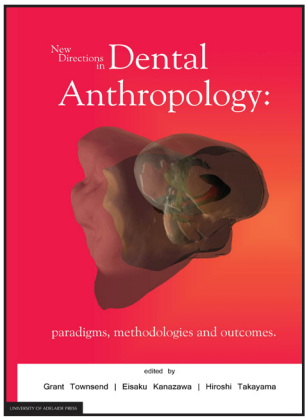


Welcome to the electronic edition of *New Directions in Dental Anthropology*.

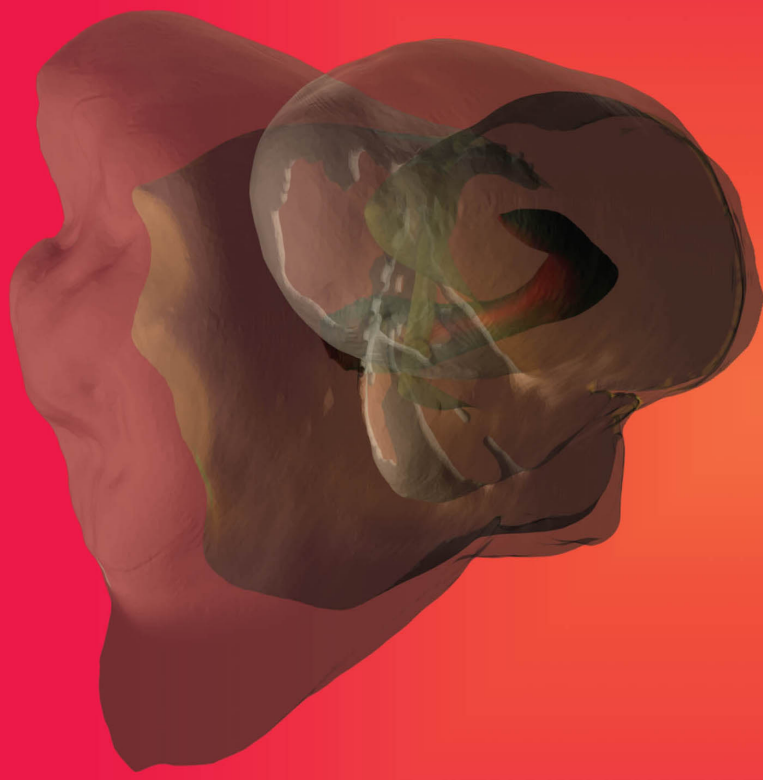
The book opens with the bookmark panel and you will see the contents page. Click on this anytime to return to the contents. You can also add your own bookmarks.

Each chapter heading in the contents table is clickable and will take you direct to the chapter. Return using the contents link in the bookmarks.

The whole document is fully searchable.

Enjoy.

New Directions in Dental Anthropology:



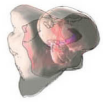
paradigms, methodologies and outcomes.

edited by

Grant Townsend | Eisaku Kanazawa | Hiroshi Takayama

UNIVERSITY OF ADELAIDE PRESS

Cover design by Emma Spoehr



New Directions in Dental Anthropology: paradigms, methodologies and outcomes.

edited by Grant Townsend, Eisaku Kanazawa and Hiroshi Takayama.

This book contains papers arising from a symposium held during a combined meeting of The International Union of Anthropological and Ethnological Sciences (IUAES), The Australian Anthropological Society (AAS) and The Association of Social Anthropologists of Aotearoa New Zealand at the University of Western Australia from July 5-8th, 2011. It follows on from a recently published Special Issue Supplement of Archives of Oral Biology, Volume 54, December 2009 that contains papers from an International Workshop on Oral Growth and Development held in Liverpool in 2007 and edited by Professor Alan Brook. Together, these two publications provide a comprehensive overview of state-of-the-art approaches to study dental development and variation, and open up opportunities for future collaborative research initiatives, a key aim of the International Collaborating Network in Oro-facial Genetics and Development that was founded in Liverpool in 2007.

The aim of the symposium held at The University of Western Australia in 2011 was to emphasise some of the powerful new strategies offered by the science of dental anthropology to elucidate the historical lineage of human groups and also to reconstruct environmental factors that have acted on the teeth by analysing dental morphological features. In recent years, migration, as well as increases and decreases in the size of different human populations, have been evident as a result of globalisation. Dental features are also changing associated with changes in nutritional status, different economic or social circumstances, and intermarriage between peoples. Dental anthropological studies have explored these changes with the use of advanced techniques and refined methodologies. New paradigms are also evolving in the field of dental anthropology.

When considered together with the recent special issue of Archives of Oral Biology that highlighted the importance of research approaches focused at both the molecular and phenotypic levels, it is clear that we have now reached a very exciting stage in our ability to address key questions and issues about the normal and abnormal development of the dentition, as well as the diseases that commonly affect our teeth and gums.

UNIVERSITY OF ADELAIDE PRESS | THE UNIVERSITY of ADELAIDE

New Directions in Dental Anthropology

New Directions in Dental Anthropology:
paradigms, methodologies and outcomes

edited by

Grant Townsend
School of Dentistry, The University of Adelaide, Adelaide, South Australia, Australia
Centre for Orofacial Research and Learning, The University of Adelaide
International Collaborating Network in Oro-facial Genetics and Development

Eisaku Kanazawa
Department of Anatomy, Nihon University School of Dentistry at Matsudo, Chiba, Japan

Hiroshi Takayama
Laboratory of Anthropology, Faculty of Letters, Keio University, Tokyo, Japan

UNIVERSITY OF ADELAIDE PRESS
THE UNIVERSITY of ADELAIDE

Published in Adelaide by

University of Adelaide Press
The University of Adelaide
Level 1, 230 North Terrace
South Australia
5005
press@adelaide.edu.au
www.adelaide.edu.au/press

The University of Adelaide Press publishes externally refereed scholarly books by staff of the University of Adelaide. It aims to maximise the accessibility to its best research by publishing works through the internet as free downloads and as high quality printed volumes on demand.

Electronic Index: this book is available from the website as a down-loadable PDF with fully searchable text. Please use the electronic version to serve as the index.

For the full Cataloguing-in-Publication data please contact the National Library of Australia: cip@nla.gov.au

New directions in dental anthropology: paradigms, methodologies and outcomes / edited by Grant Townsend, Eisaku Kanazawa and Hiroshi Takayama.

1 Dental anthropology — Congresses

I Townsend, Grant
II Kanazawa, Eisaku
III Takayama, Hiroshi

ISBN (electronic) 978-0-9871718-7-0
ISBN (paperback) 978-0-9871718-8-7

Cover design: Emma Spoehr
Cover photograph supplied by Dr Jeremy Deverell from the South Australian node of the Australian National Fabrication Facility (Ian Wark Research Institute, University of South Australia) under the National Collaborative Research Infrastructure Strategy.
Book design: Zoë Stokes
Paperback printed by Griffin Press, South Australia

Foreword

This book contains papers arising from a symposium held during a combined meeting of The International Union of Anthropological and Ethnological Sciences (IUAES), The Australian Anthropological Society (AAS) and The Association of Social Anthropologists of Aotearoa New Zealand at the University of Western Australia from July 5-8th, 2011. It follows on from a recently published Special Issue Supplement of Archives of Oral Biology, Volume 54, December 2009 that contains papers from an International Workshop on Oral Growth and Development held in Liverpool in 2007 and edited by Professor Alan Brook. Together, these two publications provide a comprehensive overview of state-of-the-art approaches to study dental development and variation, and open up opportunities for future collaborative research initiatives, a key aim of the International Collaborating Network in Oro-facial Genetics and Development that was founded in Liverpool in 2007.

The aim of the symposium held at The University of Western Australia in 2011 was to emphasise some of the powerful new strategies offered by the science of dental anthropology to elucidate the historical lineage of human groups and also to reconstruct environmental factors that have acted on the teeth by analysing dental morphological features. In recent years, migration, as well as increases and decreases in the size of different human populations, have been evident as a result of globalisation. Dental features are also changing associated with changes in nutritional status, different economic or social circumstances, and intermarriage between peoples. Dental anthropological studies have explored these changes with the use of advanced techniques and refined methodologies. New paradigms are also evolving in the field of dental anthropology.

The papers published in this book cover several exciting new areas within dental anthropology. The first three papers introduce some new concepts and approaches to clarifying how genetic, environmental and epigenetic factors influence phenotypic variation within the dentition. The first paper by Alan Brook and his son Matthew Brook O'Donnell introduces dental anthropologists to complexity theory and its potential as a means of modelling the multidimensional, multilevel and multifactorial interactions that occur over time during the development of the dentition. The second paper by Grant Townsend and colleagues summarises some of the key findings of the studies of twins and their families carried out in the Craniofacial Biology Research Group at The University of Adelaide over the past 25

years or so, and emphasises the value of different research models involving twins, including the monozygotic co-twin model and the dizygotic opposite-sex twin model. The third paper by Toby Hughes and Grant Townsend explores in detail how sophisticated mathematical modelling approaches using data from twins can be applied to explore relationships between dental features and also how these models can incorporate molecular marker data to identify genes of major influence. Modelling of data from monozygotic co-twins is also considered as a way of examining the role of the epigenome on dental development.

The next group of three papers report on recent findings from studies being undertaken by postgraduate students in the Craniofacial Biology Research Group at Adelaide. The paper by Chan and others from Adelaide looks at the question of whether feeding practices, gestation length, and birth weight affect the timing of emergence of the first primary tooth. Extremely premature infants and those with very low birth weights displayed delayed tooth emergence but, generally, the primary dentition appeared to be well 'protected' against environmental disturbances. The paper by Ribeiro and colleagues shows that a 2D image analysis system can be used to record a range of quantitative dental phenotypes accurately and precisely. The patterns of variation displayed within and between the sexes were found to be consistent with some influence of hormones during development and the authors indicate that they plan to apply the opposite-sex dizygotic twin model to further elucidate the effects of male hormones on dental development in utero. The paper by Ashar and others demonstrates the great potential of applying 3D scanning systems to dental models for both anthropological and forensic purposes.

The next paper by Taduran reports the findings of a study of sex determination in Filipinos based on crown measurements obtained from canine teeth. The results contradict previous studies that have claimed there is no significant sexual dimorphism in the permanent dentition of Filipinos. The paper by Kanazawa and Matsuno explores the morphological dental features of Papua New Guinea Highlanders and concludes that the unique set of dental features characteristic of Papua New Guineans has been acquired by a process of morphological reduction from an original Australian type of dental features and by admixture with South Asian and Pacific peoples. The paper by Kato, Takayama and Townsend presents, for the first time in English, the concepts and implications of Kato's Main Occluding Area. Some recent findings in an Australian sample are presented and a new interpretation of the evolution of this feature is provided.

The final two papers in the book consider new technologies for the analysis of tooth wear (Ranjitkar and colleagues) and a new holistic model to explain 'mineral maintenance' of dental structures in both caries and erosive tooth wear (Kaidonis and colleagues). These papers highlight the value of applying an anthropological perspective to issues of major public dental health and clinical importance in modern societies. The novel paradigms and new technologies presented in these papers should have major significance in future approaches to both the prevention and treatment of common dental diseases that are prevalent in contemporary societies.

Overall, the group of 11 papers in this publication provide an exciting perspective of

the rapidly evolving field of dental anthropology. All have been peer-reviewed by international experts.

When considered together with the recent special issue of Archives of Oral Biology that highlighted the importance of research approaches focused at both the molecular and phenotypic levels, it is clear that we have now reached a very exciting stage in our ability to address key questions and issues about the normal and abnormal development of the dentition, as well as the diseases that commonly affect our teeth and gums.

Grant Townsend, Eisaku Kanazawa and Hiroshi Takayama
Editors

Contents

1 Modelling the complexity of the dentition

Alan Brook[1,2,3,4] and Matthew Brook O'Donnell[2,5]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]International Collaborating Network in Oro-facial Genetics and Development
[3]Centre for Orofacial Research and Learning, The University of Adelaide
[4]Dental Institute, Queen Mary University of London, London, United Kingdom
[5]University of Michigan, Ann Arbor, Michigan, United States of America

ABSTRACT

The major components and phases of development needed to form a mature dentition are identified and outlined. This information is then examined against the general characteristics of Complex Adaptive Systems. It is concluded that the dentition in development and maturity has the characteristics of a Self Adaptive and Self Organising Complex System. This exploration provides the basis for future investigations of this model.

Introduction

The dentition, both in development and in maturity, is an intricate system of multiple interacting components. But is it a complex rather than a complicated system? We consider a Complex System to be a dynamic system in which interacting components at a lower level give rise to higher level emergent phenomena, whereas in a complicated system the various elements maintain a degree of independence from one another. Fortunately, in examining this question about the dentition there is much hard data now available concerning the molecular, cellular, soft tissue and mature mineralised components. The aim of this paper is to examine key characteristics of the dentition in development and its mature form against the characteristics of a Complex Adaptive System. Our method is to:

1. Identify and outline the major components and phases of development of the dentition into a mature functioning system.

2. Examine this information against the recognised general characteristics of Complex Adaptive Systems.

The dentition in development and maturity

The development of each tooth begins with the action of genes at specific sites in the adjacent tissues of the epithelium and the mesenchyme in the oral cavity. At the molecular level there are over 300 genes (Thesleff, 2006) . The functional genes are influenced by interaction with other genes, epigenetic and environmental factors (Brook, 2009). These interactions switch them on and off at critical times and their productivity may be upregulated or downregulated. The controlling genes may be part of signalling pathways (Brook, 2009; Tarcea et al., 2009). The epigenetic factors may be specific in their action, for example, demethylation or broader in effect e.g. testosterone in utero. Similarly, the environmental factors may be specific, for example, trauma to an individual developing tooth, or more general such as nutritional deficiency or systemic infection (Brook, 2009).

At the cellular level, cell-cell and cell-matrix interactions occur (Lesot and Brook, 2009). At the tissue level reciprocal interactions are seen between the epithelium and mesenchyme.

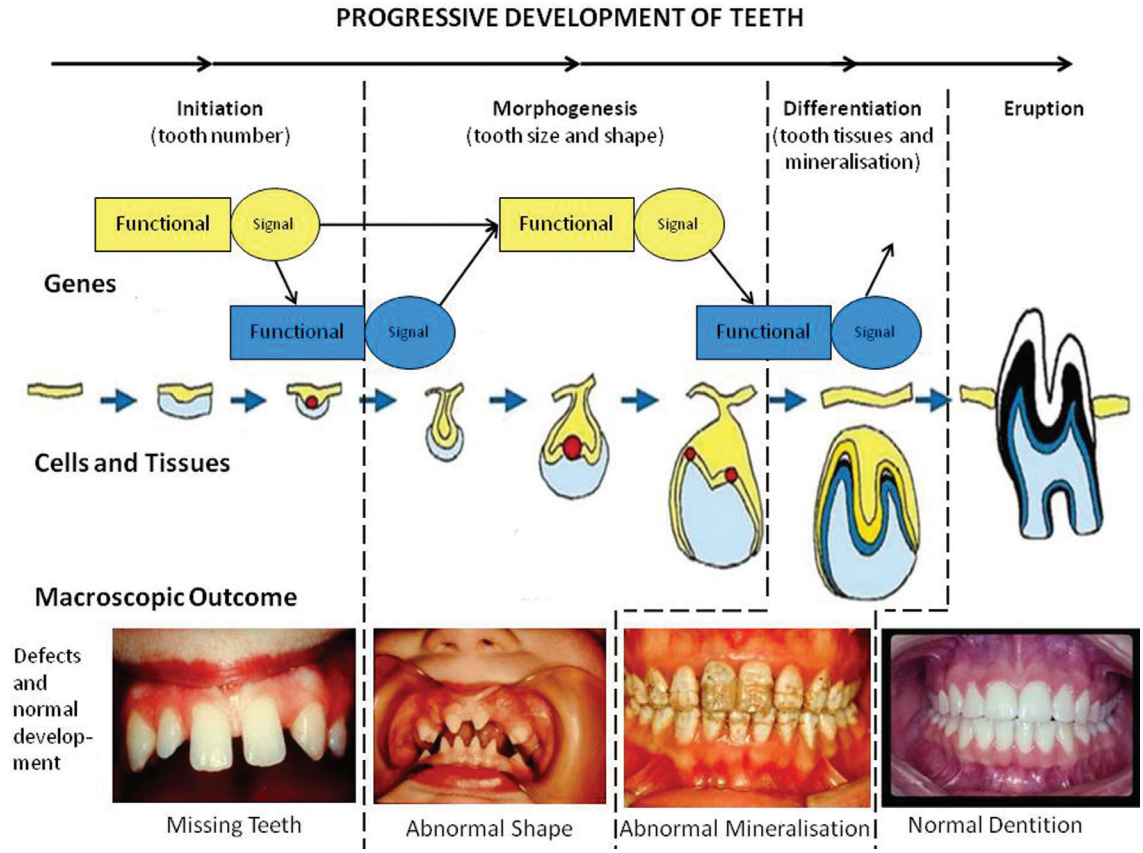


Figure 1. The multilayered developmental process of tooth formation.

The discrete tooth germs, which are initiated by these actions at specific sites in morphogenetic fields, develop through bud, cap and bell stages morphologically. As the tooth germs advance in morphogenesis, the primary and secondary enamel knots are important in determining size and shape (Lesot and Brook, 2009). The tooth germs develop in the three dimensions of space and over the fourth dimension of time. Within the morphogenetic fields at any given time the development of individual germs is at different stages. Interactions between these developing germs may result in variations in size and shape of the teeth, particularly those forming later at the end of morphogenetic fields (Townsend et al., 2009).

At the bell stage the cells undergo differentiation and ameloblasts and odontoblasts are formed. Different genes are switched on and structural matrix is secreted. Mineralisation commences, initiating dentinogenesis and amelogenesis. As mineralisation proceeds, the matrix is removed to allow denser deposition of mineral (Brook, 2009). The outcome of this intricate and prolonged developmental process, which in humans lasts from six weeks in utero to some 20 years of age, is two dentitions, primary and permanent. Each dentition is an effective functional system, made up of groups of teeth of different number, size and shape formed from the different morphogenetic fields. Each group of teeth contributes a complementary function to enhance the efficiency of the total system.

The dentition in relation to the general characteristics of a Complex Adaptive System

There are many different types of Complex Systems, e.g. biological systems, the World Wide Web and collective behaviours, but they all have a high number of properties in common (Mitchell, 2009; Camazine et al., 2003). As a Dynamic System with lower level interacting components from which higher level structures are formed, a Complex Adaptive System demonstrates such characteristics as: self organisation and emergence; self adaptation; and particular statistical concepts. In this section aspects of the developing and mature dentition are examined for conformity to these characteristics.

Self organisation and emergence

Initiation and morphogenesis stages

At the molecular level there are interactions between genes in which the functional genes are switched on by the action of signature sequences that have been activated by the release of regulatory proteins from regulatory 'master' genes. The antagonistic action of Fgfs and Bmps influence both the extent and the location of expression of Pax9, a paired box transcription factor, which is stimulated by Fgf8 and inhibited by Bmp2 and Bmp4. The action of functional genes is also influenced by specific and general epigenetic factors as in the regulation of dental stem cell differentiation by histone demethylase. In addition, local and general environmental factors affect these interactions.

Reciprocal and sequential interactions between the ectodermal and mesenchymal cells and transcription factors are controlled by multigene signalling pathways, such as Fgf, Bmp, Shh, Wnt and Tnf. In addition to these intracellular links, extracellular effects occur as in the modulation of the extracellular integration of cell signalling pathways by Lrp4.

This series of interactions regulates the initiation and morphogenesis of tooth germs, thereby determining their number, the region of the dental arch (the morphogenetic field) in which they develop, and the type, size and complex shape of each tooth (Brook, 2009; Wang and Thesleff, 2006).

Differentiation

With the underlying pattern and dimensions of the cusps determined, the dentine forming cells (the odontoblasts) and the enamel forming cells (the ameloblasts) differentiate, controlled by different genes. Pleiotrophin is expressed in both odontoblasts and ameloblasts. Tgf-B signalling controls Dspp expression in maturing odontoblasts: over expression of Tgf-B1 causes decreased expression of Dspp (Brook, 2009). Through an epigenetic mechanism, the histone demethylase Jmjd3 influences the expression of extracellular dentine matrix. The ameloblasts secrete the enamel protein matrix to which the amelogenin gene (Amel) contributes the greatest percentage of protein, but the enamelin gene (Enam) provides the protein which controls the initiation of enamel mineralisation. As mineral deposition advances the proteases, Enamelysin (Mmp20) and Kallikrein (Klk4), act to remove matrix proteins and allow increased mineralisation. Amelogenesis determines the final outline size and shape of the tooth with different thicknesses of enamel on different aspects of the tooth (Brook, 2009).

Multitasking

Multitasking occurs during dental development as different genetic pathways act simultaneously and in parallel. Further, certain functional genes and signalling pathways act reiteratively controlling different stages of development, e.g. p21, Msx2, Lef1, as functional genes in ectoderm, Msx1, Barx1, Dlx1-2, Pax9, as functional genes in mesenchyme, and Bmp, Fgf, Shh, Wnt as signalling pathways between these two tissues from the initiation stage to the end of morphogenesis (Brook, 2009). Multitasking is also seen later during the differentiation phase in enamel as simultaneously mineralisation increases and enamel proteins undergo breakdown and removal, allowing mineralisation to advance.

Summary of evidence for self organisation and bottom-up emergence

From these interactions at the lower level, i.e. molecular, cellular and developmental soft tissue interactions, emerge at the higher level the macroscopic mineralised teeth. The individual teeth have been organised under the influence of morphogenetic fields into spacially distributed groups to form an integrated, effective, functioning system. The mature individual teeth and the dentition bear no resemblance to the earlier components from which they have emerged by self-organisation. The multitasking seen during odontogenesis is characteristic of such complex adaptive systems.

Self adaptation

Three characteristics of complex self adaptive systems are diversity, critical phases and robustness.

Diversity

Self adaptation is seen in the diversity of the dentitions which are present in different species. This diversity arises not so much from different number or type of genes, which are similar in the different species, but rather from the genetic switches that are used to turn genes on and off. Diversity also occurs within species as seen in the variation in tooth number, size, shape and mineralisation. This range of variation could allow adaptation to different masticatory demands and to changes of the skeletal basis in which the dentition develops and functions. Substantial differences between four human ethnic groups in mesiodistal tooth dimension and in the relative sizes in different tooth types has been shown (Brook et al., 2009).

Interaction between the soft tissue tooth germs as they are developing adds to the diversity of the formed teeth. Within the morphogenetic fields the later forming teeth are more variable, with some evidence from the murine dentition that the dimensions of the earlier forming teeth influence the size of later forming teeth and even whether a supernumerary tooth will develop.

Critical phases

There are a series of critical phases during dental development which determine whether a mature tooth will be formed, and, if it is formed, whether it will have a developmental defect or not. Transcription factors in the Msx, Dlx and Lhx families are necessary both for initiation of the tooth germ and for progression from the initiation stage into morphogenesis (Brook, 2009). If progression does not occur at a critical phase, the tooth germ may undergo apoptosis. During the differentiation stage partial or complete failure in the secretion of enamel matrix proteins leads to hypoplastic enamel defects in the mature tooth of varying types and severity. Later in amelogenesis failure to remove the secreted matrix proteins leads to hypomineralisation defects of varying degrees.

Robustness

Although it incorporates many detailed interactions with critical phases, another aspect of dental development is the robustness of the process and the relative efficiency of the outcome system even with variations and mild or moderate anomalies. During development when a gene has a mutation which decreases or prevents its function, compensation may sometimes occur between different members of the same gene family. In the mature dentition adequate mastication is possible with some variation in tooth size and shape and in the presence of mild or moderate mineralisation defects.

Summary of evidence for self adaptation

Diversity is seen in the markedly different dentitions present in different species of animals. Within species dental variations also allow adaptation to different environmental challenges. Dental development has multiple critical phases, interference with which lead to a range of dental anomalies. Even so, the process and outcome have a degree of robustness.

Statistical models and Complex Adaptive Systems

Several statistical models have been linked with aspects of findings in Complex Adaptive Systems.

Random network model

Statistically a normal distribution is characteristic for Random Networks. From many measurement studies of different populations, the mesiodistal and buccolingual dimensions of teeth have been found to have a normal distribution with the range of values described by a mean and standard deviations (Brook, 1984).

Threshold model

This model has a normal distribution of an underlying, continuously varying parameter, on which is superimposed one or more thresholds beyond which a seemingly discontinuous, different character is expressed. This model is often termed the Quasi-Continuous Model. Brook (1984) proposed such a model based on the normal distribution of tooth size with

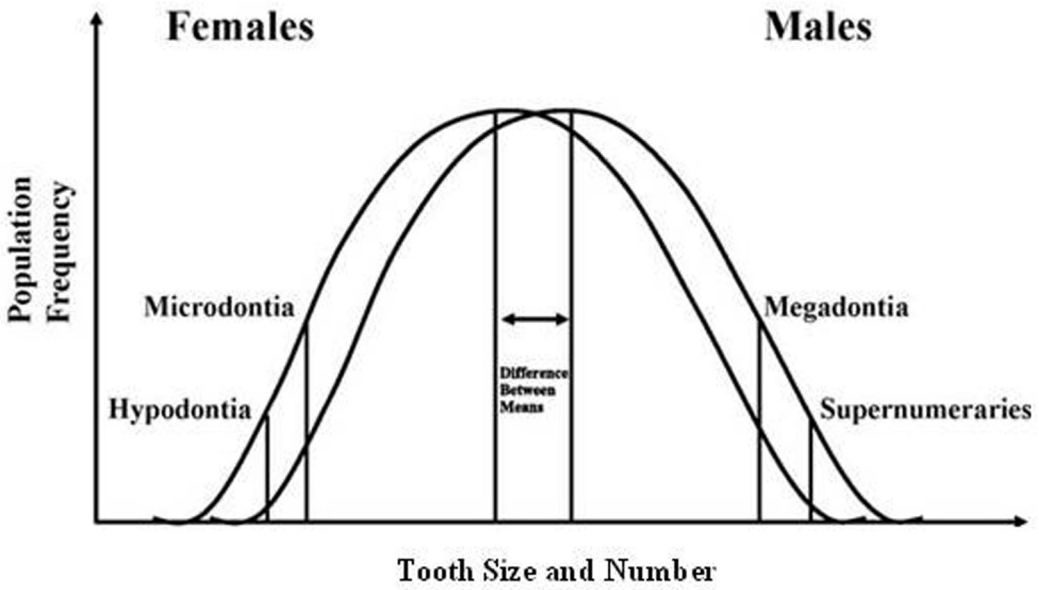


Figure 2. The threshold model of Brook (1984), based on the different normal distributions of females and males for tooth size, with thresholds determining the dental anomalies of number and size.

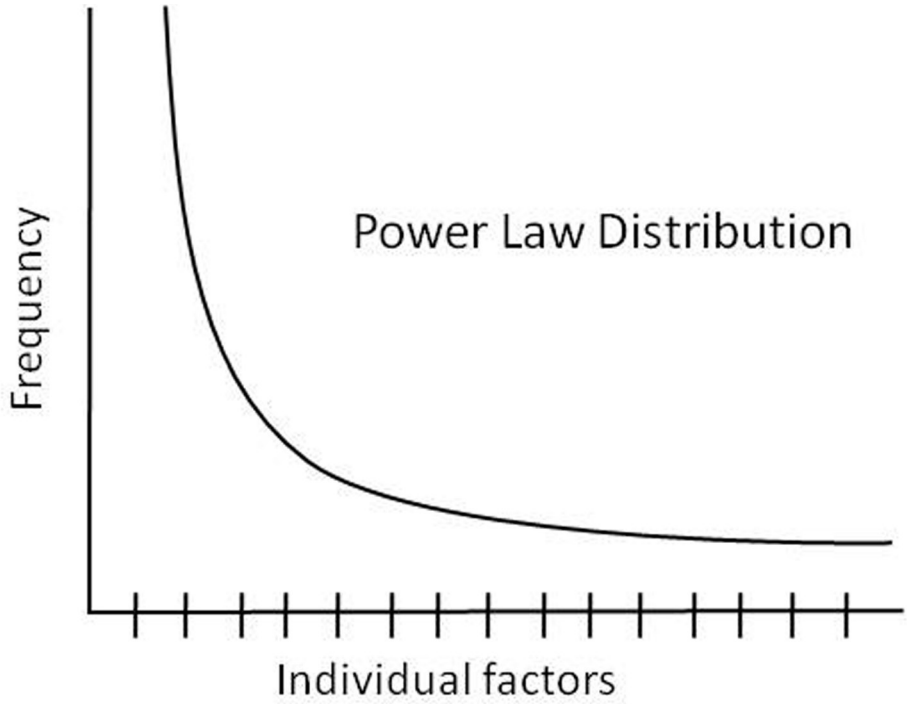


Figure 3. A typical power law distribution. The frequency with which factors occur is on the vertical axis.

thresholds at the lower end describing microdontia and hypodontia and at the upper end megadontia and supernumerary teeth.

Females have smaller teeth and a higher frequency of hypodontia and microdontia; males have larger teeth and a higher frequency of megadontia and supernumeraries. The subsequent genetic and histogenetic findings of some of the mechanisms by which these anomalies arise at critical phases during development has provided the developmental rationale for that model which was based on statistical analysis of clinical and epidemiological findings.

Scale free network model

This distribution is based on the finding that of all of the different examples of a parameter being measured, e.g. individual words in a scientific paper, most will be used infrequently but some occur with a high frequency. When such findings are plotted graphically a Power Law Distribution (often termed a Zif Distribution in linguistic studies) results.

The highly frequently occurring entities are the hubs and the many infrequent ones are the nodes.

In a study of upper incisor teeth multiple dimensions were measured using 2D image analysis and customised software. Principal Components Analysis was applied to the findings for the 34 variables measured for each incisor. Seven factors accounted for 94% of the total variance in the upper central incisor measurements with the Scree Plot of the 34 factors showing Power Law Distribution (Khalaf et al., 2009).

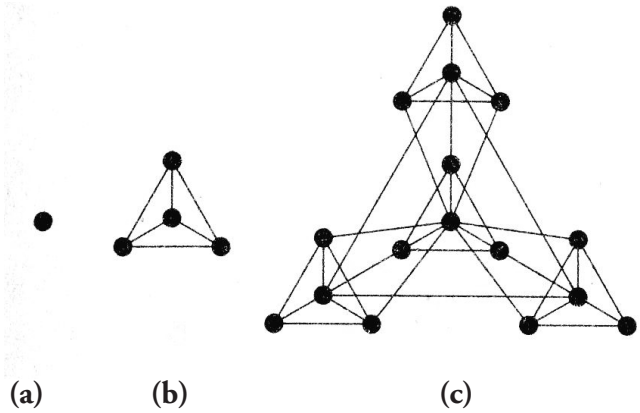


Figure 4. A typical hierarchical network, based on Barabasi (2003). (a) node (b) nodes joined to a hub to form a module (c) models hubs joined to 'super-hubs' to form a hierarchical network.

Hierarchical network model

Each individual entity, e.g. gene, is a node and is connected to one or more other nodes. A few nodes have multiple connections to other nodes and act as hubs. The hub and the nodes connected to it form a module. Such modules can be joined together by 'super hubs' to create a Hierarchical Network (Barabasi, 2003). The repeated actions of a few genes during dental development suggest that this type of model may be valuable in further studying odontogenesis.

Conclusions and future work

The dentition, both in development and in mature form, has the general characteristics of a Self Organising and Self Adaptive Complex System. This exploration provides the foundation for future investigations of this model. As a basis for these computational studies there are published genetic databases, extensive histogenetic investigations, and accurate macroscopic phenotyping data from 2D, 3D and 3D superimposition studies.

References

Barabasi A.L. (2003) Linked. Plume Books. Penguin Group New York.

Brook A.H. (1984) A unifying aetiological explanation for anomalies of tooth number and size in humans. Archives of Oral Biology, 29: 373-378.

Brook A.H. (2009) Multilevel complex interactions between genetic, epigenetic and environmental factors in the aetiology of anomalies of dental development. Archives of Oral Biology, 54: S3-S17.

Brook A.H., Griffin R.C., Smith R., Townsend G.C., Kaur G., Davis G.R., and Fearne G. (2009) Tooth size patterns in patients with hypodontia and supernumerary teeth. Archives of Oral Biology, 54: S63-S70.

Camazine S., Deneubourg J-L., Franks N.R., Sneyd J., Theraula G., and Bonabeau E. (2003)

Self-organization in biological system. Princetown, NJ: Princeton University Press.
Khalef K., Smith R.N., Elcock C., and Brook A.H. (2009) Multiple crown variables of the upper incisors in patients with supernumerary teeth compared with controls. Archives of Oral Biology, 54: S71-S78.
Lesot H., and Brook A.H. (2009) Epithelial histogenesis during tooth development. Archives of Oral Biology, 54: S25-S33.
Mitchell M. (2009) Complexity. A guided tour. New York: Oxford University Press.
Tarcea V.G., Weymouth T., Ade A., Bookvich A., Gao J., Mahavisno V., Wright Z., Chapman A., Jayapandian M., Özgür A., Tian Y., Cavacoli B., Mirel B., Patel J., Radev D., Athey B., States D., and Jagadish H.V. (2009) Michigan Molecular Interactions (MiMI) r2: From interacting Proteins to Pathways. Nucleic Acids Research, 37: D642-D646.
Thesleff I. (2006) The genetic basis of tooth development and dental defects. American Journal of Medical Genetics Part A, 140: 2530-2535.
Townsend G., Harris E.F., Lesot H., Clauss F., and Brook A.H. (2009) Morphogenetic fields within the human dentition: A new, clinically relevant synthesis of an old concept. Archives of Oral Biology, 54: S34-S44.
Wang X-P., and Thesleff I. (2006) Tooth development. In: Unisicker K. and Kriegistein K. (eds), Cell signalling and growth factors in development. Weinhein: Wiley-VCH, pp 719-754.

Correspondence

Professor Alan Brook
Dental Institute, Queen Mary University of London
London, United Kingdom
Email: alan.brook@adelaide.edu.au

2 New approaches to dental anthropology based on the study of twins

Grant Townsend,[1,2,3] Michelle Bockmann,[1,2,3] Toby Hughes,[1,2,3] Suzanna Mihailidis,[1,2,3] W Kim Seow[4] and Alan Brook[1,2,3,5]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development
[4]School of Dentistry, The University of Queensland, Queensland, Australia
[5]Dental Institute, Queen Mary University of London, London, United Kingdom

Abstract

Studies of twins carried out over the past 25 years by the Craniofacial Biology Research Group at the University of Adelaide have provided insights into the roles of genetic, environmental and epigenetic influences on human dento-facial growth and development. The aim of this paper is to review some of the main findings of these studies and to highlight the value of using different twin models, including the monozygotic (MZ) co-twin design. We also introduce the concept of 'dental phenomics' whereby modern 2D and 3D imaging systems are now enabling biologically-meaningful, dental phenotypes to be quantified in order to provide detailed descriptions of the size and shape of teeth. We propose that developments in the field of 'dental phenomics', with linking of the data generated to large-scale genome sequencing approaches, should enable us to further unravel the mysteries of how genetic, environmental and epigenetic factors interact to produce the extensive range of morphological variations evident within the human dentition and face.

Introduction

The Craniofacial Biology Research Group at the University of Adelaide has been involved in studies of the teeth and faces of twins for over 25 years (Townsend et al., 2006). Our main aim is to clarify the roles of genetic, environmental and epigenetic influences on human dento-facial growth and development. Three cohorts of twins have been recruited to enable different objectives and specific hypotheses to be addressed. The first cohort of twins comprises mainly teenage twins living in Adelaide, South Australia. Over 300 pairs of twins participated in this cross-sectional study which focuses on partitioning variation observed in permanent dental crown size into genetic and environmental components. Zygosity determination was achieved by comparison of various serum enzymes and proteins in the blood. In addition to obtaining alginate impressions from which stone dental models were made, other records include facial and intra-oral photographs, palm- and finger-prints, and information about functional lateralities.

The second cohort contains approximately 300 pairs of twins who were seen on at least three occasions: first when they displayed a full primary dentition at around 3-5 years of age; then at the mixed dentition phase around 8-11 years of age; and then again at around 12-14 years of age when most of the permanent teeth had emerged, except for the third molars. Siblings and other family members were also included whenever possible. Buccal cells were obtained to enable analysis of DNA for zygosity determinations of these twins. Other records, including finger-prints and laterality data, were also obtained. The focus of this study was to fit genetic models to data derived from both the primary and permanent dentitions of individuals, to gain further insights into the role of genetic factors on dental development over time.

The third cohort comprises over 600 pairs of twins and their families Australia-wide. For this study we are focussing on the timing and sequence of primary tooth emergence and oral health in young twins. Parents are recording the timing of emergence of each of the primary teeth of their twins on specially designed sheets or on-line. They are also obtaining buccal swabs for DNA analysis, as well as saliva and dental plaque samples for assessment of the presence of Streptococcus mutans, an important microorganism in the development of dental caries. Detailed questionnaires are also being filled out by parents about the physical growth, and general and oral health, of their twins. We have also commenced clinical examinations of the twins to assess dental caries experience and the prevalence of other developmental dental anomalies.

Phenotypic variation in the oro-facial region

To date, our studies of twins have explored phenotypic variation within the oro-facial region at different levels of complexity. For example, consideration has been given to: variation in the dental tissues, e.g. enamel, dentino-enamel junction (DEJ) and dentine; variation in dental crown size and shape, e.g. mesiodistal and buccolingual crown diameters, intercuspal dimensions, cusp areas, Carabelli trait; variation in the size and shape of the dental arches;

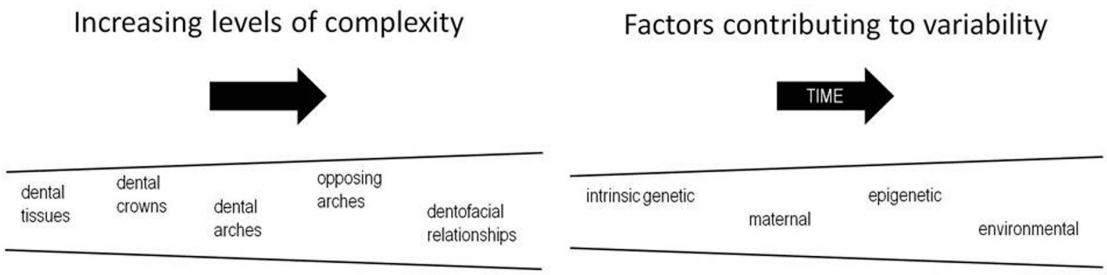


Figure 1. Different levels of complexity that can be studied in the oro-facial region and the factors contributing to observed variation.

variation between opposing dental arches, e.g. anterior overbites and overjets; and variation in aspects of craniofacial morphology. For each of these different levels of complexity, we have been interested in determining how intrinsic genetic, maternal, epigenetic and environmental sources of variation contribute to observed variation. In addition to the three spatial dimensions, the fourth dimension of time and its effect on the relative contributions of genetic and environmental factors to phenotypic variation has also been explored to a limited extent. Figure 1 summarises the different levels of complexity we are studying in the oro-facial region, as well as the factors contributing to observed variation.

Types of twins and twin models

The traditional or classical twin model involves comparisons of selected features in monozygotic (MZ) twin pairs with those in dizygotic (DZ) twin pairs. MZ twins are assumed to share all the same genes, although more recent studies have shown that this is not always correct (Martin et al., 1997). In contrast, DZ twins share only 50% of their genes on average (similar to other sibling pairs). Assuming that environmental effects are similar in each zygosity group, comparisons between MZ twin pairs and DZ twin pairs enables an estimate of the importance of genetic influences to be made. Traditionally, so-called heritability estimates, ranging from 0 to 100%, were calculated to show how much of the observed variation within the study group could be attributed to genetic influences. While estimates of heritability have been useful to provide insights into the importance of genetic and environmental contributions to observed variation in many physical and behavioural features within human populations, there was a tendency in the past to consider the contributions to be either genetic or environmental, hence the phrase 'nature versus nurture'. More recent studies have demonstrated that such a dichotomy is inappropriate and that a more apt phrase is 'nature via nurture' (Ridley, 2003). Indeed, advances in the field of epigenetics, have confirmed the dynamic nature of the interactions between the genome and the environment.

Apart from the traditional twin model, there are several other twin models that are available to researchers. These include: the twins reared apart model; the investigation of twins and other family members; the MZ half-sibling model; the MZ co-twin model; and the DZ opposite sex model (Townsend et al., 2009a). Each of these approaches has its

advantages and disadvantages, but as a group they provide a very powerful suite of approaches for addressing questions relating to genetic, environmental and epigenetic contributions to phenotypic variation in humans. Table 1 lists the five main types of twin models that can be used by researchers.

One of the outcomes of our previous studies involving twins has been the finding that the relative contributions of genetic factors to observed variation differs between different orofacial structures, with evidence of phenotypic correlations and pleiotropic effects (Townsend et al., 2009a). Figure 2 summarises some of the trends that are evident, with relatively strong genetic influences on variation in dental crown size and morphology (including mesiodistal crown diameters and Carabelli trait) but lower heritabilities for occlusal traits, such as anterior overbite and overjet.

The MZ co-twin model and epigenetics

Commonly, twin studies have tended to focus on the similarities between MZ co-twins rather than their differences. We have examined the dentitions of a large sample of MZ twin pairs from our cohorts and found many examples of differences or discordances, some relatively minor and others more distinct. For example, we found that 21 pairs of MZ co-twins were

Monozygotic (MZ) twin pairs versus dizygotic (DZ) twin pairs - the classical twin model for estimating the contribution of genetic and environmental factors to phenotypic variation
Monozygotic twin pairs reared apart (MZA) versus dizygotic twin pairs reared apart (DZA) - a model that minimises the confounding effects of common environmental influences
Twins and other family members (siblings and parents) - a model that increases power to detect genetic and environmental factors
Monozygotic half-sibling model - takes advantage of the genetic relationships between the children of MZ co-twins who are genetically half-siblings
Monozygotic co-twin model - enables comparisons of twins discordant for selected features, e.g. for missing or extra teeth, providing insights into epigenetic and environmental influences
Opposite-sex dizygotic (OSDZ) twin pairs – enables testing for possible hormonal influences in utero

Table 1. The five main types of twin models that can be used by researchers to study genetic, epigenetic and environmental contributions to phenotypic variation.

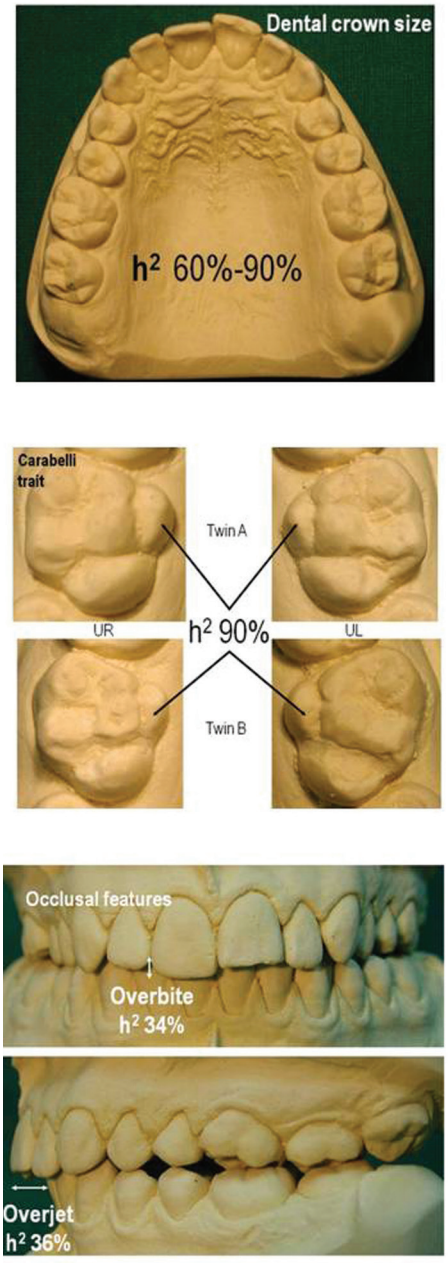


Figure 2. Trends in heritability estimates, h^2, for selected dental phenotypes, including dental crown size (top), Carabelli trait (middle), and anterior overbite and overjet (bottom).

discordant for number or position of missing maxillary lateral incisors or second premolars from a total of 24 pairs of MZ twins who showed the anomalies. Furthermore, 8 pairs of MZ twins were discordant for number of supernumerary teeth (mesiodentes) from a total of 9 pairs who displayed the feature (Townsend et al., 2005; Townsend et al., 2009b). Given the strong underlying genetic basis to hypodontia and supernumerary teeth, the finding that such a high proportion of MZ twin pairs was discordant for these features supports the view that epigenetic factors have an important role in dental development.

