the advanced-dental quantile decreases. However, the decrease does not occur continuously, as it does for Model 1. The dental development of the three dental quantiles was compared by a repeated measure ANOVA (Table 8a). As was the case with Models 1 and 2, Model 3 was significant ($F=154.32$; $p<0.0001$). Corresponding to the observed significance of Model 1, in Model 3 age was significant, as was the interaction of age and dental quantile. The R-squared was 0.9848. As Model 3 was significant, Tukey's HSD was again run. The results of the Tukey's HSD demonstrated that all three pairwise comparisons between the dental quantiles were significant (Table 8b). This consistent significance of the pairwise comparisons is similar to Model 1, in which the skeletal development was compared between the skeletal quantiles.

Model 2: Skeletal Developmental Trajectories of Dental Quantiles
Figure 2b depicts the skeletal developmental trajectories of those individuals whose dental development was delayed, average, or advanced at age three. This mixed combination of systems is similar to Model 2, although Model 4 includes the same individuals as Model 3. The repeated measure ANOVA of Model 4 (Table 9a) found that the model was again significant ($F=98.95$; $p<0.0001$). The pattern of significance for Model 4 matches that of Model 2. Age was significant, while the interaction between age and dental quantile was not ($p=0.39665$). Of the four models, Model 4 has the lowest R-squared (0.9769), although the R-squared value is still quite high. Tukey's HSD was required as the model was significant (Table 9b). Of the pairwise comparisons in Model 4, only that between the average and advanced quantiles was significant. The delayed quantile mean was not significantly different than either the average or advanced quantiles.

Discussion
The null hypothesis, that the developmental trajectories do not vary between delayed, average, and advanced individuals, failed to be rejected univer-

Table 8. Model 3: Dental development between dental quantiles. Pt.ID: individual; Age: patient's chronological age; Dental.Q: patient's dental quantile.

(a) Repeat Measure ANOVA

Number of observations =211			R-squared =0.9848		
Root MSE =0.0361			Adj R-squared =0.9784		
Source	Partial SS	df	MS	F	Prob > F
Model	12.4365	62	0.2006	154.32	0
Pt.ID	0.2711	53	0.0051	3.94	0
Age	12.0967	3	4.0322	3102.22	0
Dental.Q	0	0			
Age # Dental.Q	0.0363	6	0.0060	4.65	0.0002
Residual	0.1924	148	0.0013		
Total	12.6289	210	0.0601		

Between-subjects error term:	Age # Dental.Q
Levels:	12 (6df)
Lowest b.s.e. variable:	Age
Covariance pooled over:	Dental.Q (for repeated variable)
Repeated variable:	Pt.ID

(b) Tukey's HSD

studentized range critical value (.05, 3, 148) = 3.3483

uses harmonic mean sample size = 70.33

quantile vs quantile	quantile means		mean dif	HSD-test
delayed vs average	0.5846	0.6146	0.0299	6.9620*
delayed vs advanced	0.5846	0.6327	0.0480	11.1732*
average vs advanced	0.6146	0.6327	0.0181	4.2112*

Table 9. Model 4: Skeletal development between dental quantile. Pt.ID: individual; Age: patient's chronological age; Dental.Q: patient's dental quantile.

(a) Repeat Measure ANOVA

Number of observations = 208			R-squared = 0.9769		
Root MSE = 0.0399			Adj R-squared = 0.9670		
Source	Partial SS	df	MS	F	Prob > F
Model	9.7891	62	0.1579	98.95	0
Pt.ID	0.6146	53	0.0116	7.27	0
Age	8.9928	3	2.9976	1878.65	0
Dental.Q	0	0			
Age # Dental.Q	0.0100	6	0.0017	1.05	0.3965
Residual	0.2314	145	0.0016		
Total	10.0205	207	0.0484		

Between-subjects error term:	Age # Dental.Q
Levels:	12 (6df)
Lowest b.s.e. variable:	Age
Covariance pooled over:	Dental.Q (for repeated variable)
Repeated variable:	Pt.ID

(b) Tukey's HSD

studentized range critical value (.05, 3, 145) = 3.3490

uses harmonic mean sample size = 69.33

quantile vs quantile	quantile means		mean dif	HSD-test
delayed vs average	0.6175	0.6056	0.0119	2.4799
delayed vs advanced	0.6175	0.6261	0.0087	1.8052
average vs advanced	0.6056	0.6261	0.0206	4.2851*

sally. When the quantiles were defined based on the skeletal system, the skeletal developmental trajectories clearly differ between the delayed, average, and advanced quantiles. This is evident in the continuously decreasing differences between the delayed and advanced quantiles, as depicted in Figure 1a. The significance of the interaction terms in Model 1 indicates that the trajectories are different; they are not parallel versions simply offset from each other. This means that the rates of skeletal development differ, as do the absolute age-specific developmental percentages quantiles. The null hypothesis was, therefore, rejected based on the significance of the Tukey HSD test of Model 1.

However, when the dental development of these same individuals was considered in Model 2, the three quantiles did not follow significantly different developmental trajectories; only the advanced quantile was significantly different from the other two. The non-significance of the interaction term from Model 2's repeated measure ANOVA indicates that the trajectories are parallel. Therefore, while the rate of dental development is similar for the three quantiles, those who were skeletally advanced begin and remain relatively advanced dentally.

This difference in significance is unexpected. Growth charts, such as those released by the World Health Organization (WHO) and Center for Disease Control (CDC), show that percentiles diverge as individuals age (WHO Multicenter Growth Reference Study Group, 2006). The Greulich & Pyle logarithmic development graphs are suggestive of different developmental trajectories (Greulich &

