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## Crown and Cusp Dimensions of the Maxillary First Molar: A Study of Sexual Dimorphism in Indian Jat Sikhs

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ABSTRACT The human first maxillary molar provides clues about evolution and is functionally important. Crowns of maxillary molars have four main cusps, each having an independent growth pattern and different evolutionary background. The study aims to quantify the morphometric criterion for the maxillary first molar giving a special emphasis to sexual dimorphism. Measurements of the first maxillary molar were taken on 100 casts of Jat Sikh students (50 males, 50 females) studying in the local medical college in the age group of 17-21 years. The Jat

Sikh community of Punjab is endogamous at the caste level. Unpaired t-tests were used to compare the samples for males and females. There is statistically significant sexual dimorphism (P < 0.01) for the maxillary first molar's crown and cusp components in the Jat Sikhs. The sequence of dimorphism in cusp dimensions corresponds to the order of formation of the cusps. The percentage sexual dimorphism for the hypocone is high (right 7.2%, left 7.4%). *Dental Anthropology* 2010;21(1):1-6.

Teeth exhibit the least cellular turnover of the body's structure, and they are readily accessible for examination. Tooth size standards based on odontometric investigations can be used in age and sex determination (Black, 1902). The variations in tooth size are influenced by genetic and environmental factors. Whenever it is possible to predict the sex, identification is simplified because then only missing persons of one sex need to be considered. In this sense identification of sex takes precedence over age (Camps, 1976). Various features like tooth morphology and crown size are characteristic for males and females (Dayal *et al.*, 1998).

Among Sikhs, sub-castes have been grouped into several categories like Jats, Aroras, Khatris, Ramgarhias, Majhabis, Rajputs and Namdharis. Historically, Jat Sikhs are landowners, farmers, and warriors. Traditionally, the Jat Sikhs have been endogamous at caste level and exogamous at the (gotra) sub-caste level (Sidhu, 2003). These are divided into numerous clans like Aulak, Bains, Bajwa, Bal, Bath, Bhullar, Chahal, Dhaliwal, Dhillon, Dosanjh, Gill, Grewal, Hundal, Kang, Randhawa, Sahota, Sidhu and Virk. There are 31 million Jats in South Asia. The majority of the 11 million Jat Sikhs in India live in Punjab, a state in northern India. The Jat Sikhs are believed to be the merged descendants of the original Indo-Aryans and a later addition of Indo-Scythian tribes (Dhillon, 1994).

The present study establishes the morphometric characterizations of the first maxillary molar in Indian Jat Sikhs. The study has been conducted with a special emphasis on the impact of sex factor on the morphometry of the first maxillary molar.

Crowns of maxillary molars have four main cusps,

namely the paracone, protocone, metacone and hypocone. Each cusp has an independent growth pattern (Kraus and Jordan, 1965) and a different evolutionary background (Osborn, 1907). The paracone is the first to appear both ontogenetically and phylogenetically and is regarded as the successor of the single cone of the reptilian haplodont dentition (Patterson, 1956). The hypocone tends to develop latest in terms of ontogeny and phylogeny, and it differentiates from the lingual cingulum (Kraus and Jordan, 1965). Odontometric characteristics of each molar crown are thought to represent a cumulative effect of individual cusp dimensions (Kanazawa et al., 1985), so analysis based on measurement of cusp dimensions promises to be more meaningful biologically than conventional measurements of whole crowns.

Teeth that develop later in ontogeny are expected to display greater sexual dimorphism due to the increasing differences in sex hormone production between males and females (Gingerich, 1974). In mandibular molars, sexual dimorphism values were shown to be greater in talonid dimensions than in the trigonid, suggesting that sexual dimorphism is larger in the later developed crown units (Yamada, 1981). The present study focuses on the sexual dimorphism of cusp diameters in the first maxillary molar and tests the hypothesis that the later developed distal cusps should display greater dimorphism than earlier developing mesial cusps.

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#### MATERIALS AND METHODS

#### **Selection Criteria**

One hundred subjects (50 males, 50 females) in the age interval of 17-21 years were selected for the study because attrition is considered to be minimal in this age group. The study was conducted on the students enrolled in the Government Medical College, Patiala, India, and the Government Medical College, Amritsar, India. Consent of the subjects was obtained, and the study casts were made with the help of resident doctors, senior residents, and senior technicians at the local Government Dental College.

Only those Jat Sikh students were selected whose upper and lower arches fulfilled the following inclusion criteria.

- · Healthy state of gingiva and peridontium,
- caries free teeth,
- normal overjet and overbite,
- · absence of spacing in the anterior teeth,
- · normal molar and canine relationship, and
- clearly distinguishable central pit of first maxillary molar.

#### Odontometry

Measurements were taken with a vernier caliper with a precision of 0.02 mm. The following parameters were measured and computed: (A) the mesiodistal and buccolingual crown diameters and cusp diameters (Fig. 1); (B) the mesiodistal (Fig. 2) and buccolingual (Fig. 3) crown diameters and cusp diameters (Fig. 4). Each cusp diameter is defined as the diagonal distance from

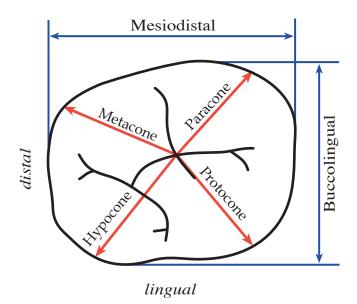
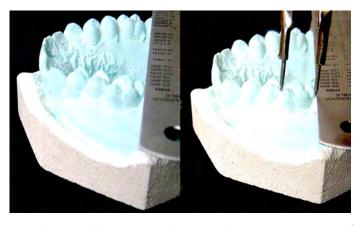


Fig. 1. Illustration of the measurement of crown dimensions.



**Fig. 2**. Illustration showing the measurement of maximum mesiodistal crown dimension.

the central pit to the most prominent convexity on the crown outline corresponding to the relevant cusp, taken perpendicular to the axis of the tooth (Kondo, 1985).

Three additional variables were calculated for each of these dimensions:

The crown area provides a measure of overall crown size:

Crown area = 
$$MD \times BL$$

where MD is the mesiodistal width and BL is the buccolingual length.

The cusp index quantifies cusp size relative to overall crown size:

Cusp index = 
$$\frac{\text{Cusp diameter}}{\sqrt{\text{MD x BL}}} 100$$

And, sexual dimorphism:

Sexual dimorphism = 
$$\frac{M - F}{M} 100$$

where M and F are the mean values in males and females. This formula is applicable for computing sexual dimorphism in mesiodistal width, buccolingual length, and crown area.

#### **Statistical Analysis**

Descriptive statistics, including distribution parameters, were calculated using Origin 6.1 software (Origin Lab Corporation, USA, version 6.1052 for Windows). Unpaired t-tests were used to compare the dimensions measured for males and females, and a table of the t distribution was consulted. Attainment of statistical significance was set at alpha = 0.01.

#### **RESULTS**

The results have been depicted in Tables 1, 2, 3 and 4. The study quantifies the morphometric criterion for the maxillary first molars in Jat Sikhs. In general the morphometric parameters were found to be quantitatively higher for the left side.

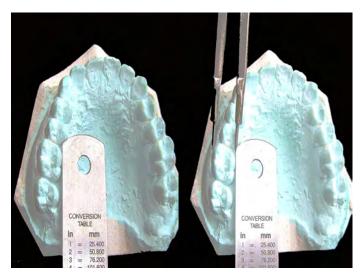
The study establishes the existence of statistically

TABLE 1. Descriptive statistics and tests for sexual dimorphism between males and females

Parameter	Side	Sex	Mean	sd	t-test	P-value <sup>1</sup>
Mesiodistal Width	Right	Males	11.33	0.078	-19.88	< 0.01
		Females	10.88	0.142		
	Left	Males	11.39	0.195	-13.53	< 0.01
		Females	10.87	0.187		
Buccolingual Length	Right	Males	12.53	0.078	-19.51	< 0.01
		Females	11.98	0.192		
	Left	Males	12.60	0.192	-18.26	< 0.01
		Females	11.98	0.142		
Crown Area	Right	Males	142.07	1.859	-24.83	< 0.01
		Females	130.29	2.789		
	Left	Males	143.54	4.617	-17.33	< 0.01
		Females	130.25	2.849		
Paracone Diameter	Right	Males	5.82	0.118	-8.73	< 0.01
	O	Females	5.63	0.124		
	Left	Males	5.84	0.138	-11.19	<0.01
		Females	5.64	0.089		
Protocone Diameter	Right	Males	5.88	0.119	-13.39	< 0.01
	C	Females	5.59	0.108		
	Left	Males	5.90	0.089	-16.83	< 0.01
		Females	5.60	0.078		
Metacone Diameter	Right	Males	5.68	0.117	-13.48	< 0.01
	0	Females	5.39	0.088		
	Left	Males	5.70	0.102	-16.39	< 0.01
		Females	5.40	0.079		
Hypocone Diameter	Right	Males	6.98	0.122	-21.19	< 0.01
J 1	C	Females	6.51	0.104		
	Left	Males	7.00	0.102	-25.80	< 0.01
		Females	6.52	0.092		
Paracone Index	Right	Males	48.82	1.114	2.46	n.s.
	1118111	Females	49.31	0.902		11.51
	Left	Males	48.74	0.982	3.72	n.s.
		Females	49.43	0.862		
Protocone Index	Right	Males	49.32	1.102	-1.77	n.s.
	0	Females	48.96	0.901		
	Left	Males	49.24	0.983	-0.91	n.s.
		Females	49.08	0.853		
Metacone Index	Right	Males	47.65	1.089	-2.17	n.s.
		Females	47.23	0.882	<b>—.</b> ,	11.0.
	Left	Males	47.59	0.982	-1.46	n.s.
		Females	47.32	0.845	1.10	11.0.
Hypocone Index	Right	Males	58.45	1.104	-7.12	< 0.01
11) Pocone much		Females	57.14	0.908	7.12	.0.01
	Left	Males	58.55	1.137	-6.46	< 0.01

<sup>&</sup>lt;sup>1</sup>Statistical significance was set at P < 0.01; ns = not significant (P > 0.01).

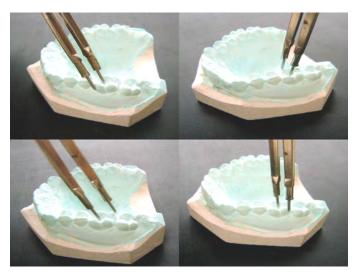
significant sexual dimorphism (P < 0.01) for the maxillary compared, they are found to be statistically insignificant. first molars in Jat Sikhs. From Table 1, it is evident that From these findings, it can be inferred that there exists the parameters as measured for males and females when a definite statistically significant sexual dimorphism for compared are found to be statistically significant. Further the maxillary first molar in Indian Jat Sikhs (P < 0.01). in males or females individually, i.e. within the same sex The percentage sexual dimorphism calculated came out (Tables 2 and 3) when these parameters as measured, are to be higher for the buccolingual dimension (4.6% for



**Fig. 3.** Illustration showing the measurement of maximum buccolingual crown dimension.

the right side and 5.2% for the left side) as compared to the mesiodistal dimension. Among the various crown dimensions, crown area displays the maximum dimorphism (9.0% for right side and 10.2% for left side).

The maximum cusp size in decreasing order came out to be hypocone > protocone > paracone > metacone for these Indian Jat Sikhs. The sex dimorphism in the cusp dimensions corresponds to the order of cusp formation, namely, hypocone > metacone > protocone > paracone. The percentage sexual dimorphism for the hypocone (right 7.2%; left 7.4%) is quite high in present study as compared to the other cusps. The cusp index exhibited the sequence: hypocone index > protocone index > paracone index > metacone index. The cusp indices (except for the hypocone index) did not exhibit statistically significant sexual dimorphism.



**Fig. 4**. Depiction of the method of measuring cusp diameters. Clockwise from the upper left are the paracone, protocone, metacone, and hypocone.

#### **DISCUSSION**

Dental morphological characteristics are useful for providing information for phylogenic and genetic studies and understanding variation within and among species. The crown characteristics are known to differ among racial groups; for example, Australian aborigines have larger teeth, Indians have smaller teeth, while whites have teeth intermediate in size (Tedeschi, 1977).

The Jat Sikhs are an endogamous group at caste level. They have distinct customs, traditions and food habits. As such the present study defines the criteria of the first molar tooth size for the Jat Sikhs. In general the morphometric parameters were found to be quantitatively higher for the left side. This observation holds true also for all the maxillary anterior teeth in North Indians (Agnihotri and Jain, 2008) but not in South Indians (Nair *et al.*, 1999). The crown dimensions for the first molar are comparable to those of the Jats (Kaul and Prakash, 1984) in Haryana. The Jats of Haryana constitute an agriculture-based community in North India.

It is a combination of environmental factors and inheritance that controls the mesiodistal and buccolingual dimensions. The dimensions obtained for the male teeth are definitely on the higher side as compared to those for females. This can be explained on the basis of the shape of the first molar tooth, which is controlled by the genetic constitution of the individual. Thus, the male teeth are usually larger in size as compared to the female teeth. It is the Y chromosome that seems to contribute most in the size of teeth by controlling the thickness of dentine, whereas the X chromosome seems to be responsible for modulating thickness of the enamel. The sexual dimorphism in tooth morphology is attributable to the presence of relatively more dentine in the crowns of male teeth (Iscan and Kedici, 2003).