The term 'epigenetics' tends to be used nowadays by molecular geneticists to refer to processes such as methylation and acetylation of DNA that occur within the nucleus and lead to alterations in gene expression without any change in DNA sequence. However, we prefer to use the term in its broadest sense, more aligned to the original concept proposed by Waddington (1957). Based on this broad perspective, the development of the dentition can be viewed as comprising a series of stages that involve spatial and temporal interactions between epithelial and ecto-mesenchymal tissues. The migration, division and differentiation of odontogenic cells are controlled by epigenetic events occurring at the local tissue level, with various signalling molecules and growth factors being expressed at different times and by different cells. It would seem that relatively minor spatio-temporal variations or perturbations in the process of odontogenesis can have significant effects on final phenotypic expression, for example, whether a tooth is fully-formed or not.

The opposite sex DZ model

The opposite-sex DZ model, offers the opportunity to clarify how the pre-natal environment influences dental development. There is evidence that the development of females belonging to opposite-sex DZ twin pairs may be influenced in-utero by male hormones from their co-twin. We have shown that permanent tooth size

in females from opposite-sex DZ twin pairs tends to be larger compared with females from MZ twin pairs or from same-sex DZ pairs, consistent with male to female diffusion of hormones (Dempsey et al., 1999). A PhD student in our research group, Dr Daniela Ribeiro, is exploring this issue at present and some of her preliminary findings are included in one of the accompanying papers in this book (Ribeiro et al., 2012).

A multifactorial threshold model for dental development

The multifactorial threshold model proposed by Brook (1984, 2009) to explain observed relationships between presence and absence of teeth and the size of teeth in males and females has proved to be very useful in considering how the inter-relatedness of different clinical dental features reflects the pleiotropic effect of genes during dental development. The complex nature of the process of odontogenesis is discussed in the paper by Brook and Brook O'Donnell in this book (Brook and Brook O'Donnell, 2012). A recent genome-wide association study of primary tooth development, involving an international team of researchers, has shown that several genetic loci are involved in determining the time at which the first primary tooth emerges into the mouth and also the number of teeth present at one year of age. Interestingly, the loci identified by the researchers included several genes that are already known to be involved in dental development and also the development of other organs. Furthermore, genes at four of the identified loci have been implicated in the development of ovarian, breast and colo-rectal cancer. The researchers also found that a variant in the HOXB gene cluster was associated with alterations in dental occlusion of subjects in the study that necessitated orthodontic treatment in their adult years (Pillas et al., 2010).

Dental phenomics

The Human Genome Project involved a huge investment of resources world-wide, but most researchers now agree that this investment was worthwhile. Recently, there have been calls to undertake a similar process involving large-scale phenotyping, referred to as phenomics, to complement the large-scale genome sequencing that occurred in the Human Genome Project (Houle et al., 2010). These calls are linked to our increasing appreciation of the fact that phenotypic variation reflects the complex interactions between the genotype and the environment and that more detailed phenotypic data are required to enable us to unravel these interactions (Houle et al., 2010).

Traditionally, the size of teeth has been determined by measuring the maximum mesiodistal and buccolingual diameters of dental crowns with hand-held callipers. Although these measurements can be made with good precision and accuracy, they only provide a very general indication of the overall size of dental crowns and provide little information about shape. There have been studies where other measures, apart from the traditional crown diameters, have been used, for example, intercuspal distances, as well as cusp areas and volumes. However, these approaches have not been adopted widely and there are few reference data available for these variables to enable inter-population comparisons. We propose that more time and effort needs to be expended in defining additional dental phenotypes that will

Physical properties	Macroscale			Microscale			Nanoscale		
Quantitative assessment	Method	Surface features	Sample destruction	Method	Surface features	Sample destruction	Method	Surface features	Sample destruction
Profile	Contacting & non-contacting profilometer	1	(usually -)	Contacting & non-contacting profilometer	1	(usually -)	Nano CT	2	+
				Micro CT	2	-			
Mechanical properties	Flexural and tensile strength	2	+	Vickers or Knoop hardness	2	+	Nanohardness	2	+
Surface roughness	Surface roughness detector	1	-	Surface roughness detector	1	-	Atomic force microscopy	1	+
Colour	Shade guide	1	-	Qualitative light fluorescence	1	-	-		
Tissue thickness	Radiography	2	-	Micro CT	2	-	Nano CT	2	+
Crystal structure	-			X-ray diffraction	2	+	X-ray diffraction	2	+
							High resolution SEM	1	+
Qualitative assessment									
Surface features	Optical microscopy	1	-	SEM	1	+	High resolution SEM	1	+
							TEM	1	+
3D reconstruction	-			Confocal microscopy	2	+	Nano CT	2	+
				Micro CT	2	-			

1 = surface features; 2 = sub-surface features; + = requires cutting/polishing and/or sample coating; sample altered during the test; or sample surface is usually inappropriate to conduct further tests; - = does not require destruction; FIB-SEM: Focussed ion beam-scanning electron microscopy; AFM: Atomic force microscopy; CT: Computed tomography; SAM: Scanning acoustic microscopy; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy

Table 2. Physical properties of teeth that can be studied at macro-, micro- and nano-levels.

provide much more detailed descriptions of the size and shape of teeth. The term we propose for this new field of dental research is 'dental phenomics'.

New approaches to dental phenotyping

The development of new equipment for measuring teeth in both 2-dimensions (2D) and also 3-dimensions (3D) is now opening up possibilities for dental researchers to define new, and hopefully more biologically meaningful, phenotypes. One of the outcomes of the establishment of the International Collaborating Centre in Oro-facial Genetics and Development in 2007 has been the setting up of similar imaging systems for obtaining 2D and 3D measurements of teeth and other oro-facial structures in research laboratories at the University of Liverpool and also the University of Adelaide. The accuracy and precision of the systems has been tested and verified to be suitable for research projects aimed at clarifying how genetic, environmental and

epigenetic factors contribute to morphological variation in the dentition (Smith et al., 2009). These imaging facilities have already led to collaborative research projects between researchers in the UK and Australia and, due to standardisation in procedures for data acquisition across the laboratories, there is great potential for building up both intensive and extensive data-sets that will provide quantitative descriptions of the dentition. A new era of 'dental phenomics' is now at-hand and international collaborations will provide the opportunity to carry out large-scale genome-wide association studies (GWAS) to locate and then identify the genes involved in dental development and morphology. The potential for future GWAS involving twins is discussed in more detail in the paper by Hughes and colleagues in this book (Hughes and Townsend, 2012).

Apart from characterising the surface morphology of teeth using scanning systems, advances in the fields of micro- and nano-imaging offer huge potential for future assessment of the physical and chemical properties of the dental tissues which make up teeth, including the enamel, dentine and their interface at the dentino-enamel junction. Table 2 shows some of the phenotypes that can be quantified at macro-, micro- and nano- levels using sophisticated equipment.

Examples of some of the new phenotypes that can be measured with these pieces of equipment are also provided. Figure 3 shows 3D models of individual teeth and dental

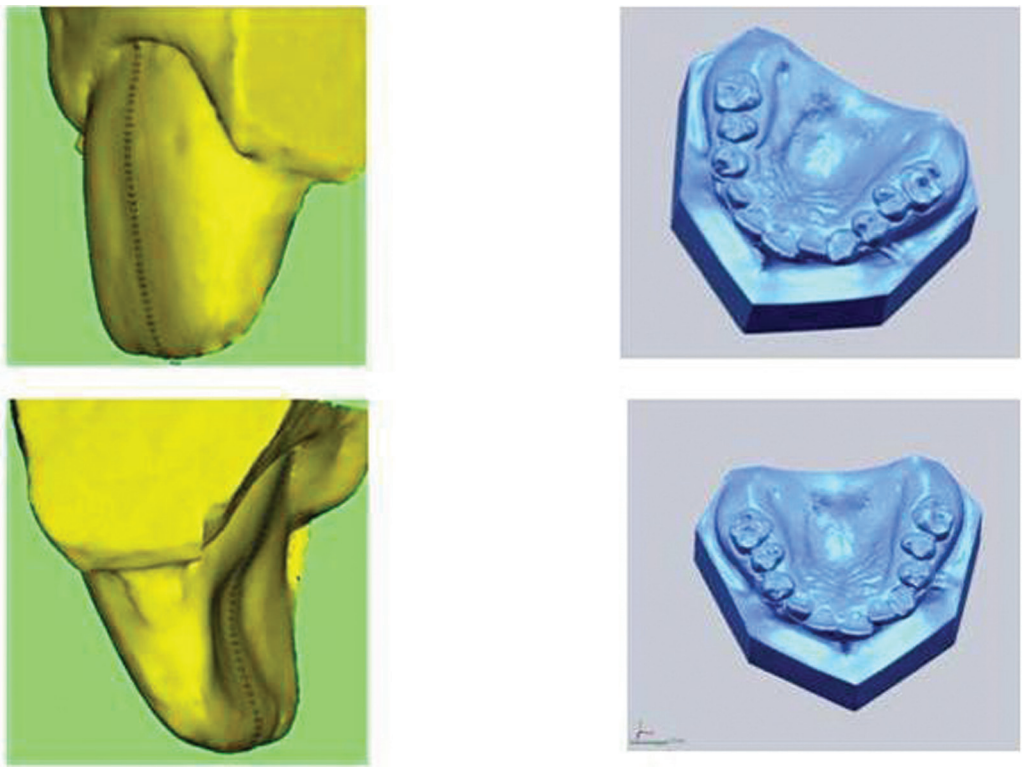


Figure 3. Examples of new dental phenotypes obtained using a 3D laser scanner. Images of individual teeth generated by Dr Richard Smith and Prof Alan Brook, and those of dental arches by Dr Atika Ashar.

models generated using 3D laser scanning equipment available in research laboratories in the Universities of Liverpool, UK, and Adelaide, South Australia. The paper by Ashar and colleagues in this book provides more information and data generated using the 3D laser scanner (Ashar et al., 2012).

Tooth emergence in twins

Our research group is currently involved in a study of primary tooth emergence and oral health in young Australian twins. Following training, parents are recording the times at which primary teeth appear in the mouths of their children. We have validated our approach by clinically examining a sub-sample of twins, and the data being collected provide much more precise times of tooth emergence than would be possible if the twins were being examined every 3 to 6 months (Hughes et al., 2007). Figure 4 provides an example of a completed recording sheet for a pair of twin girls and demonstrates the high degree of similarity in times of emergence of primary teeth in a pair of MZ twins. However, it also shows that there is some variation, both within and between twins, in the timing of emergence of antimeric teeth.

We have recently calculated descriptive statistics for the timing of primary tooth emergence in a sample of over 200 Australian children, with each of the children being one member of a twin pair (Woodroffe et al., 2010). These data provide new references for primary tooth emergence in Australian children and show that the pattern, based on average times of emergence, tends to be: central incisors, lateral incisors, first molars, canines and second molars. The lower central incisors tend to be the first teeth to emerge into the oral cavity at around 8.6 months, somewhat later than is commonly thought, and the second molars emerge around 27 months.

The values for heritability estimates for the timing of primary tooth emergence are generally very high, indicating that genetic factors contribute substantially to variation in this aspect of dental development (Hughes et al., 2007; Bockmann et al., 2010). Given that emergence into the oral cavity represents a single point of time in the extended process of tooth eruption, these findings tend to support the view that there is over-riding genetic control of this process. Whether this genetic control extends throughout the entire period of eruption of the primary dentition or whether the process unfolds in a self-propagating manner after an initial triggering genetic signal, remains to be determined.

Conclusion

This paper has reviewed some of the approaches being undertaken by the Craniofacial Biology Research Group at the University of Adelaide to study the dentitions of twins. Developments in the application of different twin models, the availability of new measuring systems, and the potential provided by new mathematical methods for morphometric analysis of complex shapes such as teeth, are all bringing an exciting new era to dental anthropology. By developing the field of 'dental phenomics' and then linking the data generated to large-scale genome scanning, we are moving closer to unravelling the mysteries of how genetic, environmental

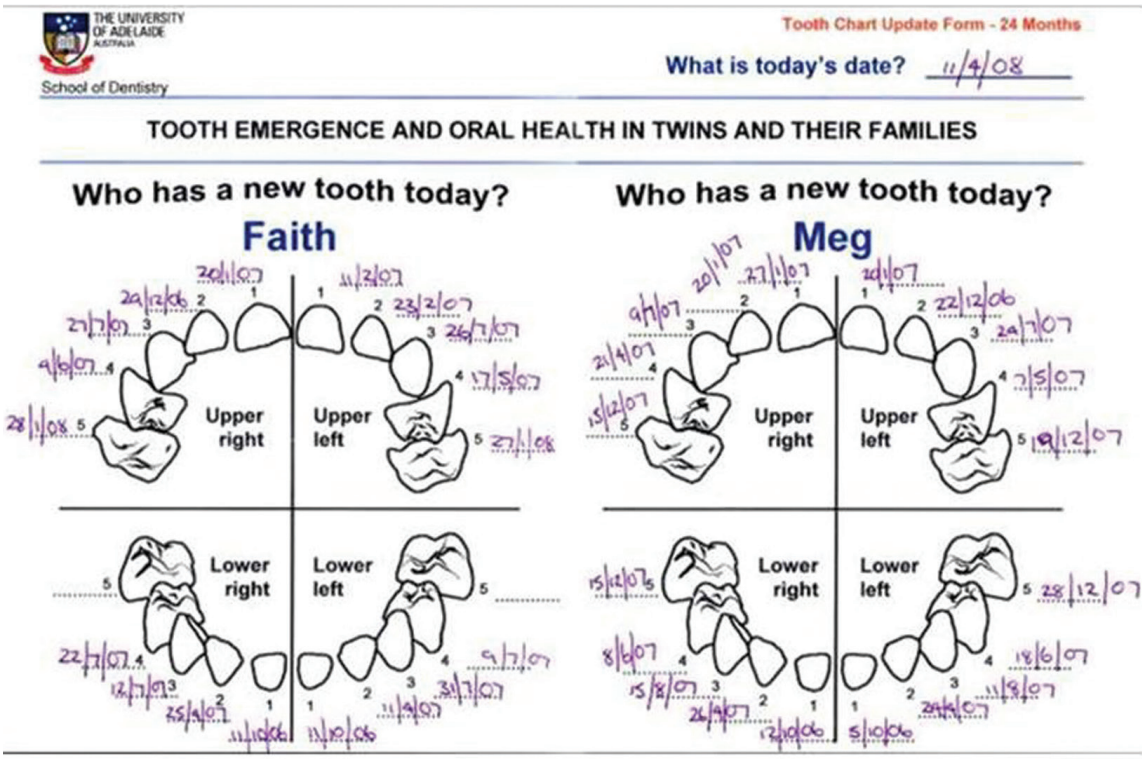


Figure 4. Example of recording sheets completed by parents showing timing of primary tooth emergence in a pair of monozygotic (MZ) twins.

and epigenetic factors interact to produce the extensive range of morphological variations evident within the dentofacial structures.

Acknowledgements

We wish to thank all of the twins and their families who have agreed to participate in our studies that have been approved by the University of Adelaide Human Research Ethics Committee (H-07-1984A and H-78-2003).

We also wish to acknowledge the support of the NHMRC of Australia, The Australian Dental Research Foundation, The University of Adelaide, The Financial Markets Foundation for Children and Colgate Australia.

References

Ashar A., Hughes T., James H., Kaidonis J., Khamis F., and Townsend G. (2012) Dental crown and arch size in Europeans, Australian Aborginals and Malaysians: 2D and 3D approaches. In: New Directions in Dental Anthropology: paradigms, methodologies and outcomes. Townsend G., Kanazawa E., Takyama H. (Eds.) University of Adelaide Press, Adelaide.

Bockmann M., Hughes T., and Townsend G. (2010) Genetic modelling of primary tooth emergence: a study of Australian twins. Twin Research and Human Genetics, 13: 573-581.

Brook A.H. (1984) A unifying aetiological explanation for anomalies of human tooth number and size. Archives of Oral Biology, 29: 373-378.

Brook A.H. (2009) Multilevel complex interactions between genetic, epigenetic and environmental factors in the aetiology of anomalies of dental development. Archives of Oral Biology, 54S: S3-S17.

Brook A.H. and Brook O'Donnell M. (2012) Modelling the complexity of the dentition. In: New Directions in Dental Anthropology: paradigms. methodologies and outcomes. Townsend G., Kanazawa E., Takyama H. (Eds.) University of Adelaide Press, Adelaide.

Dempsey P.J., Townsend G.C., and Richards L.C. (1999) Increased tooth size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. American Journal of Human Biology, 11: 577-586.

Houle D., Govindaraju D.R., and Omholt S. (2010) Phenomics: the next challenge. Nature Review Genetics, 11: 855-866.

Hughes T., Bockmann M., Seow K., Gotjamanos T., Gully N., Richards L., and Townsend G. (2007) Strong genetic control of emergence of human primary incisors. Journal of Dental Research, 86: 1160-1165.

Hughes T., and Townsend G. (2012) Genes for teeth — drawing inference from family data. In: New Directions in Dental Anthropology: paradigms. methodologies and outcomes. Townsend G., Kanazawa E., Takyama H. (Eds.) University of Adelaide Press, Adelaide.

Martin N., Boomsma D., and Machin G. (1997) A twin-pronged attack on complex traits. Nature Genetics, 17: 387-392.

Pillas D., Hoggart C.J., Evans D.M., O'Reilly P.F., Sipila K., Lahdesmaki R., et al. (2010) Genome-wide association study reveals multiple loci associated with primary tooth development during infancy. PLoS Genetics, 6: 1-7.

Ribeiro D.C., Sampson W., Hughes T., Brook A.H., and Townsend G. (2012) Sexual dimorphism in the primary and permanent dentitions of twins: an approach to clarifying the role of hormonal factors. In: New Directions in Dental Anthropology: paradigms, methodologies and outcomes. Townsend G., Kanazawa E., Takyama H. (Eds.) University of Adelaide Press, Adelaide.

Ridley M. (2003) Nature via Nurture — genes, experience and what makes us human. Fourth Estate, London.

Smith R., Zaitoun H., Coxon T., Karmo M., Kaur G., Townsend G., and Brook A. (2009) Defining new dental phenotypes using 3-D image analysis to enhance discrimination and insights into biological mechanisms. Archives of Oral Biology, 54S: S118-S125.

Townsend G., Richards L., Hughes T., Pinkerton S., and Schwerdt W. (2005) Epigenetic influences may explain dental differences in monozygotic twin pairs. Australian Dental Journal, 50: 95-100.

Townsend G.C., Richards L.C., Messer L., Hughes T., Pinkerton S., Seow K., Gotjamanos T., Gully N., and Bockmann M. (2006) Genetic and environmental influences on

dentofacial structures and oral health: studies of Australian twins and their families. Twin Research and Human Genetics, 9: 27-732.

Townsend G., Hughes T., Luciano M., and Brook A. (2009a) Genetic and environmental influences on human dental variation: limitations and advantages of studies involving twins. Archives of Oral Biology, 54S: S45-S51.

Townsend G., Hughes T., Bockmann M., Smith R., and Brook A. (2009b) How studies of twins can inform our understanding of dental morphology. In: Koppe T., Meyer G. and Alt K.W. (Eds.), Comparative Dental Morphology. Front Oral Biol, Karger, Basel, vol 13, pp. 136-141.

Waddington C.H. (1957) The strategy of genes: a discussion of some aspects of theoretical biology. Allen & Unwin, London.

Woodroffe S., Mihailidis S., Hughes T., Bockmann M., Seow K., Gotjamanos T., and Townsend G. (2010) Primary tooth emergence in Australian children: timing, sequence and patterns of asymmetry. Australian Dental Journal, 55: 245-251.

Correspondence

Professor Grant Townsend
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
Email: grant.townsend@adelaide.edu.au

3 Genes for teeth — drawing inference from family data

Toby Hughes[1,2,3] and Grant Townsend[1,2,3]

[1]School of Dentristry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development

Abstract

Development of the human dentition, a complex, self-organising system, is underpinned by a series of reiterative steps involving a number of key gene pathways, supplemented by smaller influences of a polygenic background. Modelling familial data of dental phenotypes can help to unravel genetic and environmental influences. This paper presents a review of a number of model-based approaches that can be useful analytically, with a focus on twins as the familial structure to elaborate genetic complexity. Genetic modelling is methodologically robust, and provides a framework within which to locate evidence of gene effects from modern, high-throughput genotyping approaches. The twin family structure is particularly well-suited to this approach, and provides a number of distinct advantages analytically, particularly in the presence of population stratification.

Introduction

The human dentition is of significant anthropological interest when considering variation within and between modern populations. It is also a useful tool for examining evolutionary change over time in response to changes in culture, diet, etc. Teeth provide a (relatively) stable indirect source of information about processes occurring during pre- and early post-natal development. They are also one of the most stable sources of information in the fossil record, both morphologically, and as a repository of ancient DNA sequence information (Adler et al., 2010). Furthermore, variation in tooth form and function can provide opportunities for examining inter-individual variation as a means for forensic identification. More recently,

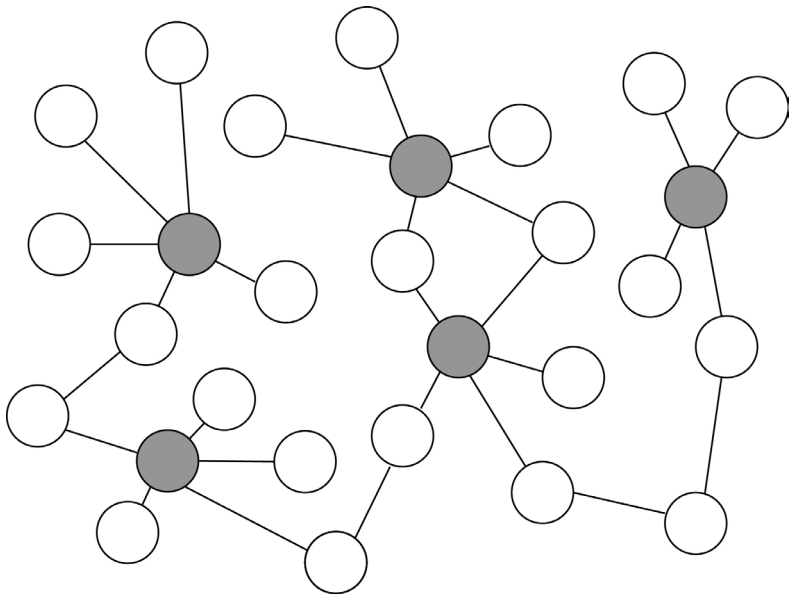


Figure 1. A scale free network in which the larger hubs are highlighted.

evolutionary models of oral microbial ecology have relied upon extraction of microbial DNA from deposits on tooth surfaces (Preus et al., 2011).

Dental development, a function of many interactions between a range of factors at multiple levels, has many network-like features. Many biological networks display substantial non-trivial topological features, with patterns of connection between their elements that are neither purely regular nor purely random. Figure 1 illustrates one conceptualization of such a complex biological system, the scale-free network. A network is named scale-free if its degree distribution, i.e., the probability that a node selected uniformly at random has a certain number of links (degree), follows a particular mathematical function called a power law. The most notable characteristic in a scale-free network is the relative commonness of vertices with a degree that greatly exceeds the average. The highest-degree nodes are often called 'hubs', and are thought to serve specific purposes in their networks. The human dentition can be considered a complex system (Brook and Brook O'Donnell, 2011) in which lower-level, interacting components give rise to higher level, emergent phenomena. The system is comprised of a hierarchical organisation of functional subunits, including cells, tissues, organs, and, if the concept is taken to its logical end point, organisms and populations. This 'self-adaptive' system has the capacity to react to change through time, both within an individual and trans-generationally.

Understanding the developmental processes that give rise to morphological variation in the human dentition is a goal of a diverse range of disciplines, including physical anthropology, evolutionary biology, comparative anatomy, forensic odontology and clinical dentistry. The human dentition demonstrates significant variation in development, form and function. This

variation exists within and between individuals, families, sexes, ethnic groups and populations. It has been attributed to temporal effects acting at the level of the individual (within a lifetime) and the population (across generations). Such variation poses three fundamental questions for dental anthropologists:

1. How does the plasticity of the genome give rise to population adaptation to a particular environment?
2. How do genes and the environment interact to produce a specific phenotype?
3. How does our understanding of the interaction between genes and the environment fit within the broader context of the dentition as a complex system?

The first two questions can be addressed using family studies; the former through use of population modelling of traits that exhibit familial aggregation; the latter through the use of linkage and association analyses to elucidate the role of specific genes in trait development. More recently, the role of the epigenome in dental development and patterns of trait transmission has become of interest to the dental anthropologist. This, too, can be addressed through judicious use of family data. The final question requires a conceptual framework in which the actions of the genome, the epigenome and the environment are linked through a scale-free network of genes and gene products, in which some factors (hubs) have a greater significance in terms of the final phenotype, whilst others (nodes) are bit players.

This review will illustrate how studies of families, and particularly twin families, can be used to partition population variation into genetic and environmental components using mathematical models of the twin relationship. It will explore how such models can reveal information about relationships between dental features, and how these family models can advantageously incorporate molecular marker data to identify genes of major influence. The review will examine how studies of monozygotic twins can be used to examine the role of the epigenome in dental development. Finally, the author will attempt to consolidate the role of genes and environment in a broader conception of the dentition as a complex system.

Phenotypic variation

At maturity, the human dentition is a highly organised, dynamic system that has a major role in the maintenance of homeostasis. A self-organising system with multiple symmetries (left-right; maxillary-mandibular), the dentition arises from a complex series of interactions occurring at multiple organisational levels, initiated in the first trimester pre-natally, and not maturing fully until around 20 years of age. Evidence of reiterative systems operating throughout dental development is further evidence that it is a truly complex, as distinct from a complicated, system (Jernvall and Thesleff, 2000).

In recent decades, the model of phenotypic variation arising as a result of conflict between genome and environment ('nature' vs 'nurture') has been supplanted by a more complex model that encapsulates the complex interaction (and in many cases association) between genes and the external organismal environment ('nature' via 'nurture'). Despite

evidence of strong genetic regulation of both developmental timing (Hughes et al., 2007) and morphological characteristics (Dempsey and Townsend, 2001; Hughes et al., 2000; Townsend and Martin, 1992), there is still a great deal of unexplained phenotypic variation in the human dentition, both within and between populations.

The role of the genome in dental development is similar to that of many human conditions. A number of features are influenced by only a single or a few genes and show a very simple pattern of inheritance. These are most commonly disease states, and may be the result of specific allelic variants 'tipping' an individual over a phenotypic threshold in the presence of a polygenic background (Brook, 1984).

Most features of interest are due to the additive effects of many genes and/or the environment (classical heritability). They characteristically show a distinct distribution (most commonly normal) within a population, and can be considered multifactorial. These features provide the most challenge to elaborate aetiologically.

Some genes act on multiple dental phenotypes pleiotropically. These are commonly homeobox-like genes, which regulate expression of structural genes, and often play a role reiteratively during development. They can be considered the hubs in a gene-centric form of a scale-free network, and will be discussed in more detail later.

Other effects may complicate the outcome, including allele interactions at the same locus (e.g. genetic dominance), allele interactions between loci (e.g. epistasis) and the interaction of genes with their environment (e.g. epigenetics).

Experimental and analytic techniques

The dental anthropologist has a range of analytic techniques available to explore the factors that contribute to observed variation in the form and function of the human dentition. Many of the most powerful rely on a detailed understanding of the complex interactions between the genome and the environment during development, maturity and senescence. Quantitative genetics, the application of intensive statistical models to deconstruct the observed phenotypic information from a population, is one such tool. It works hand-in-glove with modern, high-throughput molecular genetics approaches to provide dental anthropologists with a detailed understanding of the role of functionally significant genes in the dentition of modern human populations. It also allows researchers to draw inferences about gene flow through populations, and hence to elaborate putative models of dental evolution.

Quantitative genetics

The birth of quantitative genetics, the mathematical description of population variation in a genetic framework, is widely credited to a 1918 paper by then student, Ronald Fisher (Fisher, 1918). Elaborated by the likes of Falconer (Falconer and Mackay, 1996), it has been further developed to deal with the complexities associated with studying the human organism — namely, the requirement to capitalise on naturally-occurring mating systems. A 'top-down' approach to describing complexity, quantitative genetics seeks to apportion observed phenotypic variation into that due to the additive and non-additive effects of genes, and that

due to the environment. More powerful still, are those approaches that seek to understand the interaction between these two 'latent' sources of variation.

Quantitative genetics is complementary to the domain of the molecular biologist, seeking to provide a framework of genetic variation within which can be located models of specific gene action. Together, the two disciplines provide an opportunity to better understand the interplay between components of complex systems. Quantitative genetics relies on the development of theoretical models from a sound understanding of the biological system under analysis. These models may then be validated using real-world data, often using likelihood-based approaches. This requires collection of both intensive and extensive phenotypic data in order to substantiate the conceptual model.

A key feature of quantitative genetic analysis in humans is a reliance on known or inferred familial relationships. Knowledge of these relationships, and the transmission of alleles via meiosis, enables the dental anthropologist to develop models of trait transmission that predict the phenotypic outcome of genes segregating in families. The models are then compared to the observed trait transmission in the same families to estimate goodness-of-fit.

How can twins help us understand dental development and morphology?

First documented in the scientific literature in Francis Galton's 1875 paper, 'History of Twins' (Galton, 1875), the twin relationship is a particularly powerful familial structure for quantitative genetic analysis. Twin pairs can be either monozygous (MZ), or dizygous (DZ). Monozygous twin pairs arise from the fertilisation of one ova by one sperm, followed by an extra round of post-meiotic division resulting in two genetically identical zygotes that subsequently implant and grow. Dizygous twin pairs arise from the concurrent release of two ova, which are fertilised independently by two sperm, and subsequently implant and grow. As

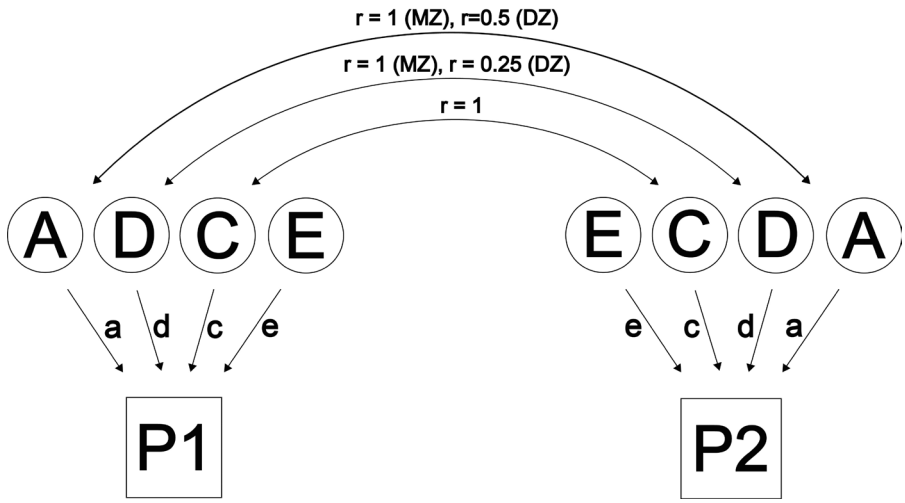


Figure 2. Path diagram representing the twin relationship.

a consequence of the phenomena of crossing-over of non-sister chromatids prior to the first meiotic division, and of genes assorting independently during the second meiotic division, DZ twins only share, on average, 50 percent of their alleles identical by descent, the same as non-twin siblings. The dental anthropologist can capitalise on these two distinct genetic relationships to draw inferences about possible genetic aetiologies of dental development.

The twin relationship, as distinct from other genetic relationships, is useful for a number of reasons. Twins are matched for age and, when reared together, share similar pre-natal and early post-natal environments (placental arrangement can complicate this generalisation).

There are a number of manifestations of the twin relationship that appear within models of familial aggregation. These include the 'traditional' twin model, which assumes that differences between MZ twin pairs reared together reflect environmental effects unique to individual twins within a pair, whereas differences between DZ twin pairs reared together reflect both genetic and environmental differences, and is the primary focus of this discussion. Other designs of note include twin adoption studies (twins reared apart), twins and their families, the MZ half-sib design, DZ opposite-sex studies (useful for drawing inference about sexual dimorphism), and the MZ co-twin design. This last model is particularly useful for examining environmental and epigenetic influences on trait mean and variance, and will be examined later.

Figure 2 presents a simple path diagram of a structural equation model (SEM) representing the twin relationship for a single trait. Variation in the observed twin phenotypes (squares) is influenced by a number of latent (unmeasured) variables (circles). Broadly-speaking, these are the additive effects of an individual's genes (A), the non-additive effects (dominance, epistasis) of an individual's genes (D), the influence of the environment shared by co-twins (C) and the unique environment experienced by an individual twin (E). This last also encapsulates experimental error.

Using likelihood-based approaches, the model completely decomposes observed variation into a number of discrete linear relationships between latent and measured variables, related by a series of parameters (a, d, c and e). The 'structural' elements of the model (intra-pair correlations, r) capitalise on the observer's knowledge of biology underpinning the relationships between latent variables. To this end, additive genetic effects have a correlation (r) of 1 in MZ twins, and 0.5 in DZ twins, and, unsurprisingly, the correlation between shared environments is 1 regardless of zygosity — twin pairs experience the same shared environment.

Given an observed covariance matrix from the raw data, parameter estimates for the model are derived using a multinomial implementation of the likelihood function, maximising the likelihood iteratively to produce a model that best-approximates the real-world data (with judicious use of good starting values). Structural equation modelling software such as Mx (Neale et al., 2006), now implemented in R (R Development Core Team, 2011) is ideal for this purpose. Invariably, models of this nature fit well, being essentially a transformation of the data. The focus then switches to whether simpler models may also be fit to the data without a significant decrease in model fit. Simpler models can be compared to more complex models using appropriate statistics or information criteria to reach the most parsimonious

explanation of the observed data (Neale and Cardon, 1992).

For twins reared together, even this simple model is underidentified (too many parameters for too few observations), necessitating that the observer settle first on a model incorporating either ADE or ACE — this can be assisted by observing intra-pair correlations within zygosity in the first instance.

The univariate model presented above can also be extrapolated to the multivariate case to answer more explicit questions regarding the structure of the data – do genetic effects change through time; is there sexual heterogeneity for trait variance; are there pleiotropic influences of individual genes. Figure 3 illustrates a multivariate model of the mesiodistal size of the permanent dentition in a cohort of male European twins from earlier work by our group (Hughes et al., 2007). Of note is the increased complexity of the covariance structure. Variation in adult male incisor tooth size is best described by a model incorporating a single general genetic effect on all teeth, as well as specific genetic factors acting on lower antimeres, and lateral incisors respectively (illustrated for one half of the twin pair). Sexual heterogeneity in variance necessitates a subtly different structure for female incisor tooth size. Figure 4 illustrates a longitudinal model of arch breadth in the same cohort. The simplex model for longitudinal data allows for innovation elements at each time point (ζa, ζe) and directional transmission elements between time points (βa, βe), as well as an estimate of experimental

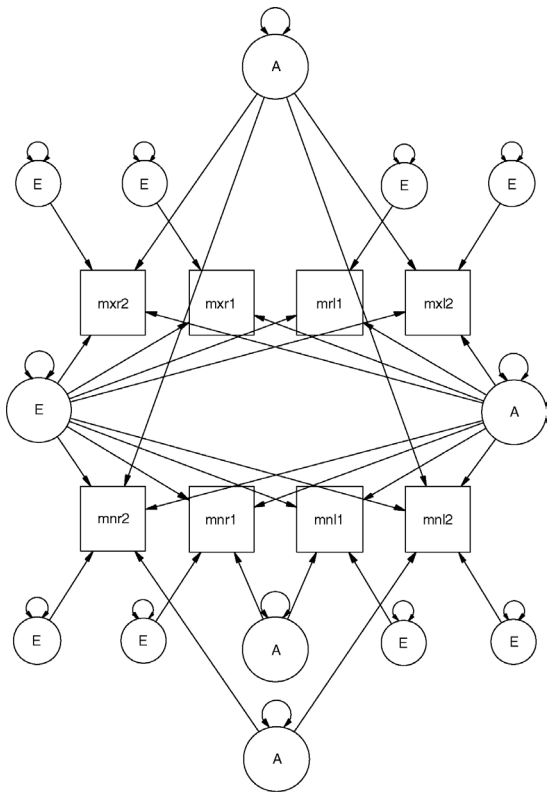


Figure 3. A multivariate model of the mesiodistal dimension of incisors in adult male twins of European descent.

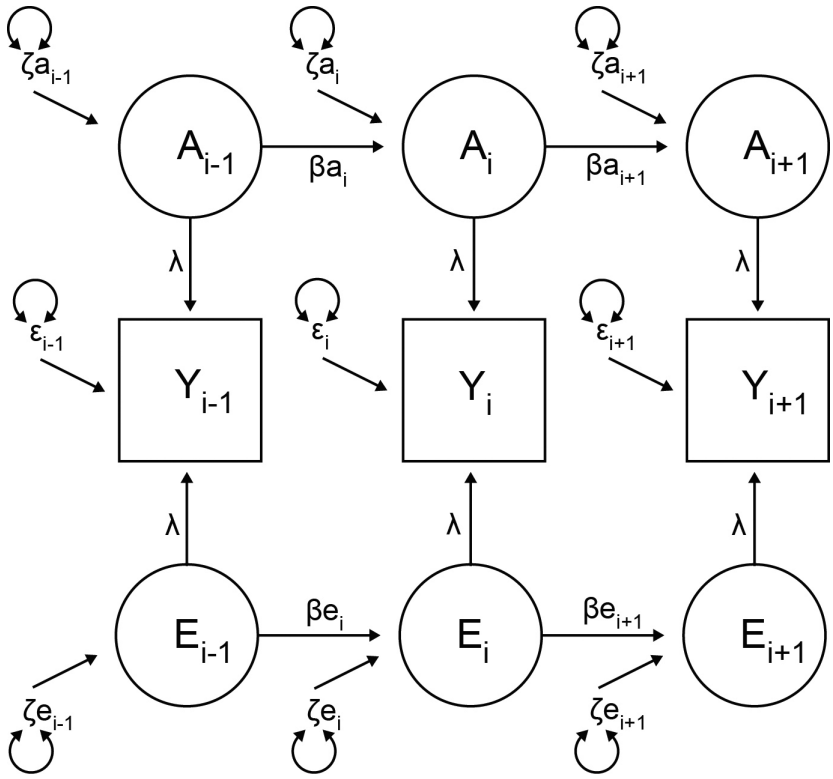


Figure 4. A general AE simplex model of arch shape through time.

error (ε, constrained equal across time) that is now independent of unique environmental effects, $E_{i.}$ Factor loadings (λ) are fixed at 1 for model identification. This model allows for genetic elements acting at discrete time-points, as well as transmissible elements that account for variation through time.

Molecular genetics

One of the appeals of the structural equation modelling approach is that it is flexible enough to enable incorporation of molecular genetic data in order to establish the putative influence of key genes. This is true regardless of whether one is using a genetic linkage-based approach or a genetic association-based approach.

These methods emphasize the utility of using familial data for modelling gene action. Linkage analysis, by definition, requires information on the co-transmission of traits and genetic markers between family members, and hence relies on family-based approaches. Dizygotic twins are one such group who may be utilised for linkage. Monozygotic twins, on the other hand, are uninformative for linkage unless data is available from other family members. Linkage can localise complex trait loci with 1-10 Mbp resolution, however the locus effect size needs to be more than ten percent of the trait genetic variance to be detectable. Quantitative trait loci detected by linkage can be considered the hubs of a complex

system due to their large influence on trait variation. Because of the natural randomization induced by segregation during meiosis, linkage is robust to confounding. Figure 5 illustrates a path diagram incorporating a putative quantitative trait locus (QTL). The intra-pair QTL correlation (pi hat) is an estimate (not all relationships are fully informative) of allele sharing identical by descent between DZ twins (Martin et al., 1997). Linkage can be tested by dropping Q from the model and examining the change in model fit — a significant decrease in model fit is suggestive of linkage.

Association (candidate gene) analysis extracts information from the co-occurrence of traits and markers within individuals. These approaches have traditionally utilised unrelated case/control (or similar) population samples. A key liability with this type of cohort is that underlying population stratification may result in spurious association. Familial structures (and particularly twins), whilst generally more expensive to genotype, allow for family-based approaches (within/between transmission disequilibrium testing) which are robust to the presence of population stratification. The localization of complex trait loci using whole-genome approaches is usually at the 0.01-0.1 Mbp resolution, provided the locus effect size is more than one percent of the genetic variance. Such loci can be considered nodes within the complex system framework. Association analysis is less robust to confounding than linkage analysis.

The flexibility of the SEM approach allows both linkage and association to be modelled simultaneously in familial data sets. Figure 6 illustrates a combined model of linkage and association in sibling pairs for a phenotype for which molecular marker data are available, allowing for possible population stratification. Latent variables for family resemblance F, QTL variance Q, and individual-specific variance E cause the phenotypes of two siblings, P1 and P2. S represents half the sum of the sibling pair's genotypic effects, and D represents half

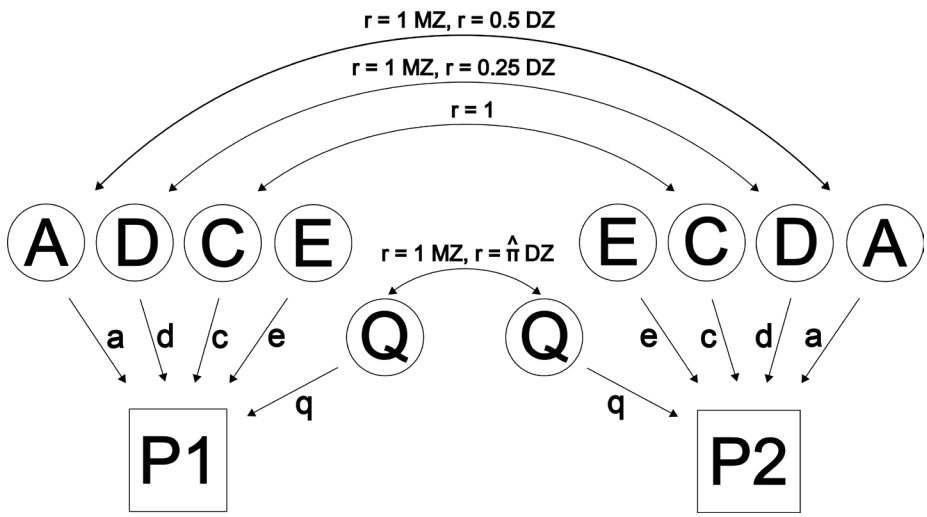


Figure 5. A simple univariate model of the twin relationship, incorporating a genetic marker at a specific locus in order to test for linkage.

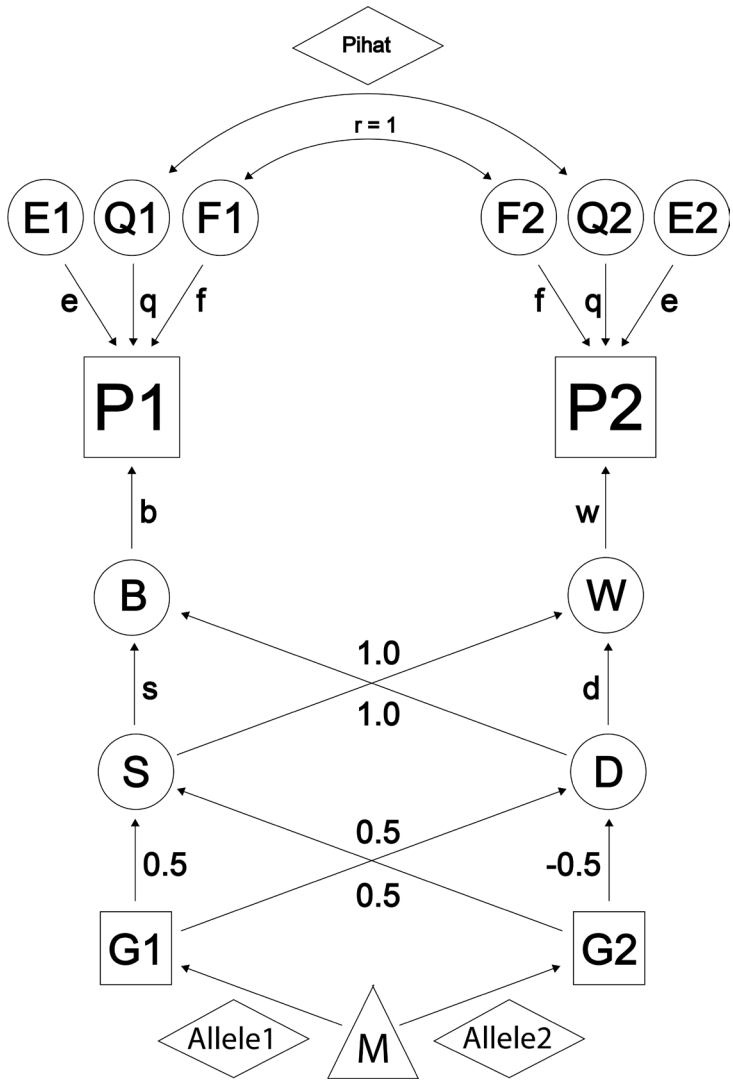


Figure 6. A twin model incorporating both linkage and association.

their difference. These components contribute to between (B) and within pair effects (W) via parameters b and w respectively. In the absence of stratification, b and w are expected to be equal. Genuine association with observed genotypes G1 and G2 will decrease the size of the linkage-based QTL effect, q.