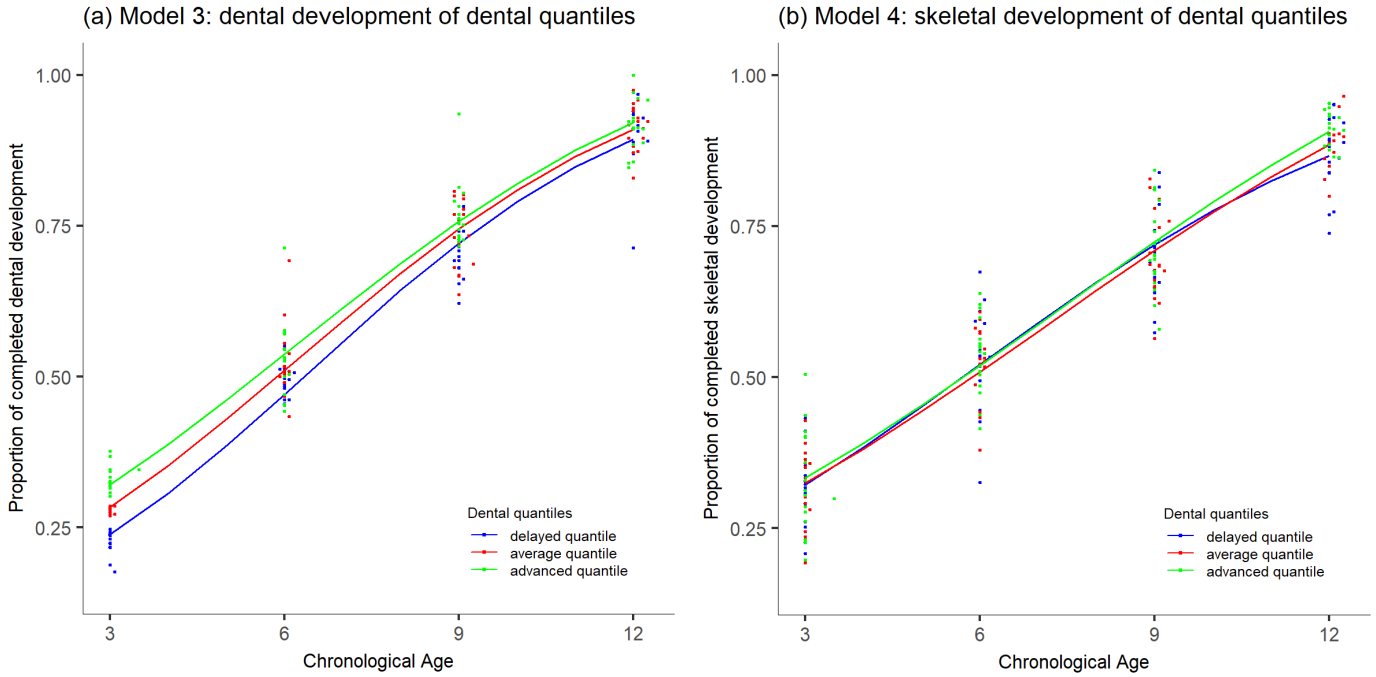


Figure 2. Logistic growth curves of dental quantiles: chronological age versus development. Plots of chronological age by proportion of completed dental (a) or skeletal (b) development based on dental development at age three, the delayed, average, and advanced dental quantiles. Logistic growth curves depict the developmental trajectories taken by those within each quantile.

Pyle, 1959), which also influenced the hypothesized difference between quantiles. If the two systems are correlated, and the body's approach to the development of both systems is the same, then it would be expected that the developmental trajectories of the dental system would also be significantly different, given the skeletal system's trajectories. That the dental system in general varies less than the skeletal system does not seem to be sufficient explanation for why the advanced quantile followed significantly different developmental trajectories than the delayed and average quantiles.

From the analysis of dental quantiles, the null hypothesis again was rejected as the dental developmental trajectories (Tukey HSD test of Model 3) were all significantly different and not parallel. As with Model 1, this finding is consistent with existing maturation charts such as those by Moorrees *et al.* (1963). It is interesting and noteworthy that while the dental developmental trajectories of the dental quantiles (Model 3) are all significantly different from one another, the delayed and average skeletal quantiles (Model 2) do not follow significantly different dental developmental trajectories.

Differences in skeletal development among the dental quantiles also reject the null hypothesis, although only the average and advanced dental quantiles followed significantly different skeletal developmental trajectories from each other. As the interaction term from Model 4 was not significant, it is apparent that these two quantiles followed different, yet parallel, trajectories. As depicted in Figure 2b, the difference between the average and advanced quantiles, while significant, is not great. Given this small difference, the variation of the delayed quantile shows an erratic pattern between the other two quantiles without being significantly different from either.

While the advanced subgroup is the only one that was consistently different throughout the analyses, these four models demonstrate that the relative relationship between the skeletal and dental systems are not the same throughout the range of IVDT.

This study did not take into consideration possible stressors that might influence the skeletal or dental development. It is possible that future research that considers such stressors will offer insight into possible tradeoffs occurring between the systems that might explain these unexpected results from skeletal based quantiles.

Conclusions

This research has demonstrated the importance of considering the possibility that those individuals towards the extremes of normal IVDT may follow different developmental trajectories than is fully characterized by the sample mean. We have shown

that for skeletal and dental development, the trajectories are significantly different between those who are delayed, average, and advanced early in life. That this significance varies, and that the trajectories are occasionally parallel when the opposite system is considered, suggests that the relationship between the development of the skeletal and dental systems is more complicated than has been previously explored.

It is important to note that while the skeletal and dental quantiles were assigned independently, there are 32 individuals who fall into the quantiles of interest for both systems (see Table 3c). Of these individuals, less than a third were classified in the same level of quantile for both systems (5 delayed, 1 average, 4 advanced). Slightly over a quarter of the individuals who were delayed in one system were advanced in the other (3 delayed skeletal, 5 delayed dental). Based on the plethora of research finding a positive, and often significant, correlation between the systems, this discrepancy of a quarter of the individuals is surprising and warrants further investigation.

The variation between the systems' developmental trajectories has been shown to vary between individuals who were delayed, average, or advanced in their development at an early age, and additional research is needed to further explore the full range of IVDT.

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Dental Molding Compounds and Casts: Use in Non-Laboratory Environments

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Keywords: dental casts, dental morphometrics, molding compound

ABSTRACT Dental casts are invaluable research tools. There are a variety of molding compounds available, all having temperature, humidity, and timing guidelines to ensure a precise replica of dentition. However, not all field research conditions allow for adherence to environmental guidelines requiring longer wait times prior to pouring epoxy for casting. This study tests a common molding compound in non-controlled environments and over varying time intervals, testing the integrity of the dental molds in producing precise replicas of original teeth. Five hundred and eight molds were created under three varying environments: room temperature, hot/humid, and cold/dry. Molds were removed from these environments in two-week intervals over twelve weeks. The resulting casts were measured to determine timing limitations for producing accurate dental casts under varying environments. Molds stored at room temperature retained their shape and size for the complete twelve weeks. Molds kept in a hot and humid environment, however, only maintained their shape and size up to four weeks, whereas molds in a cold and dry environment showed significant changes by the end of the second week. These findings provide additional tools for researchers working in a variety of field conditions allowing casts to be taken of specimens that cannot be transported off site.

