DENTAL MORPHOLOGY ’98

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Foreword

Hyvät Ystävät, Hyvät Naiset ja Herrat

Now, as we have reached the time to initiate the 11th International Symposium on Dental Morphology, I am most happy to have you here in Oulu, Finland and I hope that you will enjoy every minute, every aspect of symposium science as well as the social atmosphere.

The very nature, the core, of the symposium scientific substance has been from the beginning over thirty years ago, and still is, its multidisciplinary approach. Among others, paleontologists, histologists, oral biologists, archaeologists, anthropologists, zoologists, anatomists, geneticists and dentists have come together for informative talks and an exchange of ideas, having the same common interest, the exploration of tooth morphology. The range of the study species has been, and is, enormously large, from fossil forms of life to modern man. Personally, I have found this multidisciplinary approach to be very stimulating and fruitful; it has opened new avenues at many levels of the research process. By the same token, in a social context, the symposium has connected people from different disciplines, which given the state of things in these times of superspecialization has an exhilarating effect. Here in our eleventh symposium we have participants from nineteen countries; scientists from Australia, Belgium, Canada, Czech Republic, Croatia, France, Germany, India, Israel, Italy, Japan, New Zealand, Norway, Poland, South Africa, Sweden, United Kingdom, United States of America and Finland. A total of 84 reports will be delivered during the symposium.

In an historical sense we also remember that the art of science has since ancient times brought people together in a peaceful co-existence independent of nationality, race or other sometimes divisive factors. Even today, in spite of huge advancements in communication technology in general there still exist individual paths within our global scientific community to find creative contacts.

From the beginning of the Symposium a man from the University of Chicago, among others, was a driving force in creating, developing and maintaining it; the late Dr. Albert A. Dahlberg. His wife Thelma has also been keenly engaged in scientific activities and today I am glad to tell you that Mrs. Albert A. Dahlberg is with us. Also, I would like to introduce you our distinguished keynote speakers; Professors Percy Butler, Edward Kollar, Hervé Lesot and Steinar Risnes. To everyone here, I bid you very welcome to Oulu, with all the best wishes for an enjoyable and memorable Symposium.

With these words I, as President of the Symposium, opened the proceedings. Now, it is my honour to do the same for the published proceedings. The editors have structured the volume to reflect as closely as possible the flavour of the meetings; the papers are in approximately the same order as at the meeting and this Foreword as well as the first paper by Mayhall have been retained to allow the reader to feel as if he/she were a participant. I want to thank the authors of the 55 papers included in this volume who took the time to revise their presentations for publication. It is my sincere wish that you, the reader, will find as much stimulation and new knowledge in this volume as I and the other participants found at the meeting.

Lassi Alvesalo

Introduction

You may wonder about the title of this volume. As you will read in one of our papers, the International Symposia of Dental Morphology have been held eleven times over the last thirty years and in almost every case a proceedings volume has resulted. Unfortunately, the volumes have had differing titles and publishers. This has created a situation where a researcher wanting to follow the development of dental morphology needs to have extrasensory perception to locate some of the volumes. In collaboration with Alan Brook, who will be organizing the next symposium, and taking the example of the *Circumpolar Health* volumes, we have begun the process of using a common name for each of the subsequent volumes. (The titles of past volumes are noted in the paper entitled, “Thirty Years Old and Still Not Organized: the International Dental Morphology Symposia” in this volume.) We hope that this will simplify searching by future researchers who may want to follow the progress of dental morphological studies.

This new title implies that these volumes reveal the state of dental morphological studies, a bold assertion but one that we believe is accurate. Since the initiation of the triennial symposia in 1968 it is fair to state that most of the leading researchers in the field have attended the meetings, always held in Europe or the Middle East, and that an analysis of the papers presented in the past, and present, volumes reveals the leading edge of dental morphological research. What impresses us is the wide variety of papers and expertise that we find at the symposia and the subsequent volumes; anthropologists, histologists, oral biologists, geneticists, archaeologists, palaeontologists, anatomists…. A perusal of the present volume will reveal that “dental morphology” means many things to many people and we applaud this wide diversity of meanings and expertise. The symposia bring together many who would not otherwise meet at the annual meetings of the particular organization with which one identifies. The participants have often noted that the symposia are the only meetings they attend regularly and in which they sit through all the papers, many in areas of interest that we would not normally attend.