Using appropriate model specification, SEM can be extrapolated to modern, whole-genome approaches, which, in the case of association, can identify causal variants (Vieira et al., 2008). This enables the dental anthropologist to capitalise on comprehensive marker data arising from high-throughput, chip-based approaches to ascertaining large numbers of markers simultaneously. There is, however, a, concomitant increase in the numbers of statistical tests required, necessitating consideration of the experiment-wise error rate. MERLIN (Abecasis

et al., 2002), a Multipoint Engine for Rapid Likelihood Inference, is a purpose-built piece of software whose capabilities include linkage analysis (variance components, non-parametric linkage, parametric linkage, clustered marker data), association, haplotyping, information content, error detection (most SNP typing errors are Mendelian consistent) and simulation.

Epigenetics

In the last ten years, with the completion of the Human Genome Project (Collins et al., 2003), the generation of progressive iterations of the Human Hapmap (International Hapmap Consortium, 2005), and with the rapid publication of many large-scale, high-powered whole-genome association studies of human phenotypes, focus has shifted from variation in the genetic code *per se*, to how gene expression is modulated. There is a growing appreciation that epigenetic factors can have a major influence on trait expression, and have been implicated in changes over life course (Poulsen, 2007). In its broadest sense, epigenetics refers to differential modification of gene effects, due to stochastic variation in the local genetic milieu. A more narrow interpretation is the influence of (potentially heritable) changes in local chemical mediators of gene transcription or translation (CpG methylation, histone deactylation, X inactivation, etc.). Monozygous twins provide an ideal model for the role of epigenetic factors in trait variance, and there are numerous publications that have provided evidence of epigenetic discordance between MZ twins. Our group is currently investigating the influence of methylation on MZ discordance for tooth number (missing/extra teeth).

The dentition as a complex system

The concept of the development of the dentition as a complex system has been dealt with explicitly elsewhere in this book (Brook et al., 2012). The methodologies outlined in this article provide the mathematical framework within which to test associations and interactions between different subunits that contribute to dental variation at both the organismal and population level. Genes, signalling pathways, epigenetic factors and environmental factors may each play one or more roles as a node, hub, or module in a complex system. Elaborating the interconnections between these subunits will enable the development of predictive models of dental development, and will improve the capacity to fine tune phylogenetic relationship data, to aid in modelling evolutionary change.

Future directions

To-date, our research group has examined a large range of morphological and oral-health phenotypes from a series of three Australian twin cohorts, some of which are reported elsewhere in this special issue. We aim to continue intense phenotyping of cohort three, whilst further extracting data from records already available for cohorts one and two. Having already reported extensive heritability estimates for a range of phenotypes, we now plan to develop robust multivariate models of orofacial variation. High density genetic profiling of the three twin cohorts is currently underway, supplemented by data from collaborators. We aim to integrate molecular marker data with our current models to identify putative

QTLs for further fine-mapping and identification of causal variants, with the ultimate aim of developing predictive models of oral phenotypes. Epigenetic profiling of discordant MZ pairs has become a recent focus of our research group, and we are currently investigating the role of differential methylation in tooth number discordance. Finally, we will seek to replicate our initial findings in other datasets.

Conclusion

Genetic modelling offers a methodologically robust approach for exploring the complexities of dental development and evolution, and articulates with a conception of the dentition as a complex system. It provides a framework within which to locate evidence of gene effects from high-throughput genotyping. It capitalises on familial structure, of which twins provide a number of distinct advantages analytically.

Acknowledgements

The authors would like to acknowledge all twin participants and their families, funding bodies (NHMRC, ADRF, the Financial Markets Foundation for Children and Colgate Australia), and collaborators.

References

Abecasis G.R., Cherny S.S., Cookson W.O., and Cardon L.R. (2002) Merlin — Rapid analysis of dense genetic maps using sparse gene flow trees. Nature Genetics, 30: 97-101.

Adler C.J., Haak W., Donlon D., and Cooper A. (2010) Survival and recovery of DNA from ancient teeth and bones. Journal of Archaeological Science, 38: 956-964.

Brook A.H. (1984) A unifying aetiological explanation for anomalies of tooth number and size. Archives of Oral Biology, 29: 373-378.

Brook A.H., and Brook O'Donnell M. (2012) Modelling the complexity of the dentition. In: New Directions in Dental Anthropology: paradigms, methodologies and outcomes. Townsend G., Kanazawa E., Takyama H. (Eds.) University of Adelaide Press, Adelaide.

Collins F.S., Morgan M., and Patrinos A. (2003) The Human Genome Project: lessons from large-scale biology. Science, Apr 11 2003: 286

Dempsey P.J., and Townsend G.C. (2001) Genetic and environmental contributions to variation in human tooth size. Heredity, 86: 685-693.

Falconer D.S., and Mackay T.F.C. (1996) Introduction to quantitative genetics. 4th ed. Essex, England: Addison Wesley Longman Limited.

Fisher R.A. (1918) The correlation between relatives on the supposition of Mendelian inheritance. Philosophical Transactions of the Royal Society of Edinburgh, 52: 399-433.

Galton F. (1875) The history of twins, as a criterion of the relative powers of nature and nurture. Fraser's Magazine, 12: 566-576.

Hughes T.E., Bockman M.R., Seow K., Gully N., Richards L.C., and Townsend G.C. (2007)

Strong genetic control of emergence of human primary incisors. Journal of Dental Research, 86: 1160-1165.

Hughes T., Dempsey P., Richards L., and Townsend G. (2000) Genetic analysis of deciduous tooth size in Australian twins. Archives of Oral Biology, 45: 997-1004.

Jernvall J., and Thesleff I. (2000) Reiterative signalling and patterning in mammalian tooth morphogenesis. Mechanisms of Development, 92: 19-29.

Martin N., Boomsma D., and Machin G. (1997) A twin-pronged attack on complex traits. Nature Genetics, 17: 387-392.

Neale M.C., Boker S.M., Xie G., and Maes H.H. (2006) Mx: statistical modelling. 7th rev. ed. Richmond, VA: Department of Psychiatry, Virginia Commonwealth University.

Neale M.C., and Cardon L.R. (1992) Methodology for genetic studies of twins and their families. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Poulsen P., Esteller M., Vaag A., and Fraga M.G. (2007) The epigenetic basis of twin discordance in age-related diseases. Pediatric Research, 61: 38R-42R.

Preus H.R., Marvik O.J., Selvig K.A., and Bennike P. (2011) Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. Journal of Archaeological Science, 38: 1827-1831.

R Development Core Team. (2011) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

The International Hapmap Consortium. (2005) A haplotype map of the human genome. Nature, 437: 1299-1320.

Townsend G.C., and Martin N.G. (1992) Fitting genetic models to Carabelli trait data in South Australian twins. Journal of Dental Research, 71: 403-409.

Vieira A.R., Marazita M.L., and Goldstein-McHenry T. (2008) Genome wide scan finds suggestive caries loci. Journal of Dental Research, 87: 435-439.

Correspondence

Doctor Toby Hughes
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
Email: toby.hughes@adelaide.edu.au

4 Do feeding practices, gestation length, and birth weight affect the timing of emergence of the first primary tooth?

Emmanuel Chan,[1] Michelle Bockmann,[1,2,3] Toby Hughes,[1,2,3] Suzanna Mihailidis[1,2,3] and Grant Townsend[1,2,3]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development

Abstract

Recent studies of twins have confirmed that there is a strong genetic contribution to variation in timing of primary tooth emergence. Although environmental factors, such as severe nutritional deficiency of the infant, may affect primary tooth emergence, the roles of other environmental factors remain unclear. This study aimed to determine whether newborn feeding practices, gestation length, and birth weight affect the emergence time of the first primary tooth. Data were collected from questionnaires and parental records as part of an ongoing longitudinal study of Australian twins and their families. The sample comprised 217 twin pairs. Most commonly, a mandibular central incisor was the first tooth to emerge, with the next being a maxillary central incisor. F- and t-tests were performed, comparing variables and mean values between groups, and statistical significance was set at $p<0.05$. No statistically significant difference was found in age at first primary tooth emergence between breast-fed and bottle-fed babies, with mean emergence times of 8.0 and 8.6 months respectively. Extremely premature infants (gestation <30 weeks) had a significantly later mean emergence time compared with infants born full-term (gestation ≥37 weeks) (10.7mo compared with 7.6mo). Significantly greater variation in timing was also observed with

preterm infants. Very low birth weight babies (<1500g) also displayed a significant delay in tooth emergence compared with normal birth weight babies (>2500g) (10.1mo compared with 7.9mo). These findings indicate that the development of the primary dentition is well 'protected' against environmental disturbances, with only extreme prematurity or very low birth weight leading to significant delays in emergence.

Introduction

Considerable research has been undertaken to examine the timing and sequence of primary tooth emergence over the past decades. Despite this, primary tooth emergence remains an area of investigative interest and focus due to its biological, anthropological and clinical significance.

The timing of emergence of the primary dentition is variable. The observed variability is of multifactorial aetiology and considered to be the result of the complex interaction between genotype and environment (Robinow, 1973). Recent studies of twins have confirmed that the variation in timing of emergence of the primary dentition is mainly under genetic control (Hughes et al., 2007; Bockmann et al., 2010). Although studies have confirmed the significant effect of environmental factors such as severe nutritional deficiency (Jellife and Jellife, 1973; Rao et al., 1973) and low socio-economic status (Enwonwu, 1973) on the emergence of the primary dentition, the influence of other factors remain unclear.

Based on the association between nutritional status and the timing of emergence of the primary dentition, early childhood feeding practices have been proposed to be a significant factor in explaining the variation in primary tooth emergence times (Holman and Yamaguchi, 2005). Considering breastfeeding status as a possible source of variation in the timing of emergence, it is well recognized that breast milk is beneficial for infant growth and health by providing adequate nutrition and immunological factors in the early months of life to the child (Diaz et al., 1995; Onayade et al., 2004). In addition, breastfeeding has been thought to exert a positive effect on the development of dental occlusion due to the suckling mechanisms involved in the feeding practice (Palmer, 1998; Viggiano et al., 2004). Few researchers have sought to examine the effects of early childhood feeding practices on the timing of primary tooth emergence (Holman and Yamaguchi, 2005; Folayan et al., 2007; Sahin et al., 2008) and the findings of these few investigations have been entirely inconsistent. Holman and Yamaguchi (2005) and Sahin et al. (2008) reported appreciable associations between breastfeeding status and emergence times of primary teeth, while Folayan et al. (2007) reported no association.

The effect of preterm birth (gestational age<37 weeks) and low birth weight (birth weight<2500g) on the timing of emergence of the primary dentition has been widely examined. Most authors observe that subjects born preterm and of low birth weight exhibit a lag in the emergence of the primary dentition which diminishes with age (Lysell et al., 1962; Seow et al., 1988; Fadavi et al., 1992; Viscardi et al., 1994). Interestingly, the timing of primary tooth emergence is fairly consistent between preterm and full term subjects and subjects of different birth weights when measured from conception (Golden et al., 1981).

Thus, the delay in chronological age at primary tooth emergence exhibited by preterm and low birth weight subjects has been mainly attributed to reduced gestation length (Seow et al., 1988).

Given the lack of conclusive evidence, the aim of this longitudinal study was to examine the effect of new born feeding patterns, gestational age and birth weight on the timing of emergence of the first primary tooth in a sample of Australian twins.

Materials and methods

Study population

The data presented in this study were collected as part of a larger investigation of Australian twins undertaken at the University of Adelaide to explore the genetic and environmental contributions to variations in dental and facial features. This study involved 217 twin pairs comprising 92 pairs of monozygotic twins, 67 pairs of dizygotic same-sex twins and 58 pairs of dizygotic opposite-sex twins for whom tooth emergence data had been collected. All twins were of European ancestry and their zygosities were determined by DNA analysis. All subjects were healthy individuals with no history of genetic disorders which may affect the timing of tooth emergence.

Recording methods

Primary tooth emergence times were recorded by parents for each twin using specially designed dental charts. The charts included a photograph and a schematic representation of the primary dentition for recording the date of tooth emergence. A tooth was considered emerged at the time at which any part of the crown became visible through the soft tissues. Parents were instructed to record the date at which each tooth was first visible in the mouth and also how to palpate the tooth if in doubt about its emergence. Updates regarding tooth emergence were sought from parents at 6 monthly intervals from 12 months of age to approximately 36 months or until all teeth had emerged in the jaws. Clinical examinations were conducted on 10% of the sample population who were randomly selected to confirm the accuracy of the tooth emergence data (Hughes et al., 2007). Data for feeding practices, gestational age and birth weight were collected from two questionnaires completed by parents once at 3 months of age and another at 2 years of age.

Statistical analyses

Data on the timing of emergence of the first primary tooth, irrespective of tooth type, were available for all 217 twin pairs. The timing of tooth emergence was measured in days by subtracting date of birth from date of emergence. Sample sizes for the various analyses varied depending on the data available for the co variables; feeding behaviour, birth weight and gestational age. Tooth emergence and zygosity data were available for all 217 twin pairs, while data on feeding behaviours were available for 188 twin pairs. Data on birth weights were available for 189 twin pairs, and data on gestational age were available for 175 twin pairs. The

disparity in sample sizes was mainly attributable to variation in participant response rates to questions within questionnaires. In addition, sample sizes were reduced in some analyses due to the exclusion of twin pairs who lacked appropriate paired data.

Twins tend to be more highly correlated for many features compared to singletons as they share between 50-100% of their genes, and also a common environment if reared together. As a consequence, the data for one randomly selected twin from each pair were used for analyses to prevent bias in the results. Inferential analyses examined the emergence of the first primary tooth. This was defined as the tooth with the earliest emergence date irrespective of tooth type. F- and t-tests were performed, comparing variances and mean values between groups, and statistical significance was set at $p<0.05$.

Feeding practices

The timing of tooth emergence of 'exclusively breastfed' subjects was compared with that of 'exclusively bottle fed' subjects. 'Exclusively breastfed' subjects were defined as subjects who were solely breastfed from birth and never bottle fed until the emergence of the first primary tooth. 'Exclusively bottle fed' subjects were subjects who were solely bottle fed from birth and never breastfed until the time of emergence of the first primary tooth. In addition, correlation analyses were performed to examine the relationship between the duration of breastfeeding and the timing of emergence of the first primary tooth.

Gestational age

The effect of gestational age on timing of emergence was examined by grouping subjects by their gestational age into five groups (Table 1). A similar classification was employed by Golden et al. (1981), with the exception that gestational age was recorded in whole weeks by the authors. The grouping was modified in this investigation to account for the fact that gestational age was measured as weeks and days. The timing of emergence of the first primary tooth of preterm groups 1, 2, 3 and 4 was compared with full term subjects of group 5. The 'post-conception age' at emergence of the first primary tooth was calculated for each subject

Group 1	< 30 weeks and 6 days inclusive
Group 2	31 weeks to 32 weeks and 6 days inclusive
Group 3	33 weeks to 34 weeks and 6 days inclusive
Group 4	35 weeks to 36 weeks and 6 days inclusive
Group 5	37 weeks or greater (full term)

Table 1. Categorisation of subjects based on gestational age.

Very low birth weight (VLBW)	≤1.5 kg
Low birth weight (LBW)	1.5-2.5 kg
Normal birth weight (NBW)	≥2.5 kg

Table 2. Categorisation of subjects based on birth weight.

by adding their gestational age to their chronological age at tooth emergence (Golden et al., 1981). Analyses were performed using tooth emergence data calculated based on both chronological and post-conception age.

Birth weight

The effect of birth weight was examined by grouping subjects according to their birth weight into three groups (Table 2) based on a classification described by Seow et al. (1988). The timing of emergence of the first primary tooth was compared between subjects of different birth weight groups. Analyses were performed using tooth emergence data calculated based on both chronological and post-conception age.

Results

The mean timing of first primary tooth emergence, irrespective of tooth type, was 8.0 months with a standard deviation of 1.8 months. The timing of emergence of the first primary tooth varied considerably from 3.8 months to 14.2 months. The mandibular central incisor was usually the first primary tooth to emerge, while the maxillary central incisor was noted to emerge first in 7% of cases studied.

Feeding practices

In this study, 57 subjects were exclusively breastfed until the emergence of the first primary tooth and 20 subjects were exclusively bottle fed until the emergence of the first primary tooth. Almost all subjects who were breastfed commenced breastfeeding from birth. The duration of breastfeeding was variable, ranging from 0.25 to 28 months with the mean breastfeeding duration being 7.8 months. No significant differences were noted in age at first primary tooth emergence between exclusively breastfed and exclusively bottle fed subjects, with mean emergence times of 8.0 and 8.6 months respectively ($p = 0.4$). In addition, there was no correlation between breastfeeding duration and tooth emergence times.

Gestational age

In this study, 98 twin pairs were classified as being born preterm and 77 twin pairs were born full term. The mean chronological and post-conception ages at emergence of the first

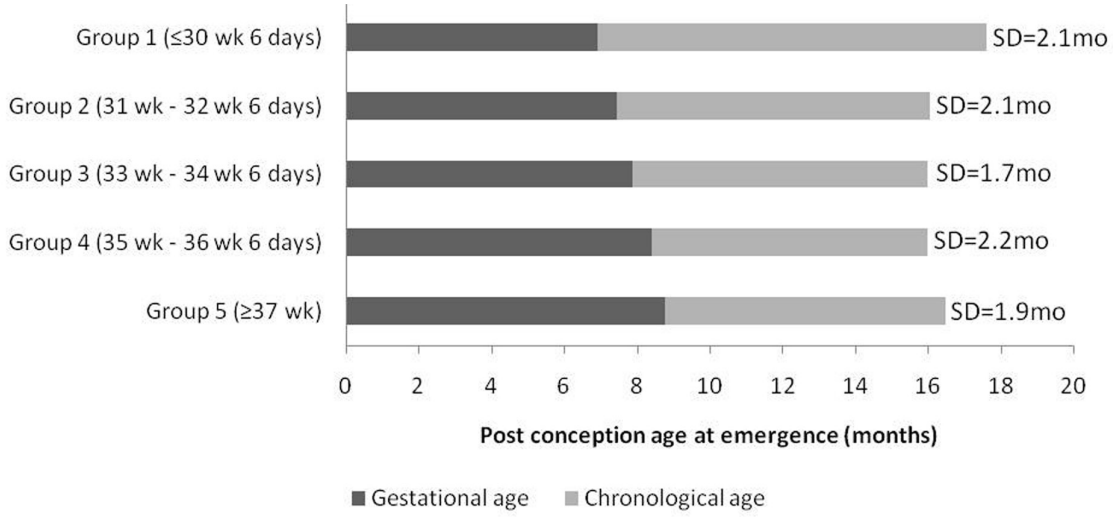


Figure 1. Post conception age at emergence of the first primary tooth by gestational age and chronological age for increasing gestational age groupings.

primary tooth of each group are illustrated in Figure 1. Severely preterm subjects of group 1 (gestation< 31 weeks) displayed significantly greater chronological age at first primary tooth emergence than full term subjects. Significantly greater variation in timing was also observed with preterm infants ($p = 0.03$). However, no differences were observed between preterm and full term subjects in mean tooth emergence time and variance when post-conception age was considered.

Birth weight

The mean emergence time of the VLBW group was significantly greater than that of the LBW group ($p = 0.02$) and the NBW group ($p = 0.007$). No significant differences were observed when comparing the variance and tooth emergence times in terms of post-conception age between the different birth weight groups. The mean chronological and post-conception ages at emergence of the first primary tooth of each group are illustrated in Figure 2.

Discussion

The results of this study indicate no significant association between feeding behaviours and the timing of emergence of the first primary tooth. Comparing the timing of emergence of the first primary tooth of exclusively breastfed subjects and exclusively bottle fed subjects yielded no statistically significant differences in terms of mean emergence times. The results in this study contribute to the mixed findings concerning childhood feeding practices and the timing of emergence of the primary dentition.

The findings of this study are consistent with those of Folayan et al. (2007) who also reported no significant differences in the timing of emergence of the primary dentition between subjects who were exclusively breastfed and subjects who were not exclusively breastfed. In

contrast, Holman and Yamaguchi (2005) reported significant associations between feeding practices and the timing of emergence of the primary dentition, in which breastfed subjects exhibit advanced emergence of the maxillary incisors but delayed emergence of the maxillary second molars. Sahin et al. (2008) reported that subjects who were fed cow's milk were 1.87 and 1.95 times more likely not to have a tooth emerge by 6 and 9 months respectively, compared to children who were breastfed exclusively.

Inter-study differences in methodology may contribute to the variation in study findings. A factor to consider is the variation in classifications of feeding practices between investigations. Unlike other studies, this study examined the influence of exclusive breast feeding compared to that of exclusive bottle feeding. In the study by Folayan et al. (2007), a classification of exclusive breastfeeding and non-exclusive breastfeeding was employed; however, the effect of a diet devoid of breast milk was not examined as all subjects were breastfed. Similarly, Sahin et al. (2008) examined a sample in which almost all subjects (99%) were breastfed and subjects were grouped based on the addition of cow's milk or formula to their diet. Holman and Yamaguchi (2005) examined historical data collected in 1914 and 1924 of Japanese subjects who were categorized as 'fully breastfed', 'partially breastfed' and 'not breastfed'. Notably, no descriptions were provided regarding the assignment of these rankings in the original tooth emergence histories.

It was evident in this study that breastfeeding status did not significantly influence the timing of emergence of the first primary tooth. Given that the first primary tooth to emerge was most commonly the mandibular central incisor, with the variation in its timing predominantly genetically determined (Bockmann et al., 2010), it is possible that future research employing emergence times for other primary teeth may improve our understanding

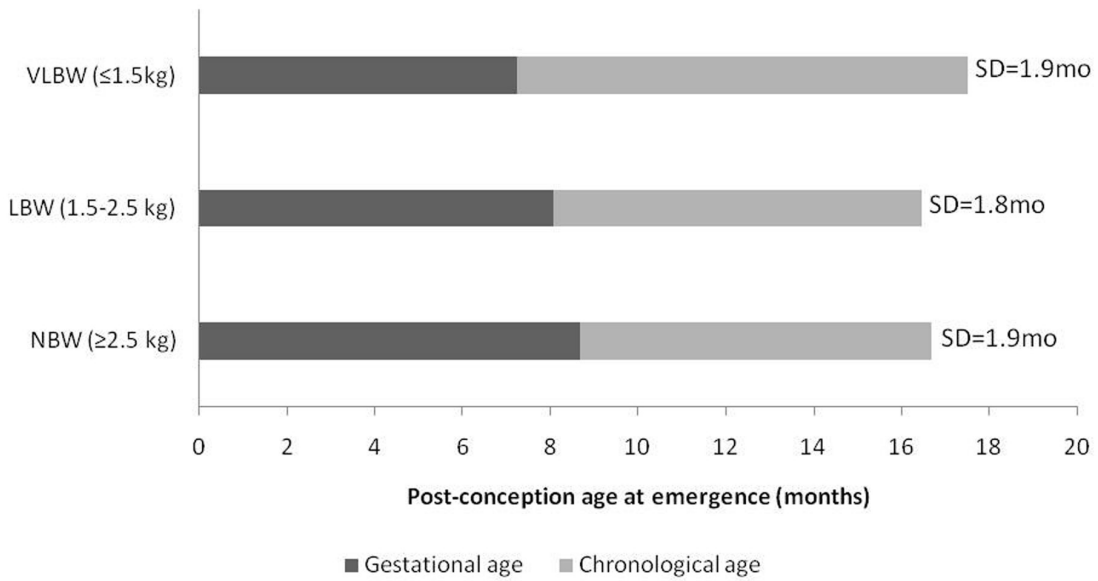


Figure 2. Post conception age at emergence of the first primary tooth by gestational age and chronological age for VLBW, LBW and NBW groups.

of the effect of feeding practices on the emergence of the primary dentition. This is further justified by the findings of Holman and Yamaguchi (2005) regarding the effect of breastfeeding, which was only significant when considering the emergence of the primary maxillary incisors and second molars. Thus, it appears that the expansion of our investigation to include other primary teeth in the future is warranted.

Various aspects of dental development, including tooth crown size and structure (Aine et al., 2000; Rythen et al., 2010; Vello et al., 2010), prevalence of dental defects (Seow et al., 2005), and dental eruption (Seow et al., 1988) are reportedly affected by preterm birth. Although the mean tooth emergence time of preterm subjects as a group was not significantly greater than that of full term subjects in this study, a significant difference was observed between full term subjects and subjects with a gestational age less than 31 weeks. In addition, the timing of emergence of preterm subjects was significantly more variable than that of full term subjects. Notably, when considering the timing of emergence of the first primary tooth as measured from conception, no significant differences were observed between preterm and full term subjects, as well as between subjects born of different gestation lengths. Thus, the variation in chronological age at tooth emergence amongst subjects with different gestational ages may be attributed to the variation in the timing of birth. Theoretically, birth could be considered as one event along the continuum from conception to tooth emergence. This is illustrated in Figure 1 in which the timing of birth is represented by the junction of gestational age and chronological age at tooth emergence.

Despite the difference not being statistically significant, it appears that the mean post-conception age at first primary tooth emergence of subjects with very reduced gestational age (group 1) is markedly greater than that of other subjects in groups 2, 3, 4 and 5. Given that there were only 7 subjects in group 1, this finding may warrant further investigation to determine whether subjects with a severely reduced gestation length i.e. less than 31 weeks, may exhibit a degree of retardation in dental development as a result of a severely reduced prenatal development period. Viscardi et al. (1994) proposed that neonatal complications in the form of prolonged intubation in prematurely born children may further contribute to the delay in primary tooth emergence. Thus, a greater propensity to neonatal problems may also explain the greater post-conception age at tooth emergence of subjects with severely reduced gestation length.

The results of this investigation support the notion that the timing of emergence of the first primary tooth is significantly associated with birth weight. The chronological age at dental emergence of VLBW subjects was significantly greater than that of LBW and NBW subjects. However, no significant differences were found when comparing post-conception tooth emergence times between the different birth weight groups. This finding is consistent with that of Seow et al. (1988), who attributed the differences in chronological age at dental emergence between different birth weight groups to the correlation between gestational age and birth weight. Although this relationship was also observed in our study, the post-conception age of the VLBW group was noted to be appreciably greater than LBW and NBW groups as illustrated in Figure 2. Despite the difference between the birth weight groups not being statistically significant, this presents an interesting finding which may suggest that the

delay in tooth emergence seen in VLBW subjects may not be entirely explained by the effects of reduced gestation. Interestingly, a study by Sajjadian et al. (2010) also observed that timing of emergence of the first primary tooth was negatively and linearly correlated with birth weight. However, all subjects in that investigation were born full term, which indicates that the effect of low birth weight on timing of emergence is not necessarily dependent on reduced gestational age.

Aside from reduced gestation, low birth weight may be related to genetic factors as well as prenatal environments (Rice and Thapar, 2010). Potentially, any defect in biological activity in the foetus, such as placental insufficiency, can result in intrauterine growth restriction and consequential low birth weight (Brodsky and Christou, 2004). Therefore, a delay in timing of emergence of the first primary tooth in VLBW subjects may also reflect a systemic delay in prenatal growth and development.

The results of this study indicate that the effects of low birth weight on the timing of emergence may be largely explained by the direct link between birth weight and gestational age. However, other factors including intrauterine growth restriction should also be considered. In this study, only 11 subjects were regarded as having VLBW. Therefore, an increase in the number of VLBW subjects in future studies may enable a greater understanding of the effect of VLBW on the timing of emergence of the primary dentition.

Conclusion

The results of this study indicate that the emergence of the first primary tooth is fairly robust to the effects of early childhood feeding practices. However, severe prematurity and very low birth weights were significantly associated with delayed tooth emergence.

Acknowledgements

We wish to thank all of the twins and their families who have agreed to participate in our studies that have been approved by the University of Adelaide Human Research Ethics Committee (H-78-2003). We also wish to acknowledge the support of the NHMRC of Australia, The Australian Dental Research Foundation, The University of Adelaide, the Financial Markets Foundation for Children and Colgate Australia.

References

Aine L., Backström M.C., Mäki R., Kuusela A.L., Koivisto A.M., Ikonen R.S., and Mäki M. (2000) Enamel defects in primary and permanent teeth of children born prematurely. Journal of Oral Pathology and Medicine, 29: 403-409.

Bockmann M.R., Hughes T.E., and Townsend G.C. (2010) Genetic modelling of primary tooth emergence: a study of Australian twins. Twin Research and Human Genetics, 13: 573-581.

Brodsky D., and Christou H. (2004) Current concepts in intrauterine growth restriction. Journal of Intensive Care Medicine, 19: 307-319.

Díaz S., Herreros C., Aravena R., Casado M.E., Reyes M.V., and Schiappacasse V. (1995)

Breast-feeding duration and growth of fully breast-fed infants in a poor urban Chilean population. American Journal of Clinical Nutrition, 62: 371-376.

Enwonwu C.O. (1973) Influence of socioeconomic conditions on dental development in Nigerian children. Archives of Oral Biology, 18: 95-107.

Fadavi S., Punwani I.C., Adeni S., and Vidyasagar D. (1992) Eruption pattern in premature low birth weight children. Journal of Dentistry for Children, 59: 120-122.

Folayan M., Owotade F., Adejuyigbe F., Sen S., Lawal., and Ndukwe K. (2007) The timing of eruption of primary dentition in Nigerian children. American Journal of Physical Anthropology, 134: 443-448.

Golden N.L., Takieddine F., and Hirsch V.J. (1981) Teething age in prematurely born infants. American Journal of Diseases of Children, 13: 903-904.

Holman D.J., and Yamaguchi K. (2005) Longitudinal analysis of primary tooth emergence: IV Covariate effects in Japanese children. American Journal of Physical Anthropology, 126: 352–358.

Hughes T.E., Bockmann M.R., Seow K., Gotjamanos T., Gully N., Richards L.C., and Townsend G.C. (2007) Strong genetic control of emergence of human primary incisors. Journal of Dental Research, 86: 1160-1165.

Jelliffe E.F., and Jelliffe D.B. (1973) Primary dental eruption, nutrition and age assessment. Journal of Tropical Pediatrics and Environmental Child Health, 19: 193-248.

Labbok M.H., and Hendershot G. (1987) Does breast-feeding protect against malocclusion? An analysis of the 1981 Child Health Supplement to the National Health Interview Survey. American Journal of Preventative Medicine, 3: 227-232.

Lysell L., Magnusson B., and Thilander B. (1962) Time and order of eruption of primary teeth. Odontologisk Revy, 13: 217-234.

Onayade A.A., Abiona T.C., Abayomi I.O., and Makanjuola R.O.A. (2004) The first six month growth and illness of exclusively and non-exclusively breast-fed infants in Nigeria. East African Medical Journal, 8: 146-154.

Palmer B. (1998) The influence of breastfeeding on the development of the oral cavity: A commentary. Journal of Human Lactation, 14: 93-98.

Rao K.V., Susheela T.P., and Swaminathan M.C. (1973) Association of growth status and primary teeth eruption among rural Indian children. Journal of Tropical Pediatrics and Environmental Child Health, 19: 223-227.

Rice F., and Thapar A. (2010) Estimating the relative contributions of maternal genetic, paternal genetic and intrauterine factors to offspring birth weight and head circumference. Early Human Development, 86: 425-432.

Robinow M. (1973) The eruption of the primary teeth (factors involved in timing). Journal of Tropical Pediatrics and Environmental Child Health, 19: 200-205.

Rythén M., Sabel N., Dietz W., Roberston A., and Norén J.G. (2010) Chemical aspects on dental hard tissues in primary teeth from preterm infants. European Journal of Oral Sciences, 118: 389-95.

Sahin F., Camurdan A.D., Camurdan M.O., Olmez A., Oznurhan F., and Beyazova U. (2008) Factors affecting the timing of teething in healthy Turkish infants: a prospective cohort

study. International Journal of Paediatric Dentistry, 18: 262-266.
Sajjadian N., Shajari H., Jahadi R., Barakat M.G., and Sajjadian A. (2010) Relationship between birth weight and time of first deciduous tooth eruption in 143 consecutively born infants. Pediatrics and Neonatology, 51: 235-237.
Seow W.K., Humphrys C., Mahanonda R., and Tudehope D.I. (1988) Dental eruption in low birth-weight prematurely born children: a controlled study. Pediatric Dentistry, 10: 39-42.
Velló M.A., Martínez-Costa C., Catalá M., Fons J., Brines J., and Guijarro-Martínez R. (2010) Prenatal and neonatal risk factors for the development of enamel defects in low birth weight children. Oral Diseases, 16: 257-262.
Viggiano D., Fasano D., Monaco G., and Strohmenger L. (2004) Breast feeding, bottle feeding and non-nutritive sucking: effects on occlusion in deciduous dentition. Archives of Diseases in Childhood, 89: 1121-1123.
Viscardi R.M., Romberg E., and Abrams R.G. (1994) Delayed primary tooth eruption in premature infants: relationship to neonatal factors. Pediatric Dentistry, 16: 23-28.

Correspondence

Ms Michelle Bockmann
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
E-mail: michelle.bockmann@adelaide.edu.au

5 Sexual dimorphism in the primary and permanent dentitions of twins: an approach to clarifying the role of hormonal factors

Daniela Ribeiro,[1,3,4] Wayne Sampson,[1,3] Toby Hughes,[1,3,4] Alan Brook[1,2,4] and Grant Townsend[1,3,4]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Dental Institute, Queen Mary University of London, London, United Kingdom
[3]Centre for Orofacial Research and Learning, The University of Adelaide
[4]International Collaborating Network in Oro-facial Genetics and Development

Abstract

This study aims to quantify the amount of sexual dimorphism in primary and permanent tooth crown size in a sample of Australian twins and to explore the role of hormonal factors in human dental development. We hypothesise that the magnitude and patterning of sexual dimorphism within and between the primary and permanent dentitions of the same individuals will reflect associations between the timing of initial stages in the process of odontogenesis and the timing of hormonal surges during pre-natal and peri-natal development. Serial dental models of the primary, mixed and permanent dentitions of 88 males and 91 females from monozygotic and dizygotic same-sex twin pairs were used. Mesiodistal crown diameters (MD), buccolingual crown diameters (BL), crown heights (CH), and intercuspal distances (IC) of all primary teeth and the permanent central incisors, lower lateral incisors, canines, second premolars, first and second molars were measured to an accuracy of 0.1mm using a 2D image analysis system. Mean values, standard deviations, coefficients of variation, percentages of sexual dimorphism, and correlation coefficients were calculated for all variables. Overall, males presented larger tooth crown dimensions than females, with the primary dentition displaying less sexual dimorphism compared with the

permanent dentition. Intercuspal distances tended to show the least sexual dimorphism whereas crown heights showed the most, reflecting differences in the timing of formation of these dimensions during odontogenesis. These results are consistent with some hormonal influence during tooth development, but further studies of twins, including opposite-sex dizygotic pairs, are needed to clarify the nature of this hormonal effect.

Introduction

Comparisons of tooth size and shape within and between related individuals provide a particularly valuable means of assessing genetic, epigenetic and environmental influences on dental variation (Garn et al., 1965a; Townsend, 1976; Townsend and Brown, 1978; Townsend, 1978; Brook, 1984; Townsend et al., 2005; Townsend et al., 2009). The human dentition is especially suitable for genetic studies because teeth start to form around 4 - 6 weeks after conception and continue to develop until around 21 years after birth (Townsend et al., 1994; Hillson, 1996; Townsend et al., 2009; AlQahtani et al., 2010).

Sexual dimorphism is evident in human tooth crown size (Garn et al., 1965b; Garn et al., 1967) and its magnitude and patterning varies within and between populations (Moorrees et al., 1957; Garn et al., 1965b). On average, males present larger tooth crown dimensions than females and this is evident for both primary (Black, 1978; Harris and Lease, 2005; Adler and Donlon, 2010) and permanent dentitions (Garn et al., 1966; Schwartz and Dean, 2005), with the latter being more dimorphic than the former. Sexual dimorphism also differs according to the tooth and dimension studied but is present in both mesiodistal (Garn et al., 1965b; Garn et al., 1967; Hanihara and Ishida, 2005; Harris et al., 2005) and buccolingual (Garn et al., 1966) crown dimensions, although little or no evidence of sexual dimorphism has been found for crown components such as intercuspal distances (Biggerstaff, 1975; Townsend, 1985).

Sexual dimorphism has been reported in the dentitions of monkeys, with males presenting larger canines than females (Lucas et al., 1986). Prenatally androgenised female Rhesus monkeys show larger upper and lower permanent canines when compared to normal females, suggesting that high levels of male hormone available in utero before tooth bud development may play a role in the masculinising of the dentition (Zingeser and Phoenix, 1978). Moreover, later-forming teeth seem to present more sexual dimorphism than teeth that form earlier as a result of increased sex hormones produced between males and females (Gingerich, 1974). Moreover, tooth dimensions that form earlier, i.e. intercuspal distances, seem to display less sexual dimorphism than dimensions that form later during tooth development (Townsend, 1985; Kondo and Townsend, 2004; Kondo et al., 2005).

Primary and permanent dentitions start to form at different times in utero, therefore, both dentitions would be exposed to different levels of intrauterine hormones once the first surge of testosterone occurs soon after testicular differentiation in males, around 7-9 weeks of gestation (Reyes et al., 1974; Knickmeyer and Baron-Cohen, 2006). The extent of any sex hormone contribution to differences in tooth size between males and females is still to be definitively established, although some researchers have indicated that the development

of sexual dimorphism occurs mainly due to the effects of the sex chromosomes (Guatelli-Steinberg et al., 2008; Alvesalo, 2009).

Studies in animals have demonstrated that hormones can diffuse across the amniotic membranes between fetuses and/or transfer through the placenta from the male fetus into the maternal circulation (Miller, 1994; Ryan and Vandenbergh, 2002). In addition, intrauterine position seems to influence litters differently in relation to prenatal hormone diffusion (Miller, 1994; Ryan et al., 2002). For example, female rats that cohabitate the uterus with a male, or develop between males, show more masculine effects of some physiological, behavioural and morphological traits such as a decrease in the number of litters and pregnancies, increased aggressiveness and sense of territoriality, and increased anogenital distance, when compared to normal females (Ryan et al., 2002). It is suggested that the same effect occurs in humans (Miller, 1994), as females who share the uterus with a co-twin brother have been shown to display a decrease in physiological oto-acoustic emission sensitivity (McFadden, 1993), an increase in aggressiveness and adventure-seeking behaviour (Resnick et al., 1993; Miller and Martin, 1995), decreased 2D:4D finger length ratios (Putz et al., 2004; van Anders et al., 2006) and an increase in brain volumes (Peper et al., 2009) when compared to other females. Evidence of increased permanent tooth crown size has also been reported in females from opposite-sex dizygotic twins compared to normal females (Dempsey et al., 1999).

Hand-held caliper measurement is a long-established method used in odontometric studies because of ease of use and transportation and this approach has shown acceptable levels of accuracy and reproducibility (Moorrees et al., 1957; Hunter and Priest, 1960; Smith et al., 2009). However, the sharp beaks of calipers can damage dental casts thereby altering measurements and leading to an increase in systematic errors of measurement (Hunter et al., 1960). An alternative way of measuring tooth crown dimensions is by using a 2D image analysis system, which is a standardized photographic system that consists of obtaining high resolution images using a digital camera under standardized illumination (Brook et al., 1999; Brook et al., 2005). This system has been shown to be accurate and reliable in obtaining images with high resolution from both occlusal and facial views of dental casts and it allows more efficient acquisition of data in comparison to the manual technique (Smith et al., 2009).

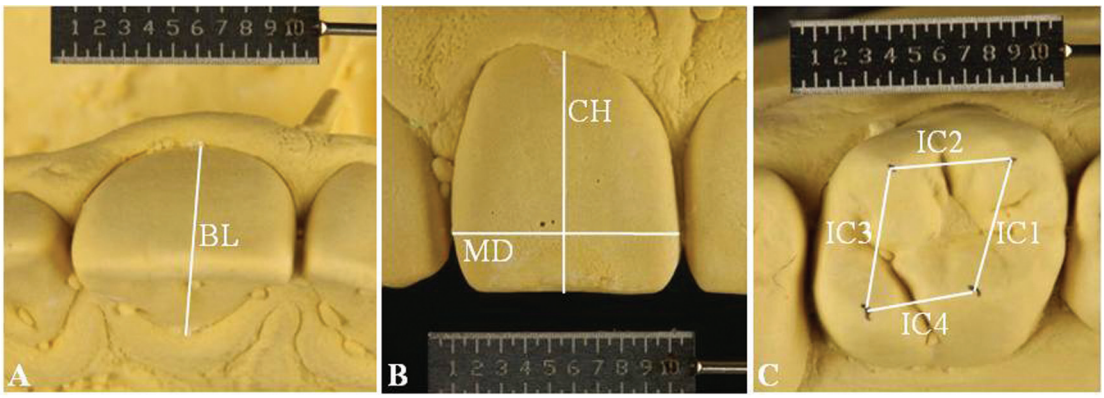
Given the advantages of using dental dimensions to make inferences about the effects of pre-natal and peri-natal factors on development, and given the lack of information on hormonal influences on tooth formation, the present study aims to fill a major gap in current understanding. This paper addresses some key aspects of a broader study on tooth size in twins and hormonal influences on dental development and will be focused on the methodology adopted and the presentation of basic descriptive statistics and correlation coefficients between the dental variables measured. It is planned to explore tooth size variability in opposite-sex dizygotic twins in a subsequent study. Specifically, the aims of this study are to: 1) describe tooth size variation in the primary and permanent dentitions of males and females from monozygotic (MZ) and dizygotic (DZ) same-sex twin pairs enrolled in an ongoing study of dentofacial development of Australian twins and their families in the School of Dentistry at The University of Adelaide, 2) to explore associations between mesiodistal crown diameters

(MD), buccolingual crown diameters (BL), crown heights (CH) and intercuspal (IC) distances obtained from a sample of MZ and DZ twin pairs of the same-sex, and 3) to determine whether the magnitude and patterning of sexual dimorphism observed in the primary and permanent dentitions of the same individuals shows any association with testosterone surges known to occur pre- and peri-natally.

Material and methods

The study sample consisted of monozygotic (MZ) and dizygotic same-sex (DZSS) twin pairs enrolled in an ongoing study of dentofacial development of Australian twins and their families being undertaken in the School of Dentistry at the University of Adelaide. Serial dental casts of the primary, mixed and permanent dentitions of the same individuals were used. The sample was divided by zygosity and gender into four groups as follows: 52 MZ same-sex female pairs, 46 MZ same-sex male pairs, 39 DZSS female pairs, and 42 DZSS male pairs. Zygosity was determined by analysis of highly polymorphic DNA markers of 10 chromosomes extracted from the cells of the cheek which has been shown to be a very effective, efficient and precise. The probability of dizygosity given concordance is less than 1% (Hughes et al., 2007). All participants were of European ancestry with no relevant medical and dental history. Ethical approval was obtained from The University of Adelaide Human Research Ethics Committee (H-07-1984A).

One co-twin from each pair of MZ and DZSS twins was randomly selected in this study to avoid bias that would be introduced by inclusion of tooth size data from both co-twins who share, on average, all (MZ co-twins) or half (DZSS co-twins) of their genes. Measurements were obtained from all primary teeth and for the permanent central incisors, lateral incisors (lower only), canines, second premolars, first and second molars. Excluded teeth were: upper permanent lateral incisors which vary greatly in size and shape, all first



A) buccolingual (BL) dimension obtained on a central incisor from the occlusal view; - B) mesiodistal (MD) and crown height (CH) dimensions obtained on a central incisor from the facial view; - C) intercuspal distances (IC1=mesio-buccal and mesio-lingual cusps; IC2=mesio-buccal and disto-buccal cusps; IC3=disto-buccal and disto-lingual cusps; IC4=mesio-lingual and disto-lingual cusps) obtained on a first molar from the occlusal view.

Figure 1. Tooth crown dimensions measured in this study.

premolars because previous results using the same sample reported no differences in MD and BL diameters greater then 0.1mm for both males and females (Dempsey et al., 1999), and all third molars because impressions were taken before eruption of these teeth. Teeth that were not fully erupted, had carious lesions or restorations, or were crowded and/or exhibited any evidence of tooth wear or model damage were excluded from this study.

The maximum mesiodistal (MD) crown dimensions were assessed according to Moorrees et al. (1957), Brook et al. (1999) and Brook at al. (2005) as being the maximum distance between the mesial and distal proximal surfaces of the tooth crown. The maximum labiolingual or buccolingual (BL) crown dimension was defined as the greatest distance between buccal and lingual surfaces of the crown perpendicular to and bisecting the line defining the mesiodistal dimension (Brook et al., 1999; Brook et al., 2005). Crown height (CH) was defined for the incisors as the maximum distance between the middle point in the incisal portion of the tooth crown and the middle point in the cervical line of the tooth crown, for the canines and premolars as the maximum distance between the buccal cusp tip to the cervical line of the tooth crown, and for molars as the maximum distance between the mesio-buccal cusp tip to the cervical line of the tooth crown. Intercuspal dimensions (IC) were defined as the distance between the buccal and lingual cusp tips of premolars and molars and comprised one measurement in premolars (ICP) and four measurements in molars. Intercuspal dimensions in molars included the distances between the mesio-buccal and mesio-lingual cusps (IC1), the mesio-buccal and disto-buccal cusps (IC2), the disto-buccal and disto-lingual cusps (IC3), and the mesio-lingual and disto-lingual cusps (IC4) (Figure 1).