Adequately documenting archaeological and paleontological dental remains in the field can be a problem when the dentition cannot be removed from their archaeological site, museum collection, or country of origin. Macroscopic analysis, such as enamel hypoplasia and microwear studies, rely on visual inspection of the dentition. Instead of relying solely on notes, sketches, and photographs, it is ideal to make replicas of the teeth that could then be taken home for further analysis such as studies on microwear and dental enamel hypoplasia (Egocheaga, 2004; Mihlbachle, Foy, & Beatty, 2018; Stynder et al., 2018; Ungar, Livengood, & Crittenden., 2019; Ungar & M'Kiera, 2013; Ungar & Williamson, 2000). The process of making an accurate replica of a tooth requires using a molding compound to create a mold and then the use of either an epoxy or stone casting material filling the mold, to produces an accurate cast. In order to produce casts with exactly the same dimensions as the original tooth, the guidelines provided with the molding and casting compounds should be followed precisely. While many field research opportunities require one to be away from climate-controlled workspaces for weeks or months at a time, most molding compounds require casts to be made from their products within one week of forming the mold. Often times archaeological or paleontological dental remains are not allowed to leave their country of origin, compelling an alternate method for research to continue after returning to one's home location. Field research can last from only a few days to a few months, which could make following the material guidelines problematic in many field research settings. Additionally, it is recommended that the molding compound and subsequently created molds be kept at room temperature (~72˚F) (Coltène Whaledent, 2018), which is not always attainable for extended periods in field

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research conditions.

In many cases, making dental molds to transport back to one's home research location is more advantageous than making the molds and casts in the field for several reasons. If the field research location is in a remote area flying with casting material can be difficult. The excessive physical weight of dental stone before and after it has been cast can be a limiting factor for air travel and shipping, as well as its relative fragile nature once cast. Additionally, 2-part epoxy components contain both a Class 8 Corrosive Liquid and a Class 9 Hazardous material. Flying with these components is against the Federal Aviation Administration regulations and shipping can be problematic, requiring special labeling and specific delivery locations. Therefore, traveling with the lighter inert components of the molding compounds would be advantageous.

However, is it still a viable option to use these molding compounds when research conditions are less than ideal? What happens when field sites are in more extreme environmental conditions and research facilities have little or no environmental controls, requiring molding compounds and molds to be used and stored outside the material temperature and humidity guidelines? To determine the range of conditions under which the integrity of the molds can be maintained, we tested a commonly used molding compound, President Putty Soft (Grine & Kay, 1987; Mahoney, 2006; Nystrom, Phillips-Conroy, & Jolly, 2004; Teaford & Oyen, 1989; Ungar, 1996), in a variety of environments for varying lengths of time. Molds were made and placed in three environments chosen to imitate potential field conditions: room temperature, hot /humid, and cold/dry. Molds were removed for epoxy casting in two-week intervals to determine if and when the molds become compromised and cast dimensions deemed unreliable.

Materials and Methods

For this study, the commonly used molding compound Presidential Putty Soft (Coltène-Whaledent, 2018) (Figure 1) was tested for its ability to maintain integrity over time in differing environments. Disposable paraffin embedding molds were used in two sizes to contain the molding material throughout the project, rectangular 22mm x 40mm x 20mm deep held two tooth impressions and 22mm x 22mm square x 20mm deep held one tooth impression (Polysciences, 2019). Twelve maxillary premolars were used to make 49 impressions each for a total of 588 tooth molds within a two-hour time frame (Figure 2). The molds were then equally divided into groups of 196 and placed in three separate environments: room temperature, hot/humid and cold/dry. After removal from the test environments Epotek 301 (Epoxy Technologies, 2019) was poured into each mold to form a cast of the individual tooth. Epoxy was chosen over a dental stone casting material like gypsum due to its durability and common use in the field (Egocheaga, 2004; Mihlbachle, Foy, & Beatty, 2018; Stynder et al. 2018; Ungar Livengood, & Crittenden, 2019; Ungar & M'Kiera, 2013; Ungar & Williamson, 2000).

Figure 1. Molding compound

Figure 2. Dental mold

Three artificial environments were constructed to simulate nonenvironmentally controlled environmental field conditions. The first set of 196 molds was placed in a typical indoor climate controlled environment with the environmental controls set to 72° Fahrenheit and a relative humidity (RH) of approximately 50% (ASHRAE, 2017). The second environment was designed to simulate

field conditions in places like the highlands of Peru, the Alps, and Siberia, so a set of 196 molds was placed in a refrigerator with a drying agent, a 10oz container of calcium chlorite moisture absorber, mimicking the effects of a cold and dry environment; the average temperature was 32°F with a variance with a RH of approximately 33% (Figure 3). The final set of 196 molds was placed in an insulated aquarium with a heat source, a reptile under tank heater, set to 95°F and kept the bottom of the tank covered with water between a ¼ of an inch to 1 inch of water to attain an average temperature of 95°F and an approximate RH of 99% (Figure 4). This hot and humid test environment was designed to replicate field conditions found in Central America, Southeast Asia, and parts of Oceania. Air temperature and relative humidity were monitored in each environment by placing HOBO automatic data logger sensors placed directly beside the molds throughout the entirety of the study. Each of the three sensors was set to record the air temperature and relative humidity of the study environment every 6 hours to ensure that conditions were maintained.

Figure 3. Cold dry environment

Figure 4. Hot humid environment

Table 1 provides the summary data for the three test environments. The HOBOs showed that the temperature in the in "Room Temperature" test environment averaged 70.5°F with a maximum temp of 78°F and a minimum of 65.6°F and a relative humidity averaging 43.1% with a maximum of 58.7% and a minimum of 37.1% relative humidity. The HOBO readings from within the "Cold and Dry" environment showed that the average temperature was 32°F with a maximum of 34.9°F and a minimum of 30.1°F. The relative humidity in the "Cold and Dry" test environment averaged 31.1% with a maximum of 45.2% and a minimum of 23.9% relative humidity. The "Hot and Humid" environment's average temperature was 94.2°F with a maximum of 99.1°F and a minimum of 88.3° F. The "Hot and Humid" test environment relative humidity average was 95% with a maximum of 98.6% and a minimum of 87.2% according to the environmental HOBO.