The present study indicates that there exists a definite statistically significant sexual dimorphism for the maxillary first molar in Indian Jat Sikhs (P < 0.01). This is in concordance with the work done on Taiwan Chinese (Kondo, 1998) and on Jordanian subjects (Hattab *et al.*, 1996). While dental difference between the sexes in several human groups has been found highly dimorphic, it was not found so in Turks (Iscan and Kedici, 2003), where the lack of dimorphism comes from male subjects. This validates the perception that sexual dimorphism is population specific.

The percentage sexual dimorphism calculated came out to be higher for the buccolingual dimension (4.6% for the right side and 5.18% for the left side) as compared to the mesiodistal dimension. This is consonant with the findings for American white (Garn *et al.*, 1966) and South Indian (Nair *et al.*, 1999) subjects. Since size dimorphism was consistently greater for the buccolingual tooth diameter, its more extensive use is indicated in like-sex and unlike-sex sibling and parent-child comparisons for tooth size. Among the various crown dimensions, crown

Parameter	Side	Mean	sd	Side	Mean	sd	t-test	P-value
Mesiodistal Width	Left	11.39	0.195	Right	11.33	0.078	1.96	>0.01
Buccolingual Length	Left	12.60	0.192	Right	12.53	0.078	2.13	>0.01
Crown Area	Left	143.54	4.617	Right	142.07	1.859	2.01	>0.01
Paracone Diameter	Left	5.84	0.138	Right	5.82	0.118	0.91	>0.01
Protocone Diameter	Left	5.90	0.089	Right	5.88	0.119	0.92	>0.01
Metacone Diameter	Left	5.70	0.102	Right	5.68	0.117	-0.90	>0.01
Hypocone Diameter	Left	7.00	0.102	Right	6.98	0.122	0.96	>0.01
Paracone Index	Left	48.74	0.982	Right	48.82	1.114	-0.35	>0.01
Protocone Index	Left	49.24	0.983	Right	49.32	1.102	-0.36	>0.01
Metacone Index	Left	47.59	0.982	Right	47.65	1.089	-0.32	>0.01
Hypocone Index	Left	58.55	1.137	Right	58.45	1.104	-0.48	>0.01

TABLE 2. Descriptive statistics and tests for left-right side differences in males

side; 10.2% for left side).

The present pioneer study on the maxillary molar tooth in Indian Jat Sikhs provides data useful for anthropological, genetic, odontologic, and forensic investigations. This is particularly so since tooth morphology is known to be influenced by cultural, environmental, and racial factors (Agnihotri and Gulati, 2008).

The maximum cusp size in decreasing order came out to be hypocone > protocone > paracone > metacone. This order has been found to differ among populations. For the Japanese, Kondo et al. (2005) found the sequence to be: protocone > hypocone > paracone. For American whites, Biggerstaff (1976) reported the order to be protocone> metacone > paracone > hypocone. The sexual dimorphism in the cusp dimensions corresponds to the order of cusp formation, namely hypocone > metacone > protocone > paracone. Thus, the ontogenetic hypothesis, that later forming structures show greater sexual dimorphism than earlier forming structures, can apparently be extended to dental crown components.

The hypocone is considered to be the key innovation in mammalian evolution (Hunter and Jernvall, 1995).

area displayed the maximum dimorphism (9.0% for right Mammals that developed the hypocone became preadapted for masticating fibrous plants and subsequently demonstrated a markedly increased species diversity. The percentage sexual dimorphism for the hypocone (right 7.2%; left 7.4%) is high in present study as compared to the other cusps. This value is in fact comparable to the values for canine in North Indian population (right 7.3%; left 8.1%). The canine is known to exhibit the largest sexual dimorphism in the human dentition.

> The cusp index exhibited the sequence: hypocone index > protocone index > paracone index > metacone index. However in the present study the cusp indices except the hypocone index did not demonstrate a statistically significant sexual dimorphism. the cusp index sequence follows the same pattern in the Japanese (Kondo et al., 2005), the hypocone index in them is statistically significant. This can be attributed to differences in ethnicity. The hypocone index and hypocone diameter are the most dimorphic parameters for the Jat Sikh population.

#### **CONCLUSION**

The study quantifies the morphometric criterion and

TABLE 3. Descriptive statistics and tests for left-right side differences in females

Parameter	Side	Mean	sd	Side	Mean	sd	t-test	P-value
Mesiodistal Width	Left	10.87	0.187	Right	10.88	0.142	-0.13	>0.01
Buccolingual Length	Left	11.98	0.142	Right	11.97	0.192	0.13	>0.01
Crown Area	Left	130.25	2.849	Right	130.29	2.789	-0.08	>0.01
Paracone Diameter	Left	5.64	0.089	Right	5.63	0.124	0.69	>0.01
Protocone Diameter	Left	5.60	0.078	Right	5.59	0.108	0.68	>0.01
Metacone Diameter	Left	5.40	0.079	Right	5.39	0.088	0.57	>0.01
Hypocone Diameter	Left	6.52	0.092	Right	6.51	0.104	0.59	>0.01
Paracone Index	Left	49.43	0.862	Right	49.31	0.902	0.64	>0.01
Protocone Index	Left	49.08	0.853	Right	48.96	0.901	0.65	>0.01
Metacone Index	Left	47.32	0.845	Right	47.23	0.882	0.55	>0.01
Hypocone Index	Left	57.03	0.989	Right	57.14	0.908	0.55	>0.01

Parameter	Right side	Left side
Mesiodistal width	4.14%	4.78%
Buccolingual length	4.68%	5.18%
Crown area	9.04%	10.20%
Paracone	3.37%	3.55%
Protocone	5.19%	5.36%
Metacone	5.38%	5.56%
Hypocone	7.22%	7.36%

TABLE 4. Sexual dimorphism for the crown and cusp dimensions

establishes the existence of a statistically significant sexual dimorphism (P < 0.01) for the maxillary first molars in Jat Sikhs. This study suggests that the hypocone index and hypocone diameter are the most dimorphic parameters Kanazawa E, Sekikawa M, Akai J, Ozaki T. 1985. Allometric for the Jat Sikh population.

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## Carabelli Trait in Australian Twins: Reliability and Validity of Different Scoring Systems

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ABSTRACT We assessed the intra- and inter-observer reliability of two methods of scoring or categorizing Carabelli trait in both primary and permanent dentitions (Hanihara, 1961; Dahlberg, 1963). By using dental casts obtained from twins, we also compared the expression of Carabelli trait within and between monozygotic (MZ) co-twins to clarify the ontogenetic processes leading to different forms of trait expression. While intra-observer concordance rates were generally good (70 - 90%), inter-observer concordance rates were poor (35 - 60%). This indicates that considerable caution is needed when comparing data for Carabelli trait derived from different samples by different researchers. By comparing

categories or scores for Carabelli trait in both dentitions of MZ co-twins, we found inter-relationships between groove and cuspal forms of the feature. Although the Arizona State University system developed by Turner is commonly used nowadays to score the Carabelli trait, we would encourage researchers interested in clarifying genetic influences and ontogenetic processes in both dentitions to refer to the often over-looked plaque of Hanihara and also Dahlberg's plaque P12B. This should improve the reliability and validity of data obtained by helping to clarify the inter-relationships between the different phenotypic expressions of Carabelli trait. *Dental Anthropology* 2010;23(1):7-15.

Carabelli trait is expressed on the mesio-palatal surface of human maxillary molar crowns, particularly primary second and permanent first molars, and the feature shows a quasi-continuous pattern of expression (Harris, 1977). Investigations of Carabelli trait, one of many so-called nonmetric dental crown traits, are usually based on classifying or scoring the feature with reference to standard plagues, leading to calculations of its frequency of occurrence and degree of expression. Although most investigations of Carabelli trait have used standard plaques, there are distinct differences in reported frequencies of the trait in similar population groups, probably resulting more from observational inconsistencies than true variation (Scott, 1980). Misclassification of the trait is further compounded by the relatively large number of different classification methods available to the researcher (Kieser and Merwe, 1984). Recently, the effect of inter-observer errors was reported when using dental morphological features to calculate genetic distances in ancient Mayans, with different 'cut points' for determining presence and absence of traits, such as Carabelli trait, influencing the outcomes of the analyses (Cucina and Wrobel, 2008). Although Carabelli trait has been studied extensively within and among human populations, there is still uncertainty about the validity of the different methods of classification, including which is the most suitable to use in primary and permanent dentitions.

Twin studies provide a valuable approach for clarifying the relative contributions of genetic and environmental effects to phenotypic variability (Eaves, 1982; Townsend *et al.*, 2009). Indeed, a study of Carabelli

trait in the permanent dentition of South Australian twins indicated a very strong genetic contribution to observed variation, with an estimate of heritability around 90% (Townsend and Martin, 1992). Pinkerton *et al.* (1999) extended this earlier investigation by analysing the expression of Carabelli trait in both the primary and permanent dentitions of a large sample of Australian twins, highlighting the importance of genetic influences on Carabelli trait variation and disclosing patterns of variation in trait expression between dentitions.

The aim of our present study was to explore the reliability and validity of two methods for classifying Carabelli trait (Hanihara, 1961; Dahlberg, 1963), by scoring the feature in both primary and permanent dentitions of a sample of Australian twins. By examining trait expression within and between the dentitions of monozygotic (MZ) twin pairs we also aimed to gain some insight into the underlying causes of observed variation, and to clarify which phenotypic forms of Carabelli trait might be more closely related in terms of their ontogeny. Given the strong genetic influence on variation in Carabelli trait,

<sup>\*</sup>Dr. Hasegawa performed this research while on sabbatical leave as a Visiting Lecturer in the School of Dentistry, The University of Adelaide.

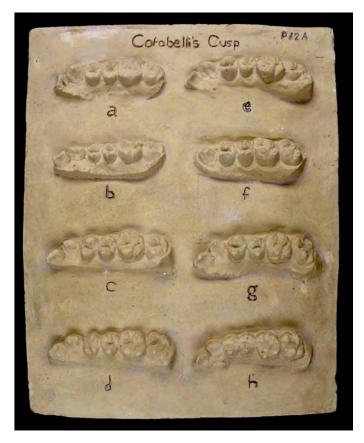
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it was hypothesized that any differences in phenotypic expression of the trait within and between MZ co-twins would tend to be small, reflecting environmental and/or epigenetic influences operating during odontogenesis. By comparing classifications or scores for Carabelli trait using the different methods, we aimed to shed light on the validity of the systems, including their ability to reflect ontogenetic processes.

#### MATERIALS AND METHODS

A total of 200 sets of dental casts, representing 50 pairs of monozygotic (MZ) and 50 pairs of dizygotic (DZ) twins, were examined and scored for Carabelli trait, using the systems of Hanihara (1961) and Dahlberg (1963). The dental casts were selected from a collection of over 600 pairs housed in the School of Dentistry at the University of Adelaide. The twins were all of European ancestry and their ages ranged from 8.3 years to 11.5



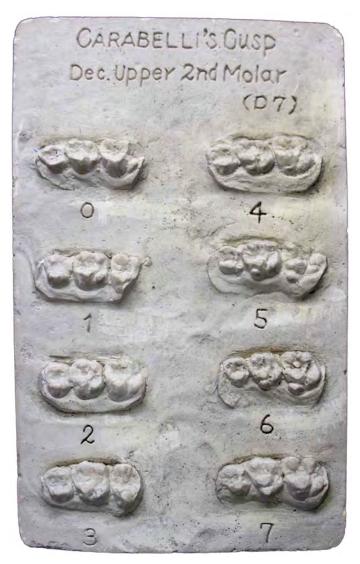
**Fig. 1a.** Dahlberg's plaque P12A for classifying Carabelli trait, highlighting increasing size of the feature. There are eight categories represented: (a) no expression; (b) a furrow; (c) a pit; (d) double grooves; (e) a Y-shaped groove; (f) a small cusp; (g) a larger cusp; (h) a large cusp.

years, with a mean age of 9.5 years. Zygosities were confirmed either by comparison of genetic markers in the blood or by DNA analysis of buccal cells (Townsend and Martin, 1992). The probability of monozygosity, given concordance for all the systems that were analysed, was greater than 99.0%. The ongoing study of teeth and faces of Australian twins was approved by the Committee on the Ethics of Human Experimentation, The University of Adelaide (Approval No. H/07/84A), and all participants have provided informed consent.

The methods of Hanihara (1961) and Dahlberg (1963) were used to classify Carabelli trait on primary maxillary second molars and permanent maxillary first molars, respectively. Plaster replicas of the standard plaques provided by Dahlberg (1956) and Hanihara (1961) were used to facilitate standardization in scoring. Dahlberg originally produced two plaques, P12A and P12B, with the 'P' denoting 'preliminary' (Figs. 1a, b). The former



**Fig. 1b.** Dahlberg's less known plaque P12B for classifying Carabelli trait, highlighting groove-cusp morphology. There are eight categories represented: (a) a groove around a large cusp (same as 'h' in P12A); (b) a groove on the mesial of a small cusp (same as 'e' in P12A); (c) a groove on the distal of a cusp (no equivalent in P12A); (d) a groove on both sides of a cusp (same as 'f' in P12A); (e) an elevation of cusp but no grooves (no equivalent in P12A); (f) a pit (same as 'c' in P12A); (g) completely smooth surface (same as 'a' in P12A).