You may be surprised at the rather long list of contributors and their addresses. We have included these because of one of the guiding principles adopted at the first Symposium; it was agreed that the symposia and the resulting volumes would act as a vehicle, “to build upon each succeeding session by interchange and correspondence….”.

We hope that you will feel free to contact the authors to initiate an exchange that will lead to fruitful collaborations. In this way the symposia can continue between actual meetings and set the stage for the next meeting.

Rather than summarizing the contents of *Dental Morphology '98* here, we invite you to dip into the volume wherever your interests take you but while you are doing this why not read a paper or two outside your "area"? We hope that this collection of papers, most of the papers presented at the meeting, will convince you that dental morphology in its widest sense is a dynamic field that through the efforts of a wide variety of researchers is helping to unravel the mystery of the shapes of teeth.

We want to thank the "president" of the symposium, Professor Lassi Alvesalo (there is no organization of "dental morphologists" nor are there officers of a scientific body; the organizing committee is headed by whomever agrees to host the meeting), for providing the participants with not only an outstanding venue for the meetings but for patiently listening to our sometimes crazy requests. Oulu, Finland and the University of Oulu are dynamic entities in a beautiful setting conducive to not only academic discussions but also to the enjoyment of the special environment that is Finland. He and his staff endured long hours to allow for a meeting that was "transparent"; everything was so well organized that we didn't notice. This was the second meeting in Finland and Lassi has been involved in the organization of both. Our sincerest thanks to the staff of the Institute of Dentistry, University of Oulu as well. We look forward to the next meeting in Sheffield, England in 2001 and we hope that many of you, students, professors and/or researchers, that haven't attended any of the previous meetings will make an effort to participate in the 12th International Symposium on Dental Morphology.

Very seldom do authors thank those who probably contribute more to our success than any other institution, our home universities. The University of Toronto and the University of Oulu have provided space, time and encouragement to us to allow us to undertake the editing of this volume. We are most grateful for their support.

John T. Mayhall and Tuomo Heikkinen.

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Thirty Years Old and Still Not Organized: the International Dental Morphology Symposia

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THE BEGINNINGS OF THE SYMPOSIA

How do write about an "organization" that is not organized nor has any members? The closest that the International Dental Morphology Symposia have come to having an organization have been the different organizing committees for each of the eleven Symposia that have made an appearance in Europe, Scandinavia and the Middle East. (The group has only flirted with a meeting in North America but has yet to take the plunge.) How did this remarkable series of symposia begin? A. A. Dahlberg (1967), writing in a special issue of the Journal of Dental Research (Pedersen et al., 1967) where the papers from the first Symposium were published, recalls that the nidus for the Symposia was himself and P.O. Pedersen with the subsequent addition of Verner Alexandersen. Before the first meeting in 1965 there had been consistent dental sessions at the meetings of the American Association of Physical Anthropologists, and the Society for the Study of Human Biology had had two meetings about the study of mammalian dentitions. The London meeting of the S.S.H.B. culminated in the volume, *Dental Anthropology,* edited by Don Brothwell (1963)

The first meeting in Fredensborg, Denmark was attended by about 65 participants. The first day dealt with the phylogenetic aspects of the dentition; the second day covered ontogenetic aspects with the final day introducing dental morphology and genetics. The three organizers must have laboured extremely hard since they were able to circulate, a month in advance, a 400 page folio containing 65 papers. This folio was an assemblage of reports, abstracts, contributions and previously published materials that were germane to the subjects covered in Denmark. Dahlberg (1967) concludes his introduction to the first volume to come from the Symposia with," At the close of the session, it was unanimously voted to have another symposium of the same composition and character 3 years hence". At the 11^{th} Symposium we still have followed the same general topics; a great tribute to the foresight of the first organizers. The numbers have grown over the years but dental morphology has always been considered in its widest sense. This has led to an important appreciation of the contributions of palaeontologists, embryologists, anatomists, geneticists and odontologists as well as allowing the interplay of ideas from individuals that would not normally come into contact with each other. The first photo (Figure 1) of the participants of the Danish symposium includes some of the participants at the eleventh Symposium that is the basis of this volume.