Assuming an average summer field season of three months, twelve weeks was used as our total experimental period. According to the President Putty Soft Instructions for Use (2018) casting mate-

Table 1. Environmental test conditions

Test Environment	Average Temperature (°F)	Temperature Maximum (°F)	Temperature Minimum (°F)	Average % Relative Humidity	% Relative Humidity Maximum	% Relative Humidity Minimum
Room Temp	70.5	78	65.59	31.1	45.2	23.9
Cold/Dry	32	34.9	30.1	43.1	58.7	37.1
Hot/Humid	94.2	99.1	88.3	95.3	98.6	87.2

rial can be poured into the molds as soon as thirty minutes after they are made and should remain dimensionally stable for up to 7 days. Within twelve hours of making the molds, Epo-Tek 301 (Epoxy Technology, Inc., Billerica, MA) epoxy was poured into twenty-eight molds left at room temperature to form the control tooth casts. In two-week increments twenty-eight molds were removed from each of the three test environments. The molds were given twelve hours to return to room temperature before casts were poured using Epo-Tek 301 two-part epoxy. Returning the molds to room temperature was designed to simulate returning to a climate controlled research environment to pour the casting material. The Epo-Tek 301 requires approximately 24 hours to harden at which point the casts were removed from the molds for measuring (Figure 5). Due to stretching and damage sustained while removing the dental casts, none of the removed and casted molds were returned to their test environments. A new set of 28 molds were removed for each subsequent two-week casting.

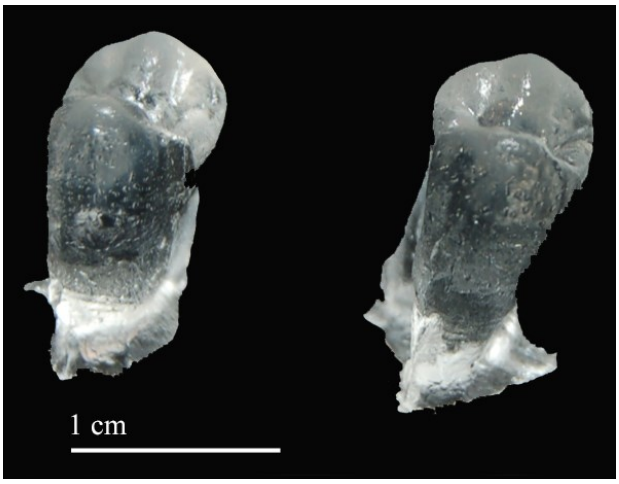

Figure 5. Epoxy casts

Bucco-lingual length, mesio-distal length, and crown height are standard dental measurements used in a variety of research methodologies (Buikstra & Ubelaker, 1994). Due to the relatively small size of teeth, a slight variation in these measurements can create statistical significance and therefore it is imperative that the casted replicas be a completely accurate representation of the original tooth. Therefore, these three measurements were used as markers of any meaningful change in the shape or size of the molds. The bucco-lingual length, mesio-distal length, and crown height of each dental cast was measured using digital calipers consistently by only one of the authors (RSK) to control for inter-observer error. Measurements were repeated for each dental cast in one-week time intervals for a total of three sets of repeat measurements to establish intra-observed reliability with analysis of variance. The observer was blind to the previously recorded measurements and environmental treatment of each casts. Results of repeated measures ANOVA to test for the intra-class correlation coefficients for the three repeated measurements of bucco-lingual length, mesio-distal length, and crown height per tooth were all above 0.90 and therefore considered highly consistent. The three repeated measurements were then averaged together to provide an averaged bucco-lingual length, mesio-distal length, and crown height for each tooth and used to determine if the size of the molds in each environment changed over time. Because the data were not normally distributed, Wilcoxon signed-rank tests were used to test for significant differences between time intervals in each environment.

Results

Table 2 provides summary statistics comparing cast measurements among environmental conditions. Those weeks that differed significantly from the null hypothesis are noted. The number (N) listed in the table refers to only those teeth (with all bucco-lingual diameter, mesio-distal diameter, and crown height measurements) used in that two-week test sample. For example, in "Room Temperature," 28 teeth with three measurements were used providing 84 compared measurements. When successive weeks were significantly smaller, this indicates that the molds and resulting casts were "shrunken" versions of the initial molds and original teeth. Significant increases in measurements in later weeks indicate that the molds and resulting casts were "swollen" versions of the originals.

As shown in the table, the room temperature molds showed no significant changes throughout the entire twelve-week period. This was an expected result; when the molding compound was used as directed, it maintained its integrity. However, this was not the case once the conditions were altered.

The hot/humid molds remained stable until the fourth week, whereupon the cast measurements became significantly larger due to the swelling of the molds than cast made at week 0. This swelling manifested as an increase in the molds in two of the three dimensions, increasing the space left by the dental impression. The statistically significant

change occurred in the fourth week with an increase in the mesio-distal and crown height measurements of the casts. Bucco-lingual changes manifest as a shrinking of the cast and became significantly different from the initial week 0 cast at the sixth-week mark. Even though the Wilcoxon signed-rank tests did not show significant differences between successive weeks compared to the initial week 0 casts until week twelve, additive changes between weeks two and four, as well as weeks 2 and 8 were also significant.

The cold/dry molds showed significant changes by week two in the bucco-lingual direction. All three cast measurements became significantly smaller due to the shrinkage of the casts. This shrinking manifested as a decrease in the overall size of the molds, including the space left by the dental impression. Wilcoxon signed-rank tests between the successive weeks indicated no additional significant differences; however, by week ten, the additive changes between weeks four and ten and weeks four and twelve became significantly different.