**Fig. 2.** Hanihara's plaque D7 developed for scoring Carabelli trait in the primary dentition. There are eight grades: (0) no expression; (1) a shallow groove; (2) a shallow depression; (3) a deeper depression or pit but no bulge; (4) similar to 3 but a slight eminence; (5) a stronger eminence but smooth; (6) a cusp encircled by a groove; (7) a strong cusp.

has been commonly used for categorizing the size of Carabelli trait in the permanent dentition, whereas the latter, less well-known plaque was designed to highlight groove-cusp morphology, following on from descriptions by Meredith and Hixon (1953). Dahlberg created plaque P12B with the intention of evaluating pits and other surface irregularities found at the sites commonly occupied by Carabelli cusp. He suggested that, for future reference, pits and grooves should be counted as features relative to Carabelli trait, and that plaque P12B might be used to provide a limited guide to the trait's development (Dahlberg, 1956).

In this study, plaque P12A was used for assessing

intra- and inter-observer reliability in scoring Carabelli trait, and plaque P12B was used to provide additional insights into variability in trait expression in selected pairs of MZ twins. Although Dahlberg stressed his plaque P12B should not be used to define classes of Carabelli trait, he emphasized that pit and grooves should be noted in addition to cuspal forms. Hanihara's Plaque D7 was also used to score Carabelli trait, with the 'D' referring to 'deciduous' (Fig. 2). It presents eight categories of Carabelli trait and has been used to interpret the relationship between pit and cuspal forms in the primary dentition.

Assessments were made by one observer for all subjects on two separate occasions, enabling an estimation of the intra-observer reliability of both methods to be made. Two broad categories, referred to as 'concavities' and 'convexities', were used to compare intra-observer concordance rates using the methods of both Hanihara and Dahlberg. The 'concavities' category included scores 0 to 3 in Hanihara's system and categories a-d in Dahlberg's P12A system. The 'convexities' category included scores 4 to 7 in Hanihara's system and categories 'e-g' in Dahlberg's P12A system. In his analysis of the American Indian dentition, Dahlberg (1963) grouped the 'b' and 'c' categories together to represent various types of grooves and pits, and then combined the categories 'd' to 'g' to represent all sizes of cusps. We have chosen to include category 'd' as a 'concavity' for the purposes of our reliability tests, reflecting the presence of two grooves or furrows.

To assess inter-observer reliability, ten pairs of twins were selected at random and classified for Carabelli trait by three observers using the methods of Dahlberg and Hanihara. These three observers had different amounts of experience in classifying Carabelli trait. Observer A was a person with considerable experience, observer B had one year of experience, and it was the first time that observer C had scored Carabelli trait. After making their observations, inter-observer concordance rates between the three observers were calculated. Chi-square tests were also performed to compare the scoring of Carabelli trait between methods with statistical significance set at an alpha level = 0.05.

After assessing reliability, Carabelli trait was reexamined in all pairs of MZ twins where co-twins showed discordant expression of the feature by referring to both of Dahlberg's plaques, P12A and P12B, as well as Hanihara's D7 plaque. Given the recognized strong genetic contribution to variation of the trait, it was considered that close examination of those MZ twin pairs who showed different degrees of expression of the feature on primary and permanent teeth, or between sides, would provide additional insights into the validity of the scoring systems and also into the underlying biological processes leading to the observed phenotypes.

TABLE 1. Concordance rates between first and second assessments (primary second molars)

		I	Ianihara						Dahll	oerg	
	Conco	rdance	Disco	rdance			Concor	dance	Disco	rdance	
	n	%	n	%	total	Significance	n	%	n	%	total
Total	305	76.3	95	23.8	400	ns	297	74.3	103	25.8	400
DZ	152	76.0	48	24.0	200	ns	138	69.0	62	31.0	200
MZ	153	76.5	47	23.5	200	ns	159	79.5	41	20.5	200
Concavity	242	78.3	67	21.7	309	ns	253	72.1	98	27.9	351
$\check{\mathrm{D}}\mathrm{Z}$	118	78.1	33	21.9	151	*	115	66.1	59	33.9	174
MZ	124	78.5	34	21.5	158	ns	138	78.0	39	22.0	177
Convexity	63	69.2	28	30.8	91	**	44	89.8	5	10.2	49
$ m  ilde{DZ}$	34	69.4	15	30.6	49	ns	23	88.5	3	11.5	26
MZ	29	69.0	13	31.0	42	ns	21	91.3	2	8.7	23

ns: not significant

#### **RESULTS**

Table 1 shows the intra-observer concordance rates for scoring Carabelli trait on two separate occasions for primary second molars. Values ranged from around 70% to 90% reflecting good intra-observer reliability. A significant difference in concordance rates between the scoring methods was noted for 'concavities' in the DZ sample. In the 'convexities' category there was a significant difference in concordance rates between the methods for MZ twins and for the total sample (Table 1).

Table 2 shows the concordance rates between first and second assessments for permanent first molars. Values ranged from 75% to 85%. No significant differences in either the 'concavities' or 'convexities' categories were found between the methods.

Table 3 indicates the inter-observer concordance rates among the three observers for scoring Carabelli trait on primary second molars and Table 4 provides similar data for permanent first molars. The concordance rates were generally low, highlighting that inter-observer reliability for scoring was relatively poor. Using the method of Hanihara, the concordance rate between observer A and C was highest, followed by the rate between observer B and C, and the rate between observer A and B was lowest for both primary second molars and permanent first molars. Using Hanihara's method, the concordance rate between observer A and C was 65% for primary second molars and 40% for permanent first molars. The concordance rates between observer B and C, and between observer A and B, were around 35% for both

TABLE 2. Concordance rates between first and second assessments (permanent first molars)

		F	Ianihara						Dahll	oerg	
	Conco	rdance	Disco	rdance			Conco		Disco	rdance	
	n	%	n	%	total	Significance	n	%	n	%	total
Total	315	78.8	85	21.3	400	ns	301	75.3	99	24.8	400
DZ	158	79.0	42	21.0	200	ns	152	76.0	48	24.0	200
MZ	157	78.5	43	21.5	200	ns	149	74.5	51	25.5	200
Concavity	224	78.6	61	21.4	285	ns	229	73.6	82	26.4	311
ĎΖ	106	78.5	29	21.5	135	ns	110	72.8	41	27.2	151
MZ	118	78.7	32	21.3	150	ns	119	74.4	41	25.6	160
Convexity	91	79.1	24	20.9	115	ns	72	80.9	17	19.1	89
$\tilde{\mathrm{DZ}}$	52	80.0	13	20.0	65	ns	42	85.7	7	14.3	49
MZ	39	78.0	11	22.0	50	ns	30	75.0	10	25.0	40

ns: not significant

<sup>\*0.05 &</sup>gt; P > 0.01; \*\*P < 0.01

<sup>\*0.05 &</sup>gt; P > 0.01; \*\*P < 0.01

	Obse	erver A	Obser	rver B	Obse	erver C
	Hanihara's method	Dahlberg's method	Hanihara's method	Dahlberg's method	Hanihara's method	Dahlberg's method
Observer A concordance	g <b></b>		13 (32.5%)	19 (47.5%)	26 (65.0%)	19 (47.5%)
discordance			27 (67.5%)	21 (52.5%)	14 (35.0%)	21 (52.5%)
Observer B concordance	e 13 (32.5%)	19 (47.5%)			15 (37.5%)	25 (62.5%)
discordance	27 (67.5%)	21 (52.5%)			25 (62.5%)	15 (37.5%)
Observer C concordance	e 26 (65.0%)	19 (47.5%)	15 (37.5%)	25 (62.5%)		
discordance	14 (35.0%)	21 (52.5%)	25 (62.5%)	15 (37.5%)		

TABLE 3. Concordance rates among three observers (primary second molars)

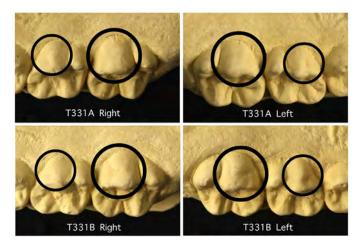
(n = 40; sum of left and right sides)

primary second molars and permanent first molars. Using the method of Dahlberg with plaque P12A, the concordance rate between observer B and C was 62.5% and the rates between observer A and B and between observer A and C were each 47.5% for primary second molars. The concordance rate between observer A and B was 47.5% and the rate between observer A and C was 45%, but the rate between observer B and C was only 35% for permanent first molars.

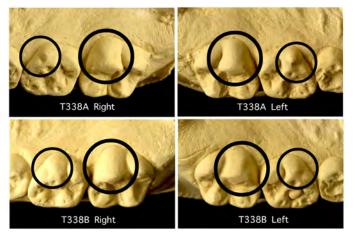
There were differences between observers regarding the interpretation of what constituted a groove or an eminence in both Hanihara's and Dahlberg's systems. The observers also had difficulty in classifying both the pit and Y-shaped categories using Hanihara's system, and there were differences in interpretation between the groove, Y-shaped, and cuspal grades in Dahlberg's system. Where there were differences in classification or scoring of Carabelli trait within or between MZ cotwins, the differences tended to be small, as we had hypothesized. By examining closely the cases where there were differences between sides or between primary and

permanent dentitions within an MZ twin, or differences between MZ co-twins, we were able to gain some insight into the ability of the different classification systems to reflect the phenotypic variation observed, and also to clarify how each category or score related to others.

Figures 3 and 4 represent two pairs of MZ twins who were selected because they showed discordant expressions of Carabelli trait that assisted in considering the validity of the Dahlberg and Hanihara systems. Table 5 shows the categories and scores for the trait, based on Dahlberg's plaques P12A and P12B, and also using Hanihara's plaque, for both the primary second molars and the permanent first molars in these two pairs of twins. The results provided in Table 5 were obtained by three observers each scoring the feature independently, then reaching a consensus on which category or score best matched the phenotypic expressions observed. It can be seen that there were differences in expression both within and between the twin pairs. For example, the primary and permanent molars for T331A were all scored as category 'b' according to Dahlberg's plaque



**Fig. 3**. A pair of MZ twins (T331A and B) showing different expressions of Carabelli trait within and between co-twins. The categories or scores are summarized in Table 5.



**Fig. 4**. A pair of MZ twins (T338A and B) showing different expressions of Carabelli trait within and between co-twins. The categories or scores are summarized in Table 5.

	Obse	erver A	Obsei	rver B	Obse	erver C
	Hanihara's method	Dahlberg's method	Hanihara's method	Dahlberg's method	Hanihara's method	Dahlberg's method
Observer A concordance			13 (32.5%)	19 (47.5%)	16 (40.0%)	18 (45.0%)
discordance			27 (67.5%)	21 (52.5%)	24 (60.0%)	22 (55.0%)
Observer B concordance	13 (32.5%)	19 (47.5%)			14 (35.0%)	14 (35.0%)
discordance	27 (67.5%)	21 (52.5%)			26 (65.0%)	26 (65.0%)
Observer C concordance	16 (40.0%)	18 (45.0%)	14 (35.0%)	14 (35.0%)		
discordance	24 (60.0%)	22 (55.0%)	26 (65.0%)	26 (65.0%)		

TABLE 4. Concordance rates among three observers (permanent first molars)

(n = 40; sum of Left and Right)

P12A, except for the permanent left first molar that was scored as category 'd'. The co-twin, T331B, displayed a 'b' category for the primary right second molar but all of the other teeth were scored as category 'e'. The corresponding categories and scores based on Dahlberg's plaque P12B and on Hanihara's D7 plaque, are also shown in Table 5. Similarly, there were differences in the categories and scores recorded for twins T338A and B. In these cases, the expression of Carabelli trait was greater on the primary molars than the permanent teeth, and there were also differences in expression between sides and between co-twins. The reader is encouraged to view the figures carefully and then to score the different teeth in both sets of twins. It becomes evident that the different phenotypic forms of Carabelli trait do seem to be linked to each other but there are many forms of the feature that are difficult to classify with any certainty.

#### DISCUSSION

The method of Dahlberg (1963) has been used commonly by many researchers to classify Carabelli trait on permanent first molars, although there have been numerous scoring methods developed over the years, including Shapiro's (1949) nine-grade classification, Goose and Lee's (1971) five-grade classification and Alvesalo et al.'s (1975) five-grade classification. Currently, the most widely used method for classifying Carabelli trait in the permanent dentition is The Arizona State University Dental Anthropology System devised by Christy G. Turner and his colleagues (Turner et al., 1991). This method is based on Dahlberg's plaque P12A but the categorical classification system of Dahlberg has been replaced by a numerical system from 0 to 7. The categories and the scores match reasonably well, although scores 3 and 4 in Turner's system refer to small and large Y-shaped

TABLE 5. Categories and scores of Carabelli trait expression in two pairs of monozygotic twins

		7	Г331A				T331B		
- -	Decidu	ous m2	Perman	ent M1	Decidu	ous m2	Perman	ent M1	
	Right	Left	Right	Left	Right	Left	Right	Left	
	side	side	side	side	side	side	side	side	
Dahlberg's plaque 12A	b	b	b	d	b	e	e	e	
Dahlberg's plaque 12B	e	e	e	NC	e	b	b	b	
Hanihara's plaque D7	1	1	1	NC	1	3	2	3	
		7	Г338A			T338B			
-	Deciduo	ous m2	Perman	ent M1	Decidu	ous m2	Perman	ent M1	
	Right	Left	Right	Left	Right	Left	Right	Left	
	side	side	side	side	side	side	side	side	
Dahlberg's plaque 12A	e	h	b	a	e	С	a	b	
Dahlberg's plaque 12B	b	a	e	h	b	g	h	e	
Hanihara's plaque D7	4	6	1	0	3	g 2	0	1	

There is no equivalent category in Dahlberg's plaque P12B or Hanihara's plaque D7 to category 'd' in Dahlberg's plaque P12A .