The 2D image analysis system

Dental casts were placed on an adjustable platform under standardised illumination, enabling movement in three planes (Figure 2). Each tooth was imaged separately from both facial and occlusal views using a digital camera (Canon EOS 50D digital SLR camera, Canon Australia) with a resolution of 15.1 megapixels and the images were displayed in an array of 4752 x 3168 pixels for analysis. A 100mm lens (Elicar macro lens) was used to capture the images. The digital camera was mounted horizontally above the dental casts on an adjustable rod and the dental casts were illuminated with four multidirectional spot lights (Figure 2). A length of steel rule marked in millimetres (Minitool Inc, USA) was placed adjacent to the tooth surface and either perpendicular to the long axis of the tooth being imaged in an occlusal view or parallel to the long axis of the tooth being imaged in a facial view (Figure 1). The camera was connected to a computer (Intel Pentium 4 CPU 3.20GHz, 3192 MHz, 1 core, 2 logical processors, Australia) and the EOS Digital Software (Canon Australia PTY. LTD) was used to acquire the images from the camera. The camera settings were set as follows: aperture (f) f16, ISO speed 160 and shutter speed 0.3 seconds, and the images obtained were saved as a JPEG format (Brook et al., 1999; Brook et al., 2005). The images were analysed using the Image J software (National Institute of Health, USA) and each image was first calibrated and then measured. Mean values, standard deviations, and coefficients of variation were derived

Figure 2. 2D image analysis system.

for all variables. Pearson's coefficient of correlation (r) was also calculated between variables and the percentage of sexual dimorphism was calculated for all variables using the formula: [(M mean-F mean)/F mean]x100 (Garn et al., 1967).

Power analysis, systematic and random errors of measurements, summary and inferential statistics

A power analysis was performed and it was determined that approximately 50 individuals per group were needed for this study. Systematic errors can arise due to impression and casting procedures, imaging procedures, and time of measurement, while random errors can arise due to object positioning in relation to the camera, camera set up, light intensity and shadows, by identifying the landmarks and due to operator skills. Double determinations were performed by two operators (DCR and TC, a PhD student from the University of Liverpool). Reproducibility was assessed by using a paired t-test (Houston, 1983; Harris and Smith, 2009). Repeatability was assessed by using Dahlberg's statistic and the Fleiss intraclass coefficient of correlation (Dahlberg, 1940; Fleiss, 1986). Dahlberg's statistic ranged from 0.02mm to 0.26mm in the primary dentition and from 0.02mm to 0.33mm in the permanent dentition for all dimensions studied. Intercuspal distances and crown heights tended to show the highest errors, probably due to difficulty in locating the landmarks. Results of Fleiss intraclass coefficients of correlation were classified as excellent for both intra-operator (96-99%) and inter-operator (86-97%) correlations (Donner and Eliasziw, 1987). A p-value of less than 0.05 was considered significant. Descriptive statistics including mean values, standard deviations (SD) and coefficients of variation (CV) were calculated for all variables and were grouped according to zygosity, gender, dentition, maxillary and mandibular arches, and right and left sides. Mean values and variances were compare using t-tests and F-tests respectively,

with p values <0.05 being considered statistically significant. The relative difference between male and female means was reported as the percentage of sexual dimorphism (Garn et al., 1967).

Results

Sample sizes varied across all variables because some participants did not have full primary or permanent dentitions when impressions were obtained. Tooth wear and teeth that were not fully erupted also resulted in smaller sample sizes for some variables. Analysis of histograms showed that all variables were normally distributed.

No systematic differences between sides were noted, so Table 1 shows values of MD dimensions of primary and permanent teeth (right side) for MZ male and female twins and Table 2 shows values of MD dimensions of primary and permanent teeth (right side) for DZSS male and female twins.

Overall, mean values of MD crown dimensions were greater in males compared to females for all teeth and zygosities studied. However, the magnitudes of the differences and relative variation varied across teeth studied. Overall, the primary dentition displayed smaller differences between males and females in comparison to the permanent dentition, showing a smaller percentage of sexual dimorphism in both MZ and DZSS twins. Primary upper lateral incisors showed no difference in MD dimensions in MZ twins while primary upper canines presented the highest percentage of sex dimorphism (6.1%) in the same group (Table 1). No differences were found in MD dimensions between DZSS males and females for primary central incisors, upper canines and upper second molars, while lower lateral incisors showed the highest percentage of sex dimorphism (2.2%) in the primary dentition of DZSS twins (Table 2). For this group, upper lateral incisors presented a negative value for sexual dimorphism (-1.9%) in DZSS twins, with females being larger than males for MD dimension. For the permanent dentition, lower central incisors showed the smallest percentage of sexual dimorphism (1.9%) for MD dimensions for both MZ and DZSS twin pairs, while lower canines showed the highest values of sexual dimorphism in both MZ and DZSS twins (9.1% and 6.0% respectively). Permanent lower second molars showed no sexual dimorphism possibly due to a small sample size.

Calculations of the percentage of sexual dimorphism were performed for all MD, BL, CH and IC variables for MZ and DZSS twins. Average percentages of sexual dimorphism were smaller for MD dimensions, followed by BL dimensions and then for CH dimensions (MD<BL<CH) and this pattern occurred in both MZ and DZSS twins. Overall, the primary dentition presented smaller percentages of sexual dimorphism on average than the permanent dentition for all variables studied in both MZ (Table 3) and DZSS (Table 4) twins. Intercuspal distances showed a smaller percentage of sexual dimorphism in the primary dentition compared to the same dimensions in the permanent dentition. Negative percentages of sexual dimorphism found for IC may also reflect a small sample size.

Correlation coefficients were calculated between all tooth size variables for MZ and DZSS twins. Pearson's coefficient of correlation (r) was high between antimeric pairs of

		MZ Male				MZ Female				% Sexual
		n	mean	SD	CV	n	mean	SD	CV	Dimorphism
Primary	Upper									
	i1	29	6.4*	0.40	6.2	40	6.2	0.39	6.3	3.2
	i2	34	5.1	0.30	5.8	44	5.1	0.33	6.5	0.0
	c	45	7.0*	0.35	5.0	51	6.6	0.44	6.6	6.1
	m1	44	7.2*	0.39	5.5	51	6.8	0.36	5.3	5.9
	m2	45	9.0*	0.42	4.7	52	8.5	0.36	4.3	5.9
	Lower									
	i1	22	4.1*	0.32	7.8	27	3.9	0.29	7.4	5.1
	i2	33	4.6	0.32	6.9	41	4.4	0.33	7.5	4.5
	c	46	6.0*	0.35	5.9	51	5.8	0.27	4.7	3.4
	m1	43	8.0*	0.39	4.9	51	7.6	0.35	4.7	5.3
	m2	45	10.1*	0.44	4.3	51	9.8	0.37	3.8	3.1
Permanent	Upper									
	I1	45	8.7*	0.56	6.4	52	8.4	0.53	6.2	3.6
	C	29	8.1*	0.41	5.1	33	7.6	0.34	4.5	6.6
	PM2	30	6.9*	0.23	3.4	38	6.7	0.32	4.8	3.0
	M1	43	10.5*	0.47	4.4	50	10.0	0.47	4.6	5.0
	M2	19	10.5*	0.57	5.4	9	9.7	0.54	5.3	8.2
	Lower									
	I1	43	5.4*	0.39	5.2	49	5.3	0.29	5.5	1.9
	I2	39	6.0*	0.37	6.2	48	5.8	0.37	6.3	3.4
	C	33	7.2*	0.47	6.6	38	6.6	0.37	5.7	9.1
	PM2	32	7.5*	0.40	5.3	36	7.1	0.40	5.6	5.6
	M1	39	11.4*	0.66	5.8	45	10.7	0.58	5.4	6.5
	M2	16	11.0*	0.45	4.1	15	10.1	0.41	4.1	8.9

i1=primary central incisor; i2=primary lateral incisor; c=primary canine; m1=primary first molar; m2=primary second molar; I1=permanent central incisor; I2=permanent lateral incisor; C=permanent canine; PM2=permanent second premolar; M1=permanent first molar; M2=permanent second molar. (*mean values differ between the sexes at $p<0.05$).

Table 1. Descriptive statistics for mesiodistal dimensions (MD) of primary and permanent teeth (right side) in monozygotic (MZ) females and males.

		DZSS Male				DZSS Female				% Sexual
		n	mean	SD	CV	n	mean	SD	CV	Dimorphism
Primary	Upper									
	i1	32	6.3	0.34	5.4	30	6.3	0.45	7.2	0.0
	i2	35	5.1	0.38	7.4	35	5.2	0.36	6.9	-1.9
	c	42	6.8	0.42	6.1	39	6.8	0.36	5.3	0.0
	m1	42	7.0	0.49	7.0	39	6.9	0.49	7.1	1.4
	m2	41	8.7	0.54	6.2	39	8.7	0.44	5.1	0.0
	Lower									
	i1	23	4.0	0.26	6.3	25	4.0	0.30	7.6	0.0
	i2	34	4.6	0.33	7.0	33	4.5	0.38	8.4	2.2
	c	41	5.8	0.40	6.9	39	5.7	0.34	6.0	1.8
	m1	39	7.8	0.57	7.4	38	7.7	0.38	5.0	1.3
	m2	40	10.0*	0.49	4.9	39	9.8	0.45	4.6	2.0
Permanent	Upper									
	I1	40	8.7*	0.47	5.4	38	8.4	0.52	6.2	3.6
	C	21	8.0	0.32	4.0	24	7.8	0.62	8.0	2.6
	PM2	26	6.8	0.36	5.3	20	6.6	0.50	7.6	3.0
	M1	37	10.3*	0.55	5.3	36	10.0	0.48	4.8	3.0
	M2	4	10.0	0.53	5.3	8	9.8	0.65	6.6	2.0
	Lower									
	I1	38	5.4*	0.26	4.8	37	5.3	0.37	6.9	1.9
	I2	38	6.0*	0.32	5.4	36	5.8	0.39	6.8	3.4
	C	28	7.1*	0.34	4.8	28	6.7	0.43	6.4	6.0
	PM2	28	7.4*	0.39	5.3	25	7.1	0.46	6.4	4.2
	M1	38	11.2*	0.60	5.3	34	10.8	0.66	6.1	3.7
	M2	3	10.4	0.81	7.8	12	10.4	0.66	6.3	0.0

i1=primary central incisor; i2=primary lateral incisor; c=primary canine; m1=primary first molar; m2=primary second molar; I1=permanent central incisor; I2=permanent lateral incisor; C=permanent canine; PM2=permanent second premolar; M1=permanent first molar; M2=permanent second molar. (*mean values differ between the sexes at $p<0.05$).

Table 2. Descriptive statistics for mesiodistal dimensions (MD) of primary and permanent teeth (right side) in dizygotic same-sex (DZSS) females and males.

		MD		BL		CH		ICP		IC1		IC2		IC3		IC4	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Primary	Upper																
	i1	69	3.2	74	6.1	53	5.7										
	i2	78	0.0	81	4.3	59	8.5										
	c	96	6.1	96	3.3	45	16.7										
	m1	95	5.9	96	5.9	57	11.9			50	7.3						
	m2	97	5.9	98	4.1	67	10.5			67	2.1	68	0.0	69	0.0	67	0.0
	Lower																
	i1	49	5.1	48	8.3	35	4.2										
	i2	74	4.5	73	2.3	60	3.9										
	c	97	3.4	97	1.8	55	6.9										
	m1	94	5.3	94	5.9	53	8.5			47	-7.7						
	m2	96	3.1	97	7.3	55	8.3			50	0.0	49	3.0	52	4.3	54	-2.2
	Average		4.2		4.9		8.5				0.4		1.5		2.1		-1.1
Permanent	Upper																
	I1	97	3.6	93	2.8	88	9.9										
	C	62	6.6	57	7.7	43	12.3										
	PM2	68	3.0	68	6.5	61	10.7	68	3.8								
	M1	93	5.0	94	7.2	87	13.0			86	3.3	87	2.1	88	5.0	86	8.9
	M2	28	8.2	30	9.1	26	11.1			29	10.0	29	6.3	17	8.3	17	2.4
	Lower																
	I1	92	1.9	89	6.8	77	5.1										
	I2	87	3.4	86	6.5	72	6.7										
	C	71	9.1	66	8.3	58	7.2										
	PM2	68	5.6	68	4.8	63	7.0	63	4.8								
	M1	84	6.5	91	7.1	63	11.1			60	4.0	56	14.0	56	5.4	62	0.0
	M2	31	7.8	39	10.2	27	4.7			29	0.0	28	10.6	28	4.0	28	13.5
	Average		5.5		7.0		9.0		4.3		4.3		8.2		5.7		6.2

i1=primary central incisor; i2=primary lateral incisor; c=primary canine; m1=primary first molar; m2=primary second molar; I1=permanent central incisor; I2=permanent lateral incisor; C=permanent canine; PM2=permanent second premolar; M1=permanent first molar; M2=permanent second molar. MD=mesiodistal; BL=buccolingual; CH=crown height; ICP=intercuspal distance in second premolar; IC1=mesio-buccal and mesio-lingual cusps; IC2=mesio-buccal and disto-buccal cusps; IC3=disto-buccal and disto-lingual cusps; IC4=mesio-lingual and disto-lingual cusps.

Table 3. Sexual dimorphism percentage for mesiodistal (MD), buccolingual (BL), crown height (CH), and intercuspal distances of primary and permanent teeth (right side) in monozygotic (MZ) twins.

		MD		BL		CH		ICP		IC1		IC2		IC3		IC4	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Primary	Upper																
	i1	62	0.0	65	2.0	43	15.4										
	i2	70	-1.9	72	2.1	54	4.2										
	c	81	0.0	81	1.7	31	1.8										
	m1	81	1.4	80	0.0	45	7.0			44	6.1						
	m2	80	0.0	80	3.1	55	5.1			51	4.3	52	0.0	52	6.3	51	0.0
	Lower																
	i1	48	0.0	49	0.0	31	4.4										
	i2	67	2.2	68	2.4	48	8.0										
	c	80	1.8	80	1.8	33	0.0										
	m1	77	1.3	78	2.9	42	12.5			34	4.2						
	m2	79	2.0	81	2.4	45	8.1			38	5.7	38	8.8	40	6.5	40	6.5
	Average		0.7		1.8		6.7				5.1		4.4		6.4		3.3
Permanent	Upper																
	I1	78	3.6	76	2.9	67	7.7										
	C	45	2.6	44	2.5	34	6.9										
	PM2	46	3.0	47	3.3	44	7.3	46	5.8								
	M1	73	3.0	72	3.6	64	4.1			61	6.9	61	2.1	62	3.3	61	4.4
	M2	12	2.0	15	7.2	15	2.0			14	5.1	15	10.6	5	11.7	5	19.5
	Lower																
	I1	75	1.9	76	1.7	49	10.4										
	I2	74	3.4	73	1.6	52	11.1										
	C	56	6.0	51	4.2	36	5.8										
	PM2	53	4.2	52	6.1	47	1.8	48	12.5								
	M1	72	3.7	75	6.1	47	6.5			44	8.3	42	2.3	43	9.3	45	3.5
	M2	15	0.0	20	2.0	18	2.5			19	2.0	18	10.4	18	12.0	18	5.3
	Average		3.0		3.7		6.0		9.1		5.6		6.4		9.1		8.2

i1=primary central incisor; i2=primary lateral incisor; c=primary canine; m1=primary first molar; m2=primary second molar; I1=permanent central incisor; I2=permanent lateral incisor; C=permanent canine; PM2=permanent second premolar; M1=permanent first molar; M2=permanent second molar. MD=mesiodistal; BL=buccolingual; CH=crown height; ICP=intercuspal distance in second premolar; IC1=mesio-buccal and mesio-lingual cusps; IC2=mesio-buccal and disto-buccal cusps; IC3=disto-buccal and disto-lingual cusps; IC4=mesio-lingual and disto-lingual cusps.

Table 4. Sexual dimorphism percentage for mesiodistal (MD), buccolingual (BL), crown height (CH), and intercuspal distances of primary and permanent teeth (right side) in dizygotic same-sex (DZSS) twins.

	MD	BL	CH	ic1	ic2	ic3	ic4
MD	1.00	0.75	**0.20**	0.53	**0.23**	0.62	0.41
BL		1.00	0.41	0.60	**0.16**	0.65	0.54
CH			1.00	**0.07**	**-0.29**	**0.15**	**0.13**
ic1				1.00	0.60	0.82	0.78
ic2					1.00	0.44	0.71
ic3						1.00	0.73
ic4							1.00

MD=mesiodistal; BL=buccolingual; CH=crown height; ic1=mesio-buccal/mesio-lingual cusp distance; ic2=mesio-buccal/disto-buccal cusp distance; ic3=disto-buccal/disto-lingual cusp distance; ic4=mesio-lingual/disto-lingual cusp distance. r-values in bold are significantly different from zero.

Table 5. Values of correlations between all variables (MD, BL, CH and IC) in the same tooth in MZ male twins: primary upper right second molar (n=19-46).

	MD	BL	CH	ic1	ic2	ic3	ic4
MD	1.00	0.41	**0.09**	**0.46**	**0.29**	**0.30**	**0.24**
BL		1.00	**0.13**	**0.19**	**0.26**	**-0.11**	**-0.14**
CH			1.00	**0.04**	**-0.20**	**0.02**	**0.22**
ic1				1.00	0.71	**0.02**	**-0.27**
ic2					1.00	**-0.34**	-0.60
ic3						1.00	0.60
ic4							1.00

MD=mesiodistal; BL=buccolingual; CH=crown height; ic1=mesio-buccal/mesio-lingual cusp distance; ic2=mesio-buccal/disto-buccal cusp distance; ic3=disto-buccal/disto-lingual cusp distance; ic4=mesio-lingual/disto-lingual cusp distance. r-values in bold are significantly different from zero.

Table 6. Values of correlations between all variables (MD, BL, CH and IC) in the same tooth in DZ same sex male twins: primary upper right second molar (n=15-41).

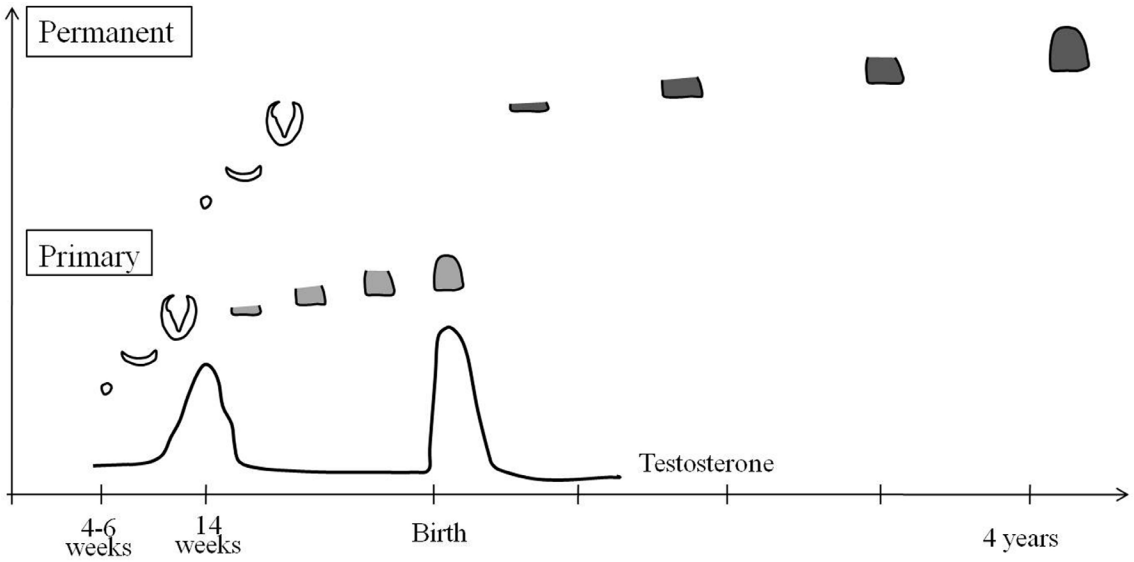


Figure 3. Schematic representation of stages of formation of the upper central incisor and surges in testosterone production. *Graph showing testosterone levels derived from data provided by Reyes et al. (1974) and Knickmeyer and Baron-Cohen (2006).

permanent teeth for MD dimensions in MZ female twins (n=9-52), with values ranging between 0.83 and 0.90. Values of correlation coefficients between isomeric pairs of permanent teeth for MD dimensions in MZ female twins were moderate to high (range: 0.61 to 0.82, n=9-52). Correlations between primary and corresponding successional permanent teeth for MD dimensions in MZ female twins (n=23-41) were moderate to low, with values ranging between 0.36 and 0.51. When correlations between all variables (MD, BL, CH and IC) within the same tooth (primary right upper second molar) were considered, MZ male twins (n=19-46) showed a moderate correlation between MD and BL dimensions (r=0.75) (Table 5) while DZSS male twins (n=15-41) showed moderate to low correlations between MD and BL (Table 6). Low correlation was found between CH and IC in MZ and DZSS male twins (Table 5). Buccolingual dimensions showed moderate to low correlations with CH and IC distances in MZ male twins and low correlations in DZSS male twins. Crown height (CH) showed low correlations with all variables, except for BL dimension in MZ male twins. Overall, correlations between intercuspal distances were moderate to high (Table 5) in MZ male twins, but a moderate to low correlation between IC were found in DZSS male twins (Table 6). Negative values found in both groups may have occurred due to small sample sizes and errors of the methods.

Discussion

Many studies have indicated that sex hormones may be important factors in explaining the differences observed between males and females in various physical features (Cohen-Bendahan et al., 2005; Hines, 2006; Knickmeyer et al., 2006; Vuoksimaa et al., 2010), even though some researchers have claimed that the development of sexual dimorphism occurs mainly due

to the effects of the sex chromosomes (Guatelli-Steinberg et al., 2008; Alvesalo, 2009).

Studies on levels of steroid hormones in normal males have shown that three surges of testosterone occur during male development. The first surge occurs in utero soon after testicular differentiation, around 7-9 weeks of gestation. The levels of testosterone are highest between weeks 10 and 20 post-conception, peaking around week 14, and the values are comparable to the levels in normal adult males (Reyes et al., 1974; Knickmeyer et al., 2006). A second testosterone surge occurs in males soon after birth due to the inhibition of oestrogen levels produced by the placenta, and a third surge in testosterone occurs at puberty during adolescence (Larsen et al., 2003). In this study, the percentage of sexual dimorphism in the permanent dentition was higher than in the primary dentition for both MZ and DZSS twins and this may be associated with greater hormonal influence on permanent teeth that form over a longer period of time than primary teeth.

The two human dentitions start to form at different times in utero. Primary teeth start to develop around 4 - 6 weeks post-conception and continue their crown development until around one year after birth, while permanent teeth start to form around 14 weeks post-conception, and continue their formation until third molar crown calcification is completed, at around 14 years of age (AlQahtani et al., 2010). During odontogenesis, the developing tooth passes through different stages of formation, such as thickening and specialization of the dental lamina, bud, cap, and bell stages before crown calcification starts (Nanci, 2003). The peak of the first testosterone surge occurs around 14 weeks post-conception, when primary teeth have already passed through all soft tissue stages of tooth formation but before commencement of calcification, while at this time permanent teeth are just starting to develop (Figure 3). In addition, the primary dentition develops much faster, i.e. over a shorter period of time compared to the permanent dentition and, consequently, is less exposed to the effects of hormones either in utero or neonatally.

The magnitude and patterning of sexual dimorphism on tooth size varies according to dentition and tooth studied. In this study, the primary dentition displayed smaller percentages of sexual dimorphism than the permanent dentition and this was evident for both MZ and DZSS twin pairs and also for all variables studied, agreeing with a previous study on sexual dimorphism in primary and permanent dentitions (Moorrees et al., 1957). Permanent lower canines showed the highest percentage of sexual dimorphism and lower central incisors the lowest for MD dimensions in both MZ and DZSS twins, corroborating previous findings that permanent lower canines are the most dimorphic teeth and lower central incisors the least for MD dimensions in the human dentition (Garn et al., 1967; Harris and Nweeia, 1980). The high percentage of sexual dimorphism found on permanent second molars might reflect a small sample size. It seems that the whole process of permanent tooth development occurs under relatively high levels of testosterone influence and this may be an explanation for the difference in sexual dimorphism found between primary and permanent dentitions of the same individuals.

The tooth calcification process starts from the incisal/cuspal tip portion of the crown and proceeds all the way to the root apex, so different crown dimensions are defined at different times during tooth formation. For example, in an upper central incisor, the MD

dimension is determined soon after calcification starts, while the BL dimension and CH are not determined until calcification of the entire tooth crown is completed, some four years after the MD dimension is defined. In this study, mesiodistal dimensions generally displayed the smaller percentages of sexual dimorphism, followed by BL dimensions and CH dimensions, and this pattern was evident for both twin groups studied. This means that earlier-forming crown dimensions (MD) showed lower percentages of sexual dimorphism when compared to tooth dimensions that form later in odontogenesis such as BL and CH. Hence, BL dimensions also presented less sexual dimorphism when compared to CH dimensions. This suggests that dimensions that form over a longer period of time tended to display more sexual dimorphism than early-forming dimensions, possibly because later-forming dimensions are more exposed to tooth-size increasing hormones than dimensions that form early during tooth development (Table 3 and Table 4). Although previous reports showed no sexual dimorphism for IC dimensions (Townsend, 1985), the present study found a small percentage of sexual dimorphism for IC dimensions for both dentitions with the primary dentition showing smaller percentages than the permanent dentition. This suggests that even structures that form early on during odontogenesis may be influenced by hormones.

Although crown dimensions are formed at different times, MD dimensions still showed a relatively moderate to high correlation with BL dimensions, but only a moderate to low correlation with CH and IC dimensions (Table 5 and Table 6). A possible explanation for this is that MD and BL crown dimensions reflect overall tooth crown size in the same plane, thus being closely related to each other, while CH dimensions are defined just before root formation and IC dimensions become established very early on during the folding of thinner enamel epithelium before crown calcification starts.

Some researchers have stated that IC dimensions seem to be affected according to the order of cusp calcification (Kondo et al., 2004; Takahashi et al., 2007). In this study, correlations between IC dimensions in MZ twins were moderate to high with IC1 presenting a high correlation with IC3, but a moderate correlation with IC2 and IC4 (Table 5). However, a moderate to low correlation was found for all IC distances in DZSS twins (Table 6).

Crown height dimensions (CH) showed the lowest correlations with MD and IC dimensions but a moderate correlation with BL dimension. This low correlation may have occurred due to the difficulty in locating the landmarks as this dimension is affected by the position of the gingiva, by the inclination of the tooth and by tooth wear.

The question of what produces sexual dimorphism of tooth size remains unanswered. Whether the sex chromosomes themselves are responsible for producing sexual dimorphism in tooth size or a combination of sex chromosomes and hormones produce the variation within the dentition observed between males and females is yet to be determined. Nevertheless, the preliminary findings of our study of tooth size in twins are consistent with an influence of male hormones on the human dentition, leading to greater sexual dimorphism in the permanent dentition than the primary dentition and greater sexual dimorphism of certain teeth and tooth dimensions. We now plan to explore whether the surge in testosterone in utero has an effect on the females from opposite-sex dizygotic twin pairs.

Conclusion

This study has shown that there is a tendency for increased tooth crown size (MD, BL, and CH) in both dentitions of MZ and DZSS male twins compared with females of the same groups. This study has shown that both dentitions present sexual dimorphism, with the primary dentition showing less sexual dimorphism than permanent dentition. The magnitude and patterning of sexual dimorphism varied between teeth studied, with permanent lower canines showing the highest percentage of sexual dimorphism compared with other teeth and permanent lower central incisors the least. Different tooth crown dimensions displayed different percentages of sexual dimorphism, with early-forming dimensions in odontogenesis being the least dimorphic and the later-forming dimensions the most. Dimensions that are associated with overall crown size, such as MD and BL, were more highly correlated with each other than dimensions that are determined earlier (IC) or later (CH) during tooth development. Even early-forming structures such as IC dimension present some degree of sexual dimorphism and may be influenced by circulating male hormones, either in utero and pre-natally, during tooth development.

Acknowledgements

This study is supported by the NHMRC, Australian Dental Research Foundation, Centre for Orofacial Research and Learning (CORAL), The University of Adelaide, Colgate Australia, Australian Society of Orthodontics Foundation for Research and Education, Australian Twin Registry, and Australian Multiple Birth Association. We are particularly thankful for all participants enrolled in this twin study. We thank Dr Tom Coxon for his assistance with the double determinations.

References

Adler C.J., and Donlon D. (2010) Sexual dimorphism in deciduous crown traits of a European derived Australian sample. Forensic Science International, 199: 29-37.

AlQahtani S.J., Hector M.P., and Liversidge H.M. (2010) Brief communication: the London atlas of human tooth development and eruption. American Journal of Physical Anthropology, 142: 481-490.

Alvesalo L. (2009) Human sex chromosomes in oral and craniofacial growth. Archives of Oral Biology, 54S: 18-24.

Biggerstaff R.H. (1975) Cusp size, sexual dimorphism, and heritability of cusp size in twins. American Journal of Physical Anthropology, 42: 127-140.

Black T.K.I. (1978) Sexual dimorphism in the tooth-crown diameters of the deciduous teeth. American Journal of Physical Anthropology, 48: 77-82.

Brook A., Smith R., Elcock C., Al-Sharood M., Shah A., and Karmo M. (1999). The measurement of tooth morphology: development and validation of a new image analysis system. In: Mayhall J. and Heikkinen T. (Eds.) Oulu: Oulu University Press, pp. 380-387.

Brook A.H. (1984) A unifying aetiological explanation for anomalies of human tooth number and size. Archives of Oral Biology, 29: 373-378.

Brook A.H., Smith R.N., Elcock C., Al-Sharood M.H., Shah A.A., Khalaf F., Robinson D.L., Lath D.L., and Karmo M. (2005) The measurement of tooth morphology: validation of an image analysis system. 13th International Symposium of Dental Morphology. In: Zadzinska E. (Ed.) Lodz: University of Lodz Press, pp. 475-482.

Cohen-Bendahan C.C.C., Buitelaar J.K., Van Goozen S.H.M., Orlebeke J.F., and Cohen-Kettenis P.T. (2005) Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. Hormones and Behavior, 47: 230-237.

Dahlberg G. (1940) Statistical methods for medical and biological students. George Allen and Unwin, London.

Dempsey P.J., Townsend G.C., and Richards L.C. (1999) Increased tooth crown size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. American Journal of Human Genetics, 11: 577-586.

Donner A., and Eliasziw M. (1987) Sample size requirements for reliability studies. Statistics in Medicine, 6: 441-448.

Fleiss J. (1986) Reliability of measurements. John Wiley and Sons Inc, Columbia, USA.

Garn S.M., Lewis A.B., and Kerewsky R.S. (1965a) Genetic, nutritional, and maturational correlates of dental development. Journal of Dental Research, 44: 228-242.

Garn S.M., Lewis A.B., Kerewsky R.S., and Jegart K. (1965b) Sex differences in intraindividual tooth-size communalities. Journal of Dental Research, 44: 476-479.

Garn S.M., Lewis A.B., and Kerewsky R.S. (1966) Sexual dimorphism in the buccolingual tooth diameter. Journal of Dental Research, 45: 1819.

Garn S.M., Lewis A.B., Swindler D.R., and Kerewsky R.S. (1967) Genetic control of sexual dimorphism in tooth size. Journal of Dental Research, 46: 963-972.

Gingerich P. (1974) Size variability of the teeth in living mammals and the diagnosis of closely related sympatric fossil species. Journal of Paleontology, 48: 895-903.

Guatelli-Steinberg D., Sciulli P.W., and Betsinger T.K. (2008) Dental crown size and sex hormone concentrations: another look at the development of sexual dimorphism. American Journal of Physical Anthropology, 137: 324-333.

Hanihara T., and Ishida H. (2005) Metric dental variation of major human populations. American Journal of Physical Anthropology, 128: 287-298.

Harris E., and Smith R. (2009) Accounting for measurement error: a critical but often overlooked process. Archives of Oral Biology, 54S: 107-117.

Harris E.F., and Nweeia M.T. (1980) Dental asymmetry as a measure of environmental stress in the Ticuna Indians of Colombia. American Journal of Physical Anthropology, 53: 133-142.

Harris E.F., and Lease L.R. (2005) Mesiodistal tooth crown dimensions of primary dentition: a worldwide survey. American Journal of Physical Anthropology, 128: 593-607.

Hillson S. (1996) Dental Anthropology. Cambridge University Press, Cambridge.

Hines M. (2006) Prenatal testosterone and gender-related behaviour. European Journal of

Endocrinology, 155: S115-121.
Houston W.J.B. (1983) The analysis of errors in orthodontic measurements. American Journal of Orthodontics, 83: 382-390.
Hughes T., Bockmann M., Seow K., Gotjamanos T., Gully N., Richards L., and Townsend G. (2007) Strong genetic control of emergence of human primary incisors. Journal of Dental Research, 86: 1160-1165.
Hunter W.S., and Priest W.R. (1960) Errors and discrepancies in measurement of tooth size. Journal of Dental Research, 39: 405-414.
Knickmeyer R.C., and Baron-Cohen S. (2006) Fetal testosterone and sex differences. Early Human Development, 82: 755-760.
Kondo S., and Townsend G. (2004) Sexual dimorphism in crown units of mandibular deciduous and permanent molars in Australian Aborigines. Journal of Comparative Human Biology, 55: 53-64.
Kondo S., Townsend G.C., and Yamada H. (2005) Sexual dimorphism of cusp dimension in human maxillary molars. American Journal of Physical Anthropology, 128: 870-877.
Larsen P.R., Kronemberg H.M., Melmed S., and Polonsky K.S. (2003) Disorders of the testes and the male reproductive tract. Saunders, Philadelphia.
Lucas P., Corlett R., and Luke D. (1986) Sexual dimorphism of tooth size in anthropoids. Human Biology, 1: 23-39.
McFadden D. (1993) A masculinizing effect on the auditory system of human females having male co-twins. Proceedings of the National Academy of Science, 90: 11900-11904.
Miller E.M. (1994) Prenatal sex hormone transfer: a reason to study opposite-sex twins. Personality and Individual Differences, 17: 511-529.
Miller E.M., and Martin N. (1995) Analysis of the effect of hormones on opposite-sex twin attitudes. Acta Geneticae Medicae et Gamellologiae, 44: 41-52.
Moorrees C.F.A., Thomsen S.O., Jensen E., and Yen P.K. (1957) Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. Journal of Dental Research, 36: 39-47.
Nanci A. (2003) Ten Cate's oral histology: development, structure and function. Mosby, St. Louis.
Peper J.S., Brouwer R.M., van Baal G.C.M., Schnack H.G., van Leeuwen M., Boomsma D.I., Kahn R.S., and Hulshoff Pol H.E. (2009) Does having a twin brother make for a bigger brain? European Journal of Endocrinology, 160: 739-746.
Putz D.A., Gaulin S.J.C., Sporter R.J., and McBurney D.H. (2004) Sex hormones and finger length. What does 2D:4D indicate? Evolution and Human Behavior, 25: 182-199.
Resnick S.M., Gottesman I.I., and McGue M. (1993) Sensation seeking in opposite-sex twins: an effect of prenatal hormones. Behavior Genetics, 23: 323-329.
Reyes F.I., Boroditsky R.S., Winter J.S.D., and Fairman C. (1974) Studies on human sexual development. II. Fetal; and maternal serum gonadotropin and sex steroid concentrations. Journal of Clinical Endocrinology and Metabolism, 38: 612-617.
Ryan B.C., and Vandenbergh J.G. (2002) Intrauterine position effects. Neuroscience and biobehavioral reviews, 26: 665-678.

Schwartz G.T., and Dean M.C. (2005) Sexual dimorphism in modern human permanent teeth. American Journal of Physical Anthropology, 128: 312-317.

Smith R., Zaitoun H., Coxon T., Karmo M., Kaur G., Townsend G., Harris E.F., and Brook A. (2009) Defining new dental phenotypes using 3-D image analysis to enhance discrimination and insights into biological processes. Archives of Oral Biology, 54S: 118-125.

Takahashi M., Kondo S., Townsend G.C., and Kanazawa E. (2007) Variability in cusp size of human maxillary molars, with particular reference to the hypocone. Archives of Oral Biology, 52: 1146-1154.

Townsend G. (1976) Tooth size variability in Australian Aboriginals. PhD Thesis, The University of Adelaide, Adelaide, SA.

Townsend G., and Brown T. (1978) Inheritance of tooth size in Australian Aboriginals. American Journal of Physical Anthropology, 48: 305-314.

Townsend G. (1985) Intercuspal distances of maxillary pre-molar teeth in Australian aboriginals. Journal of Dental Research, 64: 443-446.

Townsend G., Dempsey P., Brown T., Kaidonis J., and Richards L. (1994) Teeth, genes and the environment. Perspectives in Human Biology, 4: 35-46.

Townsend G., Richards L., Hughes T., Pinkerton S., and Schwerdt W. (2005) Epigenetic influences may explain dental differences in monozygotic twin pairs. Australian Dental Journal, 50: 95-100.

Townsend G., Hughes T., Luciano M., Bockmann M., and Brook A. (2009) Genetic and environmental influences on human dental variation: a critical evaluation of studies involving twins. Archives of Oral Biology, 54S: 45-51.

Townsend G.C. (1978) Genetics of tooth size. Australian Orthodontic Journal, 5: 142-147.

Van Anders S.M., Vernon P.A., and Wilbur C.J. (2006) Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. Hormones and Behavior, 49: 315-319.

Vuoksimaa E., Kaprio J., Kremen W.S., Hokkanen L., Viken R.J., Tuulio-Henriksson A., and Rose R.J. (2010) Having a male co-twin masculinizes mental rotation performance in females. Psycological Sciences, 21: 1069-1071.

Zingeser M.R., and Phoenix C.H. (1978) Metric characteristics of the canine dental complex in prenatally androgenized female Rhesus monkeys (Macaca mulatta). American Journal of Physical Anthropology, 49: 187-192.

Correspondence

Doctor Daniela Cisoto Ribeiro
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
E-mail: daniela.ribeiro@adelaide.edu.au

6 Dental crown and arch size in Europeans and Australian Aboriginals

Atika Ashar,[1,2,3] Toby Hughes,[1,2,3] Helen James,[1] John Kaidonis,[1,2,3] Fadhli Khamis[4] and Grant Townsend[1,2,3]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development
[4]Craniofacial Sciences and Oral Biology Unit, School of Dental Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia

Abstract

Various methods have been used to measure human dental crown and arch size as a means of examining population affinities and differences. Traditionally, this has been done using hand-held calipers, however, new technology such as two dimensional and three dimensional imaging now provide alternatives for researchers. Here, we report the findings of a study to validate two new imaging techniques (2D and 3D) and to quantify differences in the dentitions of several human populations for whom dental records are available. 2D photographic imaging using a digital SLR (Canon Inc, Tokyo, Japan), and 3D laser scanning with an Optix 400S 3D laser scanner (3D DigitalCorp, Connecticut, USA) were utilized. Measurements of dental crown variables, including mesiodistal and buccolingual dimensions and interarch widths, were explored statistically. Data extracted using both 2D and 3D techniques were compared to assess the precision and accuracy of the two methods. Both 2D and 3D techniques displayed high levels of precision and accuracy, and highlighted statistically significant differences in dental crown size and arch size within and between the study populations. The methods developed offer considerable promise for the field of forensic odontology, including distinguishing individuals within populations on the basis of their dentitions.

Introduction

Many dental anthropologists have focused on non-metric traits to characterise major population groups (Hanihara, 1967; Scott and Turner, 1997), and various 'dental complexes' have been identified, including Mongoloid, Caucasoid, Negroid and Australoid (Turner, 1990; Townsend et al., 1990; Mayhall et al., 1999). In conjunction with non-metric traits, there have also been numerous metric studies of the dentitions of human populations (Hanihara, 1998; Falk and Corruccini, 1982; Kieser, 1991; Hanihara and Ishida, 2005). Based on these studies, populations have been grouped as microdontic, mesodontic, and megadontic (Hanihara and Ishida, 2005). Some researchers have claimed that non-metric qualitative characteristics are more useful than continuously variable quantitative characteristics in grouping people according to their geographic location and affinities (Lasker and Lee, 1957; Hanihara, 2008). Measurements of dental crown size and arch size, however, provide greater objectivity than scoring of traits. A combination of metric and non-metric features is likely to provide the most comprehensive and discriminatory description of human dentitions.

This study forms part of a larger investigation aimed at using a combination of metric and non-metric dental crown features, as well as arch size and shape, to develop a probabilistic model to distinguish individuals from specific human populations, particularly for forensic purposes.

Data acquisition

Traditionally, measurements of arch and tooth size have been obtained using hand-held calipers. However, advances in technology now allow researchers to use alternative approaches. Two dimensional (2D) imaging uses a camera to capture standardized images of natural teeth or dental casts. Measurements are then carried out using computer software. Care is needed to ensure that all images of dental casts obtained using this technique are appropriately standardised to allow measurements to be compared.

Three dimensional (3D) imaging is a more advanced technique for obtaining measurements from teeth and dental arches. Although the technology was developed many years ago, and has been applied in other fields, the use of 3D imaging in dental research and practice is a relatively recent innovation.

The focus of this paper is the validation of 2D and 3D imaging systems in measurement of selected dental crown and arch dimensions in several human populations for whom

	Total	Male	Female	Age (± SD)
European	35	18	17	14.8 ± 1.3
Australian Aboriginal	35	20	15	16.8 ± 3.7
Malaysian Malay	35	18	17	16.2 ± 0.8

Table 1 Demographic data of study samples.

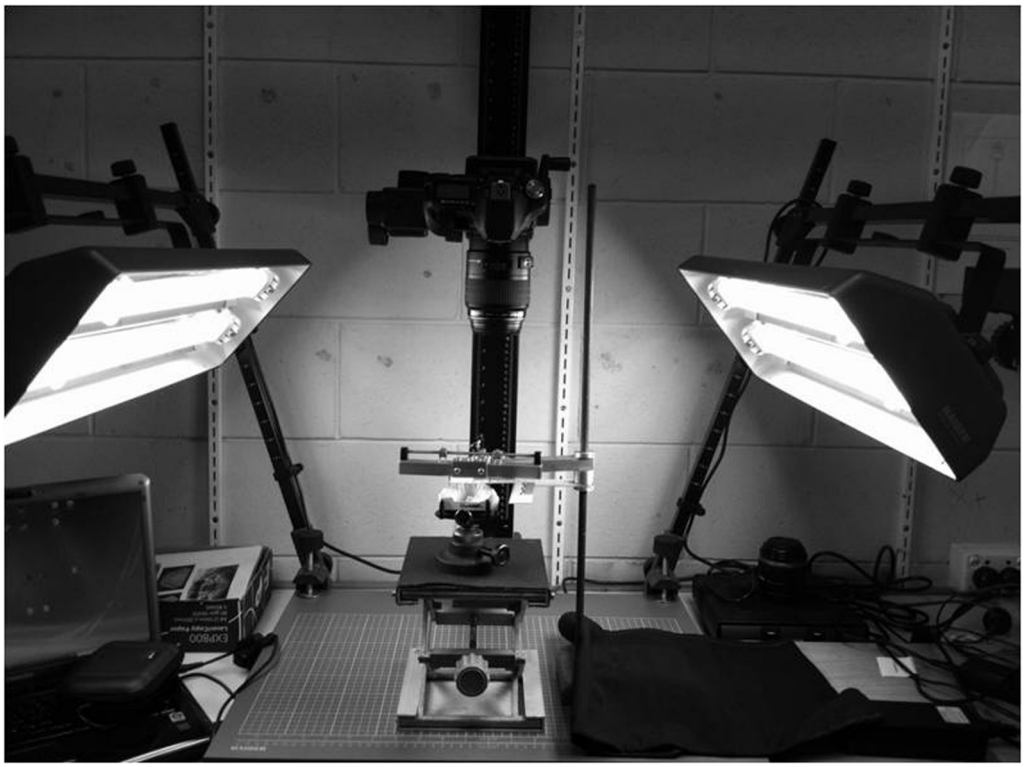


Figure 1. 2D photographic setting.

good quality dental casts were available, including Europeans, Australian Aboriginals and Malaysians.

Materials and methods

This study forms part of an ongoing investigation of dental development and morphology in twins and different ethnic groups being carried out by the Craniofacial Biology Research Group at the University of Adelaide. These studies have been approved by the Human Research Ethics Committee, University of Adelaide (Project No: H-07-1984A) 'Dentofacial variation in twins: genetic and environmental determinants' and (Project No: H-09-2-2002) 'Dental variation in Malaysian populations with application to human identification'.