Discussion and Conclusions

This study has shown that researchers have adequate time to produce dental molds over the course of a field season and return home to pour the epoxy for casts, producing reliable tooth replicas, if the molds can be kept in an environmentally controlled setting (~72°F and 50% RH). However, molds kept in a relatively cold and dry environment (~39°F and 33% RH) have been shown to shrink significantly within a short period of time (< two weeks). Therefore, molding dental remains would not be appropriate for these field conditions, as the casts would not produce reliable measurements. Using this molding product in a hot and humid environment (~95°F and 99% RH) for a short period of time would be feasible, because molds appear to remain stable for four weeks.

Making dental molds to transport back to one's home research location is more advantageous than making the molds and casts in the field for several reasons. If the field research location is in a remote area flying with casting material can be difficult. The excessive physical weight of dental stone before and after it has been cast can be a limiting factor for air travel and shipping, as well as its relative fragile nature once cast. Additionally, 2-part epoxy components contains both a Class 8 Corrosive Liquid and a Class 9 Hazardous material. Flying with these components is not allowed and shipping can be problematic, requiring special labeling and delivery locations. Considering these factors, the ability to travel with only the molding compounds greatly improves the ease and likelihood of future dental analysis from dental casts.

In summary, researchers can reliably utilize President Putty Soft as a tool for recording dental information from teeth even in a variety of environmental conditions up to a certain period of time. This method will prove especially useful

Table 2. Study results timing of cast changes between test environments

Test Environment	Number of Tooth Measurements Used in	Change (δ) in Cast Measurements From the Control Group (Week 0)	p Value	Overall Resulting Change in
Room Temperature	84	Week 0 vs. Weeks 2-12 = δ̶	0.576 (average)	No Change
Hot/Humid	82	Week 0 vs. Weeks 2-10 = δ̶	0.254 (average)	No Change
		Week 0 vs. Week 12 = δ	0.006*	Swelling
		Week 0 vs. Week 4 (crown height) = δ	.026*	Swelling
		Week 0 vs. Week 4 (mesiodistal) = δ	0.05*	Swelling
		Week 0 vs. Week 6 (bucolingual) = δ	.003*	Shrinkage
		Week 2 vs. Week 4 = δ	0.006*	Swelling
		Week 2 vs. Week 8 = δ	0.001*	Swelling
Cold/Dry	79	Week 0 vs. Weeks 2-12 = δ	0.006*(average)	Shrinkage
		Week 4 > Week 12	0.003*	

when specimens cannot be removed from the archaeological site, museum collection, or country of origin for further analysis.

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A Systematic Literature Review and Case Report of Bilateral Two-Rooted Mandibular Deciduous Canines and Their Usefulness in Forensic Identification

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Keywords: forensic dentistry, dental identification, dental anthropology, deciduous tooth, canine teeth, two-roots

ABSTRACT A systematic review of the literature in PubMed was made by combining the terms "cuspid" and "tooth root" as MeSH health descriptors, combined with the Boolean operators "+" and "&" to obtain describing publications about two roots canines in order to sustain, on scientific evidence, the application of dental anthropology (dental morphological variations) in forensic science (forensic processes of dental identification). This literature review identified reports that describe the presence of two-rooted canines and the number and distribution of root canals for diagnostic and therapeutic purposes; and one report in which description was performed for forensic identification purposes. The descriptions corresponded to different cases of permanent maxillary canines with left unilateral expression, permanent mandibular canines with right unilateral expression, left unilateral expression with bilateral expression. There were no reports of deciduous dentition. Likewise, a case report in which skeletonized human remains were identified by the presence of bilateral two-root mandibular deciduous canines is described.

There are two maxillary and two mandibular canine teeth located on each hemi-arcade among the incisors and premolars (Kraus et al., 1969). The root of the maxillary canine is convex on its vestibular and lingual surface; its mesial and distal surfaces are broad and somewhat flattened; while the root of the mandibular canine is shorter and flatter with marked longitudinal grooves. The apical portions of the root could exhibit mesial drift, which may still have a bifurcation, making a double root (Hillson, 1996). Anatomically, the canines have a bulkier (in the vestibular-palatal or lingual) and longer root than the other teeth. This anatomy allows a strong anchor in the alveolar bone and gives a high resilience to forces generated in the masticatory cycle, depending on its high nociceptive capacity during the action of the muscles of mastication to sensory stimuli. This protection is achieved through the occlusal relationship between the maxillary and mandibular canines, in which, when the lateral movement of the mandible occurs the lower canines slide on the upper. This function is described in the literature as "canine function" or "canine key" to produce posterior teeth disclusion; hence, canines are seen as fundamental teeth of dental occlusion (Scott & Turner, 1998). These morpho-physiological traits and strategic position in the maxillaries give the canine teeth high resistance; for this reason, they are the teeth with the lowest prevalence of loss. Therefore, canines have value in forensic odontological identification processes. In this context, dental anthropology through the characterization of individuals by analysis of expression and variation of root and coronal dental morphological traits is fundamental (Rodríguez & Delgado, 2000).

In single-rooted teeth, as canines, the root sheath grows as a tube shape as radicular odontoblasts are differentiated. These odontoblasts regulate the process of dentinogenesis around the dental pulp, and are fragmented to allow the passage of cells that differentiate into cementoblasts from the dental follicle, which lead the process of cementogenesis. In multiradicular teeth, two or three primary apical foramen constitute the radicular trunk (according to the number of roots and their

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position). Each root grows longitudinally as if it were a single-rooted tooth. Therefore, the existence of a number of roots higher than normal is associated with hyperactivity of Hertwig's epithelial root sheath (HERS) (Holtzman, 1997) or its partial pathological degeneration, which causes an invagination by the dental papilla inducing an accessory root (Sohn et al., 2014).

This paper reports the case of skeletonized human remains that were identified using antemortem-postmortem dental comparison, due to the presence of a bilateral mandibular two-rooted deciduous canine. Also presented is a review of the clinical literature on two-rooted canines. The goal of this paper is to demonstrate the application of dental anthropology (i.e., dental morphological variations) in the forensic dental identification processes.