NC = no category.

depressions, whereas categories 'd' and 'e' on Dahlberg's plaque P12A represent a double groove and a Y-shaped groove, respectively.

Dahlberg's P12A plaque includes absence and seven degrees of expression of Carabelli trait, ranging from a single groove (or so-called 'furrow'), a pit, a double groove, a Y-shaped groove, to various sizes of cusps. In this scheme, categories 'f 'to 'h' represent increasing sizes of cusp. However, his P12B plaque does not address any size sequence, rather it considers pit-groove relationships. Although this plaque does not appear to have been used very widely in the past, it did assist the observers in this study to focus on the inter-relationship among pits, furrows and grooves, and cusps of various sizes. In cases where Carabelli trait was difficult to categorize, reference to P12B provided additional guidance in deciding which category to choose. Although Dahlberg's method was developed for the permanent dentition, it has been used to score Carabelli trait in both the primary and mixed dentitions (Pinkerton et al., 1999) with additional reference to the plaque of Hanihara (1961).

As this study progressed it became clearer that there were some discrepancies in the expression of Carabelli trait between the primary and permanent dentitions. The primary molars tended to display a higher frequency of Y-shaped groove forms, whereas cuspal forms were more common in the permanent dentition. This finding has been reported previously by other researchers (Saunders and Mayhall, 1982; Pinkerton *et al.*, 1999; Adler, 2006).

Kieser (1984) examined the expression of Carabelli trait on primary and permanent molars and reported a high degree of equivalence of expression of Carabelli trait in both dentitions. He hypothesized that this result was consistent with low epigenetic but high genetic influence on Carabelli trait expression. We have noted previously that, if the trait appears on the permanent first molar of an individual, it is almost always present on the primary second molar. However, if the trait appears on the primary molar, it may not be expressed on the permanent molar. Consistent with Kieser's view, we have interpreted this finding as reflecting similar underlying genetic influence for Carabelli trait in both dentitions, with environmental and/or epigenetic influences being more likely to modify trait expression on the permanent molar that forms later and develops over a longer period of time (Townsend and Brown, 1981).

The plaque D7 of Hanihara was designed specifically to score Carabelli trait in the primary dentition and, therefore, some limitations were noted when attempting to use it to score different convexity categories in the permanent teeth. Interestingly, Hanihara's description of his system does not refer to Y-shaped grooves specifically, rather the term 'depression' is used. Nevertheless, the examples of depressions provided on Hanihara's plaques do have a characteristic Y-shaped appearance. Dahlberg's P12A system provides a comprehensive categorization of the cuspal categories of the trait but it does not address

the peculiarities of the various pit/groove relationships to any extent. For example, it is often difficult to decide whether a short groove that ends in a deeper depression should be classed as a groove or a pit. It is also often difficult to determine whether double grooves lie either side of a slight elevation that would warrant a cuspal classification. Similarly, Y-shaped grooves may or may not be associated with a convexity of the lingual surface of the tooth.

Despite these difficulties, it appears that an acceptable level of intra-observer reliability can be reached for scoring Carabelli trait using the methods of either Dahlberg or Hanihara. We achieved concordance values in the range of 70-90%. Observers tend to develop their own internal calibration for classifying difficult examples of the trait that is based on their interpretation of the system of classification being used. It would appear that it is probably best to use the Dahlberg system when classifying Carabelli trait in the permanent dentition and the Hanihara system in the primary dentition, while acknowledging that each method has its limitations. However, the level of inter-observer reliability was very low whichever method was used in either dentition. Our concordance values were in the range of only 35-60%. This finding reinforces the view that considerable caution is needed when making comparisons of data for Carabelli trait derived from different samples by different researchers.

For studies of the mixed dentition, where a uniform system of classifying Carabelli trait on both primary and permanent molars is desirable, it is suggested that a modified system could be used that draws on the methods of both Hanihara and Dahlberg. It is interesting that the Arizona State University (ASU) system for classifying Carabelli trait in the permanent dentition is slightly different from the system proposed originally by Dahlberg, with the 'double groove' category of Dahlberg replaced by a 'Y-shaped groove' category (Turner et al., 1991; Dahlberg, 1963). Even though it was developed for the permanent dentition, the ASU system, with its use of scores rather than categories and its modification of the original Dahlberg system, provides an additional very useful perspective for attempting to classify the range of expression of Carabelli trait in both dentitions.

Although distinguishing and classifying minor differences in phenotypic expression of Carabelli trait may not be as important in population-based anthropological studies as deciding whether the trait is present or not, we contend that fine discrimination in phenotypic expression is desirable in genetic studies and also in clarifying ontogenetic processes. We would propose for these types of studies that all available reference sources should be considered, including Dahlberg's plaque P12B, to assist in describing and then recording the rather complex inter-relationships between grooves and cusps.

The variations in expression of Carabelli trait demonstrated in the two pairs of MZ twins reported

in this paper highlight the wide range of expressions Ribeiro is also greatly appreciated. of the trait that are possible and confirm that no single scoring system is likely to be able to capture all possible phenotypic forms. The two examples we have provided also support the view that, despite a strong over-riding genetic influence on observed variation, relatively minor modifications in environmental and/or epigenetic influences within or between co-twins can apparently lead to different phenotypic expressions in Carabelli trait.

The types of expressions of Carabelli trait observed within the MZ co-twins, particularly in terms of the expression of different groove forms, confirm that there is an inter-relatedness between groove forms and cuspal forms of the trait. Our findings in twins suggest that increasing expression of Carabelli trait follows a continuum from simple grooves, to pits, to double grooves, to Y-shaped grooves, and then to cusps of various sizes, in a similar order to that represented in Dahlberg's plaque P12A. Even though Carabelli trait has probably been studied by dental anthropologists more than any other dental feature, there is still much to learn about the nature of the ontogenetic mechanisms that lead to its various expressions on primary and permanent molar teeth. We would strongly encourage researchers who are planning to study Carabelli trait to refer to the the plaque of Hanihara and plaque P12B of Dahlberg prior to commencing any study, as these earlier, often over-looked works, provide valuable insights into the rationale and limitations of the classification systems used most commonly nowadays, for example, the ASU system which is based on Dahlberg's plaque P12A.

One area that deserves further exploration is comparison of the expression of Carabelli trait on the external surface of dental crowns with its expression at the dentino-enamel junction, a structure that reflects the folding of the internal enamel epithelium of the developing tooth. Researchers such as Kraus (1952), Korenhof (1963), Sasaki and Kanazawa (1999), Avishai et al. (2004) and Skinner et al. (2009) have all explored the morphology of the dentino-enamel junction using different approaches. We plan to extend these studies by applying micro-CT scanning to exfoliated molar teeth of MZ twins where there are differences in phenotypic expression of Carabelli trait within and between co-twins.

In conclusion, we would like to reiterate the comments of Mayhall (1999) who emphasized the need for "more and better genetic studies" of dental morphological traits and the need to improve our understanding of "why the traits we observe are as they appear."

#### **ACKNOWLEDGEMENTS**

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## Demirjian Stage Tooth Formation Results from a Large Group of Children

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ABSTRACT The aim of this study is to present further data on the timing and variation of individual permanent mandibular teeth using Demirjian stages from a large collaboration. Seven mandibular permanent teeth were assessed from dental radiographs of healthy dental patients from Australia, Belgium, Canada, England, Finland, France, South Korea and Sweden (cross-sectional study; n = 9,371, 4,710 males, 4,661 females; aged 2–18). Data are presented in three ways, namely by tooth stage for males, females, and pooled sex. Mean age at entry of each tooth formation stage (maturity data) was calculated

using logistic regression and modified for age prediction. The 51% confidence interval for age within stage of individual tooth stages was calculated for use in forensic age estimation where the burden of proof is on the balance of probabilities. Average age, standard deviation, standard error, 3rd and 97th percentile within tooth stage was calculated from a uniform age sample (171 for each year of age from 3 to 16, n = 2,394). Modified maturity data and average age within stage from the uniform age distribution are two new methods of age estimation. *Dental Anthropology* 2010;23(1):16-23.

Many studies of dental maturation during the last 50 years have described the timing of permanent tooth formation stages. Several reports remain important because they include very young children and follow individuals longitudinally (Moorrees et al., 1963) or use clearly defined stages and a large sample (Demirjian et al., 1973; Demirjian and Goldstein, 1976; Demirjian and Levesque, 1980; Demirjian, 1994), although none give full descriptive results. The ease of statistical analyses and a better understanding of age estimation have highlighted the lack of descriptive data of the timing of tooth formation stages. This paper presents detailed results from a collaboration of published cross-sectional studies organised by Nils Chaillet in Canada that has resulted in several published reports including a polynomial approach to Demirjian's dental maturity scale (Chaillet et al., 2004; Chaillet et al., 2005) and maturity data of individual tooth stages (Liversidge et al., 2006). Tooth formation data are presented here in different formats to assess maturity and estimate age using developing teeth in living children, in forensic cases, or in archaeological cases where sex is uncertain.

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#### MATERIAL AND METHODS

Tooth formation data from dental radiographs using Demirjian stages (Demirjian *et al.*, 1973; 1976; Demirjian, 1994) were combined to form the International Data Base. The sample consisted of cross-sectional data from published studies from Finland, Sweden, England, Korea, Belgium, Australia, Belgium, Canada and France (Nyström *et al.*, 1986; Nyström *et al.*, 1988; Kataja *et al.*, 1989; Liversidge and Speechly, 2001; Teivens and Mörnstad, 2001; Willems *et al.*, 2001; McKenna *et al.*, 2002; Chaillet and Demirjian,

2004). The sample studied in this paper, after cleaning was radiographic data from 4,710 males and 4,661 females aged 2 to 18 (when all individuals had reached second molar maturity) shown in Figure 1 (left). Previous results of the timing of Demirjian tooth stages of individual teeth are available for males and females for each group separately (Finland, Sweden, England, Korea, Belgium, Australia, Belgium, Canada and France) and for all groups combined for individuals from age 2 up to and including 16 years of age (Table 9 in Liversidge *et al.*, 2006).

Mean age of entering a tooth stage was calculated by logistic regression for males, females and sexes combined (Table 1). Logistic regression calculates the average age at entry of a specific formation stage and represents the age when half of children at that age, have reached or passed the stage (Taranger, 1976; Eveleth and Tanner, 1990; Cameron, 2004) and is similar to probit regression used to calculate mean age of tooth eruption (Liversidge, 2003). In this regard the mean age is identical to median age, half of children enter the stage prior to mean age, and half enter subsequent to mean age. This is an appropriate method to compare maturation between groups; it is not equivalent to the mean or median age of a child in the specific maturity stage (see below). Maturity data were modified for each stage of each developing tooth (Table 2) by adding half the interval to the next stage (see Smith, 1991). The second way of presenting results is the 51% confidence interval of age

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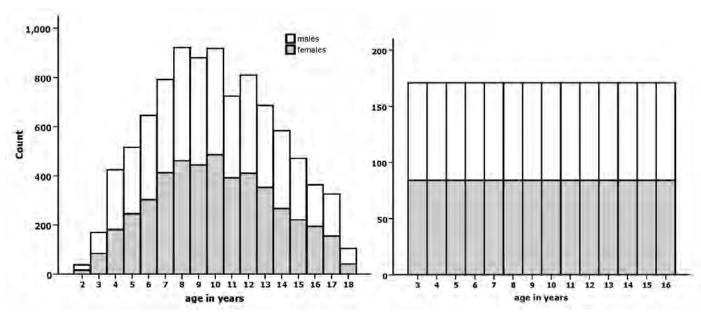
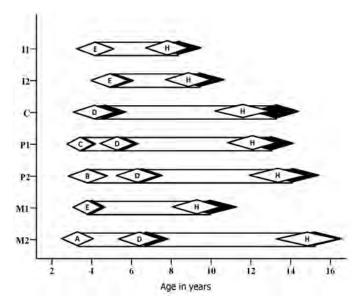


Fig. 1. Age and sex of radiographic sample (*left*), selected group for a uniform age distribution (*right*).

for each individual tooth stage. This was calculated using the 24.5th and 75.5th percentiles of average age within stage (Table 10 from Liversidge et al., 2006) and is shown in Table 3. This is slightly greater than the inter-quartile range and just over half of individuals in the specific stage will fall within this interval. The third way data are presented is average age within stage from a uniform age sample. The age cohort with the lowest number of children was 171 for three year olds (87 male, 84 female) and 87 males and 84 females were randomly selected from each year of age from 3 to 16 (total n 2,394, Figure 1 right). Descriptive statistics of age within individual tooth formation stages, including 3rd and 97th percentiles (using the normal deviate, see Cole, 2002) were calculated from this group and are shown in Table 4. This is referred to as L10a in Liversidge et al. (2010) in a comparison of dental age estimation methods using the same target sample of Maber et al. (2006).