Population

The study samples consisted of three previously collected adult cohorts (Table 1), including a cohort of Australian twins (European descent), a cohort of Australian Aborigines, and a mixed cohort of Malaysians, with approximately equal numbers of males and females. Cohorts were selected as representative of three different dental complexes ('Caucasoid', 'Australoid' and 'Mongoloid') and as three distinct groups according to tooth size (microdontic, mesodontic and megadontic).

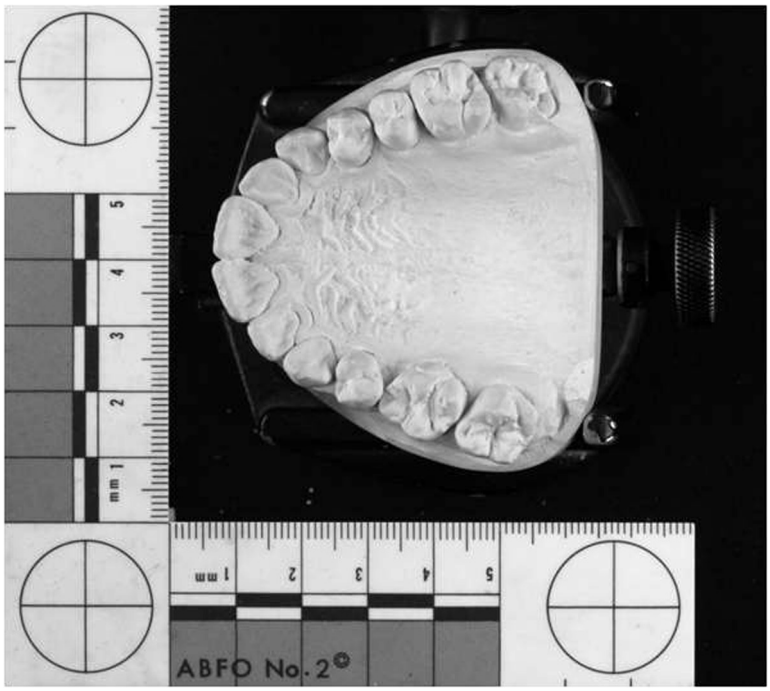


Figure 2. Final image of dental cast use for measurement.

Dental casts were obtained previously from participants using alginate impressions that were poured up with high quality dental stone. Sample sizes of 35 were established for each group by randomly selecting casts from larger collections (Table 1). Only analyses for the permanent upper right dentition are provided in this report.

Equipment

2D Imaging

Images of all dental casts were obtained using a digital SLR (Single Reflex Lens) camera which provides superior quality images over compact cameras. Unlike compact cameras, SLR cameras enable users to have total control over various camera settings at the highest quality, thus assisting in standardizing photographs as proposed by Ahmad (2009).

The 2D imaging system was adapted from the system described by Brook et al. (1999) (Figure 1). The system comprised a Canon EOS D50 digital camera with 60 mm macro lens (Canon Inc, Tokyo), laptop and copy stand (Kaiser, Odenwald). Other apparatus included a dental cast articulator to standardize dental cast position relative to the camera. The camera was connected directly to a personal computer and all images were taken by controlling the camera remotely. All images were captured using Canon EOS digital utility software and stored in RAW and JPEG format. The details of the camera setting were as follows: F-setting: F22, Exposure time: 1/20, ISO speed: 150, Focal length: 60 mm, Temperature: 4200K (Figure 1).

One occlusal view photograph was obtained of each dental cast for subsequent analysis, using a standard reference plane fixed parallel to the lens, consistently oriented using a purpose-built spirit level. The plane was defined by three reference points, including each of the central fossae of the upper first molars and the centre of the incisal edge of the left central incisor.

An American Board of Forensic Odontology (ABFO) ruler No.2 (Lightning Powder Co Inc., Oregon, USA) used in forensic photography was utilized as the reference scale. This ruler incorporates three circles that are useful in identifying and compensating for distortion resulting from oblique camera angles. Measurements within the image are then made relative to the inscribed 1cm grid lines to compensate for distortion resulting from non-parallelism between the film and object planes. The ruler was constructed from 'L' shaped laminated plastic 1mm thick. The mm markings are accurate to 0.1mm on inner edges (Figure 2).

This ruler was positioned parallel to the camera lens and at the same level as the reference plane. The following criteria were used to ensure good quality images of the dental casts: ruler and dental cast were in focus; all three circles were visible; there were no signs of over or underexposure.

3D Imaging

The Optix 400S (Figure 3) is a non-contact laser scanner. There are several varieties of 3D surface scanner available on the market, with choice depending on the application required. The scanner was purchased jointly by the Forensic Odontology Unit, Craniofacial Biology Research Group and Orthodontic Unit at the University of Adelaide. This scanner was chosen for its wide application, being suitable for scanning individual teeth, dental casts and skulls (100-200 mm range). The Optix 400S scans according to a triangulation principle. The laser

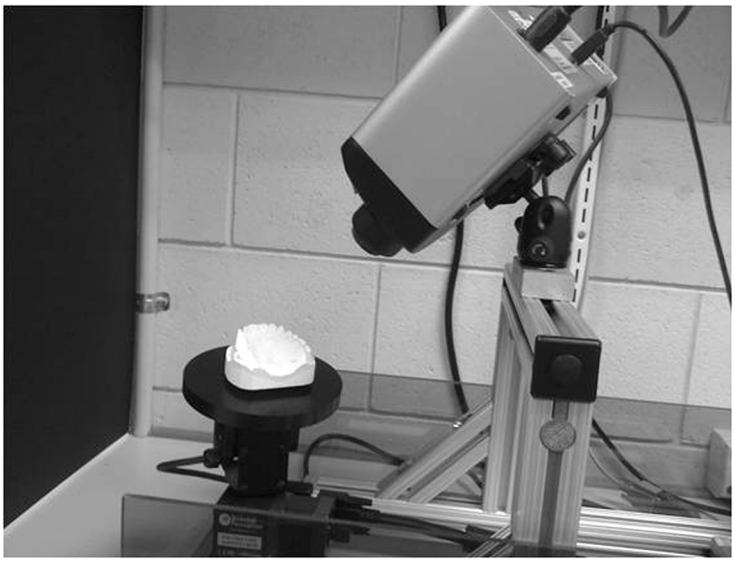


Figure 3. Optix 400S laser scanner with a rotary table.

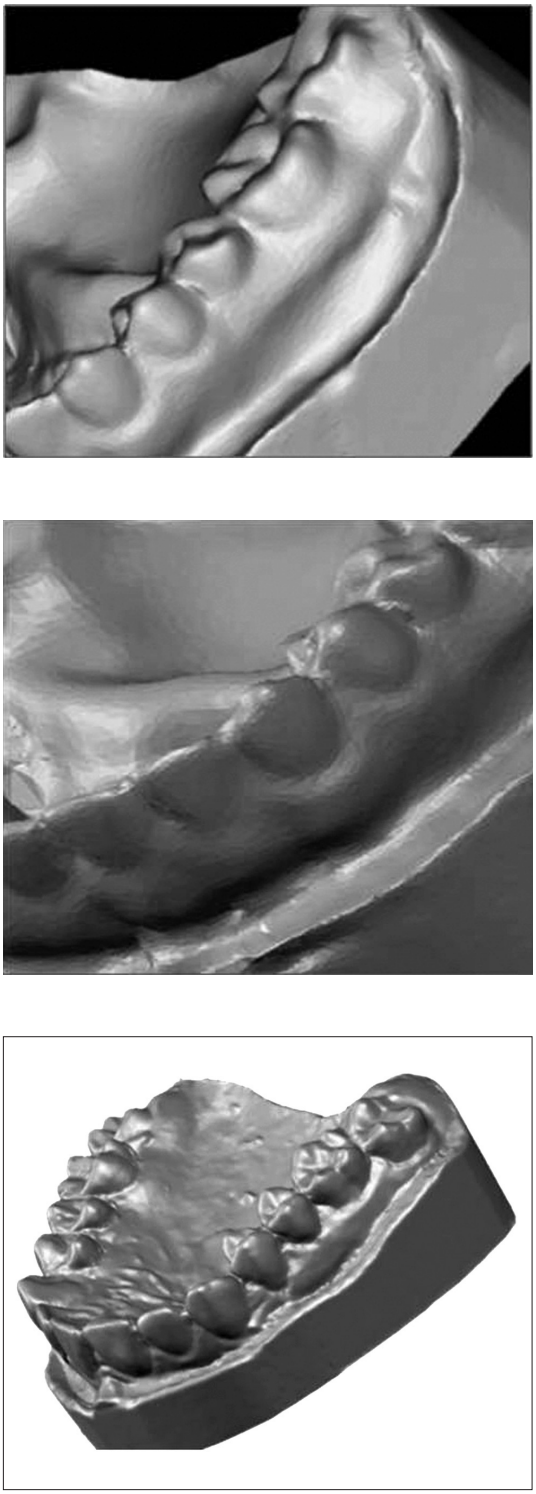


Figure 4. Dental casts scanned at a range of resolutions (A Low (top), B Medium (middle), C High (bottom)).

sweeps across the object to obtain 'point clouds' representing x, y and z coordinates. The scanner is controlled using a Windows XP based personal computer. Because the scanner can only pick up surface information within its field of view, several scans are required to obtain a complete image of a dental cast due to overhangs/ shadowed areas (Figure 3).

Various scanner settings can be controlled to ensure high quality. The best image captured at highest resolution requires more time (up to 2 hours) compared with an image captured at low resolution (up to 10 minutes). Figure 4 illustrates a range of scanned images resulting from different settings (Figure 4 A, B and C).

For our purposes, dental casts were scanned at the highest resolution. Twelve scans per cast (30 degree rotations using an automated rotary table) were obtained. Dedicated software, Slim 3D (3D-Shape, GmbH, Erlangen, Germany) provided with the 3D scanner was used to control the scanning process, for cleaning and registration, aligning and merging of the separate images. The resulting composite images were stored as both pmh and stl files.

Landmarks and measurements

The choice of appropriate landmarks is an important consideration in any quantitative morphological analysis. Amongst the many issues to consider are accuracy, reproducibility, efficacy and effectiveness. Quimby et al. (2004) defined the first two attributes (efficacy and effectiveness) as the ability of a procedure to give a favourable outcome under ideal and normal conditions, respectively. Landmarks were

chosen with these principles in mind. Landmarks were selected based on previous works and earlier practice performed using calipers to determine the most repeatable landmarks.

The following measurements were obtained using both 2D and 3D approaches.

1. Interarch width: measurement between corresponding teeth in left and right quadrants
    - distance between mesiobuccal cusp tips of permanent upper molars
    - distance between cusp tips of canines
2. Dental crown size:
    - Mesiodistal crown dimension, defined by the maximum point on the mesial and distal surfaces of the dental crown viewed occlusally
    - Buccolingual dimension, defined by the maximum buccal and lingual point of the tooth viewed occlusally

Measurements on 2D images were carried out using ImageJ software (ImageJ, National Institutes of Health, Maryland, USA). Measurements on 3D images were carried out using Rapidform software (INUS technology Inc. & Rapidform Inc., Seoul, Korea). For 3D images, the dental cast images were positioned on-screen to mimic the standardisation process used in 2D imaging. All measurements obtained from the 3D images were recorded as direct linear distances between landmarks rather than following the contours of the crown shapes. Whilst this approach limits the potential of 3D imaging, it was used to enable comparable analyses between methodologies.

Errors of the method

All measurements were obtained twice by the same author on two separate occasions. Only teeth on the right quadrant were measured for assessment. These were carried out for both 2D and 3D images. This was done to assess intra-operator reliability.

Statistical analysis

Data for all study variables were assessed for normality. Basic descriptive statistics were calculated for all variables. Comparisons between repeated measures and between methodologies were based on paired t-tests (systematic error) and Dahlberg statistics (random error). The level of statistical significance (α) was set at $p<0.05$ for all statistical tests. All analyses were performed using SPSS Version 19 (SPSS Inc., 2011).

Results

Normality testing showed that all data followed a normal distribution. Therefore, all data could be adequately described using means and standard deviations.

Error study

Tables 2 and 3 provide descriptions for the overall measurement errors. The variables that displayed systematic errors were interarch width for both intermolar and intercanine and also

mesiodistal crown size of central and lateral incisors for 2D and mesiodistal crown size of 11 and 12 for 3D. The basis of error study for both 2D and 3D were the same. These results indicate relatively high intra-operator reproducibility in measuring the variables using both methods.

Tables 4 and 5 provide descriptive statistics for dental crown size of upper teeth in the three study populations using the 2D and 3D methods. Table 6 provides descriptive statistics for interarch widths.

Australian Aboriginals showed the largest dental crown size in both mesiodistal and buccolingual dimensions using either technique. Comparisons between sexes demonstrated that males generally had larger crown size compared to females in each population. Large dental crown size was associated with interarch width, with Australian Aboriginals displaying the largest intermolar and intercanine arch values.

In order to compare the two methods, paired t-tests were used to look for systematic differences between measurements of mesiodistal and buccolingual crown size, and between interarch widths in all sample. The results are presented in Table 7. Measurements that showed significant differences are highlighted in bold. These include the mesiodistal diameter of tooth 11 and 21, and intermolar width. The findings confirmed that both systems provided comparable results under the standardised conditions used.

Tooth	n	Mean differences (mm)	Dahlberg Statistics	t-value	P-value
Mesiodistal (MD)					
11	35	0.25	0.14	1.73	0.04
12	35	0.29	0.10	2.28	0.01
13	35	0.10	0.08	0.93	0.64
14	35	0.09	0.09	1.93	0.20
15	35	0.00	0.06	0.11	0.45
16	35	0.04	0.13	1.22	0.36
Buccolingual (BL)					
11	35	0.03	0.07	0.89	0.64
12	35	0.04	0.07	0.33	0.16
13	35	0.03	0.08	0.44	0.19
14	35	0.01	0.05	0.24	0.73
15	35	0.01	0.06	1.50	0.06
16	35	0.10	0.07	0.56	0.56
Interarch width					
Intermolar	35	0.61	0.66	1.73	0.00
Intercanine	35	0.36	0.61	4.28	0.00

* Tooth is described in FDI notation

Table 2. Results for double determination of 2D system.

Tooth	n	Mean differences (mm)	Dahlberg Statistics	t-value	P-value
Mesiodistal (MD)					
11	35	0.16	0.20	2.12	0.01
12	35	0.12	0.13	1.59	0.02
13	35	0.06	0.43	0.40	0.34
14	35	0.00	0.43	2.24	0.91
15	35	0.01	0.13	2.70	0.66
16	35	0.10	0.21	1.36	0.21
Buccolingual (BL)					
11	35	0.03	0.18	2.31	0.69
12	35	0.01	0.12	0.84	0.63
13	35	0.05	0.35	0.44	0.49
14	35	0.00	0.42	1.85	0.21
15	35	0.10	0.07	1.46	0.95
16	35	0.20	0.07	1.82	0.70
Interarch width					
Intermolar	35	0.10	0.66	2.93	0.06
Intercanine	35	0.17	0.61	3.98	0.05

* Tooth is described in FDI notation

Table 3. Results for double determination of 3D system.

Discussion

Maximum mesiodistal and buccolingual crown measurements have traditionally been used to compare human populations, and to make inferences about migratory patterns and population affinities. Findings from this study confirm that Australian Aboriginals have the largest arch widths when compared with Europeans and Malaysians. Measurements obtained with calipers in a previous study of a Malaysian sample showed similar values for the mean mesiodistal and buccolingual crown size to our present study, confirming the validity of the methods adopted (Khamis et al., 2007). Differences in the mean dental crown size using three different methods ranged between 0.01-0.50 mm. Comparisons of direct and indirect mesiodistal measurements of upper permanent molars obtained from the Australian Aboriginal group also show promising results. For example, mean differences of dental crown size using 2D and 3D in comparison to calipers (from previous study) yielded values ranging from 0.01 to 0.55 mm (Brown et al., 1980).

Both of the methods employed (2D and 3D) highlighted the diversity between the three study population groups; European, Australian Aboriginal and Malaysian.

Smith et al. (2009) carried out a study using the same type of laser scanner that we used and confirmed that the 3D image system was as reliable and accurate as an established 2D system for measuring teeth using dental models. Measurements of metric variables, including

Tooth	2D									3D								
	Europeans			Australian Aboriginals			Malaysians			Europeans			Australian Aboriginals			Malaysians		
Males	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
11	18	8.7	0.47	20	**9.1**	0.55	18	8.4	0.61	18	8.5	0.61	20	**9.3**	0.41	18	8.2	0.67
12	18	7.0	0.67	20	**7.5**	0.6	18	7.1	0.63	18	7.2	0.72	20	**7.7**	0.51	18	7.4	0.44
13	18	7.8	0.39	20	**8.1**	0.54	18	8.0	0.52	18	7.8	0.57	20	**8.1**	0.63	18	**8.1**	0.35
14	18	6.9	0.37	20	**7.4**	0.46	18	7.3	0.50	18	6.7	0.38	20	**7.4**	0.47	18	6.9	0.47
15	18	6.7	0.33	20	6.9	0.50	18	**7.2**	0.06	18	6.6	0.30	20	**7.0**	0.40	18	6.8	0.27
16	18	10.4	0.53	20	**11.0**	0.49	18	10.6	0.55	18	10.3	0.67	20	**11.1**	0.55	18	10.6	0.2
Females	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
11	17	8.4	0.55	15	**8.8**	0.54	17	8.5	0.62	17	8.5	0.61	15	**9.0**	0.65	17	8.7	0.47
12	17	6.7	0.62	15	7.0	0.76	17	**7.4**	0.68	17	6.9	0.53	15	7.1	0.72	17	**7.6**	0.62
13	17	7.4	0.45	15	**7.8**	0.50	17	7.7	0.56	17	7.5	0.38	15	**8.0**	0.61	17	7.5	0.50
14	17	6.7	0.40	15	**7.2**	0.47	17	**7.2**	0.47	17	6.8	0.33	15	**7.4**	0.55	17	6.8	0.1
15	17	6.6	0.42	15	6.8	0.44	17	**7.1**	0.89	17	6.7	0.42	15	**7.0**	0.41	17	6.8	0.95
16	17	10.1	0.50	15	**10.7**	0.53	17	10.5	0.57	17	10.0	0.64	15	**10.9**	0.56	17	10.5	0.3

*Tooth is described in FDI notation
a. shaded values indicated the largest mesiodistal crown diameters in comparison to other group

Table 4. Descriptive statistics for dental crown size in three populations using 2D and 3D systems – mesiodistal crown diameters (in mm).

Tooth	2D									3D								
	Europeans			Australian Aboriginals			Malaysians			Europeans			Australian Aboriginals			Malaysians		
Males	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
11	18	8.4	1.05	20	**9.0**	0.70	18	**9.0**	0.99	18	8.3	1.12	20	**8.9**	0.77	18	8.6	1.19
12	18	7.1	1.04	20	**7.6**	0.89	18	**7.6**	0.81	18	7.2	1.06	20	**7.8**	0.9	18	7.7	1.20
13	18	8.0	1.04	20	**9.0**	0.66	18	8.1	0.72	18	7.9	0.85	20	**9.0**	0.58	18	8.2	0.62
14	18	9.4	0.47	20	**10.1**	0.60	18	9.8	0.55	18	9.1	0.51	20	**10.3**	0.65	18	9.8	0.67
15	18	9.6	0.49	20	**10.3**	0.68	18	9.6	0.63	18	9.2	0.40	20	**10.3**	0.55	18	9.3	0.57
16	18	11.7	0.46	20	**12.4**	0.6	18	11.6	0.62	18	11.7	0.60	20	**12.5**	0.65	18	11.5	0.71
Females	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
11	17	7.8	0.82	15	8.5	0.73	17	**8.7**	0.89	17	7.7	0.62	15	8.6	0.60	17	**8.9**	0.83
12	17	6.7	0.83	15	7.3	0.86	17	**7.4**	0.77	17	6.8	0.61	15	7.2	0.69	17	**7.6**	0.35
13	17	7.5	0.77	15	**8.3**	0.63	17	7.8	0.68	17	7.6	0.77	15	**8.2**	0.56	17	7.7	0.56
14	17	9.1	0.47	15	**9.8**	0.54	17	9.5	0.70	17	9.1	0.39	15	**9.9**	0.56	17	9.4	0.37
15	17	9.3	0.48	15	**9.9**	0.57	17	9.4	0.53	17	9.5	0.55	15	**10.0**	0.40	17	9.5	0.33
16	17	11.2	0.55	15	**11.8**	0.56	17	11.3	0.55	17	11.3	0.81	15	**11.8**	0.69	17	11.4	0.49

*Tooth is described in FDI notation
a. shaded values indicated the largest buccolingual crown diameters in comparison to other group

Table 5. Descriptive statistic for dental crown size in three populations using 2D and 3D systems – buccolingual crown diameters (in mm).

Tooth	2D									3D								
	Europeans			Australian Aboriginals			Malaysians			Europeans			Australian Aboriginals			Malaysians		
Males	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Intermolar	18	52.2	3.02	20	**57.0**	2.41	18	54.9	2.78	18	52.0	3.72	20	**57.4**	2.51	18	54.8	2.81
Intercanine	18	34.2	1.78	20	**38.5**	2.33	18	36.1	2.21	18	34.1	1.85	20	**38.5**	2.54	18	36.0	1.47
Females	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Intermolar	17	50.0	2.83	15	**54.3**	2.25	17	52.9	3.24	17	55.3	1.25	15	**54.7**	2.81	17	52.7	0.47
Intercanine	17	32.9	1.82	15	**37.4**	2.10	17	34.1	2.34	17	32.8	1.96	15	**37.9**	2.28	17	34.2	2.42

*Tooth is described in FDI notation
a. shaded values indicated the largest dental arch size in comparison to other group

Table 6. Descriptive statistics for dental arch size in three populations using 2D and 3D systems (in mm).

		2D		3D		2D v 3D
	Tooth	Mean	SD	Mean	SD	Paired t-test
MD	11	8.7	0.59	8.8	0.60	**0.00**
	12	7.1	0.67	7.1	0.69	**0.01**
	13	7.8	0.58	7.7	0.59	0.61
	14	7.1	0.54	7.0	0.53	0.09
	15	6.7	0.45	6.7	0.44	0.44
	16	10.6	0.53	10.6	0.62	0.53
BL	11	8.7	0.92	8.7	0.92	0.70
	12	7.4	0.80	7.4	0.82	0.17
	13	7.9	1.05	7.8	1.06	0.04
	14	9.8	0.57	9.8	0.56	0.88
	15	9.9	0.60	9.8	0.57	0.09
	16	11.7	0.58	11.8	0.59	0.40
Interarch	Intermolar	52.3	3.72	52.6	3.53	**0.02**
	Intercanine	35.4	2.92	32.3	3.05	0.05

Significant values at the p<0.05 level are highlighted
*Tooth is described in FDI notation

Table 7. Comparison between 2D and 3D method using 35 samples.

mesiodistal and buccolingual dimensions and interarch width, performed on a 3D image have also been shown to be as accurate as those obtained directly on plaster models. Comparable findings of measurements obtained using either 3D methods or direct measurement on plaster models has also been reported previously (El-Zanaty et al., 2010).

The findings from this study support the ability to obtain dental crown measurements and dental arch size measurement using either 2D or 3D methods provided that the same landmarks and protocol are utilised. Comparisons of the two methods as presented here emphasise the value of 3D imaging in this area of research and, at the same time, give a sense of confidence for researchers to move from 2D to 3D. The main obstacles incurred with the 3D method involve identifying landmarks, just as in the case of 2D imaging or when using calipers. The use of a newly created jig to help in the standardization of all photographs of the dental casts should reduce the factor of subjectivity in orientating dental casts in our planned future studies. Sources of error will most likely originate from the problem of locating landmarks.

3D approaches enable images to be manipulated and sophisticated shape analyses to be performed that promise to provide a much greater insight into morphological variation within the dentition (Smith et al., 2009). Whilst the approach utilised in this study reduced the utility of 3D imaging, it was carried out with the aim of comparing the same protocols using different methods.

The overall patterns of dental crown and arch size observed between populations were similar to previous research, with Australian Aboriginals tending to be the largest in size, Malaysians found to be intermediate and Europeans tending to have the smallest teeth (Hanihara and Ishida, 2005). The current study revealed significant differences in dental

morphology between individuals of European, Australian Aboriginal and Malaysian ancestry. This was achieved with high levels of precision and accuracy using both 2D and 3D methods.

Conclusion

3D imaging has the potential to provide detailed data representing dental crown and arch size as well as shape differences. Both 2D and 3D techniques are comparable in terms of their accuracy and precision and both imaging systems have their advantages and disadvantages. These differences can only be highlighted if the same protocols are repeated in both methods. Depending on cost and time factors, researchers can decide the best available method to employ. 3D imaging has definite advantages for researchers interested in making detailed comparisons in shapes of teeth and dental arches within and between populations.

Acknowledgements

The authors would like to thank everyone involved in contributing the dental casts used for the research; these included twins and their families, as well as the Australian Aboriginal group and Malaysian groups. We would also like to thank Prof. Alan H. Brook and Dr Richard Smith for their advice and input on the methodology. The device use for standardization of dental casts using the 2D method was manufactured by a team at the School of Mechanical Engineering led by Dr Michael Riese, Mr Bill Finch and Ms Sarah Edwards. This research has been supported by The Australian Dental Research Foundation (ADRF), the NHMRC of Australia and Colgate Australia.

References

Ahmad I. (2009) Digital dental photography. Part 1: an overview. British Dental Journal, 206: 403-407.

Brook A.H., Smith R.N., Elcock C., Al-Sharood M.H., Shah A.A., and Karmo M. (1999) The measurement of tooth morphology: development and validation of a new image analysis system. In: Proceedings of the 11th International Symposium on Dental Morphology. Mayhall J.T., Heikkinen T. (Eds.) Oulu: Oulu University Press.

Brown T., Margetts B., and Townsend G.C. (1980) Comparison of mesiodistal crown diameters of the deciduous and permanent teeth in Australian Aboriginals. Australian Dental Journal, 25: 28-33.

El-Zanaty H.M., El-Beialy A.R., Abou El-Ezz A.M., Attia K.H., El-Bialy A.R., and Mostafa Y.A. (2010) Three-dimensional dental measurements: an alternative to plaster models. American Journal of Orthodontics and Dentofacial Orthopedics, 137: 259-265.

Falk D., and Corruccini R. (1982) Efficacy of cranial versus dental measurements for separating human populations. American Journal of Physical Anthropology, 57: 123–128.

Hanihara K. (1967) Racial characteristics in the human dentition. Journal of Dental Research, 46:923-926.

Hanihara T. (1998) Metric and nonmetric dental variations of the major human populations.

In: Human dental development, morphology and pathology. Lukacs J.R. (Ed) Oregon: University of Oregon.

Hanihara T., and Ishida H. (2005) Metric dental variation of major human population. American Journal of Physical Anthropology, 128: 287-298.

Hanihara T. (2008) Morphological variation of major human populations based on nonmetric dental traits. American Journal of Physical Anthropology, 136: 169-182.

Ireland A.J., McNamara C., Clover M.J., House K., Wenger N., Barbour M.E., Alemzadeh K., Zhang L., and Sandy J.R. (2008) 3D surface imaging in dentistry — what we are looking at. British Dental Journal, 205: 387-392.

Khamis M.F., Taylor J., Samsudin A.R., and Townsend G. (2007) Variation in dental crown size within and between populations living on the Malaysian Peninsular. International Journal of Dental Anthropology, 11:25-41.

Kieser J.A., and Groeneveld H.T. (1991) The reliability of human odontometric data. The Journal of the Dental Association of South Africa, 46:267-270.

Lasker G.W., and Lee M.M.C. (1957) Racial traits in the human teeth. Journal of Forensic Sciences, 2: 401-419.

Mayhall J.T. (1999) The dental complex: a morphological smokescreen or compass? In: Townsend G.C., Kieser J. (Eds). Perspective in Human Biology. Dento-facial Variation in Perspective, Centre for human biology, The University of Western Australia, Nedlands, pp 1-7.

Quimby M., Vig K., Rashid R., Firestone A., and Mayers M. (2004) The accuracy and reliability of measurements made on computer-based digital casts. The Angle Orthodontist, 74: 298-303.

Scott G.R., and Turner G.C. (1997) The anthropology of modern human teeth. Cambridge: Cambridge University Press.

Scholts S.B., Wärmländer S.K., Flores L.M., Miller K.W., and Walker P.L. (2010) Variation in the measurement of cranial volume and surface area using 3D laser scanning technology. Journal of Forensic Sciences, 55: 871-876.

Smith R., Zaitoun H., Coxon T., Karmo M., Gurpeet K., Townsend G., Harris E.F., and Brook A. (2009) Defining new dental phenotypes using 3-D image analysis to enhance discrimination and insights into biological processes. Archives of Oral Biology, 54: S118-S125.

Stringer C.B., and Andrews P. (1998) Genetic and fossil evidence for the origin of modern humans. Science, 239: 1263-1268.

Townsend G.C., Yamada H., and Smith P. (1990) Expression of the entoconulid (six cusp) on mandibular molar teeth of the Australian Aboriginal population. American Journal of Physical Anthropology, 82: 267-274.

Turner C.G. (1990) Major features of sundadonty and sinodonty, including suggestions about East Asian microevolution, population history and Late Pleistocene relationships with Australian Aboriginals. American Journal of Physical Anthropology, 82: 295-317.

Correspondence

Doctor Atika Ashar
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
Email: noratika@gmail.com

7 Sex determination from maxillary and mandibular canines of the Filipino population

R.J. Taduran

Department of Anthropology, University of the Philippines, Diliman, Quezon City, Philippines

Abstract

The aim of this study was to derive Filipino-specific formulae that can be used as supplementary tools for sex discrimination, especially in forensic cases. Three dimensions — clinical crown height (CCH), maximum mesiodistal breadth (MMD) and maximum buccolingual width (MBL) — of the maxillary and mandibular canines were measured in 100 male and 100 female Filipino participants. CCH emerged as the most significant variable in determining sex in maxillary canines while CCH and MMD were both statistically significant in mandibular canine sex determination. Tree models were derived and 39 data sets were analysed as a test of accuracy. The accuracy ratings for the maxillary and mandibular trees were 56.41% and 74.36%, respectively. The mandibular tree is recommended for use in cases with incomplete or fragmented human remains when no other skeletal elements yielding higher accuracy estimates are available. The results of this study contradict the previous claim of no significant dimorphism in the dentition of the Filipino population.

Introduction

Sexual dimorphism has always been an interesting issue in primate and human evolution and the canine teeth are some of the most studied and debated elements. Researchers (Almquist, 1974; Crook, 1972; Clutton-Brock and Harvey, 1977; Greenfield, 1992; Guatelli-Steinberg, 2009; Harvey et al., 1978; Kay et al., 1988; Leutenegger, 1982; Leutenegger and Cheverud, 1985; Leutenegger and Kelly, 1977; Lucas et al., 1986; Oxnard et al., 1985; Plavcan, 2001,

2004; Plavcan and Ruff, 2008; Plavcan and van Schaik, 1992, 1994; Royer et al., 2009) have already shown that canine teeth exhibit sexual dimorphism and are one of the most obvious secondary sex differences among non-human primates. As teeth are the most well-preserved part of the skeleton because of their durability, withstanding both bacterial decomposition and high temperatures, they are frequently found in the fossil record, and the canine is arguably one of the most valuable and practical elements used in sexing individuals (Kelley, 1995).

As a result, research has focused on using the size of the canine tooth as a means of identifying sex of individual fossil specimens. Male primates generally have larger canines than females (Plavcan, 2004): male canines differ specifically in length, and may have up to 400% greater crown length (Plavcan and Ruff, 2008) than those of female primates (Greenfield, 1992).

Sex differences between lengths and breadths of both upper and lower canines were also seen in a study by Oxnard et al. (1985), with male canines significantly larger than those of females. In this study, the researchers compared these differences among four different genera (Gorilla, Homo, Pan and Pongo) and found that the measures have a different pattern of distribution in each genus. Pongo and Homo had similar dispersions while Pan and Gorilla had different dispersions in males and females. Gorilla and Pongo had larger differences in the mean dimensions of male and female canines than Homo and Pan. These results show that there are quantitatively different patterns in canine size between males and females in each genus.

Although research provides evidence of canine sexual dimorphism in extant anthropoid primates, limited or insignificant size dimorphism in humans has been reported (Schwartz and Dean, 2001). Schwartz and Dean (2001) provided an explanation for this result, indicating that no differences in the rate or duration of canine crown growth are exhibited by modern humans. Therefore, canine sexual dimorphism in humans is not as obvious as in extant hominoids. Yet, the same researchers, Schwartz and Dean (2005), state that, on average, males have larger tooth crowns compared to females, with varying degrees of dimorphism present within different populations. As shown by the analysis of odontometric data in their study, Moss and Moss-Salentijn (1977) found that canine teeth express sexual dimorphism. In addition, research conducted by Tompkins (1996) has shown that human canines, both mandibular and maxillary, exhibit patterns of between-sex differences.

Region-specific studies of canine sexual dimorphism have also been conducted but have produced mixed results. Isçan and Kedici (2003) studied Turks and showed that canines could correctly determine sex with an accuracy of 77%. Prathibha Rani et al. (2009) also found sexual dimorphism in bucco-lingual measurements of the canines of people from Mysore, India. However, Boaz and Gupta (2009) found no significant dimorphism in the South Indian population.

Dental morphology of the Filipino people has been the subject of only two published journal articles. Cruz (1971) tried to provide a dental profile of the Filipino population based on a study of 58 subjects. Potter et al. (1981) found no significant differences between males and females, but their study was limited to measurements of only two crown dimensions.

The present study is the first to focus specifically on the canine and the first conducted on the Filipino population in almost three decades.

Aim and objectives

Sex determination is one of the essential aspects of forensic anthropology. Teeth are very resilient, even in decomposing or burned bodies, and remain a useful tool in human identification. The aim of this investigation is to derive Filipino-specific formulae that can be used as supplementary tools for sex discrimination, especially in forensic cases.

The objectives of this study are to:

1. Identify underlying patterns of sexual dimorphism in the different dimensions of the permanent maxillary and mandibular canines of the Filipino population;
2. Describe dimensional characteristics of the permanent maxillary and mandibular canines of the Filipino population; and
3. Study the potential of the derived multivariate formulae from the three canine dimensions in sex determination of the Filipino population.

Materials and methods

All measurements were made in the Restorative Dentistry Clinic of the College of Dentistry, University of the Philippines, from 13 July to 2 August 2010. Data were recorded on an Excel spreadsheet and statistically analyzed using the R Statistical Software (R Development Core Team, 2010). One hundred male and 100 female participants' canines were measured directly with a customized sliding calliper. Blunt-tipped extensions were attached following the design of Hillson (2005) for more accurate measurements of the dental dimensions. To avoid interobserver errors, only the researcher made the measurements using the calliper with 0.1mm precision.

Significant inclusion criteria for a participant were:

1. Adult, 18 years old or above;
2. No diagnosed dental or periodontal disease;
3. No worn tips at the cervical thirds of the maxillary and mandibular canines;
4. No cultural or cosmetic modification of the teeth;
5. No restoration at the cervical thirds of the maxillary and mandibular canines; and
6. No restorations at the mesial or distal surface of the maxillary and mandibular canines.

The three different measurements were defined as follows:

1. Clinical crown height — the portion of enamel visibly present in the oral cavity (Mosby's Medical Dictionary, 2005).
2. Maximum mesiodistal diameter — the distance between two parallel planes,

tangential to the most mesial and most distal points of the crown side (Hillson, 2005).

3. Maximum buccolingual breadth — the maximum distance between two parallel planes, one tangential to the most lingual/palatal point of the crown side, and the other tangential to a point on the buccal/labial crown side (Hillson 2005).

Participants were recruited from the roster of students, faculty and staff of the University of the Philippines and walk-in clients of the College of Dentistry. The researcher wore medical gloves, goggles, a mask and a clean laboratory coat to maintain an antiseptic condition when carrying out the procedures. The calliper was sterilized with alcohol and sterilized cotton before and after every measurement.

To compare canine dimensions' means between sexes and sides, Student's t-tests were conducted and Pearsons correlation coefficient was applied to test the degree of their relationship with each other. The Step package was employed to analyse the variables and their interactions with each other thus, simplifying the model. The General Linear Model (GLM) package was utilised to determine which of the variables taken were significant in sex discrimination. The Tree Model was then used to derive a formula by producing a structured 'tree.' Thirty nine data sets were analysed using the derived trees to serve as a test.

Results

Descriptive statistics (mean, median and standard deviation) was employed to explain the dimensional characteristics of the canine teeth. Table 1 shows the basic statistics of all three measured dimensions of maxillary and mandibular canines.

Sexual dimorphism

Student's t-test was utilised to determine if means of the sexes were significantly different. With p values for all dimensions of both maxillary and mandibular canines appreciably below the 0.05 significance level, these results indicate that all means were different (Table 1).

Canine dimension	Mean		Median		Standard Deviation		T-test
	M	F	M	F	M	F	(p value)
Maxillary							
CCH	10.21	9.457	10.17	9.370	1.088	0.869	2.467e-13
MMD	8.269	8.016	8.300	8.015	0.449	0.491	1.651e-07
MBL	8.457	8.112	8.400	8.080	0.600	0.530	3.581e-09
Mandibular							
CCH	9.994	9.195	9.940	9.240	1.043	0.905	7.887e-15
MMD	7.279	6.900	7.310	6.850	0.427	0.441	2.2e-16
MBL	7.726	7.400	7.770	7.430	0.596	0.483	7.186e-09

Table 1. Descriptive statistics and t-test of the Filipino canines.

Canine dimension	Male	Female	Both
Maxillary			
CCH	0.9815	0.3668	0.5575
MMD	0.9945	0.7132	0.7478
MBL	0.479	0.3936	0.3213
Maxillary			
CCH	0.2489	0.4239	0.1876
MMD	0.3260	0.2288	0.1549
MBL	0.5808	0.3826	0.3471

Table 2. Student's t-test for left-right differences of the Filipino population canines.

Left-right differences

Student's t-test was also employed to determine any left-right differences on the canine dimensions. Table 2 presents all p values greater than 0.05, which suggests that the means of any canine dimension of opposing sides do not differ.

Correlations

Pearson's correlation was utilised to determine the relationship of canine dimensions with each other. Table 3 shows the positive correlations of all dimensions in both sexes. With p values of all relationships in both sexes less than 0.05, these results indicate that correlations were all highly significant.

Canine dimensions	Coefficient (r)		Significance (p value)	
	Male	Female	Male	Female
Maxillary				
CCH:MMD	0.2598411	0.2400981	0.0002534	0.0006567
MMD:MBL	0.5218916	0.5714576	5.995e-15	2.2e-16
MBL:CCH	0.3525301	0.4127322	4.625e-07	1.518e-09
Mandibular				
CCH:MMD	0.3472202	0.3785412	6.59e-07	4.857e-08
MMD:MBL	0.4168913	0.4927104	1.341e-09	2.542e-13
MBL:CCH	0.4100638	0.5063088	2.628e-09	4.308e-14

Table 3. Correlations table of the Filipino population canines.

Simplified models

In the Step function, R goes through a sequence of dropping the least influential variable (or interaction) and reanalysing until it arrives at the simplest model, for which it gives the formula. For the maxillary data, this simplified to:

Sex = CCH + MMD + MBL

This means that the three variables were influential enough to remain in the model, and interactions between any of these variables were not significant. Meanwhile, the resulting simplified formula for mandibular canines was:

Sex = CCH + MMD

The model simplification of the Step function indicates which variables make a significant impact on the response variable. In this case, the variables CCH and MMD were significant and their respective measurements can be very useful in determining sex.

Significant variables for sex determination

The GLM package came up with the statistical conclusion that clinical crown height (CCH) was the only significant variable in sex determination (Table 4). Thus, any tree model would be better if it were based only on this variable.

It is also shown in Table 4 that both CCH and MMD were significant in sex discrimination for the mandibular canines of the Filipino population, as determined by the GLM package of R. MBL was not included in the formula because the step function indicated that it was not significant.

Tree models

The maxillary tree (Figure 1) was constructed with the data recorded from 100 male and 100 female participants and it gave a CCH sectioning point of 9.515 mm. It was then tested on 39 separate data sets and 22 correct sex predictions, were obtained giving a 56.41% accuracy rating.

Maxillary	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-14.3024	2.2310	-6.411	1.45e-10 ***
CCH	0.6338	0.1261	5.026	5.01e-07 ***
MMD	0.5557	0.2876	1.932	0.0534
MBL	0.4268	0.2450	1.742	0.0815
Mandibular	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-16.1291	2.0483	-7.874	3.42e-15 ***
CCH	0.5887	0.1288	4.572	4.83e-06 ***
MMD	1.4790	0.2856	5.178	2.24e-07 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

Table 4. Table of coefficients of the Filipino population canines.

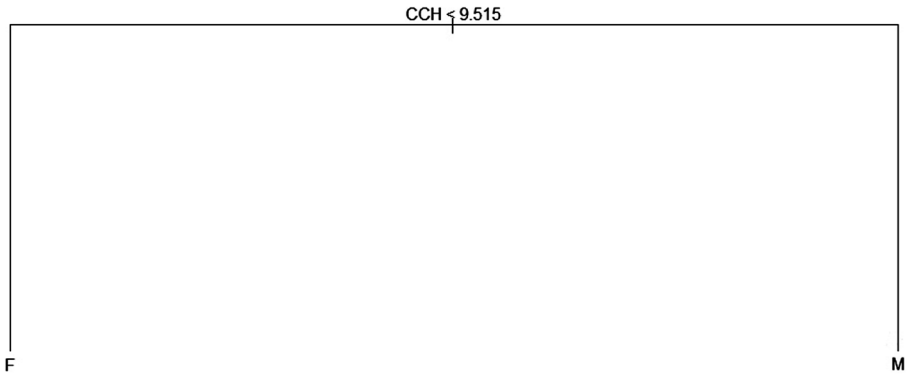


Figure 1. Tree model for the maxillary canines of the Filipino population.

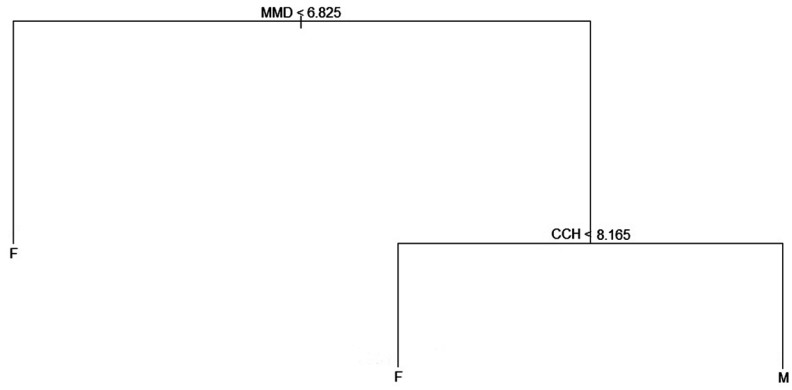


Figure 2. Tree model for the mandibular canines of the Filipino population.

The mandibular tree (Figure 2) had only two levels of binary partitioning. In this tree, the primary sectioning point was the MMD dimension with 6.825 mm. Any measurement less than or equal to that would point towards the tooth being from a female. A greater value would link to the next sectioning point, which was 8.165 mm for CCH. Measurements less than or equal to that would denote a female while greater than would imply a male.

The mandibular tree was tested on 39 data sets and obtained 29 correct sex predictions were obtained and yielding a 74.36% accuracy rating.

Discussion

This study found no significant differences in left and right dimensional measurements of both maxillary and mandibular, similar to the results of Vodanovic et al. (2007), Hashim and Murshid (1993) and specifically Potter et al. (1981) who saw 'no consistent trend for directional asymmetry' in the Filipino dentition.

However, for Iscan and Kedici (2003), sexual dimorphism was exhibited on the left maxillary canine of Turks, while Lund and Monstad (1999) reported dimorphism in the Swedish population on the right. Limiting measurements of teeth (or any other human body parts) for sex discrimination to sides could be detrimental in forensic case analysis for purposes of identification. Most forensic anthropology cases are characterised by incomplete and fragmented remains and a formula, even with 100% accuracy, is useless when the skeletal or dental part needed for examination is missing.

In this study, three dimensions of maxillary and mandibular canines from both sides of 200 participants were measured and included in data collection. Thus, each participant provided two data sets — one from the left and one from the right — which made the resulting formulae representative of the canines themselves and not their corresponding sides. Thus they could be applied in cases even with only one measurable canine available. Any left-right differences were statistically random and not related to side. Repeated measures on the individual's canines from different sides are independent and not interdependent hence, they are not replicates and the measurements cannot be called pseudoreplication (Hurlbert, 1984; Crawley, 2007).

It must be noted that this study found significant sexual dimorphism in all canine dimensions, both maxillary and mandibular, as shown by the results of the Student's t-test conducted. However, not all dimensions were relevant in sex determination—only CCH for maxillary canines and both CCH and MMD for mandibular emerged as significant.