Forensic Odontology

The process of identifying humans has particular relevance in human societies, because every single individual has an identity that must be conclusively proven at the time of death for social, cultural, religious, legal, and economic purposes. Usually, the legal life begins with a birth certificate and ends with the death certificate (Mertz, 1977). In the forensic sciences and during criminal investigations, investigators, prosecutors, and forensic experts (including the dentist) must interpret and classify the information collected. A careful examination of the soft and hard tissues of the stomatognathic system provides physical evidence that helps to establish the identity of a person (Krishan et al., 1997). Dental analyses and scrutiny of the soft and hard tissues that make up the stomatognathic system document physical evidence and/or injuries. If these are documented, it may help to establish the identity of an individual, to refute or confirm a testimony, or objectively link a victimizer with the victim and the crime scene; such as part of the comprehensive forensic analysis of a corpse and related elements within the context of each particular case (Whittaker, 1995).

Teeth are used as an identification tool in forensic odontology investigations. Their high identification value relies on the number of teeth, pathological conditions, restorations, dental materials used, prostheses, and implants. Therefore, if a set of remains is missing teeth, it can be difficult to identify the individual (Krishan et al, 1997; Whittaker, 1995; Rothwell, 2001; Pretty & Sweet, 2001a, 2001b). Overall, dental identification is based on a comparison between the antemortem and postmortem record, which provides the forensic odontologist with enough distinctive features to make a positive identification (Pretty & Sweet, 2001a, 2001b). Such characteristics are scientifically supported by the morphological individuality of the skeleton and teeth. This identification process can be comparative through analyses of antemortem dental records (medical history, dental chart, periodontal chart, radiographs, study models, cephalometric analysis, treatment plan, etc.) with postmortem and anthropological data. After performing the postmortem dental record of an unidentified individual or unknown set of human remains and having circumstantial evidence to suggest the possible identity of these, the next step is to obtain a dental medical history to collate postmortem-antemortem dental records. Which, according to the American Board of Forensic Odontology (1994) and supported by national and international law, allows the establishment of a positive (total coincidence), possible (compatibility), insufficient (inadequate information available), or exclusive (incoherence and incompatibility) identification in a particular case.

Case History

A male minor with a chronological age of 5 years was reported missing. The initial search to find this minor was unsuccessful. Despite this, enquires in rural forensic units about cases of unidentified persons were made, finding out that in one of them, a set of unknown human skeletal remains was reported. The remains were found in a sugarcane plantation on the same date. The clothing worn by the individual on the day of his disappearance was found to be compatible with those on the skeletal remains. With this circumstantial evidence, the odontological medical history was obtained. This antemortem dental history did not contain radiographic images of the individual; however, the dentist noted in the chart the presence of deciduous lower two-rooted canines. A dental comparison was performed for the process of forensic identification. During which two reliable characteristics were discovered: The presence of deciduous lower two-rooted canines (Figures 1 and 2), whose interradicular septum was evident in the alveolar process of the mandible (Figure 3). In the postmortem radiographs of the deciduous canines it was possible to observe the presence of a root canal in every root and one pulp chamber in the crown of the tooth (Figure 4). In the dental clinical setting, Vertucci (2005) classified the number of roots and the number, shape, and configuration of the root ca-

Figure 1. Deciduous lower two-rooted canines. Buccal view.

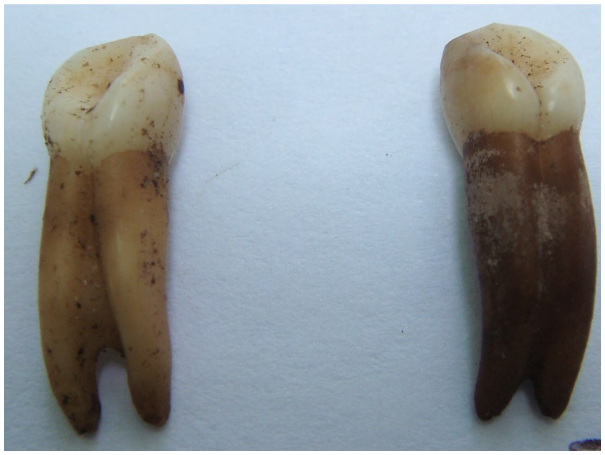

Figure 2. Deciduous lower two-rooted canines. Lingual view.

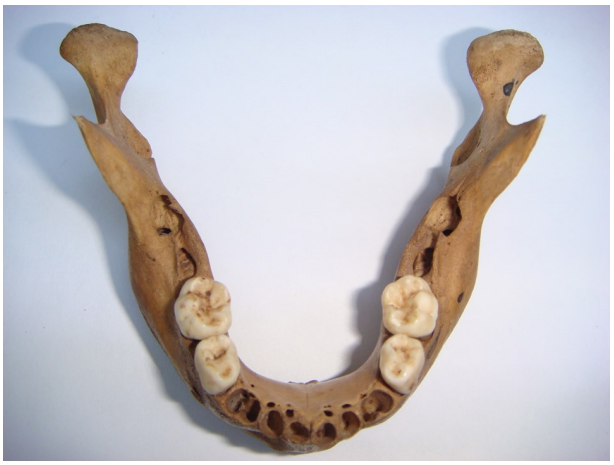

Figure 3. In alveolar process of the deciduous lower two-roots canines of the mandible is evident the interradicular septum.

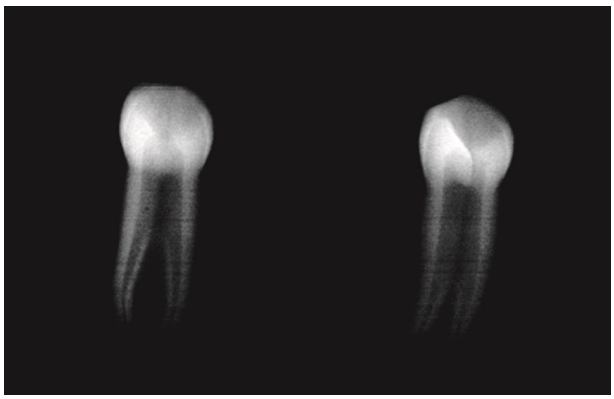

Figure 4. Postmortem radiography of the deciduous lower two-rooted canines.

nals for diagnostic and endodontic therapeutic purposes. In such a way, according to the configuration of the pulp chamber of the tooth and root canals, the case reported is classified as a type I, where each root has a single canal that ends in its own apical foramen. Also, according to Turner et al. (1991) the case was classified as a mandibular two-rooted canine. Turner et al. (1991) standardized the observation of the number of roots of the mandibular canines, in which there are one or two roots, where the second one –usually a small, conical-shaped root directed towards lingual surface– is separated from the root trunk at the middle third.