#### **RESULTS AND DISCUSSION**

Maturity data representing the average age entering a tooth formation stage (age when half of children have reached or passed the stage) are shown in Table 1. The ages when 3% and 97% of girls and boys had entered some stages including D (crown complete with initial root) and H (mature apex) are shown in Figure 2. The left and right hand edges of the open diamond are the ages when 3% and 97% of girls had reached or passed this stage. Filled diamonds are data for boys. The sex difference is smaller for earlier stages and greatest for stage H (mature apex) of the canine. This is the only appropriate measure of the final maturity stage. Smoothed cumulative frequency distribution curves are shown for M2 stages in Figure 3. This is the only tooth in this study where data are available from crypt stage to mature apex; however the variation for early tooth stages is probably unrepresentative as this collaborative study includes only 38 two year olds. Maturity data modified for age prediction are shown in Table 2 (referred to as L9a in Liversidge *et al.*, submitted). Once a tooth reaches the most mature stage (apex closed with mature periodontal ligament width) age cannot be estimated using development and this stage is omitted from tables of modified maturity data and within stage data. The second type of result is the 51% confidence interval (Table 3, Figure 4) similar to what Koningsberg *et al.* (2008) term 'coverage'. This interval is useful when estimating age in forensic cases where the burden of proof is on the balance of probabilities. Coverage also



**Fig. 2**. Timing of some Demirjian tooth stages including D (crown complete with initial root) and H (mature apex). Diamond extends from 3rd to 97th percentile, apex is mean age. Open diamonds females, solid diamonds males.

TABLE 1. Mean age of entering Demirjian tooth stages for mandibular permanent teeth recalculated from cleaned data using logistic regression of 4,710 males and 4,661 females<sup>1</sup>

		Males A	lone	Females A	lone	Sexes Poo	led
Mandibular		Mean for		Mean for		Mean for	
Tooth	Grade	prediction	sd	prediction	sd	prediction	sd
I1	E	4.39	0.44	4.24	0.42	4.33	0.43
	F	6.02	0.38	5.70	0.36	5.88	0.38
	G	6.97	0.40	6.68	0.37	6.82	0.39
	Н	8.35	0.60	7.84	0.57	8.09	0.60
I2	E	5.21	0.47	4.87	0.42	5.05	0.46
	F	6.68	0.38	6.28	0.37	6.49	0.39
	G	7.82	0.47	7.32	0.44	7.56	0.47
	Н	9.41	0.65	8.79	0.59	9.09	0.65
C	D	4.75	0.59	4.19	0.53	4.49	0.59
	E	6.60	0.45	5.99	0.44	6.31	0.47
	F	8.51	0.51	7.59	0.50	8.04	0.56
	G	11.04	0.61	9.84	0.56	10.42	0.67
	Н	13.23	0.64	11.73	0.68	12.47	0.80
P1	C	3.58	0.40	3.41	0.35	3.49	0.39
	D	5.56	0.43	5.28	0.41	5.43	0.43
	E	7.26	0.49	6.78	0.43	7.02	0.48
	F	9.19	0.53	8.70	0.49	8.94	0.53
	G	11.44	0.62	10.70	0.58	11.05	0.63
	Н	13.03	0.64	12.27	0.65	12.64	0.69
P2	В	3.81	0.57	3.80	0.55	3.81	0.56
	C	4.94	0.55	4.81	0.55	4.89	0.55
	D	6.48	0.57	6.21	0.56	6.35	0.57
	E	8.02	0.58	7.62	0.64	7.82	0.62
	F	9.83	0.69	9.35	0.60	9.58	0.65
	G	12.26	0.75	11.57	0.71	11.90	0.76
	Н	14.09	0.73	13.44	0.76	13.77	0.78
M1	E	3.99	0.32	3.81	0.38	3.91	0.35
	F	5.46	0.41	5.21	0.41	5.35	0.42
	G	6.84	0.48	6.42	0.45	6.64	0.46
	Н	9.95	0.71	9.33	0.68	9.64	0.71
M2	O	3.40	0.30	2.92	0.51	3.19	0.40
	A	3.46	0.33	3.33	0.42	3.40	0.37
	В	3.97	0.33	3.72	0.46	3.87	0.38
	C	4.91	0.44	4.85	0.44	4.88	0.42
	D	6.79	0.52	6.43	0.54	6.61	0.54
	E	8.80	0.56	8.48	0.52	8.64	0.55
	F	10.68	0.60	10.12	0.56	10.39	0.60
	G	12.18	0.60	11.57	0.60	11.86	0.63
	Н	15.22	0.71	14.75	0.77	14.99	0.75

<sup>&</sup>lt;sup>1</sup>These statistics represent the age when half of children have reached or passed the stage. Code O refers to crypt formation prior to evidence of tooth mineralization.

provides a means to test if a target sample is representative Results of this type from this study are shown in Table 4 (Konigsberg et al., 2008); 24.5% should fall below the interval, 51% within the interval and 24.5% above. The third type of result is average age within stage from a uniform age distribution (similar number of individuals in each age category). Konigsberg and Frankenberg (1992) suggest that age could be more accurately estimated using a reference sample based on a uniform age distribution.

and are referred to as L10a in Liversidge et al. (submitted). This flat age distribution, together with an adequate sample size and wide age range, are important features of a reference sample that contribute to accuracy (Konigsberg and Frankenberg, 2002; Konigsberg et al., 2008).

Understanding how maturity data differ to within stage data is challenging and Figure 5 illustrates some of these

TABLE 2. Maturity data modified for age prediction for mandibular permanent tooth stages

		Males A	lone	Females A	lone	Sexes Pool	ed
Mandibular		Mean for		Mean for		Mean for	
Tooth	Grade	prediction	sd	prediction	sd	prediction	sd
I1	E	5.24	0.44	5.01	0.42	5.11	0.43
	F	6.48	0.38	6.18	0.36	6.35	0.38
	G	7.64	0.40	7.29	0.37	7.45	0.39
I2	E	5.94	0.47	5.62	0.42	5.77	0.46
	F	7.20	0.38	6.86	0.37	7.02	0.39
	G	8.56	0.47	8.06	0.44	8.32	0.47
C	D	5.68	0.59	5.09	0.53	5.40	0.59
	E	7.64	0.45	6.83	0.44	7.18	0.47
	F	9.86	0.51	8.76	0.50	9.23	0.56
	G	12.14	0.61	10.80	0.56	11.44	0.67
P1	C	4.61	0.40	4.37	0.34	4.46	0.39
	D	6.34	0.43	5.99	0.41	6.22	0.43
	E	8.20	0.49	7.82	0.43	7.98	0.48
	F	10.32	0.53	9.83	0.49	10.00	0.53
	G	12.24	0.62	11.49	0.58	11.84	0.63
P2	В	4.41	0.57	4.30	0.54	4.34	0.56
	C	5.62	0.55	5.46	0.55	5.62	0.55
	D	7.16	0.57	6.86	0.56	7.08	0.57
	E	8.90	0.58	8.48	0.64	8.70	0.62
	F	11.04	0.69	10.50	0.60	10.74	0.65
	G	13.19	0.75	12.56	0.71	12.84	0.76
M1	E	4.80	0.32	4.58	0.38	4.63	0.35
	F	6.20	0.41	5.81	0.41	6.00	0.42
	G	8.38	0.48	7.84	0.45	8.14	0.46
M2	O	3.43	0.30	3.12	0.51	3.30	0.40
	Α	3.65	0.33	3.74	0.42	3.64	0.37
	В	4.35	0.33	4.74	0.46	4.38	0.38
	C	5.59	0.44	5.84	0.44	5.75	0.42
	D	7.45	0.52	7.78	0.54	7.62	1.05
	E	9.35	0.56	9.74	0.52	9.52	1.10
	F	10.84	0.60	11.46	0.56	11.13	1.15
	G	12.92	0.60	13.74	0.60	13.42	1.20

differences. Smoothed cumulative distribution curves for stages D, E and F of M2 (sexes pooled) are shown. These curves represent the increasing proportion of children at each age who have reached or passed the specific stage. A tooth is considered to be 'in' a stage until it enters the next stage. The shaded area shows the age interval of all individuals within stage D; ranging from the youngest (most dentally advanced) to the oldest individual in that stage (most dentally delayed). Maturity is a continuum and we arbitrarily divide this into discrete stages, even though the process of maturation is gradual. Stage D in molars is defined as crown complete with initial root spicules visible at the mesial and distal edges. These root spicules increase in length and the root bifurcation becomes visible, firstly as a dot or line, then as a semi-lunar radio-opacity. Once this occurs, the tooth is deemed to be in stage E. The three types of tooth data in this study are summarised in Figure 5. Mean age at entry for M2 stage D is shown as a dot,

maturity data modified for age prediction for this stage is shown as a triangle. The age interval for individuals 'in' stage D, extends from the youngest child in stage D, up to the age when the most delayed child leaves this stage and enters the *next* stage (when all individuals have entered stage E). The age range of individuals within stage D and E are marked. The 51% age coverage for stage D is also shown. This figure was chosen for the forensic odontologist where the burden of proof is 'on the balance of probabilities.' This is an expression of the probability of estimated age being on one side of an age threshold. If an individual of unknown age presents with a second permanent molar in stage D (crown complete with initial root), the 51% age interval (from Table 3) is 7.01 to 8.50. On the balance of probabilities, the age of this individual is older than six but younger than nine.

The existence of population differences in dental maturity is unclear and uncertain. Many studies report

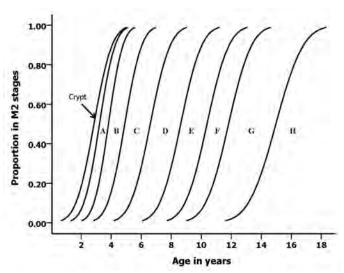
TABLE 3. Fifty one percent coverage for mandibular tooth stages<sup>1</sup>

			es (n = 4.71)	· ,	Fe	males (n =			Combined (	n = 9.371
Tooth	Grade	n	24.5%	75.5%	n		75.5%	n	24.5%	75.5%
I1	D	222	3.62	4.70	162	3.49	4.48	384	3.54	4.58
	E	425	4.73	5.91	297	4.58	5.60	722	4.67	5.80
	F	308	6.05	7.03	269	5.80	6.83	577	5.98	6.99
	G	561	7.25	8.57	460	7.01	8.09	1021	7.10	8.30
I2	C	42	3.38	4.21	29	2.83	4.05	71	3.06	4.10
	D	386	4.08	5.20	253	3.80	4.95	639	3.98	5.03
	E	425	5.39	6.60	335	5.10	6.19	770	5.23	6.46
	F	428	6.96	8.00	353	6.50	7.64	781	6.72	7.95
	G	684	8.01	9.54	643	7.67	8.97	1327	7.90	9.10
С	C	327	3.85	5.01	173	3.52	4.62	500	3.71	4.93
	D	512	5.00	6.51	370	4.72	5.96	882	4.92	6.27
	E	745	6.99	8.40	542	6.46	7.78	1287	6.79	8.06
	F	1063	8.97	10.60	995	8.00	9.58	2058	8.43	10.06
	G	800	11.02	12.97	817	10.00	11.65	1617	10.44	12.30
P1	A	20	2.57	3.34				21	2.59	3.33
	В	69	3.33	4.26	55	3.01	3.79	124	3.10	4.07
	C	433	4.38	5.44	308	4.18	5.28	741	4.28	5.40
	D	554	5.95	7.20	406	5.60	6.95	960	5.78	7.05
	E	803	7.67	9.00	795	7.20	8.53	1598	7.47	8.96
	F	918	9.40	11.00	889	8.90	10.54	1807	9.05	10.92
	G	580	11.32	12.97	657	10.63	12.10	1237	10.96	12.70
P2	O	10	2.39	4.92				16	2.67	4.52
	A	89	3.54	4.72	77	3.30	4.44	166	3.45	4.60
	В	222	4.27	5.21	160	4.19	5.33	382	4.23	5.23
	C	446	5.17	6.68	338	5.06	6.48	784	5.10	6.58
	D	570	6.70	8.10	494	6.61	7.96	1064	6.66	8.01
	E	769	8.06	9.87	744	7.86	9.05	1513	7.99	9.46
	F	945	9.98	11.97	969	9.64	11.39	1914	9.83	11.76
	G	626	12.00	13.98	708	11.32	13.29	1334	11.70	13.74
M1	D	131	3.31	4.22	100	3.22	4.03	231	3.25	4.14
	E	358	4.47	5.42	247	4.25	5.23	605	4.39	5.38
	F	432	5.66	9.53	313	5.42	6.56	745	5.56	6.90
	G	1279	7.70	9.53	1187	7.28	9.00	2466	7.50	9.20
M2	O				19	2.89	3.91	28	3.11	4.28
	A	66	3.68	4.49	42	3.54	4.56	108	3.60	4.52
	В	215	4.31	5.05	175	4.08	5.00	390	4.20	5.02
	C	562	5.39	6.78	402	5.28	6.65	964	5.32	6.72
	D	799	7.10	8.70	791	6.98	8.30	1590	7.01	8.50
	E	794	8.98	10.48	734	8.57	10.01	1528	8.80	10.25
	F	572	10.55	12.03	629	10.04	11.59	1201	10.25	11.97
	G	983	12.53	14.50	1096	11.97	13.98	2079	12.02	14.11

<sup>&</sup>lt;sup>1</sup>This age interval includes 51% of individuals within each stage. Stage included if n ≥ 10

significant differences in average age within tooth stage between groups, but this is inappropriate to compare maturity or average age at entry. For example, imagine if the minimum age of a study was 7 and the average age at entry of M2 stage D is calculated. Looking at Figure 5, this will exclude many individuals younger than 7 who have entered this stage. Looking at the cumulative incidence curve for stage D, it is clear that *more than half* of 7 years old in this large study have M2 in stage D (or later), indicating differences occur, but it is questionable if they have any

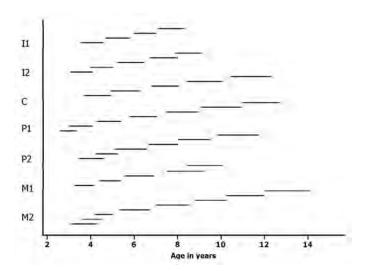
that a minimum age of 7 is too old to calculate the average age for this stage. Significant differences between groups have also been shown using a single dental maturity score such as Nolla (Nolla, 1960) or Demirjian (Demirjian et al., 1973; 1976; Demirjian, 1994). These have been interpreted as due to either a secular trend or regional differences in dental maturation but little attempt has been made to investigate this more fully. There is little doubt that these



**Fig. 3**. Smoothed cumulative distribution curves (proportion of individuals and age) for stages crypt to H for the permanent mandibular second molar (sexes pooled).

biological meaning.