Fig. 1. Participants at the 1st International Symposium on Dental Morphology, 1965, Fredensborg, Denmark. Professors P.O. Pedersen, A.A. Dahlberg and Verner Alexandersen, organizers.

It was decided at the first meeting in Denmark that there would be no formal organization involving election of officers or collection of annual dues. How many other groups do you know that voted themselves out of existence before they even existed? But an “organization” does exist in a curious way. Each organizing committee has a mailing list of those who attended the previous meeting and this forms the basis of the next Symposium’s “invitees”. One of the shortcomings of this method may be that some who could benefit and contribute to a particular discussion do not know of the existence of the symposia or may have been inadvertently excluded. (Everyone with an interest in dental morphology in the widest sense is not only welcome but urged to participate in the meetings.)

The strength of the Symposia is the fact that we do not attend the meetings to vote for officers or to decide how our dues will be used; we are here because as Dr. Dahlberg wrote , “…it was decided to have no formal organization involving election of officers or collection of annual dues.” Fine, but what has this meant for the Symposia? It means, in Dahlberg’s words,”…the essential focus of the symposia was to build upon each succeeding session by interchange and correspondence of active individuals of previous meetings.” Was this effort to concentrate on communication, scientific investigation and collaboration a success? I think it is fair to state that it is a model for other symposia.

SUBSEQUENT SYMPOSIA

The second meeting was at Royal Holloway College outside London. Percy Butler hosted that meeting in intimate Victorian surroundings that leant itself to a camaraderie that has persisted. My first meeting was the one at Royal Holloway College and I was awed, as a student, to not only meet the “important” people in my chosen field but to hear them hotly discussing many topics. Nothing was held back and there was, as the newspapers describe rambunctious meetings of heads of state, “a full and frank discussion”. I still remember two embryologists hotly discussing whether it was the mesoderm or the ectoderm that “called the shots” when it came to deciding on the form of a tooth. The first of the “stand alone” books emanated from this meeting and was edited by Dahlberg (1971).

The Brussels meeting, the third one, was one that did not produce a published volume but allowed many of us to become more familiar with the work of Brabant and his co-workers. I am sorry that we don’t have a record of this Symposium but in some ways this was atoned for by the volume that came from the succeeding meeting in Cambridge. The volume edited by Ken Joysey and Percy Butler (1978) contained 24 excellent papers. Many of the papers from this volume were controversial and thought provoking; maybe, this was part of what those at the first symposium wanted; interchange and correspondence between participants. After Cambridge we moved to Turku where, in an ideal setting arranged by Pentti Kirveskari and Lassi Alvesalo, we met at a small hotel among the sea and woods. It was hard to stay inside and attend the sessions with this beautiful land and seascape enticing us to sample more of the hospitality of Finland. We were fortunate to have Bjorn Kurtén assume the editorship of a volume resulting from this meeting (1982).

Keeping our location in Scandinavia we next met in Iceland where Gudjon Axelsson arranged a great meeting site in Reykjavik. He not only organized a good meeting but showed us the beautiful country of Iceland and introduced us to “Black Death”, a brandy that was so strong that it ate through the plastic cups in which it was served as we stood beside a bus looking at the now blurry landscape. Once this “problem” was realized we scientifically (by multiple tests) determined that one must drink the Branavin immediately upon it being served. No volume emanated from this meeting possibly because of everyone’s addled brains after the “Black Death”. In 1986 we moved to Paris where J-P Santoro, who had surprised many in Reykjavik by inviting us from the “wilds” of Iceland to the sophistication of the capital of France, made Paris and the meetings a “must see”. In the subsequent volume the Russells and Santoro, the editors, (1988) provided a reminder of the latitude of dental morphology.