These features were sufficient to constitute reliable markers to positively identify the individual, despite the absence of radiographs.

Systematized Search of the Literature

A systematic review of the literature in PubMed (a free-access search engine to MedLine database of The United States National Library of Medicine) was performed through the combination of health science descriptors: "cuspid" and "tooth root", combined with the Boolean operators: "+" and "&", which were located in the Medical Subject Headings (MeSH). Publications describing the presence of two-rooted canines were considered, in order to support the discussion of a case report of an individual with deciduous mandibular two-rooted canines.

Results

Twenty-five publications fill the inclusion criteria, and were classified by year, type of tooth (deciduous or permanent, maxillary or mandibular canines), expression (unilateral or bilateral), sex (female or male), purpose of publication, and other important considerations (Table 1). There was only one report in which a description was performed

Table 1. Reports of two-rooted canines in the literature. U=upper, L=lower

Authors	Year	Permanent Tooth	Expression	Gender	Number of roots/ canals	Objective	Considerations
Rahmatulla & Wyne	1993	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of the distribution in roots canals is performed
Heling et al.	1995	LC	No reporting	No reporting	Two roots / three canals	Case report	According to the authors this is the first time a mandibular canine three canals reported
Ouellet	1995	LC	No reporting	No reporting	Two roots / two canals	Descriptive study	806 canines were examined 95% of which has a root and 5% two roots
Orguneser & Kartal	1998	LC	No reporting	No reporting	Two roots / three canals(two apical foramen)	Case report	The description of the distribution in roots canals is performed
D´Arcangelo et al.	2001	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of root canal treatment is performed in decayed tooth
Alapati et al.	2006	UC	Unilateral (right)	Male	One root / two canals	Case report	The description of root canal treatment is performed in decayed tooth
Bolla & Kavuri	2009	UC	Unilateral (left)	Female	Two roots / two canals	Case report	The description of root canal treatment is performed in decayed tooth
Wang et al.	2009	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of the distribution in root canals and the root canal treatment in two cases is performed
Victorino et al.	2009	LC	Bilateral	Female	Two roots / two canals	Case report	The presentation of a case of bilateral mandibular canines with two canals and root canal treatment are performed
Oporto et al.	2010	LC	Unilateral (left)	Female	Two roots / two canals	Case report	The description of root canal treatment is performed in decayed tooth
Andrei et al.	2010	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of the distribution in root canals is performed
Andrei et al.	2011	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of the conventional root canal treatment and apical surgery
Fonseca et al.	2011	LC	Unilateral (left)	Male	Two canals	Case report	The description of root canals treatment of bifurcation lesion and vertical loss of cortical bone was performed
Bolla & Kavuri	2011	UC	Unilateral (left)	Female	One root / two canals	Case report	The description of root canals treatment is performed in decayed tooth
Gaikwad	2011	LC	Unilateral (left)	Female	Two roots / two canals	Case report	The description of root canals treatment is performed in decayed tooth
Bhardwaj & Bhardwaj	2011	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of root canals treatment by recurrent tooth decay due to restora-
Moogi et al.	2012	LC	Unilateral (right)	Female	Two roots / two canals	Case report	The description of root canals treatment is performed
Ferreira et al.	2012	LC	Unilateral (right)	No reporting	Two roots / two canals	Case report	Morphological description of the tooth roots for forensic identification is performed
Kaul et al.	2012	LC	Unilateral (left)	Female	Two roots / two canals	Case report	Morphological description of the dilaceration of the two roots and their respective root canals treatment is performed
He et al.	2013	LC	Bilateral	Male	One root / multiples canals	Letter to the editor	Presentation of a case of bilateral mandibular canines with multiple canals, root canals treatment and restoration of anterior teeth is performed
Ramírez-Sotelo et al.	2013	LC	Unilateral (right)	Female	Two roots / two canals	Case report	Morphological description of roots using computed tomography was performed
Fuentes & Borie	2013	LC	Bilateral	Female	Two roots / two canals	Case report	Morphological description of roots using conventional radiography was performed
Chawla et al.	2013	UC	Unilateral (left)	Female	One root / two canals	Case report	The description of root canals treatment in decayed tooth is performed
Mithunjith.& Borthakur	2013	LC	Unilateral (left)	Female	Two roots / two canals	Case report	The description of root canals treatment in decayed tooth is performed
Arora, Nikhil & Gupta	2013	LC	Unilateral (left)	Female	One root / two canals	Case report	The description of root canals treatment in decayed tooth is performed

for forensic identification purposes; the primary means of description were conventional radiography and computed tomography. The descriptions corresponded to four cases of permanent maxillary canines with left unilateral expression; the other 21 cases were permanent mandibular canines, with right unilateral expression (10 cases); left unilateral expression (9 cases) and bilateral expression (3 cases). Nineteen cases in female and three in male subjects were reported; two-root expression, each root with a canal, was predominant (18 cases). There were no reports in deciduous dentition.

Discussion

Usually, the maxillary and mandibular canines are considered as a single-rooted tooth, given the high prevalence of 93.48% of this condition (Oporto et al., 2010), However, Brothwell –cited by Ferreira et al. (2012)– collected several reports on the prevalence of two-rooted canine expression, finding no population significant differences related to ethnic pattern, bilateral expression, or sexual dimorphism. According to the literature, the morphological variation related to the number of roots is 1% to 2% for the maxillary canines, and 1.3% to 15% for the mandibular canines, mainly the two roots and two canals expression with one canal per root (Bolla & Kavuri, 2011). Exceptionally, mandibular two-rooted canines and three canals have been reported (Heling et al., 1995), as well as, two-rooted canines, three canals, and two apical foramina (Orguneser & Kartal, 1998), single-rooted canines and two canals (Arora, 2013), and single-rooted canines with multiple canals (He et al., 2014). Another important feature is that in most cases of canines with two roots these are distributed in a buccal and lingual direction (Ferreira et al, 2012); however, in this case report, the roots were distributed in a mesial and distal direction. Thus, most of the reports suggest that canines with two roots correspond to a shape abnormality of the tooth during morphogenesis, related to an alteration of the HERS. A tooth root develops from the HERS and around the dental papilla underneath the dental follicle, until it completely cover the papilla in the primary apical foramen (Thomas, 1995).