The time interval between the original study Demirjian et al. (1973) and the present collaboration is between twenty to forty years. Mean age at entry of individual tooth stages in girls from the Canadian reference (triangles apex at bottom; Demirjian and Levesque, 1980) and this study (triangles apex at top) are shown in Figure 6. The largest differences occur as a single stage difference in four teeth; the earliest stage with data for both incisors and canine and the second stage for first premolars with mean age in the recent study being later than the original reference. The mean ages in subsequent stages in these teeth are similar or close in age. A comparison of the most mature stage in all tooth types is similar or marginally earlier in the recent study compared to the reference. It is unclear what these



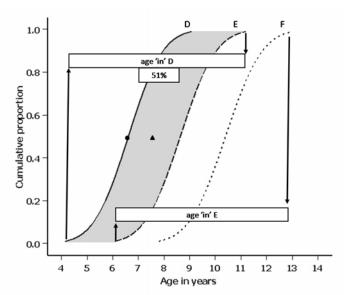
**Fig. 4.** Age interval of 51% coverage for stages A to G of permanent teeth (pooled sex).

differences and similarities indicate, but no clear pattern or developmental shift is evident.

Most studies of dental maturity are based on children of European origin. Mean age at entry for individual tooth stages was compared in the groups of this collaborative study. Children in Canada, Finland, Sweden, Belgium, England, France, Australia and a small group from Korea showed a wide age range of individuals within each tooth stage with many similarities in average age at entry (Liversidge et al., 2006). No single tooth type was consistently earlier or later and no clear pattern emerged from this analysis. A comparison of mean age at entry of tooth stages by Moorrees et al. (1963) on White and Bangladeshi children aged 2 to 22 in London, United Kingdom showed few significant differences in mean age between these ethnic groups (Liversidge 2009). The lack of published data of dental maturity from other regions of the world is sparse, but being addressed. Preliminary results from a worldwide comparative study showing stage H (apex mature) of the mandibular first molar in girls is shown in Figure 7. Smoothed cumulative distribution curves and 95% confidence interval of mean age (calculated from one year age groups using probit regression) are shown from the following regions: Australian Aboriginal (Liversidge and Townsend, 2006), Inuit, Japanese, Maori and Pacific Islanders (Moananui et al., 2008), South African Black and Cape Coloured, UK, Bangladeshi and White (Liversidge, 2009). The most advanced girls (youngest) girls in this stage are 6 years old and by 12, almost all have reached this stage. The average age at entry, when 50% of girls have reached this stage, is similar between groups, although two groups are slightly earlier than the others.

These similarities between the reference study and the present study and between world groups are supported by recent histological findings in the duration of crown formation (Reid and Dean 2006) between the past and the present. For instance, molar crowns take around 3 to 3.4 years to develop in maxillary, mandibular, first, second and third molars from Medieval Danes, northern European, South African Black and North American groups (Reid and Dean, 2006). The largest permanent crowns are found in Australian aborigines and the duration of enamel formation in molars in three first molars from this group is from 3 to 3.5 years (pers. comm. DJ Reid). Despite little documentation of the rate of dentine growth and root formation between individuals or groups, these findings suggest that the time it takes to grow a tooth is similar across time and between groups, especially at the resolution of crown and root fractions from radiographs.

Assessing maturity or estimating age from crown and root stages is usually used for an individual child. In this regard the individual is compared to reference data while, in human biology, differences between groups are of interest. Small differences in the mean age of individual tooth stages at the group level have little influence on the estimated age for an individual. Population specific reference data of radiographic tooth stages may be



**Fig. 5**. A comparison of maturity data and within stage data. Smoothed cumulative distribution curves for stages D, E and F for second molars are shown. Mean age of attainment and modified for age prediction are shown as dot and triangle respectively. Shaded area is the age interval of individuals within stage D. Age range within stage D and E are shown as well as 51% age coverage for stage D.

unnecessary and until evidence is available to show otherwise, the methods of age estimation presented here are appropriate for individuals from all groups.

#### **CONCLUSIONS**

New data on the timing of Demirjian stages from a large sample are presented in several ways. Two of these (modified maturity data, average age within stage from a uniform aged group) are new methods appropriate to estimate age from individual permanent teeth. The 51% coverage age interval for individual tooth stages are described for forensic age estimation where the burden of proof is on the balance of probabilities. These results represent the biggest data set and therefore probably the most reliable maturity reference and age estimation methods for mandibular permanent tooth formation using Demirjian stages in humans.

#### **ACKNOWLEDGMENTS**

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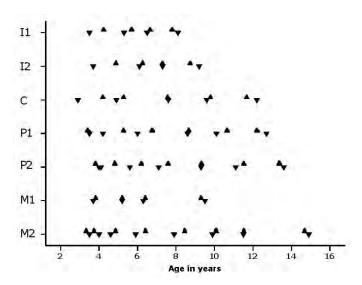
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**Fig. 6.** A comparison of mean age entering tooth stages for girls from Demirjian and Levesque (1980) triangles apex down and Liversidge *et al.* (2006) triangles apex up.

TABLE 4. Average age of children within a stage from a uniform age distribution of 87 males and 84 females for each year of age 3-16. Underscore means age not significantly different between sexes. Asterisk means age significantly different between sexes.

Tooth	Stage	u	Males (n = mean sd	(n = 1,21) sd	218) 3rd	97th	u	Females mean	s (n = 1) $sd$	1,176) 3rd	97th	The second of t	Sexes mean		Combined (n = sd sem	2,394) 3rd	97th
' I	D 0	111	3.96	0.61	2.81	5.11	108	3.85	0.57	2.78	4.92	219	3.90	0.59	0.04	1	5.01
	щ	144	5.18	0.88	3.53	6.83	120	5.01	0.81	3.49	6.53	264	$\frac{5.10}{6.25}$	0.85	0.05	3.50	6.70
	ı Ü	123	5.30 7.94*	1.13	5.82	0.00 10.06	104	0.19 7.54	0.85	5.94	7.32 9.14	10 <del>4</del> 227	7.76	1.03	0.00		7.07 9.70
	O	28	3.83	0.52	2.85	4.81	15	3.57	0.49	2.65	4.49	43	3.74	0.52	0.08		4.72
	Ω μ	166	4.38*	0.80	2.88	5.88	140	4.13	0.70	2.81	5.45	306	4.26	0.76	0.04		5.69
	ı ır	95	7.42*	1.07	4.21 5.41	7.31 9.43	127 94	7.00	0.86	5.38	7.00 8.62	249 189	7.21	0.99	0.03		9.07
	Ŋ	142	8.61	1.23	6.30	10.92	120	8.35	0.92	6.62	10.08	262	8.49	1.10	0.07		10.56
	O	143	4.16	0.83	2.60	5.72	26	3.93	0.65	2.71	5.15	240	4.07	0.77	0.02		5.52
		166	5.51*	0.98	3.67	7.35	162	5.07	0.99	3.21	6.93	328	5.29	1.01	0.06		7.19
	d Lt	222	.1C./ 9.90*	1.31	2.63 7.44	9.32 12.36	189	8.86 8.86	1.05	77.C 6.89	o.33 10.83	29/ 411	7.75 0.47	1.30	0.00		2.03 11 86
	, U	191	12.12*	1.24	9.79	14.45	149	10.91	1.24	8.58	13.24	340	11.59	1.38	0.07		14.18
	В	40	3.75	0.57	2.68	4.82	39	3.54	0.47	2.66	4.42	62	3.65	0.53	90.0		4.65
	C	163	4.59	0.82	3.05	6.13	150	4.46	0.76	3.03	5.89	313	4.53	0.79	0.04		6.01
	О	153	6.32*	0.89	4.65	7.99	131	80.9	0.99	4.22	7.94	284	6.21	0.95	90.0		8.00
	Щ	162	8.21*	1.06	6.22	10.20	166	7.85	0.92	6.12	9.58	328	8.02	1.01	90.0		9.92
	Щ	202	10.37*	1.35	7.83	12.91	160	9.83	1.12	7.72	11.94	362	10.13	1.28	0.02		12.54
	ტ .	151	12.25*	1.27	98.6	14.64	133	11.74	1.20	9.48	14.00	284	12.01	1.26	0.07		14.38
	V d	47	4.07	0.75	2.66	5.48	49	3.80	0.60	2.67	4.93	96 11	3.93	0.69	0.07		5.23
	م ر	04 144	4.44 7.70	0.72	3.03 3.93	7.65	116	4.40 5.44	0.00	2.04 3.84	0.00 7.04	260	7.43	0.79 0.95	0.00		7.74
	) (	121	7.20	1.11	5.11	9.29	133	7.15	1.01	5.25	9.05	254	7.18	1.06	0.07		9.17
	Щ	159	*80.6	1.33	6.58	11.58	143	8.60	1.16	6.42	10.78	302	8.85	1.27	0.07		11.24
	Н	216	11.11*	1.55	8.20	14.02	172	10.68	1.26	8.31	13.05	388	10.92	1.44	0.07		13.63
	Ŋ	168	13.10*	1.48	10.32	15.88	156	12.50	1.35	96.6	15.04	324	12.81	1.45	80.0		15.54
	О	86	3.94	1.00	2.06	5.82	70	3.68	0.53	2.68	4.68	159	3.83	0.83	0.02		5.39
	ΠГ	124	4.76	0.73	3.39	6.13	111	4.53	0.72	3.18	5.88	235	4.65	0.73	0.05		6.02
	ין נ	C11 286	0.20°	0.00 7.7.	СС.4 7	7.65	100	07.0	1 32	4.24 5.66	7.52 10.63	CI2 S로로	6.00 8.35	1.00	0.00		7.63
	0	700	5.5	5.1	F 0.0	04.11	2,2	3.45	0.32	2.85	4.05	17	3.61	0.41	0.10		4.38
	A	43	3.80	0.43	2.99	4.61	26	3.75	0.44	2.92	4.58	69	3.78	0.43	0.02		4.59
	В	88	4.56	0.65	3.34	5.78	88	4.39	0.83	2.83	5.95	176	4.48	0.74	90.0		5.87
	O	162	5.93	0.95	4.14	7.72	133	5.68	0.88	4.03	7.33	295	5.82	0.93	0.02		7.57
	D	166	7.80	1.19	5.56	10.04	176	7.53	1.05	5.56	9.50	342	7.66	1.13	90.0		9.78
	ШΙ	167	9.74		7.33	12.15	137	9.38	1.13	7.26	11.50	304	9.58	1.22	0.07		11.87
	ц (	135	11.56*	1.14	9.42	13.70	120	10.98	1.05	9.01	12.95	255	11.29	1.13	0.07		13.41
- 1	ַל	707	10.00	1. <del>1</del> .	10.70	10.00	±07	13.24	1.40	10.40	10.02	1770	17.44	1.40	0.00		10.22

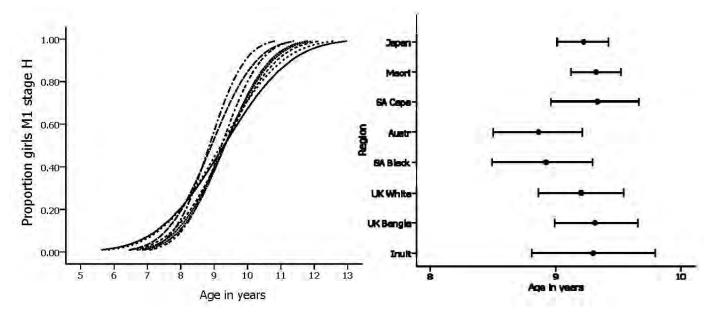


Fig. 7. Preliminary results of maturation of stage H of the mandibular first molar from world regions. Left: smoothed curves (proportion of girls vs. age). Right: 95% confidence interval of mean age for Australian Aborigine, Inuit, Japanese, Maori and Pacific Islanders, South African Black, South African Cape Coloured, UK Bangladeshi, UK White girls.