> Contrary to what their titles would lead one to believe, these symposia,…, greatly exceed the limits of merely dental morphology, as they have brought together specialists of numerous disciplines: embryology, genetics, histology, palaeontology, physiology and zoology. Indeed, teeth lend themselves eminently well to multidisciplinary studies, their multiple properties rendering them organs of unique quality; and it is not the least of the interests of these symposia that they permit the confrontation of data that would otherwise be restricted to specialized journals.”

After Paris it was on to Jerusalem where Pat Smith smiled through all our odd requests to not only participate in the symposium sessions but to tour the Holy City and the rest of Israel. Pat and Eitan Tchernov edited the volume (1992) from this meeting. One of the touching things about this volume was the obituary for Shirley Glasstone, an attendee since the beginning. Shirley was one who always added a touch of class and humour to the symposia.

The last two symposia have been on the continent. First, there was a meeting in the centre of Florence. Bruno Chiarelli and Jacopo Moggi-Cecchi organized a very stimulating meeting with over 75 participants. The meetings were continuing to grow but we have been lucky that the number of participants and papers presented has allowed for single sessions. Jacopo edited the Florence papers (1995) and we looked forward to a new venue, Berlin, in 1995. For the first time since the initial meeting the papers for discussion and presentation were published and available at the meeting. Ralf Radlanski produced not only an interesting and intense meeting but along with Herbert Renz published the “PREceedings” which contained 95 papers (1995). Ralf seemed to do what no one else had done before; he held us to a tight publishing schedule and forced us to be succinct in our papers.

THE PRESENT SYMPOSIUM

Now with the 11th Symposium there is a long heritage of 33 years of hard work by many organizers. Who would have suspected that a non-organization such as this could exist for a third of a century and still be as anticipated and vibrant as the meetings have been, are and will be? We are indebted to all the organizers of the past meetings and especially to Drs. Alexandersen, Dahlberg and Pedersen for their legacy of bringing together scientists from throughout the world and from various disciplines to discuss “dental morphology’, which as has been noted earlier is a much too restrictive term for our wide ranging discussions.

I have avoided including the names of all those who have consistently participated in these Symposia because I know that I would omit someone. The photo of the 11th Symposium (Figure 2) includes some that have attended all or nearly all of the previous Symposia as well as many “new” recruits. Rather than dwell on the past, even though it has provided us with a forum for our meetings, I want to acknowledge the participants who have presented their papers at the meeting in Oulu and who have contributed to the present volume. This volume will, hopefully, begin a new tradition of a consistent title that will make it easier to locate the volumes that emanate from the symposia. I look forward to the next meeting and am happy to state that I have been attending meetings since 1968. These have energized my research and given me an opportunity to meet the leaders in the amazingly wide field of dental morphology. We have many to thank over the past years but I believe that we should congratulate all of the participants and, especially, our founders for maintaining the interest in our non-organization. May there be more meetings like these!

PREVIOUS PROCEEDINGS VOLUMES

There have been eight volumes that contained the proceedings for all but two of the symposia. To aid your search for past papers, I have listed the editors and dates; Pedersen et al. in 1967, Dahlberg in 1971, Butler and Joysey in 1978, Kurtén in 1982, Russell et al. in 1988, Smith and Tchernov in 1992, Moggi-Cecchi in 1995 and Radlanski and Renz also in 1995. The complete citations are in the References below.



Fig. 2. Participants at the 11th International Symposium on Dental Morphology, 1998, Oulu, Finland. Professor Lassi Alvesalo, organizer.

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DENTAL ANTHROPOLOGY

The Relation of Cusp Development and Calcification to Growth

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More than 40 years have passed since I reviewed the ontogeny of the molar pattern (Butler 1956). Great progress has since been made on the molecular and cellular aspects of tooth development, especially the earliest stages, but not so much on the morphological organization of the tooth germ. Dental morphologists have been mainly concerned with evolutionary and phylogenetic problems, and they have paid less attention to the ontogenetic processes whereby teeth acquire their shapes.