This study did not only employ univariate statistics (Al Rifaiy et al., 1997; Boaz and Gupta, 2009; Potter et al., 1981; Prathibha Rani et al., 2009) of measurements as it is concerned with the three dimensions and their interactions, and which of them is most useful in predicting sex. Multivariate methods in sex determination based on teeth measurements have been explored by Ditch and Rose (1972) and Acharya and Mainali (2008) and accuracy levels range from 88.4 to 95.5% for the former and 62.3 to 83% for the latter. But the studies are multivariate not only in dimensions but also in terms of teeth — their formulae cannot be applied on human remains with incomplete or missing teeth. The present study, on the other hand, can be useful in cases even when only one canine in good condition is present.

The Tree Model is computationally intensive as it fits data using binary recursive partitioning (Crawley, 2007), which provides this research with a valuable method for sex discrimination. The tree method shows sectioning points, as presented in the tree models of maxillary canine (Figure 1) and mandibular canine (Figure 2) and it can also be user-friendly compared to the derived multivariate formulae on sex discrimination involving the teeth.

Accuracy rating for the maxillary tree was 56.41% and this result, due to the inclusion of two insignificant variables MMD and MBL in the simplified formula Sex = CCH + MMD + MBL, is so low that the value is close to 50% for each sex in sex determination. This tree is therefore not recommended to be used in human identification of any population at all.

The mandibular tree has an accuracy rating of 74.36% and can be used as a supplementary tool for sex discrimination in the Filipino population especially in cases with incomplete or fragmented human remains. It must be noted that the clinical crown height (CCH) is different from anatomical crown height — the former at times can no longer be measured in

fully decomposed or skeletonised remains. Canines have been reported to be recovered from human remains in aviation disasters and tropical cyclones (Kaushal, 2003) and this model is best applied in cases where the remaining canine's gingival line is still present, as well as in sea or land mass disasters, fire disasters and murder-mutilation (colloquially called 'chop-chop'), all of which are recurring unfortunate incidents in the Philippines. It is suggested that research on a univariate test using the MMD dimension that can be applied in cases with fully decomposed or skeletonised remains should be undertaken. It is also recommended that the mandibular tree be tested with a larger sample size.

Conclusions

Results show that there is significant sexual dimorphism in maxillary and mandibular canines of the Filipino population, contrasting with the reports of Yuen et al. (1997) and Boaz and Gupta (2009). Moreover, the findings of this study contradict Potter et al.'s (1981) previous claim of no significant dimorphism in the dentition of the Filipino population. No evidence of reverse dimorphism was formed where dimensional measurements are greater in females than in males, as reported in studies by Acharya and Mainali (2007) and Boaz and Gupta (2009). However, only the CCH dimension in maxillary canines and both CCH and MMD dimensions in mandibular canines emerged as significant variables in sex determination.

References

Acharya B.A., and Mainali S. (2007) Univariate sex dimorphism in the Nepalese dentition and the use of discriminant functions in gender assessment. Forensic Science International, 173: 47-56.

Acharya, B.A., and Mainali S. (2008) Sex discrimination potential of buccolingual and mesiodistal tooth dimensions. Journal of Forensic Science, 53:790-792.

Al Rifaiy M.Q., Abdullah M.A., Mashraf I., and Khan N. (1997) Dimorphism of mandibular and maxillary canine teeth in establishing sex identity. Saudi Dental Journal, 9: 17-20.

Almquist A.J. (1974) Sexual differences in the anterior dentition in African primates. American Journal of Physical Anthropology, 40: 359-368.

Boaz K., and Gupta C. (2009) Dimorphism in human maxillary and mandibular canine in establishment of gender. Journal of Forensic Dental Sciences, 1: 42-44.

Clutton-Brock T.H., and Harvey P.H. (1977) Primate and social organization. Journal of Zoology, 183: 1-39.

Crawley M. (2007) The R Book. Wiley & Sons: Chichester, United Kingdom.

Crook J.H. (1972) Sexual selection, dimorphism, and social organization in the primates. In: Campbell B (Ed.) Sexual selection and the descent of man 1871-1971. Chicago: Aldine, pp. 231-281.

Cruz L.C. (1971) A study of the mesiodistal crown diameters of permanent teeth among young Filipino adults. Journal of Philippine Dental Association, 23: 14-18.

Ditch L.E., and Rose J.C. (1972) A multivariate dental sexing technique. American Journal of Physical Anthropology, 37: 61-64.

Greenfield L.O. (1992) Origin of the human canine: a new solution to an old enigma. Yearbook of Physical Anthropology, 35: 153-185.

Guatelli-Steinberg D., Ferrell R.J., Spence J., Talabere T., Hubbard A., and Schmidt S. (2009) Sex differences in anthropoid mandibular canine lateral enamel formation. American Journal of Physical Anthropology, 140: 216-233.

Harvey P.H., Kavanagh M., and Clutton-Brock T.H. (1978) Sexual dimorphism in primate teeth. Journal of Zoology, 186: 474-485.

Hashim M.A., and Murshid Z.A. (1993) Mesiodistal tooth width in a Saudi population sample comparing right and left side: part 2. Egyptian Dental Journal, 39: 347-350.

Hillson S., FitzGerald C., and Flinn H. (2005) Alternative dental measurements: proposals and relationships with other measurements. American Journal of Physical Anthropology, 126: 413-426.

Hurlbert S.H. (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 54: 187-211.

Iscan Y.M., and Kedici S.P. (2003) Sexual variations in bucco-lingual dimensions in Turkish dentition. Forensic Science International, 137: 160-164.

Kaushal S., Patnaik V.V.G., and Agnihotri G. (2003) Mandibular canines in sex determination. Journal of Anatomical Society of India, 52: 119-124.

Kay R.F., Plavcan, J.M., Glander K.E., and Wright P.C. (1988) Sexual selection and canine dimorphism in New World monkeys. American Journal of Physical Anthropology, 77: 385-397.

Kelley J. (1995) Sexual dimorphism in canine shape among extant great apes. American Journal of Physical Anthropology, 96: 365-389.

Leutenegger W. (1982) Scaling and sexual dimorphism in body weight and canine size in primates. Folia Primatologica, 37: 163-176.

Leutenegger W., and Cheverud, J.M. (1985) Sexual dimorphism in primates: the effects of size. In: Jungers W.L., (Ed.) Size and scaling in primate biology. Plenum Press: New York pp. 33-50.

Leutenegger W., and Kelly J.T. (1977) Relationship of sexual dimorphism in canine size and body size to social, behavioral and ecological correlates in anthropoid primates. Primates, 18: 117-136.

Lucas P.W., Corlett R.T., and Luke D.A. (1986) Sexual dimorphism in tooth size in anthropoids. Human Evolution, 1: 23-39.

Lund H., and Monstad H. (1999) Gender determination by odontometrics in a Swedish population. Journal of Forensic Odontostomatology, 17: 30-34.

Mosby's Medical Dictionary. (2005) Mosby: St. Louis.

Moss M.L., and Moss-Salentijn L. (1977) Analysis of developmental processes possibly related to human dental sexual dimorphism in permanent and deciduous canines. American Journal of Physical Anthropology, 46: 407-414.

Oxnard C.E., Lieberman, S.S., and Gelvin, B.R. (1985) Sexual dimorphisms in dental dimensions of higher primates. American Journal of Primatology, 8: 127-152.

Plavcan J.M. (2001) Sexual dimorphism in primate evolution. Yearbook of Physical

Anthropology , 44: 25-53.
Plavcan J.M. (2004) Sexual selection, measures of sexual selection and sexual dimorphism in primates. In: Sexual selection in primates: new and comparative perspectives. Kappeler P.M., and van Schaik C.P. (Eds.) Cambridge University Press: Cambridge, pp. 230-252.
Plavcan J.M., and Ruff C.B. (2008) Canine size, shape, and bending strength in primates and carnivores. American Journal of Physical Anthropology, 136: 65-84.
Plavcan J.M., and van Schaik, C.P. (1992) Intrasexual competition and canine dimorphism in anthropoid primates. American Journal of Physical Anthropology, 87: 461-477.
Plavcan J.M., and van Schaik, C.P. (1994) Canine dimorphism. Evolutionary Anthropology, 2: 208-214.
Potter R.H.Y., Alcazaren A.B., Herbosa F.M., and Tomaneng J. (1981) Dimensional characteristics of the Filipino dentition. American Journal of Physical Anthropology, 55: 33-42.
Prathibha Rani R.M., Mahima V.G., and Patil K. (2009) Bucco-lingual dimension of teeth — an aid in sex determination. Journal of Forensic Dental Sciences, 2: 88-92.
R Development Core Team. (2010) R: A Language and Environment for Statistical Computing.
Royer D.F., Lockwood C.A., Scott J.E., and Grine F.E. (2009) Size variation in early human mandibles and molars from Klasies River, South Africa: comparison with other Middle and Late Pleistocene assemblages and with modern humans. American Journal of Physical Anthropology, 140: 312-323.
Schwartz G.T., and Dean C. (2001) Ontogeny of canine dimorphism in extant hominoids. American Journal of Physical Anthropology, 115: 269-283.
Schwartz G.T., and Dean C. (2005) Sexual dimorphism in modern human permanent teeth. American Journal of Physical Anthropology, 128: 312-317.
Tompkins R.L. (1996) Human population variability in relative dental development. American Journal of Physical Anthropology, 99: 79-102.
Vodanovic M., Demo Z., Njemirovskij V., Keros J., and Brkic H. (2007) Odontometrics: a useful method for sex determination in an archaeological skeletal population. Journal of Archaeological Science, 34: 905-913.
Yuen K.K., So L.L., and Tang E.L. (1997) Mesiodistal crown diameters of the primary and permanent teeth in southern Chinese: a longitudinal study. European Journal of Orthodontics, 19: 721-731.

Correspondence

Doctor Richard Taduran,
Department of Anthropology, University of the Philippines
Diliman, Quezon City, Philippines
Email: rjotaduran@yahoo.com

8 Non-metric dental characteristics in Papua New Guinea Highlanders and their association with molar reduction

Eisaku Kanazawa and Masanobu Matsuno

Department of Anatomy, Nihon University School of Dentistry at Matsudo, Japan.

Abstract

A study of non-metric dental traits in people in the Papua New Guinea (PNG) Highlands was carried out and the results were compared with other Asian and Pacific peoples. Dental impressions were obtained of young adults from Kasi village, Wabag, Enga Province, PNG. Frequencies of 13 dental traits were recorded using the Arizona State University Dental Anthropology System. Conspicuous characteristics in PNG Highlanders included: low frequencies of shoveling and double-shoveling of maxillary incisors, 6th cusp in mandibular first molar and Carabelli's trait, but in contrast high frequencies of hypocone reduction in maxillary second molars, 5th cusp in maxillary first molars and 4-cusped mandibular second molars. A principal coordinate plot including 39 Asian and Pacific populations for scores of these 13 traits, based on Smith's MMDs and standard deviations, showed that the PNG Highlanders belonged to the Sunda-Pacific group, but occupied an extreme position on the first axis. Many of dental characteristics of Wabag were related to morphological reduction of the molar dental crowns, especially their distal components. This suggests that their dental morphologies have changed from the original Australian type of dental characteristics to a peculiar type of morphology associated with nutritional conditions and complex genetic factors.

Introduction

Recent discoveries of archaeological site in highland Papua New Guinea (PNG) demonstrate that this area was colonized by humans almost 49,000 years ago (Gasden, 2010; Summerhays,

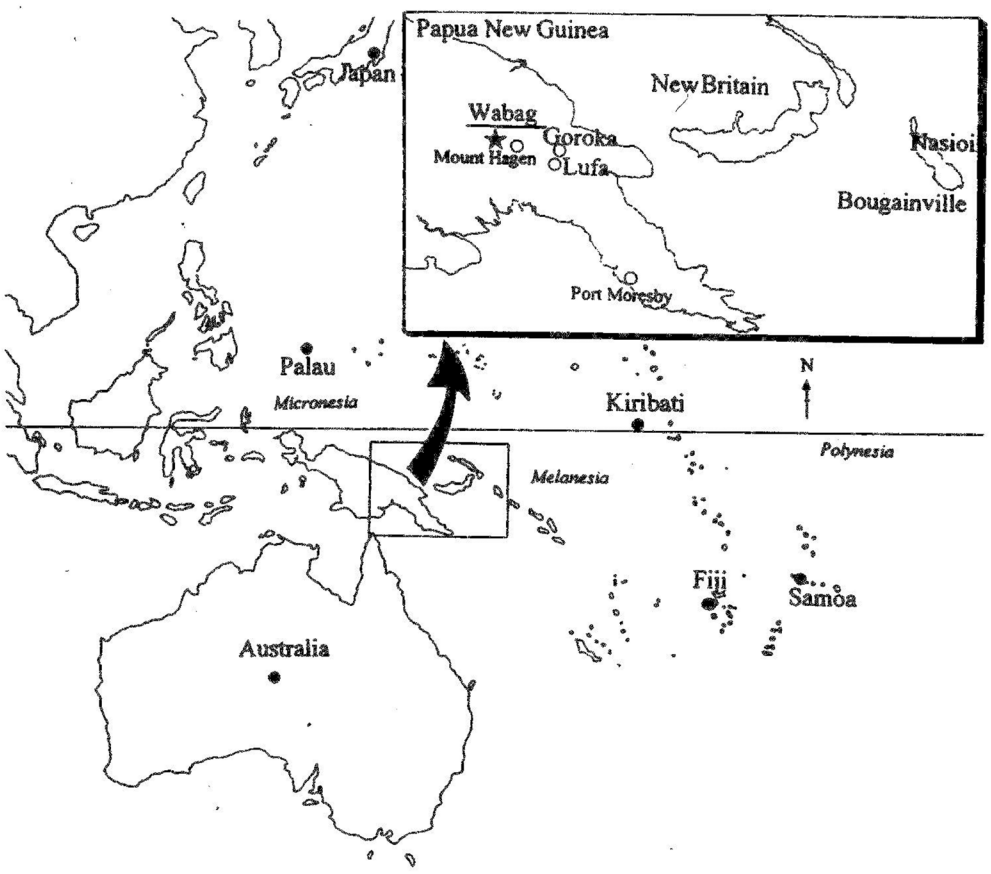


Figure 1. Map of Papua New Guinea showing region where records were collected.

2010). It has also been pointed out that people in the highlands of PNG are direct descendants of Australoids from Western Melanesia and that they have a close relationship with Australian Aborigines on the basis of morphological study (Howells, 1976; Pietrusewsky, 1983), archaeological study (Bellwood, 1989), and genetic study (Cavalli-Sforza, 1994). Dental anthropological studies of tooth size in PNG Highlanders, from Goroka and Lufa, in the Eastern highlands also support this hypothesis (Doran, 1974; 1977).

A dental anthropological survey was carried out at Kasi village, Wabag in Enga Province of PNG by a Nihon University Dental Survey Team in 1997. Enga Province is one of the separate provinces in the great highlands area of PNG with a population of 195,000 (Figure 1). People in this area are usually stockier and shorter than the coastal people, and are fragmented into small clans. They traditionally cultivate sweet potatoes, bananas, sugar cane, greens and yams, although coffee is an important local industry.

A few dental features of Highlanders have been studied by Barksdale (1972) and Kanazawa et al. (2001) including shovel-shaped incisors or basal tubercles and spines on the lingual surface of the maxillary central incisors. However, dental non-metric characters other than these characters have not been fully studied as yet. The purposes of this study were

to describe the frequencies of dental non-metric characters in PNG Highlanders in Enga Province, and to compare the results with other populations in Asian and Pacific areas.

Materials and methods

Materials were dental plaster casts of the permanent dentition collected from Kasi villagers of whom 61 were males (mean age 18.3 years) and 50 were females (mean age 20.3). Villagers having only indigenous parents and grand-parents were selected for examination. The parameters recorded in this study were 13 crown traits classified by the Arizona State University Dental Anthropology System (Turner, 1991). They were selected for enabling wide comparison among Asian-Pacific populations cited in this study. Traits were shoveling and double-shoveling of upper central incisor, mesial ridge of upper canine, odontome in upper and lower premolars, hypocone reduction of upper second molar, 5th cusp and Carabelli's trait in upper first molar, deflecting wrinkle, distal trigonid crest, 6th cusp, 7th cusp in the lower first molar, Y-groove pattern and cusp number in the lower second molar.

Frequencies of 13 traits were recorded using a 4-point scoring scale, none ( ), trace (±), present (+) and strong (++) for traits such as shoveling and double-shoveling of upper central incisor, mesial ridge of upper canine, hypocone reduction of upper second molar, 5th cusp and Carabelli's trait in upper first molar, deflecting wrinkle, 6th cusp, 7th cusp in the lower first molar, or a 3-point scale, Y, +, X for groove pattern in lower molars, or a 2-point scale, i.e., present or absent, for traits such as the odontome in upper and lower premolars, distal trigonid crest in the lower first molar, and cusp number in the lower second molar.

The frequency data from males and females were pooled, because there were no significant differences (at the 5% level) between the sexes for all 13 traits observed in this study. Using the frequencies of the 13 traits, Smith's Mean Measure of Divergence (MMD), together with the Freeman-Tukey method for inverse sine transformation, was used to determine inter-population distances (Berry and Berry, 1967; Freeman and Tukey, 1950; Green and Suchey, 1976). Affinity between PNG Highlanders and other Asian populations was expressed on 2-dimensional coordinates using the principal coordinate analysis based on Smith's MMD. The statistical tests, i.e., the MMD, the principal coordinate analysis, and the Chi-square test for sex differences, were all carried out on a personal computer using the JMP statistical package (SAS Institute Inc. ver. 6).

Results

For the purpose of comparison with 38 other Asia-Pacific populations, frequencies composed of the number of scales were reduced into dichotomized frequencies. Expression dichotomy was determined differently among 13 traits as shown in Table 1 according to previous studies (Ichikawa et al., 2008; Kanazawa et al., 2009; Manabe et al., 1997). Thus the frequencies for each parameter were expressed as percentages.

Table 2 shows the dichotomized frequencies of the 13 traits in 39 populations from Asia and the Pacific region, including PNG Wabag Highlanders at the top. Frequencies for the populations other than PNG Highlanders were taken from already published studies

Trait	Expression dichotomy	Frequency in PNG (%)
Shoveling (UI1)	(++, +)/(++〜-)	28.6
Double-shoveling (UI1)	(++, +)/(++〜-)	2.0
Mesial ridge (UC)	(++〜±)/(++〜-)	0.0
Odontomes (U-L, P1, P2)	(+)/(+, -)	3.6
Hypocone (UM2)	(4+〜3+)/(4+〜3-)	93.0
Cusp 5 (UM1)	(++〜±)/(++〜-)	58.1
Carabelli's trait (UM1)	(++, +)/(++〜-)	12.7
Deflecting wrinkle (LM1)	(++)/(++〜-)	7.3
Distal trigonid crest (LM1)	(+)/(+, -)	0.0
Cusp 6 (LM1)	(++〜±)/(++〜-)	7.9
Cusp 7 (LM1)	(++〜±)/(++〜-)	5.5
Y groove pattern (LM2)	(Y)/(Y, +, X)	13.0
Cusp number (LM2)	(4)/(4, 5)	75.0

*(Present range of grades)/(Total range of grades)

Table 1. Dichotomized frequency of 13 traits in the PNG Highlanders.

(Barksdale, 1972; Scott and Turner, 1997; Ichikawa and Matsuno, 2008; Kanazawa et al., 2009; Chikushi, 2001; Kiyosue, 2000; Jin, 2003; Fukunari, 2003; Kikuti, 2003; Yamagichi, 1996; Manabe et al., 1991; Manabe et al., 1992; Manabe et al., 1997; Turner, 1987).

In Wabag Highlanders, the frequencies of shoveling and double shovel were notably low compared with other populations. Relatively high frequencies were found in 5th cusp in the maxillary first molar. On the other hand, Carabelli's trait in the maxillary first molar, and deflecting wrinkle and 6th cusp in the mandibular first molar were not found so frequently compared with other populations. The frequency of the 4-cusp type in mandibular second molar (75.0%) was the highest of all the Asian and Pacific populations cited in this study.

The principal coordinate plot for 39 populations based on Smith's MMDs computed from frequencies of 13 traits is shown in Figure 2. Data from Sundadont and Sinodont populations were positioned differently on the first axis. The Wabag Highlanders belonged to Sundadont group including Sunda-Pacific populations and were located in the position on the right of the first axis, and in a relatively lower position in the second axis, being close to Pacific populations, i. e., Palauans.

The coefficients of correlations between each axis and the dental traits are shown in Table 3. The first component was highly correlated with shoveling and double-shoveling negatively and with cusp number of the lower second molar, positively. The second component was highly correlated with 6th cusp and deflecting wrinkle in the lower first molar and Y groove pattern in the lower second molar, positively.

Australian Aborigines (AA) were located near the center of the horizontal axis, but in a relatively high position along the vertical axis. Australians showed a low frequency of shoveling,

Traits	Shoveling		Double-shovel		Mesial ridge		Odontomes	
Teeth		UI1		UI1		UC		LP2
1.PNG wabag	105	28.6	102	2.0	111	0.0	111	3.6
2.PNG east highland	30	0.0	32	0.0	54	1.9	119	0.0
3.Australia aborigines	274	20.1	261	4.2	391	2.0	336	3.0
4.Melanesia	135	8.9	134	4.5	174	2.9	218	2.8
5.Polynesia	275	20.7	287	4.5	382	2.9	572	2.3
6.Micronesia	83	31.3	85	8.2	121	5.0	170	1.2
7.Palau	94	31.9	94	5.3	88	11.4	93	0.0
8.Miao (China)	92	60.9	94	17.0	94	12.8	97	2.1
9.Pumi (China)	76	63.2	84	10.7	80	7.5	88	1.1
10.Naxi (China)	92	64.1	97	13.4	90	11.1	97	1.0
11.Hani (China)	80	55.0	86	15.1	76	7.9	87	1.1
12.Dai (China)	89	33.7	93	45.2	90	0.0	96	1.0
13.Dafur (China)	172	51.2	172	32.0	166	11.2	167	1.1
14.Hui (China)	162	58.0	155	46.5	167	10.2	168	2.4
15.Chaoxian (China)	167	68.9	165	50.3	166	11.4	167	0.5
16.Man (China)	208	61.6	200	42.5	211	8.1	211	0.5
17.Han (China)	145	56.6	143	23.1	147	4.8	139	0.7
18.Bali	172	25.0	169	12.4	173	1.7	172	0.6
19.Bunun (Taiwan)	95	76.8	90	42.2	86	7.0	94	1.1
20.Ami (Taiwan)	146	70.5	141	33.3	135	5.9	145	2.8
21.Yami (Taiwan)	192	70.3	191	23.0	155	10.3	155	1.3
22.Lake Baikal	13	92.3	10	50.0	16	6.3	6	0.0
23.NE Siberia	44	81.4	24	25.0	90	0.0	54	0.0
24.Amur (China)	16	68.8	18	44.4	27	11.1	40	5.0
25.N China-Mongolia	200	84.0	213	30.0	255	2.4	231	3.9
26.Recent Japan	276	65.9	267	19.5	365	3.0	462	5.0
27.Jomon Japan	117	25.6	138	1.4	136	2.2	260	0.4
28.Hong Kong P	307	63.8	299	28.4	305	3.0	314	7.6
29.S China	35	74.3	33	24.2	55	3.6	94	0.0
30.Prehistric Taiwan	22	59.1	21	0.0	10	0.0	17	0.0
31.Philippines	54	42.6	29	17.2	76	2.6	116	2.6
32.Early Mainland (SeA)	99	32.3	100	10.0	120	2.5	83	1.2
33.Recent SE Asia	13	46.2	14	28.6	39	2.6	63	3.2
34.Recent Thailand P	127	37.0	111	9.0	143	7.7	189	4.2
35.Burma	15	13.3	13	0.0	33	6.1	57	1.8
36.Nepal	10	20.0	11	9.1	17	0.0	38	2.6
37.Recent Indomalaysia	49	24.5	36	11.1	82	6.1	147	0.7
38.Early Malay Archipel.	71	29.6	67	28.4	103	9.7	120	4.2
39.East Malay Archipel.	12	8.3	3	0.0	16	6.3	25	0.0

Traits	Hypocone reduction		Cusp 5		Carabelli's trait		DW	
Teeth		UM2		UM1		UM1		LM1
1.PNG wabag	100	93.0	105	58.1	110	12.7	110	7.3
2.PNG east highland	191	95.3	151	45.7	197	18.8	52	3.8
3.Australia aborigines	643	96.7	449	61.5	332	21.4	35	17.1
4.Melanesia	295	92.5	234	44.4	291	20.3	184	17.9
5.Polynesia	632	92.2	565	42.7	617	21.7	322	14.0
6.Micronesia	186	84.9	163	27.6	160	22.5	149	22.8
7.Palau	74	94.6	78	50.0	90	15.6	71	1.4
8.Miao (China)	93	100.0	83	16.9	96	10.4	80	0.0
9.Pumi (China)	78	88.5	75	24.0	85	22.4	64	1.6
10.Naxi (China)	85	78.8	87	12.6	89	5.6	68	1.5
11.Hani (China)	88	84.1	86	17.4	90	15.6	67	1.5
12.Dai (China)	95	84.2	93	29.0	93	16.1	82	1.2
13.Dafur (China)	156	78.3	159	17.0	163	35.6	158	21.5
14.Hui (China)	150	92.0	166	23.5	157	45.8	159	32.7
15.Chaoxian (China)	156	85.2	153	26.8	157	49.6	152	6.6
16.Man (China)	201	88.5	212	25.5	212	33.9	209	28.2
17.Han (China)	129	89.2	130	20.0	135	16.2	107	30.8
18.Bali	169	94.1	168	29.2	169	59.1	146	13.0
19.Bunun (Taiwan)	71	88.7	90	35.6	94	46.8	70	31.4
20.Ami (Taiwan)	129	83.7	138	26.1	146	42.5	122	31.1
21.Yami (Taiwan)	106	80.2	192	41.1	197	37.1	188	26.1
22.Lake Baikal	24	100.0	3	66.7	10	30.0	2	0.0
23.NE Siberia	138	76.1	63	3.2	109	18.3	43	39.5
24.Amur (China)	52	82.7	42	21.4	60	26.7	38	39.5
25.N China-Mongolia	406	90.4	295	28.1	374	30.5	89	29.2
26.Recent Japan	482	86.5	390	19.7	458	31.2	262	14.9
27.Jomon Japan	206	82.0	146	31.5	181	8.3	162	4.9
28.Hong Kong P	299	90.3	276	21.7	301	37.5	215	9.8
29.S China	93	86.0	62	16.1	99	25.3	39	17.9
30.Prehistric Taiwan	27	85.2	9	22.2	15	33.3	9	44.4
31.Philippines	148	83.8	132	27.3	146	37.0	74	18.9
32.Early Mainland (SeA)	189	93.1	132	37.1	140	37.1	76	31.6
33.Recent SE Asia	102	87.3	74	13.5	93	41.9	36	19.4
34.Recent Thailand P	196	89.8	143	28.7	179	40.2	80	18.8
35.Burma	95	94.7	72	33.3	93	30.1	14	0.0
36.Nepal	58	86.2	50	32.0	50	26.0	14	7.1
37.Recent Indomalaysia	215	91.2	177	36.2	207	46.4	66	10.6
38.Early Malay Archipel.	156	89.1	90	24.4	100	23.0	66	10.6
39.East Malay Archipel.	29	86.2	22	45.5	28	50.0	17	0.0

Traits	DTC		Cusp 6		Cusp 7		YGP		4-cusp	
Teeth		LM1		LM1		LM1		LM2		LM2
1.PNG wabag	109	0.0	101	7.9	110	5.5	108	13.0	104	75.0
2.PNG east highland	80	0.0	166	15.1	100	7.0	102	39.2	93	59.1
3.Australia aborigines	291	4.1	235	61.7	294	5.4	465	12.7	413	9.7
4.Melanesia	209	1.4	210	49.5	267	12.4	254	26.8	234	50.0
5.Polynesia	453	4.6	417	53.5	495	7.1	501	18.8	461	33.2
6.Micronesia	177	4.0	148	45.3	175	5.7	212	16.0	161	20.5
7.Palau	73	0.0	85	21.2	90	1.1	68	2.9	68	70.6
8.Miao (China)	82	0.0	68	35.3	89	5.6	77	10.4	68	17.6
9.Pumi (China)	66	4.5	57	21.1	77	1.3	71	4.2	72	50.0
10.Naxi (China)	65	0.0	64	23.4	81	1.2	80	5.0	76	34.2
11.Hani (China)	56	12.5	76	7.9	88	3.4	79	3.8	79	53.2
12.Dai (China)	68	1.5	86	30.2	90	4.4	90	4.4	90	48.9
13.Dafur (China)	152	7.2	150	54.0	157	10.2	160	3.8	155	32.9
14.Hui (China)	158	5.1	159	34.0	160	8.1	153	4.6	147	29.9
15.Chaoxian (China)	155	0.0	153	39.9	156	6.4	161	2.5	160	34.4
16.Man (China)	209	6.2	209	34.0	208	5.8	209	2.9	207	35.8
17.Han (China)	115	7.8	115	40.9	115	5.2	115	0.9	112	27.7
18.Bali	154	1.9	157	20.4	162	8.0	51	0.0	155	40.6
19.Bunun (Taiwan)	74	6.8	76	47.4	79	8.9	84	6.0	85	27.1
20.Ami (Taiwan)	131	10.7	137	34.3	142	12.0	146	4.8	144	40.3
21.Yami (Taiwan)	188	9.0	190	45.8	188	5.3	144	7.6	141	22.0
22.Lake Baikal	5	0.0	9	33.3	21	19.0	21	4.8	18	22.2
23.NE Siberia	83	7.2	46	50.0	96	5.2	86	3.5	89	20.2
24.Amur (China)	49	20.4	44	50.0	55	7.3	56	12.5	52	11.5
25.N China-Mongolia	158	5.7	211	37.4	341	9.4	338	6.5	258	17.1
26.Recent Japan	334	18.0	314	42.7	382	6.5	352	13.1	345	13.6
27.Jomon Japan	292	6.8	214	46.7	285	5.3	290	32.1	244	28.7
28.Hong Kong P	227	5.3	267	33.7	295	8.8	228	7.5	296	24.3
29.S China	63	7.9	60	40.0	85	10.6	80	12.5	77	19.5
30.Prehistric Taiwan	16	25.0	15	46.7	33	6.1	19	10.5	21	19.0
31.Philippines	93	9.7	98	38.8	129	6.2	123	13.0	122	27.9
32.Early Mainland (SeA)	96	6.3	136	36.8	217	9.7	187	17.1	163	38.7
33.Recent SE Asia	65	10.8	61	27.9	84	7.1	83	15.7	79	31.6
34.Recent Thailand P	128	10.2	120	28.3	178	6.2	176	19.3	163	25.8
35.Burma	19	5.3	21	52.4	35	8.6	33	6.1	28	21.4
36.Nepal	21	4.8	21	42.9	34	5.9	30	23.3	28	53.6
37.Recent Indomalaysia	81	11.1	97	36.1	137	13.1	142	18.3	134	29.9
38.Early Malay Archipel.	116	6.0	99	45.5	131	4.6	139	19.4	130	24.6
39.East Malay Archipel.	20	10.0	18	38.9	25	4.0	25	20.0	24	45.8

Data sources were shown by the literatures cited in the text. 1:present study, 2: (Barksdale 1972, Scott 1997), 3,4,5,6,23,26,27: (Scott 1997), 7: (Ichikawa 2008), 8, 9,10,11,12: (Kaanazawa 2009), 13:(Chikushi 2001), 14:(Kiyosue 2000), 15:(Jin 2003), 16: (Fukunari 2003), 17: (Kikuti 2003), 18: (Yamagich 1996), 19: (Manabe 1991), 20: (Manabe 1992), 21: (Manabe 1997), 22,23,24,25,28,29,30,31,32,33,34,35,36,37,38,39: (Turner 1987). Abbreviations were DW: Deflecting Wrinkle, DTC: Distal Trigonid Crest, YGP: Y-Groove Pattern.

Table 2. Numbers of samples and dental trait frequencies (Total sample, Present %) in Asia-Pacific population samples.

but high frequency of hypocone reduction, 5th cusp and 6th cusp. These frequency patterns are different from Wabag and also from another highland population (Goroka and Lufa) (Barksdale, 1972). The traits showing the most different frequencies were seen in 6th cusp and deflecting wrinkle in the lower first molar, and 4-cusped lower second molar between AA and two PNG Highlanders as clearly seen in the Table 2. These figures were thought to reflect a different location of these groups on the 2-dimensional expression.

Other Pacific populations were seen to be scattered on the right side of the horizontal axis, while their distribution was wide on the vertical axis such as Polynesian, Melanesian, Micronesian (Scott and Turner, 1997) and Palauan (Ichikawa and Matsuno, 2008). This demonstrated that morphological variations of the traits contributing to the second axis such as 6th cusp, deflecting wrinkle and Y groove pattern were largely diversified among Pacific groups.

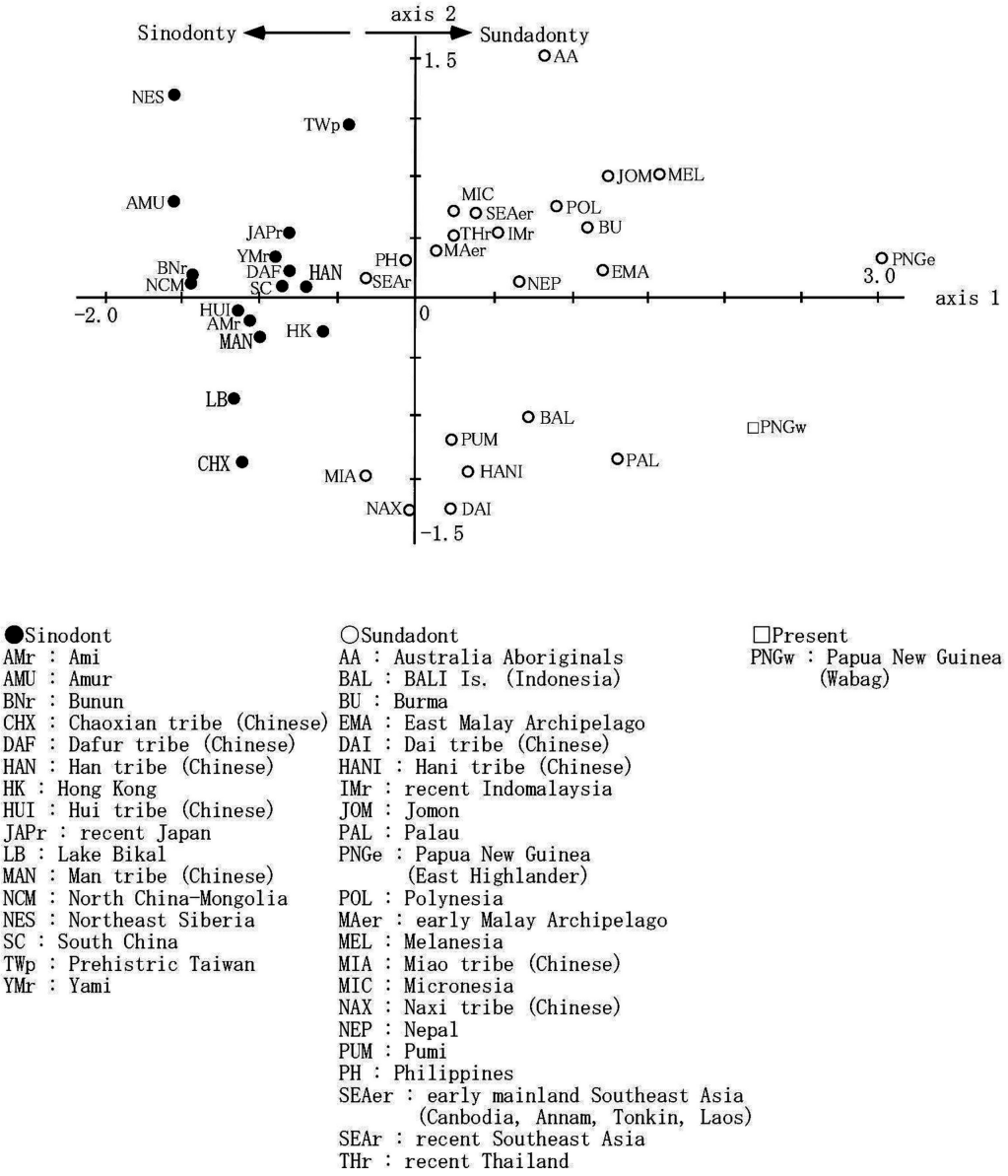
Discussion

Two major dental and morphological variations among Asian populations, are called Sinodonty and Sundadonty, respectively (Turner, 1990). These are categories defined by the combined dental characteristics of the Northeast and Southeast Asian populations. As shown in Fig. 2, where Asians and Pacific peoples were analyzed together, Pacific people are included in the

	1st	p	2nd	p
Shoveling	-0.87668	***	-0.25989	
Double-shovel	-0.77358	***	-0.26283	
Mesial ridge	-0.30527		-0.3993	*
Odontomes	-0.13853		0.131241	
Hypocone reduction	0.403162	*	0.078051	
Cusp 5	0.52226	***	0.066218	
Carabelli's trait	-0.31124		0.109477	
DW	-0.56403	***	0.564494	***
DTC	-0.36768	*	0.455774	*
Cusp 6	-0.31601		0.730602	***
Cusp 7	-0.24633		0.222275	
Cusp number	0.652119	***	-0.52831	***
YGP	0.533608	***	0.542025	***

P: significant level of probability, *: 0.05, **:0.01, ***:0.001

Table 3. The coefficient of correlations of each axis and dental trait.



●Sinodont
AMr : Ami
AMU : Amur
BNr : Bunun
CHX : Chaoxian tribe (Chinese)
DAF : Dafur tribe (Chinese)
HAN : Han tribe (Chinese)
HK : Hong Kong
HUI : Hui tribe (Chinese)
JAPr : recent Japan
LB : Lake Bikal
MAN : Man tribe (Chinese)
NCM : North China-Mongolia
NES : Northeast Siberia
SC : South China
TWp : Prehistric Taiwan
YMr : Yami

○Sundadont
AA : Australia Aboriginals
BAL : BALI Is. (Indonesia)
BU : Burma
EMA : East Malay Archipelago
DAI : Dai tribe (Chinese)
HANI : Hani tribe (Chinese)
IMr : recent Indomalaysia
JOM : Jomon
PAL : Palau
PNGe : Papua New Guinea
(East Highlander)
POL : Polynesia
MAer : early Malay Archipelago
MEL : Melanesia
MIA : Miao tribe (Chinese)
MIC : Micronesia
NAX : Naxi tribe (Chinese)
NEP : Nepal
PUM : Pumi
PH : Philippines
SEAer : early mainland Southeast Asia
(Canbodia, Annam, Tonkin, Laos)
SEAr : recent Southeast Asia
THr : recent Thailand

□Present
PNGw : Papua New Guinea
(Wabag)

Figure 2. Two dimensional expression of multidimensional scaling applied to Smith's MMD based on 13 crown traits in 39 Asia-Pacific populations.

Sundadont group. However, Sunda-Pacific and Sahul-Pacific types of dentition have also been proposed to represent the dentitions of Polynesians and Micronesians in the Sunda-Pacific, and Australian, New Guinea and other island Melanesian populations in the Sahul-Pacific (Turner, 1987; Hanihara, 1992). Although Australians and New Guineans are included in one group in these studies, a few studies have pointed out that the dental characteristics of Papua New Guinea (PNG) Highlanders are unique, being different from Australians.

One of the unique dental characters in PNG Highlanders is a tubercle-shaped maxillary

central incisor. This trait is well-developed in Pacific peoples (Yamada et al., 2000), but its frequency in Wabag Highlanders was second highest among the samples of the 7 Pacific groups studied (Kanazawa et al., 2000). On the other hand, the frequency of the shovel-shaped maxillary central incisor was the lowest among them. Their tooth size, referred to as megadont, falls in the middle of the range of some Australian samples, being larger than Melanesian Island samples (Harris, 1987). People in Goroka and Lufa in the Eastern Highlands also showed good examples of megadontia (Doran and Freedman, 1974). Kanazawa et al. (2000) reported on the tooth size of Wabag Highlanders using the same samples as the present study, and noted that Wabag's teeth were a little smaller than Goroka and Lufa, although the difference was small, and that they were altogether clustered in the same subcluster being larger than Australian Aborigines.

Dental arch shape in Highlanders was also unique. Igarashi et al. (2001) demonstrated that the dental arch breadth of the Wabag samples was the largest among six Pacific groups including Australian aborigines, while dental arch length was the shortest among them.

Kondo et al. (2005) also showed that crown dimensions were larger generally in the Australian Aborigines from Yuendumu than in the PNG Highlanders from Wabag, with differences being more evident in M2 and M3 than M1, especially in talonid dimensions, and that the trigonid, which develops early both phylogenetically and ontogenetically, tended to be relatively stable in size, whereas the later-forming talonid displayed size reduction when comparisons were made both within and between groups.

Sixth cusp in the lower molar is an important character contributing to the Australian dental complex (Townsend, 1990). The frequency is extremely high in Australian Aborigines, which was also confirmed by a quantitative analysis of cusp areas using the Moiré method (Kanazawa, 1985). This trait is also a representative character showing the development of the distal part of the molar, talonid. The underdeveloped morphology of this trait in PNG samples showed a degenerative feature of the dentition in this population.

For these morphological features, two possible causes might be considered, genetic and environmental factors. The earliest archaeological sites in New Guinea and some of the larger Melanesian islands fall in the 30,000-40,000 year range (Intoh, 1997). They are as old as those in Australia which was connected with New Guinea by a land bridge in the late Pleistocene before being cut off by rising sea levels at about 12,000 years BP. This implies close genetic relationships between New Guineans and Australian Aborigines. Human dispersal from Asia to the Pacific islands, i.e., Melanesia, Polynesia and Micronesia, was a relatively recent event taking place about 4,000 years BP (Bellwood, 1989). Coastal areas of New Guinea and its surrounding islands were inhabited by these people leading to admixture with original New Guineans. People in the highlands might have received genetic influences also from these people (Cavalli-Sforza, 1994).

Many nutritionists have become interested in nutritional adaptation of PNG Highlanders (Dennet, 1988; Koishi, 1990; Golson, 1990). Among the protein-deficient PNG Highlanders, caused by depending on sweet potato as a staple food for a long time, there may be three possible mechanisms of adaptation to low protein intake: a slow rate of growth and a small physique, a high level of urea N utilization, and fixation of gaseous

N in the intestinal tract by the action of bacterial flora (Koishi, 1990). People in Enga also displayed short stature, being about 157cm in males and 150cm in females. Small physique in Southern highland people was also reported by Kawabe (1995). It seems difficult to explain reduced morphology of the Highlanders dentition by general reduction of the highlander's physique, but there might be a relationship between tooth development and their slow rate of growth or a small physique as one of the environmental causes. Further studies will be needed in this context.

In conclusion, conspicuous characters in PNG Highlanders include: low frequency of shoveling and double-shoveling of maxillary incisors; 6th cusp in mandibular first molars; and Carabelli's trait in maxillary first molar; whereas high frequencies were noted for hypocone reduction in maxillary second molars; 5th cusp in maxillary first molars; and 4-cusped mandibular second molars. It is suggested that this unique set of dental characters in PNG was acquired by morphological reduction from the original Australian type of dental characters.

Acknowledgements

We are grateful to Dr. Masami Takahashi, Department of Anatomy, Health Sciences University of Hokkaido, for his assistance in the measurement of teeth and statistical analysis. Thanks also to Drs. Kayoko Sasaki, Yuriko Igarashi and Department of Anatomy and Physical Anthropology, Nihon University School of Dentistry at Matsudo, for their kind reading of the manuscript, and to Professor Grant C. Townsend, University of Adelaide, for his reading of the text and kind revision of the manuscript.

References

Barksdale J.T. (1972) A descriptive and comparative investigation of dental morphology. In: Physical Anthropology of the Eastern Highlands of New Guinea. Littlewood R.A. (Ed.) University of Washington Press: Seattle.

Bellwood P. (1989) Man's Conquest of the Pacific. The Prehistory of the South Asia and Oceania. Oxford University Press: London, 1978. Translated into Japanese and published from Hosei University Press.

Beppu K. (2003) Dental characteristics in the Naxi tribe of Yunnan, China. Comparative analysis between Naxi and east Asian populations. Journal of Kyushu Dental Society, 57: 191-196 (in Japanese).

Berry A. C., and Berry R.J. (1967) Epigenetic variation in the human cranium. Journal of Anatomy, 101: 361-379.

Cavalli-Sforza L.L., Menozzi P., and Piazza A. (1994) The History and Geography of Human Genes. Princeton University Press.

Chikushi S. (2001) Dental characteristics in the Dafurs tribe of inner Mongolia, China. Journal of Kyushu Dental Society, 55: 189-205 (in Japanese).

Dennet G., and Connell J. (1988) Acculturation and health in the highlands of Papua New Guinea. Current Anthropology, 29: 273-299.