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## Triple Fusion in the Primary Dentition from Law's Site, Alabama (1MS100): A Case Report

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ABSTRACT from Law's Site on Pine Island, in Marshall County, Alabama. Analysis of the remains found that MS100-14

Dental fusion of the primary dentition is presented a clear case of triple fusion of primary dentition a rare congenital anomaly. Evidence in the literature of in the maxilla. This appears to be the first case of triple bioarchaeology is scarce. Burial MS100-14 was recovered fusion reported from among prehistoric Native American remains in the Southeastern United States. Dental Anthropology 2010;23(1):25-27.

Dental fusion of the primary dentition is a rare congenital anomaly. Examples in the literature of bioarchaeology are exceedingly scarce. Skeletal remains of an infant from the Law's Site (1MS100), in Marshall County, Alabama, presents a clear case of triple fusion of the primary dentition. This is a highly unusual condition, and thus a significant find for the fields of dental anthropology and bioarchaeology.

#### LITERATURE REVIEW

Examples of dental fusion in the anthropological literature are uncommon, and texts on dental anthropology, developmental osteology, and paleopathology give the topic little or no attention (Aufderheide and Rodríguez-Martín, 1998; Hillson, 1996; Ortner, 2003; Scheuer and Black, 2000). Two fused deciduous mandibular incisors are shown in Figure 1.1 of The Anthropology of Modern Teeth (Scott and Turner, 1997: 5), but neither the defect nor the provenance of the specimen is discussed in detail. A rare "talon cusp" found on a deciduous lateral incisor was the primary topic of a case report of a juvenile skeleton excavated in England, though the report states that "the affected incisor also shows abnormal widening, probably representing a double tooth", and mentions the presence of a supernumerary permanent incisor (Mays, 2004:206). It is unclear if this "double tooth" is an actual case of dental fusion, or a case of gemination, defined as the unsuccessful or incomplete division of one tooth germ into two (Canut Brusola, 1988; Oliván Rosas et al. 2004). Some authors agree that distinction between fusion and gemination can sometimes be confusing, and the term 'double teeth" should be used when the diagnosis is inconclusive (Andlaw and Rock, 1999; Gonzalez Marquez and Mendez-Nuñez, 1993; Killian and Croll, 1990; Oliván Rosas *et al.*, 2004; Uys and Morris, 2005).

Although there is little in anthropological sources concerning the topic of dental fusion, there have been some relevant clinical studies on modern populations, and some patterns have been documented regarding this dental anomaly. These clinical studies agree that fusion in the primary dentition usually affects two teeth

unilaterally in the mandibular arch, and most often in the anterior region; that is, either two incisors, an incisor and a canine, or a supernumerary tooth fused with an incisor (Barberia *et al.*, 2001; Cheng *et al.*, 2003; Favalli *et al.*, 1998; Modrizuki et al., 1999; Oliván Rosas et al., 2004; Yonezu et al., 1997).

Studies among European, (Asian) Indian, and Turkish populations reveal a prevalence of less than 1% for double fusion in primary dentition (Aquiló et al., 1999; Barberia Leache and Boj Quesada, 2001; Boj Quesada 1990; Bruce et al., 1994; Erdem et al., 2001; Reddy and Munshi, 1999). North American populations have a prevalence ranging from 0.14 to 3% (Hagman, 1988). A study in Shenyang city, China, reported 1.52% prevalence among children there (Cheng et al., 1999). The prevalence appears to be highest in Japan, where 4.1 to 5% of children studied presented this anomalous feature, and unlike other populations, there was a tendency regarding sex, as a significantly higher proportion of boys displayed congenital dental fusion (Modrizuki et al., 1999; Yonezu et al., 1997).

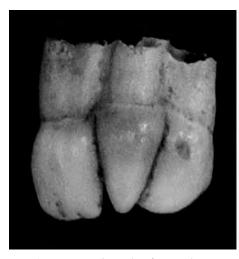
As rare as double fusion appears to be, it is not surprising that triple fusion of primary dentition is even less common. In the study of Indian children (n = 4,205), there was no case of triple fusion (Reddy and Munshi, 1999), though a separate team from India reports the case of a child with triple fusion involving two ipsilateral incisors and a supernumerary tooth (Prabhakar et al., Among Chinese children studied (n = 4,286), only one case of triple fusion was found, involving two ipsilateral incisors and the adjoining canine (Cheng et al., 1999). The prevalence of "triplication of primary teeth" among Turkish children was stated as 0.02% (Erdem et al., 2001).

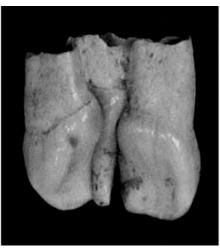
Problems associated with the congenital fusion of primary dentition may include an increased susceptibility to caries in the fused teeth (Reddy and Munshi, 1999).

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**Fig. 1**. Fused teeth of Burial MS100-14. (*Left*) Labial view with the left primary central incisor (i1) to the left of the photograph, a conical lateral incisor (i2) in the center, and the canine (c) to the right. (*Center*) Lingual view of the fused teeth, with c to the left and i1 to the right. (*Right*) Alveolar (apical) view of the formative roots with c to the left and i1 to the right of the photograph.

There is also an association between fusion of primary dentition and agenesis of the corresponding permanent teeth; with regard to this condition, various authors cite percentages of incidence ranging from 20 to 75%, the occurrence of which may depend on which teeth are fused (Aquiló *et al.*, 1999; Barberia Leache and Boj Quesada, 2001; Boj Quesada 1990; Canut Brusola 1988; Oliván Rosas *et al.*, 2004; Ostos Garrido and Peñalva Sanchez, 1996; Hagman, 1988; Reddy and Munshi, 1999).

#### **MATERIALS AND METHODS**

A standard osteological analysis was performed on the skeletal remains recovered from Law's Site (1MS100). The Law's Site was a village on the southern end of Pine Island in Marshall County, Alabama. The site was excavated in 1938 by the Works Project Administration (WPA) under the direction of Carl F. Miller (Webb and Wilder, 1951). Shortly thereafter, Pine Island was inundated when construction of Guntersville Dam was completed and the low-lying Guntersville Basin flooded to become Guntersville Lake.

The site had seen Native American occupation since the Archaic Period (about 8,000-1,000 B.C.) (Walthall, 1980); though many of the burials have been convincingly attributed to the post-contact period, between 1540 and about 1715 (Fleming, 1976; Padgett, 2007; Webb and Wilder, 1951). It is believed that the Native occupants of this latter period were the historically known Koasati, or Coushatta, tribe (Padgett, 2007; Swanton, 1985, 1989), though there is some dispute on this issue (Hudson, 1997).

#### **CASE REPORT**

Burial MS100-14 was an infant of indeterminate sex, aged about 9 months based on dental eruption. No indications of pathological infection or physical trauma were found among the remains.

Burial MS100-14 exhibited fused dentition, appearing as a block of three teeth fused side by side in their standard position. The fused set consists of the deciduous left maxillary central incisor, lateral incisor, and canine (Fig. 1). The central incisor and canine appear to be of normal dimensions, while the lateral incisor is reduced in mesiodistal width, and appears to be a conically-shaped "peg tooth."

#### **DISCUSSION**

The three teeth are fused at both the enamel and the dentin, though all three can be recognized as distinct from the others (Fig. 1). Furthermore, it is apparent that although the pulp cavities of the three teeth are continuous with each other, each tooth was maintained by its own root canal. These observations support the assertion that this is a true case of dental fusion, rather than gemination of a single tooth (Oliván Rosas *et al.*, 2004; Uys and Morris, 2005).

Some studies have found a predilection for one sex or the other regarding fused teeth or peg teeth (Wu and Feng 2005; Yonezu *et al.*, 1997); however, no evidence relating to sex can be interpreted from the remains or associated materials of Burial MS100-14.

#### **CONCLUSION**

The expression of triple fusion in MS100-14 as a dental anomaly is unusual in that it occurred among the maxillary dentition, as other researchers have found that when dental fusion occurs it is predominantly in the mandibular arch (Cheng *et al.*, 2003; Yonezu *et al.*, 1997). The peg-shaped tooth, while an anomalous feature, is typical in the respect that it is a lateral incisor (Wu and Feng, 2005). Burial MS100-14 represents a rare case in physical anthropology of triple fusion of primary dentition found in an archaeological context. Furthermore, the case

of triple fusion reported from among prehistoric Native American remains in the Southeastern United States.

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## A Rare Form of Protostylid: Review of Literature and Case Reports

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ABSTRACT Human teeth possess an active cingular zone that serves as the point of origin for specific accessory cusps. The term protostylid or protoconid is used for any additional cusps on the buccal surface of maxillary and mandibular premolars and molars. Their occurrence in maxillary and mandibular deciduous and permanent

dentition is discussed in the literature. This article presents a review of literature in relation to formation of the protostylid, and two cases of prominent protostylids are described, one each in the deciduous and permanent dentitions. *Dental Anthropology* 2010;23(1):28-31.

The protostylid exhibits a range of morphological expression ranging from a pit to a prominent cusp. However, its most common form is a surface irregularity (Dahlberg, 1963; Mayhall, 1979). Human teeth possess an active cingular zone that serves as the point of origin for specific accessory cusps (Butler, 1956). In the maxilla, this zone is active primarily on the lingual surfaces of the anterior teeth whereas in the molars, lingual tubercles and Carabelli's trait are expressed. The mandibular teeth are less likely to exhibit development from the lingual cingular zone, while a cingular trait of the lower molars, the protostylid, is sometimes present on the buccal surface of the mesiobuccal cusp. Simons (1972) points out that a correlation exists in pongids between well-developed lingual cingula of the upper molars and buccal cingula of the lower molars. This suggests that the Carabelli's traitprotostylid association in the human dentition reflects a long-term developmental relationship in hominoid phylogeny (Scott, 1978). The protostylid is also more frequent in early hominid species like those from the genus Australopithecus than in later Homo species. Among Australian Aborigines, the remarkable characters include those termed the "Mongoloid dental complex" (Hanihara, 1966, 1967, 1968, 1970) and Carabelli's cusp. The Mongoloid dental complex is composed of five crown characters, namely shovel-shape in the maxillary central incisors, cusp six, cusp seven, deflecting wrinkle, and protostylid on the mandibular first molars. This suite of characteristics is similar for deciduous maxillary incisors and mandibular second molars (Hanihara 1970).

Hanihara (1967) stated the protostylid occurs more frequently on the primary than permanent molars. According to Dahlberg (1950) and Hanihara (1961), whenever the protostylid is present on a permanent molar the trait was present on the primary second molar. However, the reverse situation does not always occur (Tongkoom, 1994). Hanihara (1961) provides a

classification consisting of seven grades of the protostylid (Table). The protostylid also can occur on the primary mandibular second molars (Tongkoom, 1994).

This cingular feature, which was first reported by Bolk (1916), is seen most frequently on the buccal surface of the mesiobuccal cusp of both primary and permanent molars, so an additional cusp on the buccal surface of a molar is referred to as a paramolar tubercle, or Bolk's cusp (Tongkoom, 1994). Broom (1937) described the protostylid feature on "a rudimentary external cingulum." Dahlberg (1945) later proposed the term protostylid or parastyle for any anomalous cusp on the buccal surface of maxillary and mandibular premolars and molars (Goaz and Miller, 1966). Dart (1948) described the feature on a molar as "a laterally-disposed enamel ridge" separated from the protoconid by a cingular furrow. Dahlberg (1950) also noted that "although the cusp had its origin as an expression of the cingulum, it is a unit structure, an entity in itself and definitely unlike the continuing cingular eminence seen on the gorilla and other anthropoids.'

The Arizona State University Dental Anthropology System (ASUDAS), which was devised for the analysis of modern human teeth, defines the protostylid as "a secondary groove that extends mesially from the buccal groove and which culminates in teeth with a marked expression of the protostylid, as a cusp with a free apex" (Turner *et al.*, 1991). Turner *et al.* (1991) described it as "a paramolar cusp found on the buccal surface of the protoconid that is normally associated with the buccal groove." As noted by Hlusko (2004), the terms

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TABLE 1. Hanihara's (1961) classification is composed of seven grades of protostylid forms



Grade 0: The mesiobuccal groove is straight and there is no trace of any irregularity.



Grade 1: No evidence of a protostylid, but its presence is suggested by the curvature and branching of the mesiobuccal groove. There may be a small but distinct pit at the lower terminus of the mesiobuccal groove separating the protoconid from the hypoconid. In such a case the buccal groove is slightly bent distalward at the point of the pit.



Grade 2: The divergence of the mesiobuccal groove is evident



Grade 3: The two branches of the mesiobuccal groove are more strongly developed than in grade 2. A small triangular area with its tip downward occurs between the branches of the buccal groove



Grade 4: A shallow groove appears at the mesial corner of the buccal surface. The area between this groove and the mesial branch of the mesiobuccal groove bulges slightly and gives a triangular shape with its tip upward.