The systematic review of the literature predominantly described canines with more than one root to demonstrate the clinical difficulty of root canal treatment of these teeth after the development of pathological processes (usually caries). This difficulty is mainly related to the identification of the longitudinal course of the canals due to superimposed radiographic images, the narrowness of the canals, complications from filling them, and apical sealing (Bolla & Kavuri, 2009). Radiographic images were used for forensic purposes, in order to identify features that allow the identification of the decedent from the shape, size, and number of roots of the teeth (Senn & Weems, 2013). Thus, Ferreira et al. (2012) reported a case of a mandibular two-rooted canine with two canals in a decomposing set of remains. However, although the victim was not identified by the dental setting, the authors state that given the low prevalence of this dental trait, could eventually become a valuable tool for forensic odontology identification.

Conclusions

In this case report, it is evident that the study of dental morphological variation from the point of view of dental anthropology –as in the case of bilateral expression of mandibular two-rooted canines– constitutes a reliable marker that allows a positive identification of an individual during antemortem-postmortem comparisons in the field of forensic odontology. In the literature, the expression of bilateral lower two-rooted canines was found to be rare. In this case, the observation and recording of the presence of a bilateral temporal expression of lower two-rooted canines contributed to the process of dental forensic identification, specifically the information registered by the dentist in the dental chart. The medical history had no radiographic records.

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BOOK REVIEW

The Tales Teeth Tell. By Tanya M. Smith. MIT Press. 2018. 296 pp., $29.95 (hard cover). ISBN: 978-0262038713.

The Tales Teeth Tell is an introduction to dental anthropology interwoven with its author's own experience of research and discovery. It takes the considerable expertise in tooth histology and imaging methods of the author and embeds them in the larger world of tooth growth and development, detailing processes at both the cellular and population level, introducing avenues of research and the questions that face the field. Professor Tanya Smith, now of Griffith University, has had a remarkable career trajectory thus far, moving from her PhD to prestigious fellowships at the Max Planck Institute for Evolutionary Biology and the Radcliffe Institute at Harvard University. Her work has concentrated on advancing histological research through innovative imaging projects. This work involved long-term collaborations with Paul Tafforeau on synchrotron imaging of dental tissue, and through other methods of understanding early life tooth growth and development, such as the collaborations with dental researchers Manish Arora and Christine Austin looking at breastfeeding signals in tooth chemistry. Her considerable expertise in dental growth and development and its evolution in our lineage has allowed her to offer a uniquely bottom-up approach to introducing dental anthropology, specifically by introducing the structure and growth of dental tissues as a way to approach questions of import to primate evolution as well as health and well-being in modern human societies.

The book is comprised of nine chapters, grouped into three sections covering major concepts in dental development, evolution, and what teeth reveal about behavior in addition to an introduction, conclusion, index, and a uniquely formatted 'notes' section that occupies a useful halfway house between endnotes (collected by chapter, though placed at the end of the text) and a formal bibliography. The flow of the book follows a path that might be expected from the author's special interest in dental structures. From Chapter one we are immediately immersed in the complications of tooth biology, and while it is a daunting subject, the explanations are clear and concise. Chapter two ties the structure of teeth to their development, while chapter three introduces the obverse of development in the form of growth disruptions and other features that reveal information about past lives such as carious lesions and malocclusion. Chapter four begins the section on evolution, and we follow from fish through to hominin fossils by Chapter five, which presents major arguments in hominin dental evolution (enamel/dentine thickness, size reduction) without being overly dogmatic. Chapter six is perhaps the most interesting of the book, as it deals directly with the author's subject of expertise, dental growth and development and the evolution of our species. The potential for new research in this area is immense, and the treatment here allows the reader to sense this.

The final section is devoted to how dental anthropology can be used to examine behavior, with a nuanced discussion in Chapter seven of what is (and isn't) possible to say about past diet from teeth alone. While some of the discussion of the interpretation of barium stable isotope ratios as a weaning signal may eventually need to accommodate a wider range of elements to fully describe the trophic dietary processes revealed in dental tissue, this chapter clearly introduces fascinating and important applications of developmentally focused dental anthropological research. Chapter eight continues with an up-to-date discussion of the possibilities and pitfalls of biomolecular analyses as well as a very brief look at a variety of other subjects including morphology, wear, and sexual dimorphism. Finally, Chapter nine introduces the many ways teeth can be culturally modified, such as for display, with wear making a reappearance in the discussion. In closing, Smith considers the future of teeth, offering a glimpse into the changing evidence of life history and adaptation to new lifestyles teeth reveal.

The main strength of this book is that it asks the reader to begin at the beginning by foregrounding the developmental process of dental tissues, an approach that provides a solid foundation for dental anthropological research. In addition, it addresses several of the bugbears of dental anthropology, with very careful attention paid to theories which may have been taught as current for decades but within narrow subfields are being challenged, such as the

idea that molar eruption in primates maps directly onto life history stages such as age at weaning or reproduction, or that the dental (and facial) reduction seen in our species in the last ~10,000 years is completely understood. It is a comprehensive and detailed introduction to dental anthropology, so much so that it is possible to wonder if the work is targeted to a public or professional audience. It is of considerable utility to the advanced student or non-specialist seeking to broaden their knowledge, but the author's willingness to share her love of anthropological science and discovery suggests a hope that it will fall into the hands of someone who does not (yet) know the fascination of dental anthropology.

It speaks to the depth of subject matter in the field that Smith's foray into accessible writing about dental anthropology comes so close on the heels of the excellent volume by Peter Ungar but still offers much of unique interest. While some basic descriptions of tissues or processes might repeat those in other texts, Smith's volume maintains a distinctive voice while uniquely presenting a cell–up perspective on dental tissues. A nuanced understanding of the processes of tooth development allow the author to relay the complicated and, frankly, difficult to digest, patterns of enamel and dentine formation in a comprehensible way. Very few undergraduates come to dental anthropology with a developmental perspective, but given the potential for research in this area to answer big questions about evolution and behavior, this seems like a timely reframing of what is necessary for the anthropologist to know about teeth. It is a rather large ask to take microhistology and make it into something that inspires wonder, but I do hope that of the many anthropology students who will eventually pick up this book at least a few catch the sense of excitement and possibility Smith so clearly feels for the tales teeth can tell.

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