Doran G.A., and Freedman L. (1974) Metrical features of the dentition and arches of population from Goroka and Lufa, Papua New Guinea. Human Biology, 46: 583-594.

Doran, G.A. (1977) Characteristics of the Papua New Guinea dentition, I. Shovel–shaped incisors and canines associated with lingual tubercles. Australian Dental Journal, 22: 389-392.

Freeman M.F., and Tukey J.W. (1950) Transformations related to the angular and square root. Annals of Mathematical Statistics, 21: 607-611.

Fukunari F. (2003) Dental characteristics in the Manchu tribe of Xin-Bin, China. Journal of Kyushu Dental Society 53: 63-88 (in Japanese).

Gasden C. (2010) When human arrived in the New Guinea Highlands. Science, 330: 41-43.

Golson, J., and Gardner D.S. (1990) Agriculture and sociopolitical organization in New Guinea Highlands prehistory. Annual Review of Anthropology, 19: 395-417.

Green R.F., and Suchey J.M. (1976) The use of inverse sine transformations in the analysis of non-metric cranial data. American Journal of Physical Anthropology, 45: 61-68.

Hanihara, T. (1992) Dental and Cranial Affinities Among Populations of East Asia and the Pacific: The Basic Populations in East Asia, IV. American Journal of Physical Anthropology, 88: 163-182.

Harris E.F., and Bailit H.L. (1987) Odontometric comparisons among Solomon Islanders and other Oceanic peoples, In: The Solomon Islander Project: A Long Term Study of Health, Human Biology and Culture Change. Friedlaender J.S. (Ed.) Oxford University Press: Cambridge.

Howells W.W. (1976) Physical variation and history in Melanesia and Australia. American Journal of Physical Anthropology, 45: 641-650.

Ichikawa H., and Matsuno, M.(2008) Dental anthropological study of the non-metric traits of the Palauans. International Journal of Oral-Medical Sciences, 6: 112-121.

Igarashi Y., Matsuno M., Majima A., Kawasaki Y., Kobayashi K., Kasai K., Aboshi H., and Kanazawa E. (2001) Short and broad dental arch in Papua New Guinea Highlanders. Anthropological Science, 109: 239-251.

Intoh M. (1997) Human dispersals into micronesia. Anthropological Science 105: 15-28.

Jin H. (2003) Dental characteristics in the Chaoxian of Liao-Ning Province, China. Journal of Kyushu Dental Society, 53: 529-537 (in Japanese).

Kanazawa E., Satake T., Sasaki K., Matsuno M., Igarashi Y., Nakayama M., Peiris R., Takahashi M., Ito T., Ito K., Guo D., Ji X., and Ma J. (2009) Dental traits in five Chinese minorities in Yunnan Province. Communication on Contemporary Anthropology, 3: 77-84.

Kanazawa E., Shirono Y., Nakayama M., Yamada H., Hanamura H., and Kondo S. (2001) Distribution of tubercle-shaped incisors in South Pacific populations. Anthropological Science, 109: 225-238.

Kanazawa E., Matsuno M., Sekiguchi H., Suzuki T., Satake T., Sasaki K., and Igarashi Y. (2000) Tooth size of people in Wabag, Papua New Guinea Highlanders and its comparison with Pacific peoples. Anthropological Science, 108: 169-181.

Kanazawa E., Sekikawa M., Akai J., and Ozaki, T. (1985) Allometric variation on cuspal areas of the lower first molar in three racial populations. Journal of Anthropological Society

of Nippon, 93: 424-438.
Kawabe T. (1995) Adult body physique of the Huli in Southern Highlands of New Guinea. Anthropological Science, 103: 188.
Kikuti N. (2003) Dental characteristics in the Han of Liao-Ning, China. Journal of Kyushu Dental Society, 51: 193-216 (in Japanese).
Kiyosue T. (2000) Dental characteristics in the Hui of Liao-Ning, China. Journal of Kyushu Dental Society, 54: 510-520 (in Japanese).
Koishi H. (1990) Nutritional adaptation of Papua New Guinea Highlanders. European Journal of Clinical Nutrition, 44: 853-885.
Kondo S., Townsend G. C., and Kanazawa E. (2005) Size relationship among permanent mandibular molars in Aboriginal Australians and Papua New Guinea Highlanders. American Journal of Human Biology, 17: 622-633.
Main P., Attenborough R., Chelvanayagam G., and Gao X. (2001) The peopling of New Guinea: Evidence from Class I human leukocyte antigen. Human Biology, 73: 365-383.
Manabe Y., Ito R., Kitagawa Y., Oyamada J., Rokutanda A., Nagamoto S., Kobayashi S., and Kato K. (1997) Non-metric tooth crown traits of the Thai, Aka and Yao tribes of Northern Thailand. Archives of Oral Biology, 42: 283-291.
Manabe Y., Rokutanda A., and Kitagawa Y. (1992) Nonmetric tooth crown traits in the Ami tribe, Taiwan Aborigines: comparisons with other East Asian populations. Human Biology, 64: 717-726.
Manabe Y., Rokutanda A., Kitagawa Y., and Oyamada J. (1991) Genealogical position of native Taiwanese (Bunun tribe) in East Asian populations based on tooth crown morphology. Journal of Anthropological Society of Nippon, 99: 33-47.
Pietrusewsky M. (1983) Multivariate analysis of New Guinea and Melanesian skulls: A review. Journal of Human Evolution, 12: 61-76.
Scott G.R., and Turner II C.G. (1997) The anthropology of modern human teeth. Cambridge University Press: Cambridge.
Summerhays G.R., Leavesley M., Fairbairn A., Mandui H., Field J., Ford A., and Fullagar R. (2010) Human adaptation and plant use in Highland New Guinea 49,000 to 44,000 ago. Science, 330: 78-81.
Townsend G., Yamada H., and Smith P.(1990) Expression of the entoconulid (sixth cusp) on mandibular molar teeth of an Australian aboriginal population., American Journal of Physical Anthropology, 82: 267-274.
Turner II C.G. (1991) Traits of the permanent dentition: The Arizona State University Dental Anthropology System. In: Advances in Dental Anthropology, Wiley-Liss: New York pp. 13-31.
Turner II C.G. (1987) Late Pleistocene and Holocene Population History of East Asia Based on Dental Variation, American Journal of Physical Anthropology, 73: 305-321.
Turner II C.G. (1990) Major features of Sundadonty and Sinodonty, including suggestions about East Asian microevolution, population history, and Pleistocene relationships with Australian Aboriginals. American Journal of Physical Anthropology, 82: 295-317.

Yamada H., Hanamura H., and Kondo S. (2000) A study of tubercle-shaped incisors of the inhabitants in the Cook Islands, Journal of Anthropological Society of Nippon, 108: 321-330.

Yamaguchi Y. (1996) Dental characteristics in Balinese. Journal of Kyushu Dental Society, 50: 663-680 (in Japanese).

Correspondence

Professor Eisaku Kanazawa
Department of Anatomy, Nihon University School of Dentistry at Matsudo
2-870-1 Sakaecho-Nishi, Matsudo
Chiba, Japan, 271-8587
Email: kanazawa.eisaku@nihon-u.ac.jp

9 The Main Occluding Area between opposing teeth during chewing: a comparison between Australians and Japanese

Hiroshi Takayama,[1] Hitoshi Kato[2] and Grant Townsend[3,4,5]

[1]Laboratory of Anthropology, Faculty of Letters, Keio University, Japan
[2]Dental Clinic of Tokyo Securities Industry Health Insurance Society, Tokyo, Japan
[3]School of Dentistry, The University of Adelaide, South Australia, Australia
[4]Centre for Orofacial Research and Learning, The University of Adelaide
[5]International Collaborating Network in Oro-facial Genetics and Development

Abstract

The Main Occluding Area (MOcA) defined by Kato (1996) has been found to almost always be located between the upper and lower first molars in Japanese. However, there have not been any reports of this feature in other human populations. In this study, the location of the MOcA was assessed in a sample of 80 Australian dental students as part of an exercise relating to dental occlusion. A piece of stopping material was used to locate the MOcA and to determine the preferred chewing side. There was no significant difference between published findings for Japanese and those for Australians in relation to the location of the MOcA, nor were there any significant differences between the ethnicities represented within the Australian sample. However, there was a difference between ethnicities within the Australian sample in the preferred chewing side, with Asians displaying a preference for the left side. We propose that the location of the MOcA is relatively stable across human populations, having been derived from the tribosphenic biting system of the earliest mammals. The difference observed in preferred chewing side between Europeans and Asians may relate to differences in the use of food utensils between these groups.

Introduction

During human chewing behaviour, only limited contact occurs between opposing surfaces of the dental crowns. Kato et al. (1996) examined the nature of this contact in Japanese and defined the region where maximum contact occurred as the Main Occluding Area (MOcA). He found that the MOcA was usually located between the functional cusps, ie supporting cusps, of the upper and lower first molars. The importance of this feature when chewing food, is that one tends to clench and begin to chew on the MOcA. An understanding of the position of the MOcA is important for dental treatment and also for placing the pattern of modern human masticatory activity into a broader evolutionary perspective.

Kato (1981) and Kato et al. (1984) proposed that, if the MOcA is moved from a small area between the functional cusps of the first molars, there may be problems with chewing food efficiently and possibly food impaction. He went on to examine what was the best way to restore normal chewing behaviour following dental treatment, including the placement of inlays and crowns. He also developed many types of special equipment and instruments to examine effective features in chewing behaviour. He developed a practical and easy method to identify the location of the MOcA by using a small piece of stopping material (Kato et al., 1996). Subsequently, Kato et al. (2005) proposed an hypothesis to explain the location of the MOcA in terms of hominioid evolution (Kato et al., 2005). This hypothesis is explored further in the Discussion section of this paper.

Kato and his colleagues have continued to examine and report on the MOcA in the Japanese population (Kato 1982, 2003; Kato et al., 1996, 1999, 2008; Tokuda et al., 2006; Nakatsuka et al., 2010; Abe et al. 2011; Goto et al., 2011), but they have not examined its position in other populations. Therefore, the aim of this study was to assess the MOcA in an Australian sample and to compare the findings with published data for Japanese. We also aimed to further develop an explanation for the expression of the MOcA in modern populations based on an evolutionary perspective.

Study sample and methods

In the 1990s, Kato noticed that a small temporary stopping material could provide a practical way to examine the MOcA (Kato et al., 1996). These materials are usually used as temporary sealings for treated carious lesions in teeth. In clinical use, they are warmed, then softened to seal the cavity. However, at normal temperatures, they are solid but chewable with no smell or taste. We used the stoppings made by GC Inc., Tokyo, Japan. Figure 1 shows a simple explanation of how to use stopping materials to judge the location of the MOcA. A piece of stopping, which is 3.4 x 4mm, is placed on the tongue. The subject is asked to start chewing once. Then the examiner checks the location of chewed stopping. It is pressed and positioned by the opposing cusps of the subject. In this research project, after verbal and written instructions had been provided, pairs of students examined chewing of the stoppings on each other and marked their location. The most common location of the MOcA was established after five separate chewing events. They also chewed a larger stopping for 20 seconds and recorded the preferred side of chewing.

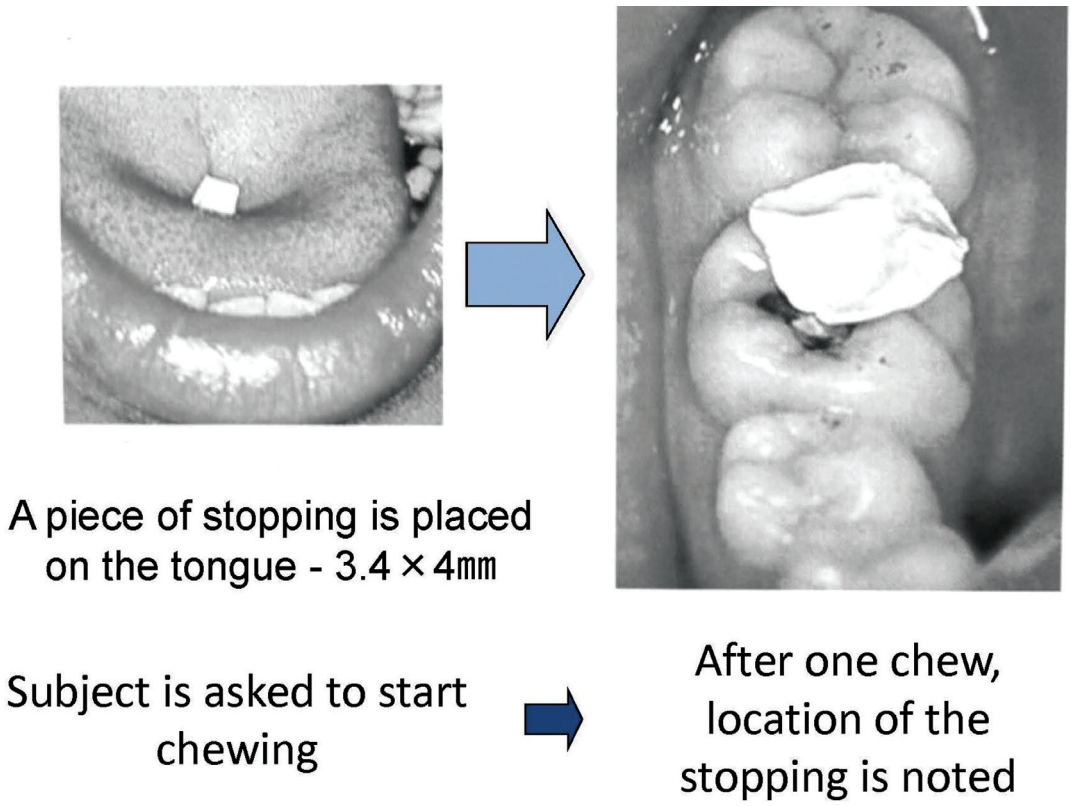


Figure 1. Use of stopping material to judge the location if the MOcA.

The number of participating students was 80 initially, but several records were incomplete and so 8 records were excluded. Final validated records are presented for 72 students (females 42, males 30). The average age of the students was 20.8 years. Their ethnicities, based on verbal verification by the students, were classified as 36 Asian, 20 European, 7 Indian and 9 others. The reasons for exclusion of subjects included previous prosthodontic care, missing teeth, presence of malocclusion, and doubtful or incomplete recordings.

Results

Intra-observer errors were checked in 8 pairs (16 students) after a one week interval. Two records were very similar and in all other cases the results coincided.

By combining the data relating to the locations of all of the 360 chewed stoppings, ie first and second premolars, and first and second molars, on both sides and in the upper and lower arches, we have provided a summary of the location of the MOcA (Figure 2).

The combined data indicate that the MOcA was found on the 1st molars 71.8% of the time. There was no significant difference between females (72%) and males (71%) ($p>0.05$, z test). This result is similar to recent reports in Japanese (Kato et al., 1996, 2003; Tokuda et al., 2006; Kato 2010; Nakatsuka et al., 2010; Goto et al., 2011) as shown in Table 1. The data

	Right	Left	Female	Male	Asian	European	Others
P1	0	2	1	1	1	2	0
P2	20	1	19	8	19	20	3
M1	135	8	140	104	130	102	33
M2	35	17	39	36	27	12	9
M3	0	6	1	6	1	1	0
Total	190	165	200	155	178	137	45

P1=first premolar, P2=second premolar, M1=first molar, M2=second molar, M3=third molar teeth

Table 1. Recorded positions on the teeth where stoppings were located after chewing left and right.

Authors (year)	Population	Ethnicity	Average age(yrs)	Position of MOcA	
This study (2011)	Australian	Mixed	21	72.8% (n=72)	1st molars
Kato et al. (2003)	Japanese	Asian	51	61.4% (n=44)	1st molars
Nakata et al. (2003)	Japanese	Asian	27	70.0% (n=30)	1st molars
Nakatsuka et al. (2010)	Japanese	Asian	51	87% (n=44)	1st molars
Goto et al. (2011:personal)	Japanese	Asian	60	80% (n=22)	1st molars

Table 2. Comparisons of position of MOcA in different studies.

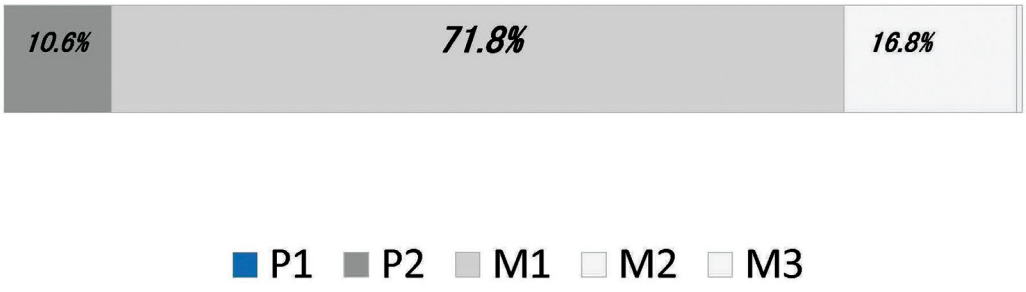


Figure 2. The positions of the MOcA (combined).

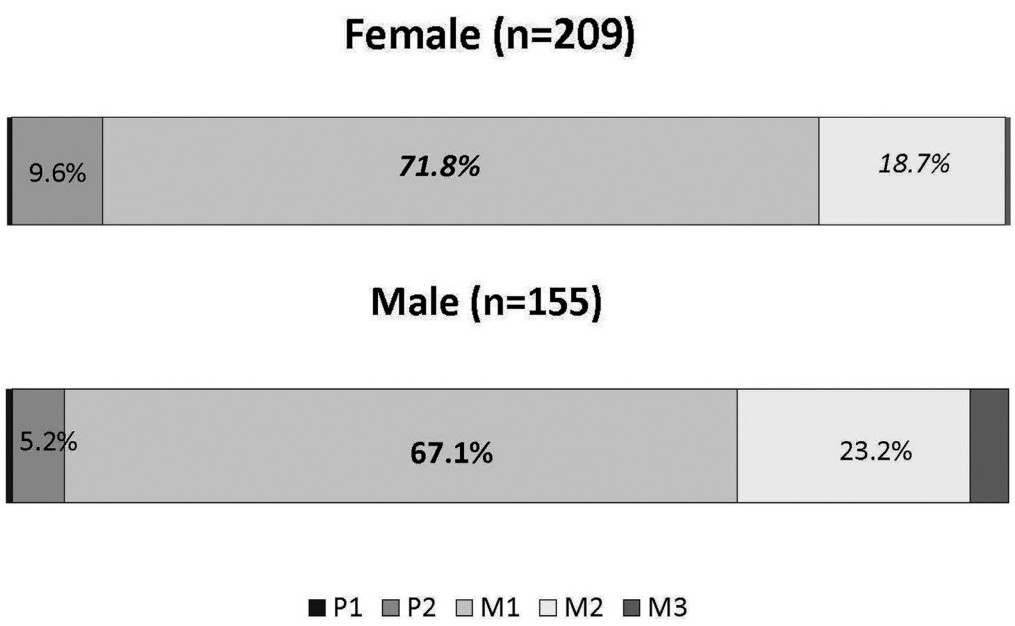


Figure 3. Location of the MOcA in males and females.

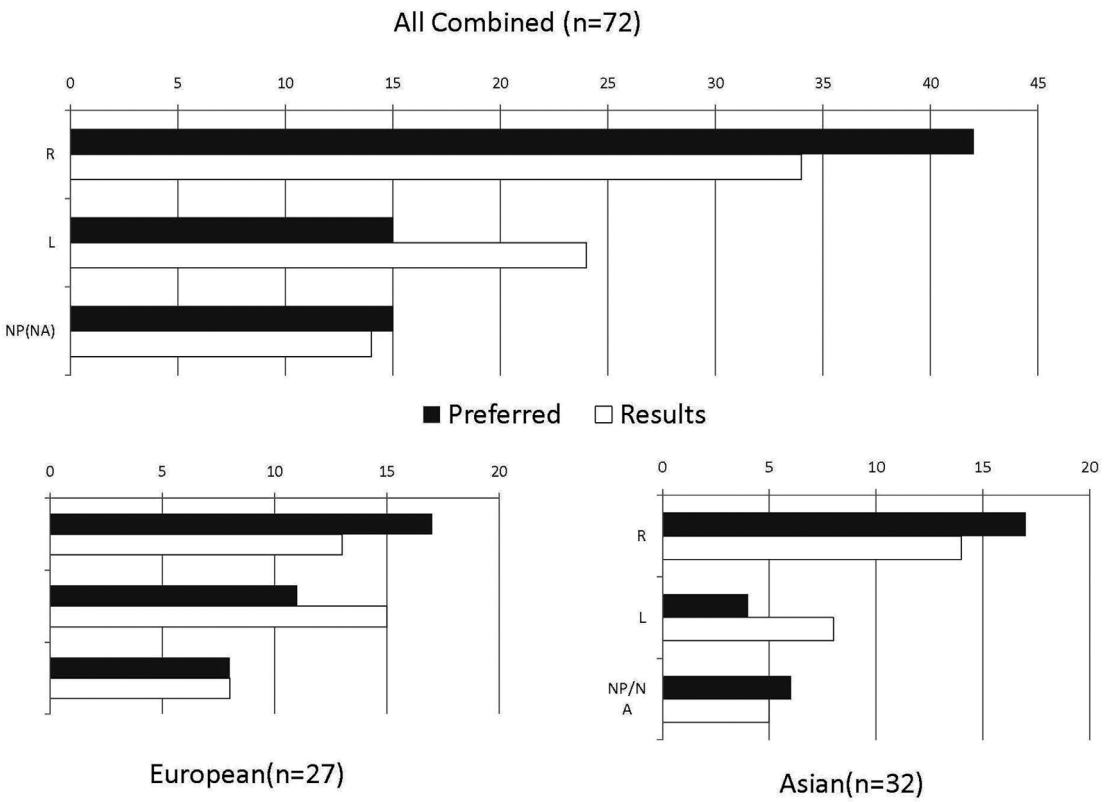


Figure 4. Self reported preferred chewing side and side that stopping was chewed most often.

reported in Japanese varied because the MOcA was examined in different age groups (from children to adults) with different occlusal status (from mostly edentulous to fully dentate) (Table 2).

Possible ethnic differences in the location of the MOcA were examined in the Australian sample. This sample was divided to two major ethnicities, Asian (East Asian: 36) and European (including Indian: 27). Figure 3 shows the combined results for the location of the MOcA, with no significant difference evident between males and females.

Figure 4 shows the self-reported preferred chewing side and the side that the stopping was chewed on most often in the Australian sample. There were no significant differences between right and left sides, or between the self-reported preferred side and the stopping side ($p>0.05$, chi-square test). There was a difference between the Asian and European (including Indian ethnicity) students in relation to preferred side of chewing. The European and Indian students preferred chewing on the right side.

Discussion

In human chewing behaviour, the first contact between the upper and lower teeth occurs most often between the upper and lower molar teeth. This feature was recognized and named by Kato et al. (1996) as the Main Occluding Area (MOcA), based on clinical examinations over a period of 30 years. Kato and his colleagues researched the position of the MOcA in Japanese and found that its position was mostly (over 70%) between the functional cusps of the upper and lower first molars. But up until now there have been no data available about the MOcA in different populations of young and healthy adults. In this study, students of the Adelaide School of Dentistry participated in our research, enabling data about the MOcA to be obtained. The University of Adelaide's Human Ethics Committee agreed that the study could be proceed as part of the student's normal clinical practice.

The positions of the MOcA in the Australian sample were similar to those reported in Japanese, with the most common location being between the upper and lower first molars. No significant differences in the location of the MOcA were found between females and males, nor between Asian and European (including Indian) students. These results suggest that the position of the MOcA is relatively stable in modern human populations, reflecting its evolutionary significance throughout hominoid evolution.

Apart from the stability in position of the MOcA, our results relating to the preferred chewing side showed an interesting outcome. There were no significant differences between the right and left sides in the total sample, but there was a difference between students of Asian and European ethnicity. Asians preferred to chew on both sides to a similar extent, but Europeans were more likely to prefer the right side to the left. We propose that different cultural practices in the use of utensils for eating food could account for these differences. Asians prefer to use chopsticks, which bring food to both sides of the mouth, whereas Europeans use a knife and folk and mainly present food to the right side of the mouth. This suggestion is speculative and further studies are needed to validate it.

To explain why the location of the MOcA is similar among different modern human

populations, we propose an hypothesis based on the evolutionary trends of the MOcA. It is proposed that the MOcA commenced with the development of tribosphenic molars in the earliest primates approximately 80My ago. Then, early hominoid fossils, i.e. *Propliopithecus* 35My ago, showed signs of the MOcA. According to the tricuspid theory of Cope and Osborn (Goto and Otaishi, 1986), the early mammalian molars were derived to perform two types of function, tribos (clenching and grinding), and sphen (cutting and shearing). During primate evolution, tribosphenic molars changed their cuspal morphology and became more flattened, with four cusps on upper molars and five cusps on lowers. As the first evidence of the MOcA, we find evidence of attrition on the molar teeth of the fossil primate, *Propliopithecus*, discovered from the *Oligocene* layer in France and dated from 35My (Hartwig, 2002). We also propose that the position of the MOcA and function of the first molars became more important during hominoid evolution from 25My ago with the development of the *Dryopithecus* occlusal pattern of lower molars. Thus, in modern human populations, the MOcA has persisted in its position between the functional and supporting cusps of the upper and lower first molars as the 'keystone' to our system of dental occlusion.

Further research on the MOcA is planned as follows:

1. The precise position of the MOcA will be checked on dental casts of the Adelaide students by replacing the chewed pieces of stopping in their correct locations.
2. Clinical dental occlusal examinations of subjects will enable more detailed assessments of masticatory function.
3. More data about the MOcA are planned to be collected from additional samples of Australians of different ethnicities for future comparisons.

Conclusion

The location of the MOcA was similar in Japanese and Australian samples, suggesting evolutionary stability in this feature over time. However, the preferred chewing side did differ between Asian and European students, perhaps reflecting differences in the use of utensils to prepare and eat food.

Acknowledgements

We would like to express our thanks to the staff and students of the School of Dentistry, the University of Adelaide, for their assistance and co-operation.

References

Abe H., Miura H., Okada D., Kato H., and Shin C. (2011) The Main Occluding Area in patients with fixed partial denture. Journal of Medical and Dental Sciences 58: 41-4.

Goto M., and Ohtaishi N. (Eds.) (1986) Comparative Odontology – morphology, function and evolution of tooth in vertebrates. Ishiyaku Publishers, Tokyo (in Japanese).

Goto T., NishinakaH., Kashiwabara T., Tomotake T., Nagao Y., and Ichikawa T. (2011) Significance of the Main Occluding Area in implant treatment. Journal of Prothodontic

Research 3: 120th supplement issue: 139.
Hartwig W.C. (Ed.) (2002) The Primate Fossil Record. Cambridge University Press, London.
Kato H. (1981) The function of the tooth supporting structures. Part 1. A method of measuring tooth displacement in two dimensions. Journal of Japan Prosthodontic Society 25:733-745 (in Japanese with English summary).
Kato H. (1982) The function of the tooth supporting structures. Part 2. The dynamics of molars in function and at rest. Journal of Japan Prosthodontic Society 26: 133-147 (in Japanese with English summary).
Kato H. (2003) Relationship between the stomatognathic function and occlusion; Part 4 The realization of functional form. Practice in Prothodontics 36: 602-622 (in Japanese).
Kato H. (2010) Practical occluding theory based on the Main Occluding Area — the long history of 1st molars indicating a landmark of occlusion. Dental Diamond, Tokyo (in Japanese).
Kato H., Sato N., Miura S., Yoshida K., Yoshida T., and Hasegawa S. (1984) A method for analyzing mastication from simultaneous measurements of molar displacement. Journal of Japan Prosthodontic Society 28: 836-843 (in Japanese with English summary).
Kato H., Furuki Y., and Hasegawa S. (1996) Observations on the Main Occluding Area in mastication. Journal of Japanese Society of Stomatognathic Function 2: 119-127 (in Japanese with English summary).
Kato H., Hasegawa S., Yoshida K., and Okada D. (1999) Observations on the Main Occluding Area in mastication. Continuation: the relation with food impaction. Journal of Japanese Society of Stomatognathic Function 5: 125-133 (in Japanese with English summary).
Kato H., H Miura H, Hasegawa S., Yoshida K., and Tanaka Y. (2003) Observations on the Main Occluding Area in mastication. Part 3: Relation between the Main Occluding Area and the tooth fracture of the inlay-restored tooth. Journal of Japanese Society of Stomatognathic Function 9: 177-184 (in Japanese with English summary).
Kato H., Tokuda A., Miura H., Okada D., Hoshino K., and Hasegawa S. (2005) The Main Occluding Area and the occlusal contacts. Journal of Japanese Society of Stomatognathic Function 12: 6-11(in Japanese with English summary).
Kato H., Miura H., Takayama H., Hasegawa S. (2008) Importance of Main Occluding Area in mastication. Journal of the Japanese Association for Dental Science 27: 50-54 (in Japanese with English summary).
Nakata S., Watanabe R., Hayazaki H., and Nakata M. (2003) The change of the Main Occluding Area at mastication by dental stage. Japanese Journal of Pedodontics 41: 252-258 (in Japanese with English summary).
Nakatsuka Y., Yamashita S., Nimura H., Mizoue S., Tsuchiya S., and K. Hashii. (2010) Location of Main Occluding Areas and masticatory ability in patients with reduced occlusal support, Australian Dental Journal 55: 45–50.
Tokuda A., Kato H., Miura H., Okada D., Hoshino K., and Hasegawa S. (2006) The Main Occluding Area in consideration of the occlusal relation. Journal of Japanese Society of Stomatognathic Function 13: 31-37 (in Japanese with English summary).

Correspondence

Professor Hiroshi Takayama
4-4-1 Hiyoshi, Kohoku-ku
Yokohama, Japan, 223-8521
Email: taky@hc.cc.keio.ac.jp

10 'Mineral Maintenance' of dental structures in caries and erosive tooth wear: an holistic model

John Kaidonis,[1,2,3] Sarbin Ranjitkar[1,2,3] and Grant Townsend[1,2,3]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development

Abstract

It is argued that dental structures within the oral environment have evolved with an ability to resist dissolution when exposed to acidic conditions, and to promote remineralisation after damage has occurred. It is hypothesised that dietary acids acted as one of the selective forces in the evolution of the oral environment. It appears that a balance was achieved in hunter-gatherer populations, with the composition and action of saliva, and associated oral biofilms, evolving to protect the teeth against dietary acids. However, in the relatively short period of time since the development of farming and especially with the adoption of modern cultural practices, changes in diet have overwhelmed the oral environment, creating an imbalance. In general, the vast increase in consumption of acidic foods and drinks has decreased the protective mechanisms of saliva. Similarly, the increased consumption of sugar has changed the ecology of oral biofilms, leading to and maintaining a lower oral pH. A combination of these factors has tipped the balance towards demineralisation and increased the risk of oral diseases, such as dental caries and erosive wear, that are so prevalent in many of today's societies.

Introduction

The Paleolithic, stone age or hunter-gatherer way of life, covers a period of over 2.5 million years of hominin evolution, culminating with the appearance of modern Homo sapiens over

200,000 years ago. This period demonstrates the most basic technological developments including the use of the most primitive stone tools (Toth and Schick, 2007).

The Neolithic period of human evolution, by comparison to the Paleolithic, is a relatively short time-span from an evolutionary perspective and involves only one species, Homo sapiens. Many anthropologists regard this period as the start of the technological age, beginning with the advent of farming about 10000 years ago, through to the widespread use of metal tools (Diamond, 1997).

Although anthropologists have given the Paleolithic and Neolithic periods a temporal association in human evolution, strictly speaking these periods represent lifestyles and a measure of technological development only. There is well documented evidence that hunter-gatherer lifestyles have existed well into the 20th century (e.g. Australian Aborigines) and the Neolithic lifestyle existed independently over different time periods in different countries around the world.

It can be argued that much of the evolution of the human oral environment occurred well before and during the Paleolithic period, where the hunter-gatherer diet played a major role in the evolution of much of the oral environment as we see it today. Simply speaking, our current 'genetic make-up' is still in-tune with the paleolithic diet.

The premise for this model is that the human oral environment (and that of many other species) has evolved in the presence of acids. That is, acids were one of the selective forces responsible for the evolution of the oral environment. The components of the oral environment, including the saliva, oral biofilms, oral physiology and the anatomical structures, evolved to not only physically protect the teeth from dietary acid, but to provide for the reparative process of dental hard tissues referred as remineralisation.

The human diet and oral adaptations

The temporal and spatial variations in diet among hunter-gatherer populations show great versatility in the ability to survive. The animal-plant food ratio of consumed food varies and was driven by seasonal variations as well as general environmental extremes across the globe. Studies of contemporary hunter-gatherers have provided some insight into past diets, and although the animal-plant ratio averages between 65% and 35%, in very cold (e.g. arctic) environments the diet is almost exclusively animal (Cordain et al., 2002).

Although the Paleolithic diet has been extensively discussed in the literature, especially from the nutrient/energy perspective, close evaluation of the pH values of different foods has been largely overlooked. We propose that the majority of food (whether plant or animal) consumed by humans during the Paleolithic period was acidic and is still acidic in contemporary populations.

For example, live muscle has a pH value of about 7.1, however after death the pH drops to about 5.5 in a number of hours. Food hunted and killed by hunter-gatherers would have been acidic. In addition, the plant component of the diet (fruits, root vegetables etc) is also acidic and can have a pH as low as 2, depending on the species of plants, the maturity, as well as the components consumed and the timing of their harvest.

There are numerous adaptations of the oral environment in response to pH. For instance, the well documented 'reflex action' of a sudden increase in the volume of saliva in response to acid on the tongue is a good example. This stimulated saliva that comes exclusively from the parotid glands produces large amounts of bicarbonate ions, a natural and very efficient buffer to acids. Alternatively, in general, bitter tasting foods in nature are poisonous (eg. alkaloids). The most sensitive taste buds in the mouth are those discerning bitter taste and they are situated on the posterior aspect of the tongue. Interesting this region of the tongue is innervated by the glossopharyngeal nerve that is associated with the gagging and vomiting reflexes, again an adaptive mechanism protecting against poisons.

Adaptations in human behaviour that were pivotal for survival were passed down many generations. Knowledge on which foods or parts of foods were safe, nutritious and seasonally available, also played a vital role. For example, some fruit, although acidic, is safe to eat even though the kernal inside is bitter and dangerous.

Overall, much of the consumed plant food of prehistoric societies ranged from near neutral to low pH values, well below the critical pH at which dental structures (hydroxyapatite) dissolve. Yet the prevalence of erosive damage caused by acids in Paleolithic societies is virtually non-existent when compared to our contemporary populations (Kaidonis, 2008). In addition to the lack of erosive pathology, the prevalence of dental caries was also so low in paleolithic populations that one would consider this pathology to be also insignificant (Kaidonis, 2008). Yet oral biofilms (dental plaque) did exist in all individuals and oral hygiene as we know it today was not practised.

Finally, apart from the breastfeeding of infants, water was generally consumed when individuals were thirsty. It was only in the late Paleolithic to early Neolithic and up to the present, that fermentation and other cultural changes permanently changed the diet.

Tooth structure and the 'theory of mineral maintenance'

It is well known that teeth existed and have evolved well before hominids came into being. Human teeth (as with other species and phyla) are biological structures that have evolved for millions of years. Their composition and morphology give them physical properties that make them more durable than bone and able to withstand high forces for long periods of time. However, this organic structure is soluble in acid.

This dilemma of having a structure that is soluble yet has to function in an acidic environment is overcome by oral adaptations that include the complex inter-relationship of biofilm, saliva and the host.

Oral bio-interactions for mineral maintenance

Our current concepts of the disparate oral interactions that occur in a healthy oral environment are not new (Marsh, 2010). What is new is the paradigm shift in our understanding of the processes from an evolutionary and holistic perspective.

Pellicle, composed of a large variety of adsorbed salivary proteins develops immediately when clean enamel is exposed to saliva. The saliva also contains a large variety of ions (eg.

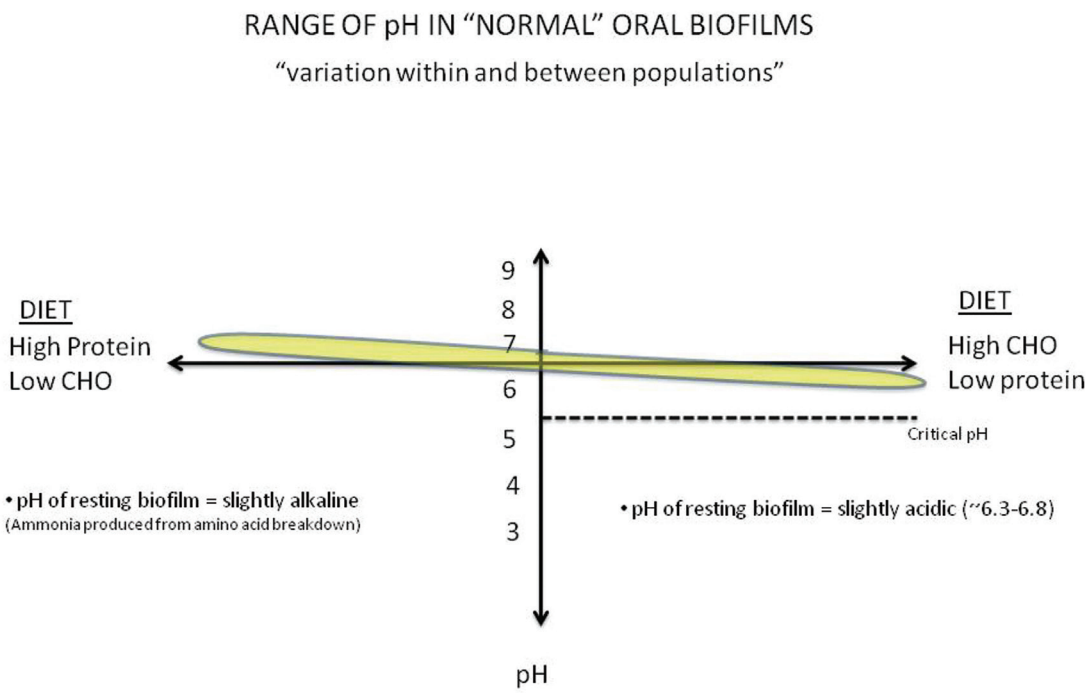


Figure 1. The resting pH range of oral biofilms is diet related and varies within and between populations. If the resting pH is above the critical pH of hydroxyapatite then the balance is towards mineral maintenance.

calcium, phosphate and hydroxyl ions) that are super-saturated with respect to hydroxyapatite, the building blocks of tooth structure. These ions are also very reactive and have the potential to precipitate (e.g. $CaPO_4$). However, specific salivary proteins such as the statharins, proline-rich proteins and other complexes or loosely surround these ions preventing them from precipitating. Again an important adaptation, preventing salivary stones from forming in salivary glands and ducts.

Almost immediately after the pellicle forms, there is the sequential colonisation of bacterial species eventually forming a 'climax' community of bio-diverse bacterial species living symbiotically with one another. The pH of this biofilm may range from sightly alkaline to slightly acidic (pH~6.8) depending on the diet consumed. Populations consuming food with a high animal to plant ratio (e.g. Inuit) will have a more neutral to alkaline pH while those with a low animal to plant ratio will have a pH below neutral (Figure 1). In addition, although there are bacteria that will metabolise sugar and produce acids, there are also bacterial species that will metabolise urea and arginine from saliva to produce ammonia and CO_2 as bi-products. These 'good' bacteria have the potential to maintain alkaline conditions in the biofilm, thereby controlling the numbers of acidogenic and aciduric organisms to a minimum. The balance of such 'healthy' biofilms tip the balance towards remineralisation.

The oral bacteria making up such biofilms are bio-diverse and live symbiotically with one another and their human host providing some benefits. Firstly, they act as physical barriers to dietary acids. Recent studies indicate that the patterns of erosion observed in

contemporary populations is closely associated with the thickness of biofilm (Hannig and Balz, 2001). Secondly, biofilms protect the tooth from demineralisation that may result from metabolic acids.

In conjunction with the consumption of dietary acids, carbohydrates forming part of the Paleolithic diet have the potential to be metabolised by bacterial species to produce organic acids. As soon as the biofilm pH drops, there are two outcomes. Firstly the statharins and proline-rich proteins described previously 'release' these ions allowing the PO_4 ions from resting saliva to react with and buffer the acids. Secondly, a more powerful HCO_3 ion buffer from stimulated parotid saliva neutralises the acid until the pH returns to normal. If however, the system becomes overwhelmed and the biofilm environment becomes unsaturated with respect to hydroxyapatite, then enamel dissolution will occur. The acid perculates inside enamel laminar pores between enamel rods until subsurface dissolution results.

Finally, all the bi-products of demineralisation (e.g. Ca, PO_4, OH ions) accumulate at the biofilm / tooth surface interface, and this, together with an increase in pH and further ions from saliva, re-establishes supersaturated conditions. This tips the balance towards remineralisation and repair of the enamel defect. In addition, if fluoride ions are present, the reforming crystal will be fluoro-apatite that is more insoluble than hydroxyapatite and will

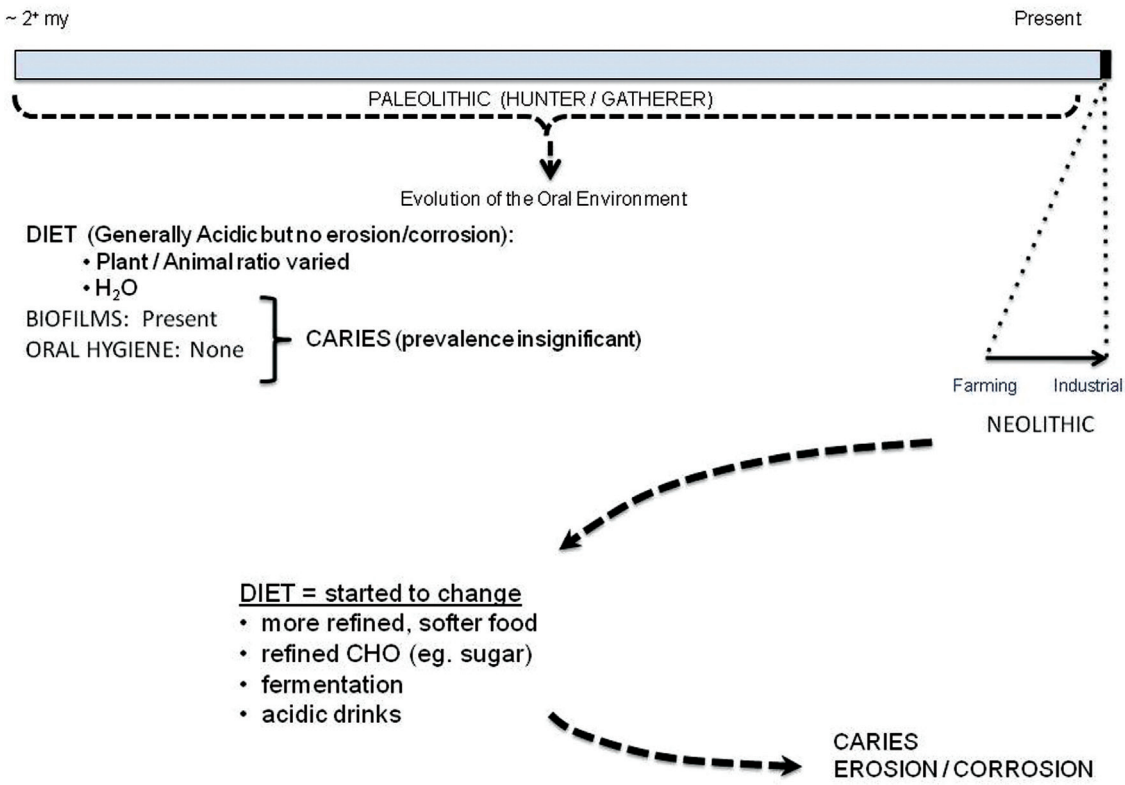


Figure 2. General comparison of the Paleolithic 'balanced' oral environment and Neolithic 'imbalanced' ways of life that has resulted in modern day diseases like caries and erosion (corrosion). The Neolithic is a very recent event by comparison to the Paleolithic geological timescale.

therefore resist further dissolution. This demineralisation/ remineralisation cycle allows for the maturation of the tooth to a more insoluble form.

This explanation is not new in the research arena. However, the understanding that this cascade of events evolved with a purpose alters our way of thinking: the 'mineral maintenance' of a structure with unique physical properties that is also inorganic and therefore soluble.

The Neolithic imbalance

Compared to the Paleolithic, new technological developments, especially the advent of farming, caused a major shift in human lifestyles that has accelerated to the present (Figure 2). Although it is not the purpose of this paper to list or discuss these changes, it is agreed that the human diet changed permanently. Apart from the increase in consumption of specific grains and legumes, there was a slow increase in the consumption of processed foods, fermentation processes and especially the refining of sugar during the Industrial Revolution. This increase in consumption of simple sugars (fermentable carbohydrates) tipped the balance of the ecosystem towards demineralisation and therefore oral diseases such as caries and erosion. It can be also argued that this change in lifestyle towards fermentable sugar consumption is also the reason for the non-communicable diseases we see in our modern populations today, such as cardiovascular disease, diabetes mellitus, certain cancers and dementia (Hujoel, 2009).