Grade 5: The triangular area is more strongly developed than in grade 4.



Grade 6: The protostylid is strongly developed so that the tooth seems to have an extra cusp on the buccal surface of mesiobuccal cusp.

"protostylid" and "protoconidal cingulum" have been used interchangeably when describing features the buccal surface of hominin lower molars.

The prevalence of the protostylid varies with race (Dahlberg, 1963). It may be present in up to 40% of a population. The protostylid can occur with or without Carabelli's trait on the maxillary molars of Arctic people. The Carabelli trait frequently appears in Caucasoids. Like Carabelli's trait, the protostylid has both a similar range of morphological variation and frequency of forms (Turner, 1967). Hanihara (1968) reported a high frequency of this character in Pima Indians. Suzuki and Sakai (1954) found fairly frequent appearance of the protostylid in the mandibular molars of Japanese. In Mongoloid groups, the protostylid trait occurs in more than 40% of individuals, while in non-Mongoloid populations the prevalence is generally below 20%.

#### **CASE REPORTS**

The following are case reports of two patients who visited the Department of Pedodontics and Preventive Dentistry of Modern Dental College and Research Centre, Indore, Madhya Pradesh, India.

The first case was a 14 year old girl with the chief complaint of decay in right and left lower back teeth. There was no history of pain or any discomfort, and she had no significant health history. Examination revealed an unusual accessory cusp in relation to the mesiobuccal cusp of the lower first permanent molars, which also exhibited a four cusp pattern (Figs. 1-3). This accessory cusp is grade 6 using Hanihara's (1961) classification (Table). The protostylid was strongly developed, giving the appearance of an extra cusp on the buccal surface. There also was a prominent cusp of Carabelli on the upper first permanent molars. The lower second premolars showed a Y-shape groove pattern.

The second case was an 11 year old girl with the chief complaint of a white spot on her upper left front tooth, and she wanted to have her teeth cleaned. She had no significant medical or dental history. Examination revealed that the patient had Turner's hypoplasia on the permanent maxillary left central incisor. An accidental finding was an accessory cusp (Fig. 4) on the mesiobuccal cusp of the deciduous maxillary right first molar classified as grade 6 using Hanihara's classification, and a pronounced bulge similar in relation to deciduous maxillary left first molar classified as grade 4 of the same (Table).

#### DISCUSSION

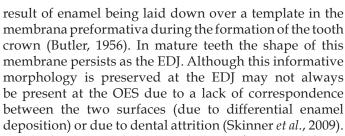
The protostylid forms during the morphogenetic phase of tooth formation, before the onset of dentinogenesis or amelogenesis. The fact that, it is actually the beginning of a cusp formation can be established by the shape of the enamodentin junction (EDJ) beneath it. These are considered outer enamel surface (OES) traits that are the



**Fig. 1**. Protostylid in relation to mesiobuccal cusp of permanent mandibular right first molar.



**Fig. 3**. Bilaterally appearing protostylid on the mandibular permanent first molars.



The location and the morphology of protostylid pits make them similar to occlusal fissures. Both features open at the bottom of the groove between the two cusps,



**Fig. 2.** Protostylid in relation to mesiobuccal cusp of permanent mandibular left first molar.



Fig. 4. Bilaterally appearing well-pronounced protostylid on deciduous maxillary left and right first molar.

and they both extend to the most concave point of the enamodentin junction. The depth of the normal fissure depends on the distance between two growth centers (Awazawa *et al.*, 1989), which is on a concavity of the EDJ, and the same probably is true for protostylid pits. Soon after the beginning of amelogenesis at this site, the enamel organ becomes increasingly constricted because of the concave EDJ. Eventually, amelogenesis at the foot of the pit ceases and the ameloblasts lose their Tomes' processes and form a layer of surface aprismatic enamel (Gaspersic, 1993).

cusp and as a remnant of a cingulum (i.e., a crestal feature). This distinction is relevant to considerations of whether the feature is a cusp or a crest, but this depends Hanihara K. 1961. Criteria for classification of crown on the definition of a cusp. The primary cusps of all primate teeth have a dentin horn, which forms early in the development of the tooth crown on the surface of the inner enamel epithelium, and is subsequently covered by enamel. This is also the case for the majority of accessory cusps, such as cusp six and cusp seven (Skinner et al., 2008), and even small features such as marginal ridge Hanihara K. 1968. Morphological pattern of deciduous tubercles on upper molars and the mammelons present on unworn incisors (Kraus and Jordan, 1965). Only in rare circumstances in extant hominoids and fossil hominins are there "enamel-only" cuspules (i.e., small cusps with no underlying dentin horn). Thus, for the purpose of defining the protostylid trait a structure defined as a cusp should exhibit an underlying dentin horn at the EDJ surface. The protostylid pit may lie between a large protoconid and a nearly negligible protostylid consisting only of dentin core (Gaspersic, 1993).

#### **CONCLUSION**

The protostylid and Carabelli's trait was found to co-occur in the two cases described. This combination is interesting because these traits occur on homologous cusps in opposite jaws (Tongkoom, 1994). The similarity in form and position of this structure in contemporary man and in prehistoric forms is considered as evidence of a relationship between these groups.

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## **Obituary Alice "Sue" Marie Frances Haeussler (1932-2009)**

Sue Haeussler passed away in November, 2009, at the age of 77, following a lengthy period of illness linked with Alzheimer's disease. Among the many reasons to remember Sue, especially for the readers of this journal, is that she was Editor of *Dental Anthropology* for 10 years (1991-2001), sharing editorship for her last issue with Edward F. Harris. During her tenure she advanced *Dental* Anthropology from being a short newsletter started by DAA founder, M. Yasar Iscan, to a professional journal in AIPA style with peer-reviewed articles, book reviews, DAA secretary and treasurer's reports, presidential addresses, and other interesting items. Sue also promoted an international membership, paying out of her own pocket the annual dues for overseas scholars she knew who were in financial need. Whoever reads this necrology will hopefully carry on Sue's helping our needy foreign dental anthropology colleagues.

Born on July 26, 1932, in Philadelphia, the city where in 1954 she earned a B.A. degree in microbiology from the University of Pennsylvania. As an undergraduate she was involved in various extra-curricular activities, notably photographic editor and feature writer for *The Pennsylvania News*, experiences that she expertly applied years later to her meticulous guidance of *Dental Anthropology*.

I first remember Sue being in an introductory anthropology class that I taught in the large auditorium at the 1930s-style Arizona State University agricultural building. The old, Depression-era concrete building had as its only architectural excess, large fronting intaglio portraits of five or six famous scientists. Only one was mostly hidden by a large tree — Charles Darwin. Sue often came up excitedly after class with a ream of questions, especially about the issue of the colonization of the New World, a subject that would eventually become the heart of her massive doctoral dissertation and her grand odyssey in the former USSR.

Later, Sue earned an M.A. in 1985 and a Ph.D. in 1996, both in physical anthropology at our explosively-growing Arizona State University campus in Tempe. Her dissertation data on dental morphology was collected traveling alone during a nine-month trip in the former USSR from December, 1990, to August, 1991. She was formally invited by scholars in various USSR Academy of Science institutes and universities housing archaeologically-derived human dental remains [St. Petersburg, Kiev (the Ukraine), Irkutsk, Novosibirsk, Tomsk, Tbilisi (Georgia), Moscow, and Krasnoyarsk], in that order, based on my examination of her more than 1,000 labeled color slides. Her grit and stamina at a mature age (58) can be appreciated by the fact that despite unrelenting back pain, she walked resolutely,



Fig. 1. Sue Haeussler (left) and Irkutsk State Univeristy archaeologist, German I. Medvedev, at the Siberian village of Mal'ta, south of Lake Baikal, March 31, 1991. The pair are standing on the frozen ground, beneath which (at about two meters depth) is the 21,000 yearold Upper Paleolithic site of the same name. The Mal'ta archaeological site is world famous for its carved ivory female figurines, carved images of birds, stone blade knives, other artifacts, and mammoth bone shelters, all strongly suggestive of a link with European Upper Paleolithic culture. This link is further strengthened by the European-like permanent incisor and molar morphology of a child found "buried" at the site. Sue and the author agree on the European character of the Mal'ta teeth, making Mal'ta the most eastern-known extension of Cro-Magnon culture and people. Photographer uncertain, but probably by Ekaterina Lipnina, Medvedev's archaeologist

with the help of a stout wooden walking stick (Fig. 1), people. And, (2) few if any molecular geneticists (paleoeven in the frigid winter months of her Russian odyssey. Her Soviet research was aided by an IREX fellowship and other sources. Her speaking and reading knowledge of Russian was gained with immense help from ASU language professor, Sanford Couch. Sue's institute visits were greatly helped by the Russians in her photos that I know also spoke excellent English, including Serghei A. Arutionov, Moscow; Alexander G. Kozintsev, St. Petersburg; Alexander K. Konopatski, Novosibirsk; and the world renowned dental anthropologist, Alexander A. Zoubov, Moscow.

My own Russian travel and research before and after that of Sue's has taken me to many of the institutes that she visited. Everyone asked how she was, and had very kind words to say about her. She was an excellent ambassador for the United States. Sue made an additional trip to Russia that I know about. She participated in an international conference held in Vladivostok. Hence, she traveled across the totality of Russia, from the Baltic (St. Petersburg), to the Sea of Japan (Vladivostok). I know of few other graduate students, or even seasoned professors, who have undertaken such an odyssey, and everywhere left so much good will.

addition to her monumental two-volume dissertation (> 750 pages), Sue also published a number of articles, abstracts, and presented posters, all at national and international meetings. A few of her more easily obtained titles are cited in the following bibliography. Her dissertation lists several papers that were waiting publication or were in progress.

As a dental morphology researcher, Sue was a careful observer. Where she and I studied the same dental collections in the USSR, we were concordant in >90% of our ranked scale and discrete observations. The prime rule governing the ASU Dental Anthropology System of trait observation is: "when in doubt, never guess." Sue followed this rule religiously. In Irkutsk, she found an example of Donald Morris' "Uto-Aztecan" premolar. While her finding was only one of two examples ever recognized outside of the New World, there is absolutely no reason to doubt her observation. The gene(s) for this trait was present but very rare in northeastern Asia, but its relatively frequent occurrence in American Indians fits nicely with the views that there was founder's effect in the crossing of Beringia, and more than one Siberian migration to the New World since the trait has never been found in Aleut-Eskimo populations. Sue's thousands of other observations fit well with the hypothesis of a northeast Asian origin of all Native Americans, and not an origin from central Asia or Europe as has been suggested on the basis of some archaeological considerations. I mention these finding to make two points: (1) Very few archaeologists concerned with the colonization of the New World have traveled to Russia to learn what archaeologists have found there. Sue traveled to see the actual teeth of late Pleistocene and Holocene Eurasian

or modern) read or acknowledge the findings of dental anthropologists. Sue's magnificent dental morphology study in the USSR will hopefully not be overlooked.

As wife of a busy psychiatrist (William B. Haeussler, M.D.), and mother, Sue balanced her professional and personal life with admirable skill, compassion, and charm, always hiding her chronic back pain. Her daily routine at ASU often started at 4:00 a.m. lasting until well after dinnertime. She usually rode the bus from her home in Phoenix to the ASU campus in Tempe. Her time in transit was usually spent editing or reading articles, a number published in Russian.

Peers and associates will greatly miss her, as will her family. At the risk of overlooking someone, Sue's close dental anthropology colleagues of whom I am aware of included Edward F. Harris; G. Richard Scott; Diane E. Hawkey; Donald H. Morris; Shara E. Bailey; C. Loring Brace; Joel D. Irish; Scott Burnett; Christine Lee; Jaime Ulinger; Heather H. Edger; Kenneth A. R. Kennedy; Korri D. Turner; Christian R. Nichol; John R. Lukacs; Alma J. Adler; Lorrie Lincoln-Babb; Edwin F. Crespo; Stephen C. Reichardt; Thelma Dahlberg; Natalya I. Khaldeyeva; Alexander A. Zubov; Simon Hillson; and the late Daris R. Swindler, Albert A. Dahlberg, and Kazuro Hanihara. Sue had close professional colleagues in other branches of anthropology or science who will also miss her. Again, those whom I know of include Serghei A. Arutiumov, Russia; Liu Wu, China; Inna Potieklhim, Ukraine; Triona G. McNamara, Ireland; Yoshitaka Manabe, Japan; and a number of others who Sue met along the way during her USSR odyssey and during her DAA editorship. I mention these names here and earlier, probably inappropriately, but to simply illustrate the fine mesh of Sue's professional and social net. A little of each of these friends of Sue, and myself, have or will die as a result of Sue's passing. I am at a loss of words to say how much my late wife, Jacqueline, and I enjoyed Sue and Bill's presence at various gatherings in our home honoring one or another graduate student's completion of his or her program, and various visits by established scholars. Sue and Bill almost always sat on the large built-in couch by the big front room window. They were older than most of the other guests, but their quiet cheerful presence always gave class and respect to the evening gathering.

> Christy G. Turner II Regents' Professor Emeritus Arizona State University

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The manuscript should be accompanied by a letter from the student's supervisor indicating that the individual is the primary author of the research and the paper. Multiple authorship is acceptable, but the majority of the research and writing must be the obvious work of the student applying for the prize. Send enquiries and submissions to the President of the *DAA*:

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