Dental caries

Currently, the most commonly accepted bacterial model is the ecological plaque hypothesis (Marsh, 1994; Takahashi and Nyvad, 2011). Simply speaking, the increased consumption of sugars in modern populations has tipped the balance of the oral biofilm towards demineralisation. Biofilms exposed to large and frequent episodes of refined sugar increase the amount of metabolic acid produced, resulting in a decrease in the resting pH of the biofilm. Maintaining a low resting pH will change the balanced bio-diversity of the oral environment. That is, bacterial species that cannot live in continual or extreme low pH conditions will be slowly eliminated. These are the species ('good bacteria') that help promote alkaline conditions. Simultaneously, this change in pH will select for and promote the proliferation of acidogenic and aciduric bacterial species producing more acid and so on. Individuals with such biofilms are at high risk of developing caries. The protective mechanisms that have evolved as part of the oral environment are overwhelmed and rampant dissolution occurs.

Dental erosion (Corrosion)

Our current 'modern' lifestyle has also imposed another major challenge to the oral environment. Instead of drinking water when thirsty as our paleolithic predescessors did, we consume a large variety and volume of acid drinks. This adds to an already increased acidic challenge from the consumption of refined sugar, further acidifying an already acidic biofilm.

Acid erosion occurs when acids not related to those of bacterial origin cause dissolution of teeth. The current literature describes erosion observed in our contemporary populations as a common pathology, where over 50% of some populations are affected, particularly in

children, teen-agers and young adults. Although the source of acids can result from gastro-oesophageal disease, the most common source is the consumption of acidic drinks.

As described previously, humans have evolved and adapted to acids, however the acids were mostly food that was consumed in the presence of a 'healthy biofilm' that provided physical protection. However, the excessive and regular consumption of acidic drinks causes the biofilm to dissolve leaving exposed dental surfaces. It is well documented that patients with active erosion often show very clean, plaque-free and stain free dentitions.

The flooding of extrinsic, strong acid in the mouth causes immediate dissolution at a rapid pace before stimulated saliva can have any affect. Even so, dietary acids displace saliva very easily due to the contact angles of the opposing liquids (Busscher et al., 2000), and by the time the acid is cleared, the dissolution of large volumes of tooth structure can occur. The dissolution bi-products are not retained but lost through swallowing leaving the ends of hydroxyapetite crystals permanently damaged. Although these ends will remineralise with the help of saliva, the loss of tooth structure during the dissolution phase is permanent. This reflects an 'open system' in contrast to the subsurface perculation of bacterial acids, and the reuse of the dissolved bi-products in a 'closed system'.

Conclusion

We argue that the oral ecosystem evolved during the paleolithic (and even earlier) into a highly balanced system, where the inorganic dental structures (e.g. enamel and dentine) were protected from permanent dissolution and where the entire system was set up for 'mineral maintenance'.

The Neolithic period is but a 'blip' on the evolutionary timescale, and reflects cultural changes and in particular dietary changes that have overwhelmed the oral environment leading to dental dissolution, and hence the diseases we see today. Adaptation from an evolutionary perspective to this sudden change will not occur. However, a better understanding and appreciation of these changes should lead to better preventive management for our patients.

References

Busscher H.J., Goedhart W., Ruben J., Bos R., and Van der Mei C.H. (2000) Wettability of dental enamel by soft drinks as compared to saliva and enamel demineralization. In: Tooth Wear and Sensitivity, Addy M., Embery G., Edgar W.M., Orchardson R. (Eds.) Martin Dunitz Ltd, London, UK, pp. 197-200.

Cordain L., Eaton S.B., Miller J.B., Mann N., and Hill K. (2002) The paradoxical nature of hunter-gatherer diets: meat based, yet not artherogenic. European Journal of Clinical Nutrition, 56 (Suppl 1): S42–52.

Diamond J. (1977) Guns, germs, and steel : the fates of human societies. W.W. Norton & Co.

Hannig H., and Balz M. (2001) Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. Caries Research, 35: 142-148.

Hujoel P. (2009) Dietary carbohydrates and dental-systemic diseases. Journal Dental Research, 88: 490-502.

Kaidonis J.A. (2008) Tooth wear: the view of the anthropologist. Clinical Oral Investigations, 12: S21-S26.

Marsh P.D. (1994) Microbial ecology of dental plaque and its significance in health and disease. Advances Dental Research, 8: 263-271.

Marsh P.D. (2010) Microbiology of dental plaque biofilms and their role in oral health and caries. Dental Clinics North America, 54: 441-454.

Takahashi N., and Nyvad B. (2011) The role of bacteria in the caries process. Ecological perspectives. Journal Dental Research, 90: 294-303.

Toth N., and Schick K. (2007) Overview of Paleolithic anthropology In: Henke H.C.W., Hardt T., Tattersall I. Handbook of Paleoanthropology, Volume 3, Berlin; Heidelberg; New York: Springer-Verlag, p. 1944.

Correspondence

Associate Professor John Kaidonis
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
Email: john.kaidonis@adelaide.edu.au

11 Emerging techniques for the analysis of tooth wear

Sarbin Ranjitkar,[1,2,3] John Kaidonis,[1,2,3] Colin Hall,[1,4] Victor Marino,[1] Lindsay Richards[1,2,3] and Grant Townsend[1,2,3]

[1]School of Dentistry, The University of Adelaide, South Australia, Australia
[2]Centre for Orofacial Research and Learning, The University of Adelaide
[3]International Collaborating Network in Oro-facial Genetics and Development
[4]Mawson Institute, University of South Australia, South Australia, Australia

Abstract

Changing patterns of tooth wear have been used extensively to obtain information about the lifestyle and culture of pre-historic and modern humans. The assessment of tooth wear in previous anthropological studies has been largely based on quantitative analysis of wear indices and qualitative analysis of micrographs. Wear indices are simple to use and can be sensitive tools, but there is a lack of international standardization in their use. Micrographic assessment of pits and scratch marks on the worn surfaces of teeth can assist in dietary reconstruction of humans, but this approach has low reliability and high observer error. This review will provide an update on a new wear index and novel nano-techniques that hold promise for improving the analysis of tooth wear. Recently, a new wear index, termed the Basic Erosive Wear Examination index, has been proposed as a standardized universal tool for diagnosing erosive tooth wear. However, its value seems to be limited when assessing the dentitions of populations, in whom tooth wear occurs predominantly by attrition and abrasion. Optical techniques involving scanning confocal microscopy combined with fractal analysis can provide an objective assessment of the worn surface. Other nanotechnology-based methods, such as nanohardness measurements, nano-computed tomography and mass spectrometry, can be also useful in physical and chemical characterization of both sound and worn teeth, but these techniques are limited to use in vitro. A combined assessment of the worn dentition using all of these techniques promises to provide the best holistic approach to analyse tooth wear.

Introduction

Anthropological studies investigating the form and function of teeth over the past century have highlighted the complexity and varying nature of tooth wear in both extinct and extant primates and hominids (Begg, 1954; Campbell, 1925; Kaidonis et al., 1993; Kaidonis, 2008; Kaifu et al., 2003; Molnar, 1972). The knowledge generated from scholarly research and academic discussions has caused a paradigm shift in the perception of tooth wear from a pathological condition to a predominantly physiological phenomenon (Molnar, 1972), except when excessive tooth wear severely compromises function and leads to pulpal and periapical pathology (Bartlett and Dugmore, 2008; Kaidonis, 2008).

Tooth wear can be caused by various mechanical and chemical processes. There are some inconsistencies in the use of terminologies describing these processes in the literature, so we will use standard terms currently used in clinical situations in this review. 'Attrition' (bruxism or tooth grinding) and 'abrasion' (wear from foreign bodies such as coarse food or tools) are mechanical wear processes, whereas 'erosion' refers to chemical demineralization of tooth structure without bacterial involvement. 'Erosive tooth wear' is a relatively recent term used to describe erosion occurring in combination with mechanical wear (Meurman and Sorvari, 2000).

There is a large body of information on the significance of abrasion in palaeontological and anthropological research. Inefficient chewing from excessive abrasive wear in large lemurs, Propithecus edwardsi, has been associated with reduced lactation, increased infant mortality and, by inference, reproductive senescence and unfavourable natural selection (King et al., 2005; Ungar, 2005). Studies of abrasion have also revealed information about the culture, lifestyle and diet of extinct hominins as well as pre-historic and modern humans (Kaifu et al., 2003; Scott et al., 2005; Ungar et al., 2006).

Severe abrasive wear was a common occurrence before agricultural development and the industrial revolution, but is not a common problem in modern humans (Kaidonis et al., 1992). Attrition is a universal wear mechanism in humans living both industrialized and non-industrialized ways of life, but it is not a major contributor to pathological tooth wear (Cash, 1988; Kaidonis et al., 1993; Springbett et al., 1999). In contrast, erosive tooth wear can result in rapid loss of tooth structure and has become a topic of growing interest in recent decades (Lussi, 2006; Taji and Seow, 2010). In this context, all three wear processes, including attrition, abrasion and erosion, are relevant to the anthropological study of humans.

The field of tooth wear research is changing rapidly, with the number of publications rising sharply since the 1970s (Lussi, 2006). This is undoubtedly related to the technological development in mineralized tissue research, leading to numerous advances in our understanding of physical and chemical properties of tooth structure (Dickinson et al., 2007; Gjorgievska and Nicholson, 2011; Hannig and Hannig, 2010; He and Swain, 2007; Kinney et al., 2003; Mahoney et al., 2003; Mann and Dickinson, 2006; Smith et al., 2011). For example, the collaboration of engineering materials science and dental anthropology has enabled researchers to gain insights into the relationship between the diet and tooth characteristics of early hominins when they abandoned their arboreal lifestyle and started walking upright

in the African planes between 2.5 and 1.5 million years ago (Mya) (Lucas et al., 2008). It was concluded that thick enamelled teeth evolved during this period after an abrasive diet was introduced in the African plains (Lucas et al., 2008; Ungar, 2008). Similarly, advances in nanotechnology and nanomaterial science hold promise to improve the diagnostic ability to detect early stages of tooth wear, and to elucidate fundamental mechanisms relating to the effects of demineralization and remineralization of nanoapatite crystals (Hannig and Hannig, 2010; Roveri et al., 2009; Yamagishi et al., 2005). Overall, technological advancement has greatly influenced our understanding of the characteristics of tooth wear in both clinical and anthropological contexts.

A number of reviews of erosive tooth wear have been published over the past decade (Attin, 2006; Azzopardi et al., 2000; Pickles, 2006), but they have focussed heavily on the diagnosis of erosive tooth wear in clinical practice. Clearly, reviews with a well-balanced coverage on both mechanical and chemical wear processes tailored to the needs of dental anthropology are needed to provide a holistic approach to the study of tooth wear. It is outside the scope of this review to describe all of the methods available for tooth wear analysis, so traditional wear indices and scanning electron microscopy will only be described briefly. We will focus on some new, promising analytical methods that have the potential to advance research in this field.

Traditional methods of assessing tooth wear

Visual examination and wear indices

Visual examination can provide qualitative information about the causes of tooth wear and can reflect the lifestyle and occupation of pre-contemporary populations, such as yarn production, weaving and basketry (Lorkiewicz, 2011). The occurrence of wear facets in European medieval dentitions has been associated with attrition, and occlusal scooping is indicative of erosion from acidic diet (Ganss et al., 2002). Numerous wear indices have been proposed to quantify gross wear features in skeletal materials, living humans and dental models. For example, the Smith and Knight (1984) index is commonly used to quantify the severity of enamel and dentine wear in humans.

Generally, visual examination and wear indices are non-destructive and can be used directly on teeth or models. However, wear indices can be subjective and have low reliability. They are unsuitable in dietary reconstructions in anthropological studies (Lee-Thorp and Sponheimer, 2006) because of their limited potential in discriminating between different degrees of wear severity (Holbrook and Ganss, 2008) and between different aetiological factors (for example, scooped dentine caused by abrasive and erosive wear) (Ganss, 2008).

Microwear assessment

Microwear features of wear facets in anthropological research are commonly assessed by using scanning electron microscopy, typically at a resolution of x40 to x500. Microwear features better reflect dietary regimes than macrowear features, and are more useful in dietary reconstructions

of primates and hominins (Lee-Thorp and Sponheimer, 2006). Varying degrees of abrasive tooth wear are also associated with different stages of human evolution. For example, the diet of the Natufian hunter-gatherers (12,500 – 10,250 bp), containing plants and animals, display some pits and scratches, but the shift of lifestyle into agriculturalists in the early Neolithic period (stone age) (10,250 – 7,500 bp) is associated with observations of larger pits and wider scratches (Mahoney, 2006). Wear facets formed by attrition are different from those of abrasion in that attrition displays parallel striations during micrographic examination (Kaidonis et al., 1992). However, information on micrographic assessment of worn surfaces of attrition and erosion is scarce in the literature, and our understanding in this area is mainly based on experimental findings (Eisenburger and Addy, 2002; Eisenburger et al., 2004; Li et al., 2011; Manton et al., 2010; Meurman et al., 1991; Ranjitkar et al., 2008; Ranjitkar et al., 2009a; Ranjitkar et al., 2009b).

Some of the major disadvantages of micrographic assessment of tooth wear are high subjectivity and low reliability (Lee-Thorp and Sponheimer, 2006). Grine et al. (2002) reported an approximately 19% variation in the quantitative assessment of micrographs of wear facets using three popular methods, differing from each other in parameters related to microscopy settings (for example, kilovoltage) and the scoring method (for example, the size and magnification of micrographs). They also reported intra- and inter-examiner errors of 7% and 9% respectively, and the quality of the micrographs was noted to be a critical factor. These error rates need to be considered while conducting micrographic assessments.

Micrographic assessment of tooth wear complements findings from examination of gross wear features, but these methods generally provide qualitative information on tooth wear. Thus, there has been a thrust in research over the past decade to enable objective quantification of the physical and chemical characteristics of tooth wear.

Emerging techniques of tooth wear assessment

This section describes some new methods that have been developed over the past decade for tooth wear analysis. These methods include a new wear index (Basic Erosive Wear Examination (BEWE) Index to assess macro-wear on teeth), three-dimensional surface profilometry (scanning confocal microscopy combined with fractal analysis to assess microwear details), physical analytical techniques (nanohardness testing, micro-computed tomography and nano-computed tomography to assess physical wear characteristics at micro- and nano-levels), and chemical analytical technique (mass spectrometry to assess surface changes in the first few atomic layers of teeth).

Basic Erosive Wear Examination (BEWE) Index

In an attempt to develop a simple, valid and repeatable tool to measure erosive tooth wear in both research and clinical situations, the Basic Erosive Wear Examination (BEWE) index was proposed recently by Bartlett et al. (2008). This index was developed from a workshop of a group of international leaders in the area and the scoring system relates to different risk levels, providing some guidance over the management of erosive tooth wear.

Tooth wear is graded into four levels according to the degree of severity:

0 = No erosive tooth wear,
1 = Initial loss of surface texture,
2 = Distinct defect, hard tissue loss < 50% of the surface area, and
3 = Hard tissue loss ≥ 50% of the surface area, with scores of 2 and 3 often involving dentine.

Teeth are divided into six sextants (i.e. anterior teeth, and left and right posterior teeth in both maxillary and mandibular arches). Scores are assigned to each of the buccal/labial, lingual/palatal, and occlusal surfaces for each tooth in a sextant, and the highest score is recorded for the sextant. The cumulative scores are graded into four risk levels, including no risk (i.e. cumulative score ≤ 2); low risk (cumulative score = 3-8); medium risk (cumulative risk = 9-13) and high risk (cumulative risk ≥ 14). Individuals at low, medium or higher risk of erosive tooth wear require some preventive counselling and regular monitoring, whereas restorative management is generally reserved for selected high risk cases.

Bartlett et al. (2008) also indicated that the BEWE index would be ideal for screening subjects for erosive tooth wear research. However, the cut-off values for risk levels were based on experience of one of the authors, backed up by research observations, and further studies were recommended to validate the method against existing data in the literature. The authors also indicated that this process should eventually lead to the development of an internationally accepted, standardized and validated index.

It is envisaged that the BEWE index would be useful for anthropological studies on erosive tooth wear, but some modification will be required to include the assessment of attrition and abrasion. An international index specific for use in anthropological research would enable consistency in data collection and facilitate comparisons by different research groups throughout the world. Such a system would be an improvement on currently existing wear indices that do not reflect the causes of tooth wear, although inherent limitations of wear indices will still remain (as discussed in the previous section). Complementary information about the physical and chemical characteristics of tooth wear from emerging analytical tools available in the field are also needed.

Scanning confocal microscopy and fractal analysis

This is essentially a three-dimensional (3D) profilometry method of assessing tooth wear objectively and it overcomes some of the problems relating to subjectivity with scanning electron microscopy. It is capable of conducting sophisticated mathematical calculations compared with standard profilometry methods that quantify tooth wear in the form of depth or volume loss (Attin, 2006). Ungar et al. (2003) specifically developed this method to automate the characterization of the microwear details of a worn surface rather than manually counting the number of pits and scratches. Epoxy replicas of worn surfaces were scanned by using a white-light source in a scanning confocal microscope at a high resolution (0.18μm in

the x- and the y-axes and 0.008μm in the z-axis) to obtain a 3D model of the surface. Then, the 3D model was subjected to fractal modelling (analysis) that involved fitting a meshed surface of tiny equilateral triangles on the reconstructed surface. Fractal analysis is commonly used in creating complex 3D structures (such as mountains) in animated movies and has broad applications in biological science, including structural analysis of natural objects made of self-repeating geometry (for example, mountains, snowflakes and fern leaves).

The characteristics of the worn surface were assessed by measuring microwear complexity (i.e. surface roughness) and microwear directionality (i.e. surface anisotropy). Microwear complexity was quantified by measuring the variation in peak to valley distances across a surface, and microwear anisotropy was calculated by plotting the relative lengths of surface roughness vectors over a given area across a 0º to 180º scale at a 5º interval (Scott et al., 2005; Ungar et al., 2003). Surfaces with deeper pits and gouges corresponded to higher microwear complexity than smoother surfaces with finer striations, and surfaces with parallel scratch marks displayed higher degree of anisotropy than surfaces abraded evenly in different directions with hard seeds (Ungar et al., 2003).

In a landmark paper, Scott et al. (2005) validated this method to identify microwear patterns associated with different diets in two species of extant New World monkeys (primates), Alouatta palliata and Cebus apaella. The diet of A. palliata comprises greater amounts of tough, pliant foods (such as leaves that require shearing), whereas C. apaella consumes more hard, brittle seeds. There was a greater anisotropy for A. palliata and a greater microwear complexity for C. apaella, suggesting significant associations between wear anisotropy and pliant foods and between microwear complexity and brittle foods. The authors extended this method in dietary reconstruction of extinct hominins, including Australopithecus africanus (2.8 - 2.4 Mya) and Paranthropus robustus (1.9 – 1.5 Mya). The findings of greater anisotropy in A. africanus and greater microwear complexity in P. robustus provided support to previous hypotheses that the former consumed more tough, pliant foods and that the latter consumed more hard, brittle foods. However, a large variation and a considerable overlap in these features suggest some common diet, with seasonal fall-back on hard or pliant foods.

The application of this method to other hominins in recent studies has led to some interesting conclusions being drawn about their diet (Ungar et al., 2008; Ungar et al., 2011). For example, the Plio-Pleistocene hominin P. boisei (2.3 – 1.4 Mya), nicknamed the 'Nutcracker Man', had robust cranial features and large, flat and thickly enamelled posterior teeth that were thought to have been adapted to eat mechanically challenging food, but the microwear complexity and anisotropy values were lower than those expected for a hard-object feeder (Ungar et al., 2008). This finding has been confirmed in a new population of P. boisei (<1.8 Mya), implying that craniofacial and dental morphology can indicate what a hominin was capable of eating but not what it ate regularly (Ungar et al., 2011). Furthermore, the analysis of microwear texture between Homo erectus and Homo habilis has also revealed that the former consumed a harder diet than the latter (Ungar et al., 2011).

Overall, scanning confocal microscopy combined with fractal analysis have been shown to be a powerful method to discriminate microwear topographies between different diets. It has also been used to detect wear patterns on stone tools used on different materials (for

example, hide, shell and wood) (Stemp and Chung, 2011). This method requires some training but is user friendly and is usually more economical than most scanning electron microscopy analysis (personal communication with Prof Peter Ungar, University of Arkansas, USA). The selection of appropriate area for analysis is critical as reconstructed surface may have some outlying spikes, so care is needed in selecting the appropriate area for analysis. Because the data on microwear complexity and anisotropy are likely to discriminate wear patterns from attrition, abrasion and erosion, this method holds promise as a diagnostic tool for tooth wear in modern humans. Further research should be encouraged in exploring its clinical and anthropological applications in modern humans. Information obtained on microwear details should be complemented with studies investigating fundamental changes in the physical and chemical characteristics of tooth wear.

Nanohardness testing

The mechanical properties of tooth structure, including compressive and tensile strengths at both macro- and micro-levels, provide information about a tooth's fracture resisting properties and have some relevance to the understanding of tooth wear by attrition, abrasion, and erosion. Microhardness testing is a more reliable method in detecting the degree of tooth softening by erosive demineralization and of hardness recovery by remineralization (Maggio et al., 2010; Seow and Thong, 2005; Srinivasan et al., 2010; Tantbirojn et al., 2007). Microhardness testing involves indenting a polished tooth surface with diamond tips, such as a rhomboid Knoop indenter or a tetra-pyramidal Vickers indenter. The size of the indentation can be measured by using a microscope and correlates directly to the severity of erosion (Attin, 2006).

Nanohardness testing is a relatively new method that is similar to microindentation, but loads applied to make indentations are smaller. Conical or spherical indenter tips are available in different sizes, ranging from around 5μm to 1μm in diameter. A tip diameter of 5μm corresponds to that of an enamel prism, whereas finer tips can be used to obtain hardness values at different parts of an enamel prism. Nanohardness testing has been used by researchers to investigate biomechanical properties of sound tooth structure (Ge et al., 2005; Mahoney et al., 2000), carious tooth structure (Angker et al., 2004; Dickinson et al., 2007; Marshall et al., 2001) and eroded tooth structure (Barbour et al., 2003a; Lippert et al., 2004; Mahoney et al., 2003).

Nanoindentation is very sensitive to changes occurring during early stages of erosion. For example, previous in vitro studies employing microhardness indentation have tended to use longer periods of demineralization (ranging from 1 hour to 48 hours) to detect measurable enamel erosion (Rees et al., 2007; Tantbirojn et al., 2007) compared with nanohardness studies that typically erode enamel for around 2 to 10 minutes (Barbour et al., 2003b; Mahoney et al., 2003). Our own preliminary findings have shown that baseline nanohardness of enamel (4.70 ± 0.37 GPa, mean ± SD) decreases after a 10 minute erosion in white wine (at pH 3.5) (3.49 ± 0.28 GPa). Although these findings are interesting, the duration of erosion of around 10 minutes still does not simulate early stages of erosive demineralization in vivo.

Recently, White et al. (2010) conducted in vitro experiments to assess human enamel

softening using nanohardness measurements and profilometry under a single drink condition (from 2 to 60 seconds of enamel erosion with citric acid at pH 3.2). No salivary protection was provided to the enamel specimens in the study. Interestingly, the authors were able to detect enamel softening after 2 seconds of erosion whereas a measurable erosion depth by profilometry required erosion to be conducted for 20 minutes. The findings of this study have provided a realistic model of early enamel erosion, although further studies should be conducted with the inclusion of salivary protection in the experimental design.

Nanohardness testing holds promise for anthropological studies of erosion in modern humans. However, this is technically demanding and requires preparation of highly polished, flat specimens. This makes it unsuitable for direct measurement of erosion in vivo, but this problem can be overcome partly by conducting in situ studies. Although nanohardness testing provides information about the degree of surface softening during early demineralization, it is unable to clarify whether the softened layer is formed by a true softened sub-surface zone or a layer of loosely organized, porous and partially eroded apatite crystals (Hannig and Hannig, 2010). To clarify this matter, it seems logical to suggest that nanohardness measurements need to be conducted with qualitative methods such nano-computed tomography (nano CT).

Micro-computed tomography (micro CT) and nano-computed tomography (nano CT)

Micro-computed tomography is a sensitive technique that uses high-resolution x-ray slices of a hard material to create its three-dimensional model. The features of the rendered model are usually qualitative but, with careful experimental design, micro CT can provide quantitative information on structural dimensions and material density. It has been used to characterize carious lesions in fossils and archeological specimens (Rossi et al., 2004) as well as in modern humans (Huang et al., 2010; Swain and Xue, 2009). Recently, Jungers and Kaifu (2011) applied this technique to study wear features in mandibular teeth of Homo floresiensis (commonly known as the 'Hobbit'), whose recent discovery of skeletal remains in Indonesia has generated a great deal of controversy over its classification as a new species (Argue et al., 2006; Baab and McNulty, 2009; Henneberg and Schofield, 2008). The mandibular teeth displayed severe wear, with a complete loss of occlusal enamel mesiodistally between the thin buccal and lingual cusps. The lost enamel was replaced by a 'matt-white' lining that was interpreted by some researchers to be a dental filling (restoration) associated with endodontic treatment, implying that the specimen was a modern day human (Henneberg and Schofield, 2008). However, the analysis of a three-dimensional micro computed tomography (micro CT) reconstruction confirmed the absence of a dental restoration (Jungers and Kaifu, 2011).

Although micro CT reconstruction can provide a high resolution surface topography of an eroded surface, its resolution does not allow reconstruction of softened zone associated with an erosion lesion. This may change with the use of nano-computed tomography (nano CT). To date, only a handful of studies has been conducted to characterize the nanostructure of human dentine (Parkinson and Sasov, 2008) and bone (Muller, 2009). Since these studies were conducted, new, more powerful nano CT equipments (for example, UltraXRM-L200, X-Radia, California, USA) have been manufactured. Information from the manufacturer

indicates that this technique is capable of constructing 3D images of structures, such as bone (a mineralized, hard tissue) and a single osteoclast cell (a non-mineralized structure), at a resolution of 50nm. This technology also offers a new way of obtaining information about ultra-high resolution structural changes associated with early demineralization and remineralization.

Nano CT is a sophisticated technique and it is not surprising that the sample preparation is highly expensive and technically demanding. To scan a sample at a resolution of around 50nm, it has to be milled into a cylindrical shape of around 50μm length and 20μm diameter. Although its application is limited to in vitro, it is likely to provide insights into the fundamental mechanisms of tooth wear. To date, there are no reports of its application in studying early demineralization and remineralization of enamel or dentine and such studies should be given a high priority.

Mass spectrometry

Erosion and remineralization are essentially chemical processes that necessitate chemical analyses to be performed in order to gain an insight into the underlying mechanisms. There are well-established techniques, such as electron probe microanalysis (EPMA) and microradiography, suitable for the analysis of the chemical characteristics of carious lesions, but they cannot detect early mineral changes associated with erosion. Erosion research requires the application of more sensitive surface techniques, such as calcium sensitive electrode and atomic adsorption spectrophotometer to assess the changes in calcium content and phosphomolybdate-malacite green procedure to assess phosphorous content (Attin, 2006). Another method, secondary ion mass spectrometry (SIMS), is a sensitive technique capable of analysing a wider range of elements or molecules, and the application of time of flight - secondary ion mass spectrometry (TOF-SIMS) in erosion research will be discussed in more detail.

Tof-SIMS can detect concentrations of various elements and molecules from only a few atomic layers (1 to 2nm) of a sample. A pulsed primary ion beam consisting of metal ions, such as Caesium (Cs^+) and Gallium (Ga^+), is used to desorb and ionize species (for example, Ca, Mg, F and PO_4 ions) from the sample surface. The ionized species are then accelerated through an electric field (voltage difference) and are identified on the basis of the time taken to reach a detector. The duration of flight corresponds to the atomic or molecular weight of the ionized species.

TOF-SIMS has been used in detecting corrosion products from orthopaedic knee implants at the site of physical wear (Lewis and Heard, 2005; Lewis et al., 2005), and in mapping the mineral content of incipient carious lesions (Dickinson et al., 2007). Barbour and Rees (2004) described their findings of a preliminary study relating to its application in mapping calcium and magnesium of an eroded enamel surface. The detection sensitivity of TOF-SIMS has improved over the years, but its full potential in elucidating fundamental processes of erosion is yet to be explored.

The application of TOF-SIMS is limited to in vitro because of the need to prepare

highly polished, flat samples in an ultra-clean environment. However, its findings may help to explain some of the observations noted in other experimental, clinical and anthropological studies on dental erosion.

Conclusions

Various methods are now available to investigate the physical and chemical changes associated with tooth wear at the macro-, micro- and nano-levels both quantitatively and qualitatively. The BEWE index is an internationally standardized, visual method designed to assess macro-changes associated with erosive wear in clinical situations. A similar international index is desirable for the assessment of attrition and abrasion. Some of the limitations with wear indices, including subjective assessment, can be overcome by conducting objective assessment of microwear details using three-dimensional profilometry (such as scanning confocal microscopy combined with fractal analysis). This method has been found to be useful in dietary reconstruction of both extinct and extant primates and hominids. In order to develop a truly holistic approach to the understanding of tooth wear, it will be important to extrapolate findings from experimental wear studies investigating fundamental physical characteristics (for example, using nanohardness testing, micro CT and nano CT) and chemical characteristics (for example, using TOF-SIMS) associated with tooth wear.

Each method assesses a specific aspect of tooth wear, and one size does not fit all. To address the limitations inherent in each method, a combined assessment of tooth wear may be indicated depending on the scope of the project. As part of continued education, researchers and clinicians need to be aware of novel developments in the field of biomaterials and mineralized tissue research. Further developments in the field may enable us to better answer one of the most fundamental of questions, 'Why do teeth wear?'

Acknowledgements

The support of the Australian Dental Research Foundation Inc and Dentsply Australia Pty Ltd for our research on tooth wear is gratefully acknowledged.

References

Angker L., Nijhof N., Swain M.V., and Kilpatrick N.M. (2004) Influence of hydration and mechanical characterization of carious primary dentine using an ultra-micro indentation system (UMIS). European Journal of Oral Sciences, 112: 231-236.

Argue D., Donlon D., Groves C., and Wright R. (2006) Homo floresiensis: microcephalic, pygmoid, Australopithecus, or Homo? Journal of Human Evolution, 51: 360-374.

Attin T. (2006) Methods for assessment of dental erosion. Monographs in Oral Science, 20: 152-172.

Azzopardi A., Bartlett D.W., Watson T.F., and Smith B.G. (2000) A literature review of the techniques to measure tooth wear and erosion. European Journal of Prosthodontics and Restorative Dentistry, 8: 93-97.

Baab K.L. and McNulty K.P. (2009) Size, shape, and asymmetry in fossil hominins: the

status of the LB1 cranium based on 3D morphometric analyses. Journal of Human Evolution, 57: 608-622.

Barbour M.E., Parker D.M., Allen G.C., and Jandt K.D. (2003a) Human enamel dissolution in citric acid as a function of pH in the range 2.30 ≤ pH ≤ 6.30 — a nanoindentation study. European Journal of Oral Sciences, 111: 258-262.

Barbour M.E., Parker D.M., and Jandt K.D. (2003b) Enamel dissolution as a function of solution degree of saturation with respect to hydroxyapatite: a nanoindentation study. Journal of Colloid and Interface Science, 265: 9-14.

Barbour M.E. and Rees J.S. (2004) The laboratory assessment of enamel erosion: a review. Journal of Dentistry, 32: 591-602.

Bartlett D. and Dugmore C. (2008) Pathological or physiological erosion — is there a relationship to age? Clinical Oral Investigations, 12 (Suppl 1): S27-S31.

Bartlett D., Ganss C., and Lussi A. (2008) Basic Erosive Wear Examination (BEWE): a new scoring system for scientific and clinical needs. Clinical Oral Investigations, 12 (Suppl 1): S65-S68.

Begg P.R. (1954) Stone age man's dentition. American Journal of Orthodontics, 40: 298-312, 373-383, 462-475, 517-531.

Campbell T.D. (1925). Dentition and palate of the Australian Aborigine. Hassell Press, Adelaide.

Cash R.C. (1988) Bruxism in children: review of the literature. Journal of Pedodontics, 12: 107-127.

Dickinson M.E., Wolf K.V., and Mann A.B. (2007) Nanomechanical and chemical characterization of incipient in vitro carious lesions in human dental enamel. Archives of Oral Biology, 52: 753-760.

Eisenburger M. and Addy M. (2002) Erosion and attrition of human enamel in vitro part I: interaction effects. Journal of Dentistry, 30: 341-347.

Eisenburger M., Shellis R.P., and Addy M. (2004) Scanning electron microscopy of softened enamel. Caries Research, 38: 67-74.

Ganss C., Klimek J., and Borkowski N. (2002) Characteristics of tooth wear in relation to different nutritional patterns including contemporary and medieval subjects. European Journal of Oral Sciences, 110: 54-60.

Ganss C. (2008) How valid are current diagnostic criteria for dental erosion? Clinical Oral Investigations, 12 (Suppl 1): S41-49.

Ge J., Cui F.Z., Wang X.M., and Feng H.L. (2005) Property variations in the prism and the organic sheath within enamel by nanoindentation. Biomaterials, 26: 3333-3339.

Gjorgievska E. and Nicholson J.W. (2011) Prevention of enamel demineralization after tooth bleaching by bioactive glass incorporated into toothpaste. Australian Dental Journal, 56: 193-200.

Grine F.E., Ungar P.S., and Teaford M.F. (2002) Error rates in dental microwear quantification using scanning electron microscopy. Scanning, 24: 144-153.

Hannig M. and Hannig C. (2010) Nanomaterials in preventive dentistry. Nature Nanotechnology, 5: 565-569.

He L.H. and Swain M.V. (2007) Enamel — a 'metallic-like' deformable biocomposite. Journal of Dentistry, 35: 431-437.

Henneberg M. and Schofield J. (2008) The Hobbit trap. Wakefield Press, Adelaide.

Holbrook W.P. and Ganss C. (2008) Is diagnosing exposed dentine a suitable tool for grading erosive loss? Clinical Oral Investigations, 12 Suppl 1: S33-39.

Huang T.T., He L.H., Darendeliler M.A., and Swain M.V. (2010) Correlation of mineral density and elastic modulus of natural enamel white spot lesions using X-ray microtomography and nanoindentation. Acta Biomaterialia, 6: 4553-4559.

Jungers W.L. and Kaifu Y. (2011) On dental wear, dental work, and oral health in the type specimen (LB1) of Homo floresiensis. American Journal of Physical Anthropology, 145: 282-289.

Kaidonis J.A., Townsend G.C., and Richards L.C. (1992) Abrasion: an evolutionary and clinical view. Australian Prosthodontic Journal, 6: 9-16.

Kaidonis J.A., Richards L.C., and Townsend G.C. (1993) Nature and frequency of dental wear facets in an Australian aboriginal population. Journal of Oral Rehabilitation, 20: 333-340.

Kaidonis J.A. (2008) Tooth wear: the view of the anthropologist. Clinical Oral Investigations, 12 (Suppl 1): S21-S26.

Kaifu Y., Kasai K., Townsend G.C., and Richards L.C. (2003) Tooth wear and the 'design' of the human dentition: a perspective from evolutionary medicine. American Journal of Physical Anthropology, 37 (Suppl): 47-61.

King S.J., Arrigo-Nelson S.J., Pochron S.T., Semprebon G.M., Godfrey L.R., Wright P.C., and Jernvall J. (2005) Dental senescence in a long-lived primate links infant survival to rainfall. Proceedings of the National Academy of Sciences of the United States of America, 102: 16579-16583.

Kinney J.H., Marshall S.J., and Marshall G.W. (2003) The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. Critical Reviews in Oral Biology and Medicine, 14: 13-29.

Lee-Thorp J. and Sponheimer M. (2006) Contributions of biogeochemistry to understanding hominin dietary ecology. American Journal of Physical Anthropology (Suppl 43): 131-148.

Lewis A.C. and Heard P.J. (2005) The effects of calcium phosphate deposition upon corrosion of CoCr alloys and the potential for implant failure. Journal of Biomedical Materials Research: Part A, 75: 365-373.

Lewis A.C., Kilburn M.R., Papageorgiou I., Allen G.C., and Case C.P. (2005) Effect of synovial fluid, phosphate-buffered saline solution, and water on the dissolution and corrosion properties of CoCrMo alloys as used in orthopedic implants. Journal of Biomedical Materials Research: Part A, 73: 456-467.

Li H., Liu M.C., Deng M., Moazzez R., and Bartlett D.W. (2011) An experiment on the attrition of acid demineralized dentine in vitro. Australian Dental Journal, 56: 63-67.

Lippert F., Parker D.M., and Jandt K.D. (2004) Susceptibility of deciduous and permanent enamel to dietary acid-induced erosion studied with atomic force microscopy

nanoindentation. European Journal of Oral Sciences, 112: 61-66.
Lorkiewicz W. (2011) Nonalimentary tooth use in the neolithic population of the Lengyel culture in central Poland (4600-4000 BC). American Journal of Physical Anthropology, 144: 538-551.
Lucas P., Constantino P., Wood B., and Lawn B. (2008) Dental enamel as a dietary indicator in mammals. Bioessays, 30: 374-385.
Lussi A. (2006) Erosive tooth wear — a multifactorial condition of growing concern and increasing knowledge. Monographs in Oral Science, 20: 1-8.
Maggio B., Guibert R.G., Mason S.C., Karwal R., Rees G.D., Kelly S., and Zero D.T. (2010) Evaluation of mouthrinse and dentifrice regimens in an in situ erosion remineralisation model. Journal of Dentistry, 38 (Suppl 3): S37-44.
Mahoney E., Holt A., Swain M., and Kilpatrick N. (2000) The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study. Journal of Dentistry, 28: 589-594.
Mahoney E., Beattie J., Swain M., and Kilpatrick N. (2003) Preliminary in vitro assessment of erosive potential using the ultra-micro-indentation system. Caries Research, 37: 218-224.
Mahoney P. (2006) Dental microwear from Natufian hunter-gatherers and early Neolithic farmers: comparisons within and between samples. American Journal of Physical Anthropology, 130: 308-319.
Mann A.B. and Dickinson M.E. (2006) Nanomechanics, chemistry and structure at the enamel surface. Monographs in Oral Science, 19: 105-131.
Manton D.J., Cai F., Yuan Y., Walker G.D., Cochrane N.J., Reynolds C., Brearley-Messer L.J., and Reynolds E.C. (2010) Effect of casein phosphopeptide-amorphous calcium phosphate added to acidic beverages on enamel erosion in vitro. Australian Dental Journal, 55: 275-279.
Marshall G.W., Jr., Balooch M., Gallagher R.R., Gansky S.A., and Marshall S.J. (2001) Mechanical properties of the dentinoenamel junction: AFM studies of nanohardness, elastic modulus, and fracture. Journal of Biomedical Materials Research, 54: 87-95.
Meurman J.H., Drysdale T., and Frank R.M. (1991) Experimental erosion of dentin. Scandinavian Journal of Dental Research, 99: 457-462.
Meurman J.H. and Sorvari R. (2000) Interplay of erosion and abrasion in tooth wear and possible approaches to prevention. In: Addy M., Embery G., Edgar W., and Orchardson R. (Eds.), Tooth wear and sensitivity: clinical advances in restorative dentistry. Martin Dunitz Ltd, London, pp. 171-180.
Molnar S. (1972) Tooth wear and culture: a survey of tooth functions among some prehistoric populations. Current Anthropology, 13: 511-526.
Muller R. (2009) Hierarchical microimaging of bone structure and function. Nature Reviews Rheumatology, 5: 373-381.
Parkinson C.R. and Sasov A. (2008) High-resolution non-destructive 3D interrogation of dentin using X-ray nanotomography. Dental Materials, 24: 773-777.
Pickles M.J. (2006) Tooth wear. Monographs in Oral Science, 19: 86-104.

Ranjitkar S., Kaidonis J.A., Townsend G.C., Vu A.M., and Richards L.C. (2008) An in vitro assessment of the effect of load and pH on wear between opposing enamel and dentine surfaces. Archives of Oral Biology, 53: 1011-1016.

Ranjitkar S., Kaidonis J.A., Richards L.C., and Townsend G.C. (2009a) The effect of CPP-ACP on enamel wear under severe erosive conditions. Archives of Oral Biology, 54: 527-532.

Ranjitkar S., Narayana T., Kaidonis J.A., Hughes T.E., Richards L.C., and Townsend G.C. (2009b) The effect of casein phosphopeptide-amorphous calcium phosphate on erosive dentine wear. Australian Dental Journal, 54: 101-107.

Rees J., Loyn T., and Chadwick B. (2007) Pronamel and Tooth Mousse: an initial assessment of erosion prevention in vitro. Journal of Dentistry, 35: 355-357.

Rossi M., Casali F., Romani D., Bondioli L., Macchiarelli R., and Rook L. (2004) MicroCT Scan in paleobiology: application to the study of dental tissues. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 213: 747-750.

Roveri N., Battistella E., Bianchi C.L., Foltran I., Foresti E., Iafisco M., Lelli M., Naldoni A., Palazzo B., and Rimondini L. (2009) Surface enamel remineralization: Biomimetic apatite nanocrystals and fluoride ions different effects. Journal of Nanomaterials, 746383.

Scott R.S., Ungar P.S., Bergstrom T.S., Brown C.A., Grine F.E., Teaford M.F., and Walker A. (2005) Dental microwear texture analysis shows within-species diet variability in fossil hominins. Nature, 436: 693-695.

Seow W.K. and Thong K.M. (2005) Erosive effects of common beverages on extracted premolar teeth. Australian Dental Journal, 50: 173-178.

Smith A.J., Scheven B.A., Takahashi Y., Ferracane J.L., Shelton R.M., and Cooper P.R. (2012) Dentine as a bioactive extracellular matrix. Archives Oral Biology, 57: 109-121.

Smith B.G. and Knight J.K. (1984) An index for measuring the wear of teeth. British Dental Journal, 156: 435-438.

Springbett S., Townsend G.C., Kaidonis J., and Richards L. (1999) Tooth wear in the deciduous dentition: a cross-cultural and longitudinal study. Perspectives in Human Biology, 4: 93-101.

Srinivasan N., Kavitha M., and Loganathan S.C. (2010) Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: An in situ study. Archives of Oral Biology, 55: 541-544.

Stemp W.J. and Chung S. (2011) Discrimination of surface wear on obsidian tools using LSCM and RelA: pilot study results (area-scale analysis of obsidian tool surfaces). Scanning, 33: 279-293.

Swain M.V. and Xue J. (2009) State of the art of Micro-CT applications in dental research. International Journal of Oral Science, 1: 177-188.

Taji S. and Seow W.K. (2010) A literature review of dental erosion in children. Australian Dental Journal, 55: 358-367.

Tantbirojn D., Huang A., Ericson M.D., and Poolthong S. (2007) Change in surface hardness

of enamel by a cola drink and a CPP-ACP paste. Journal of Dentistry, 36: 74-79.
Ungar P. (2008) Materials science: strong teeth, strong seeds. Nature, 452: 703-705.
Ungar P.S., Brown C.A., Bergstrom T.S., and Walkers A. (2003) Quantification of dental microwear by tandem scanning confocal microscopy and scale-sensitive fractal analyses. Scanning, 25: 185-193.
Ungar P.S. (2005) Reproductive fitness and tooth wear: milking as much as possible out of dental topographic analysis. Proceedings of the National Academy of Sciences of the United States of America, 102: 16533-16534.
Ungar P.S., Grine F.E., Teaford M.F., and El Zaatari S. (2006) Dental microwear and diets of African early Homo. Journal of Human Evolution, 50: 78-95.
Ungar P.S., Grine F.E., and Teaford M.F. (2008) Dental microwear and diet of the Plio-Pleistocene hominin Paranthropus boisei. PLoS One, 3: e2044.
Ungar P.S., Krueger K.L., Blumenschine R.J., Njau J., and Scott R.S. (2011) Dental microwear texture analysis of hominins recovered by the Olduvai Landscape Paleoanthropology Project, 1995-2007. Journal of Human Evolution, in press (doi:10.1016/j.jhevol.2011.04.006).
White A.J., Yorath C., ten Hengel V., Leary S.D., Huysmans M.C., and Barbour M.E. (2010) Human and bovine enamel erosion under 'single-drink' conditions. European Journal of Oral Sciences, 118: 604-609.
Yamagishi K., Onuma K., Suzuki T., Okada F., Tagami J., Otsuki M., and Senawangse P. (2005) Materials chemistry: a synthetic enamel for rapid tooth repair. Nature, 433: 819.

Correspondence

Doctor Sarbin Ranjitkar
School of Dentistry, The University of Adelaide
Adelaide, South Australia, 5005
E-mail: sarbin.ranjitkar@adelaide.edu.au