Comparative anatomy shows that there is no constant relation between cusps and roots; a cusp can occupy different positions on the crown, depending upon the relative development of the surrounding cusps. Thus, in insectivores, according to the size of the protocone, the paracone can be buccal, intermediate or lingual in position (Butler 1996). In 1956 I postulated that there are two components of tooth growth. On the one hand there is growth of the inner dental epithelium, which folds within a space restricted by the follicle. On the other hand growth of the mesenchyme pushes out the follicle in specific directions, three in maxillary molars, two in mandibular molars, related to the future position of the roots. Mesenchymal growth determines the shape of the crown as seen in occlusal view; epithelial growth determines the arrangement of cusps within that shape.

Cusp proportions differ from tooth to tooth within the series; they also vary individually in each tooth. I studied this variation in mandibular teeth from the 4th century Romano-British cemetery at Poundbury, Dorset, preserved in the Anthropology Department of the Natural History Museum, London (Farwell and Molleson 1993). The lengths of the trigonid and talonid were measured on the buccal and lingual sides of three adjacent teeth: the two deciduous molars (DM1, DM2) and the first permanent molar (M1) (Fig. 1). The proportionate length of the talonid is most variable in DM1, where it reflects the variable level of molarisation (Butler 1988). On M1 a short talonid is associated with reduction of the hypoconulid and approach of the pattern towards that characteristic of M2.

On all the teeth the coefficients of variation of the trigonid and talonid were significantly higher than those of the length and width of the whole tooth (Fig. 2). The correlations between trigonid and talonid lengths were negative or not significant. It thus appears that partition of the tooth between trigonid and talonid is independent of tooth size, as if the mesial and distal regions of the epithelium were competing for space controlled by growth of the mesenchyme.

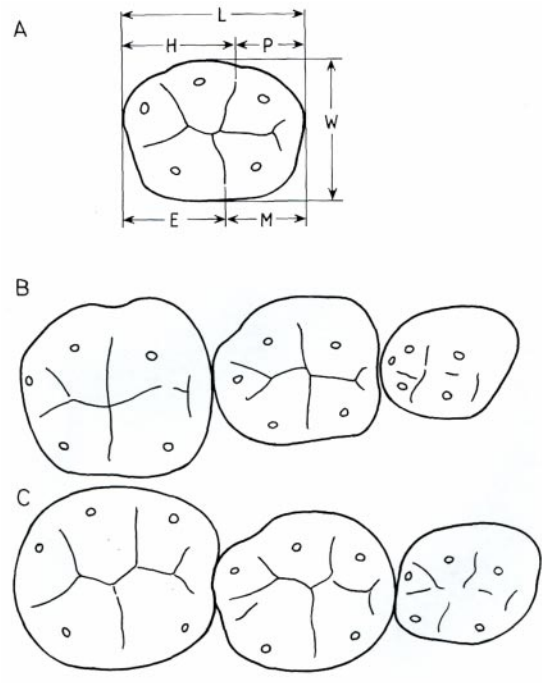


Fig. 1. A, scheme of measurements; B, C, left M1, DM2 and DM1 of two individuals, showing variable talonid/ trigonid proportions. Mesial to right, buccal above.

This however does not take account of heterochrony during ontogeny. The talonid is retarded in its development, relatively to the trigonid (Kraus and Jordan 1965; Butler 1968, 1971). At a stage when the protoconid is acute and the metaconid is dome-shaped, the talonid is represented only by the flattened distal part of the epithelium (Fig. 3). In its subsequent development. the talonid of DM1 is more retarded than those of DM2 and M1. Its cusps are frequently poorly differentiated even when the protoconid begins to calcify, and it grows in length more slowly than the trigonid. On DM2 and M1 the talonid develops more rapidly and it overtakes the trigonid in length. This difference suggests that the talonid of DM1 develops from a smaller amount of original material. One could imagine that at the cap stage the epithelium becomes partitioned between prospective trigonid and talonid, and that molarisation factors, weaker on DM1 than on DM2, increase the proportion of talonid. Tooth germs of DM1 show some variation in talonid development, relatively to that of the trigonid, foreshadowing the variable molarisation of' the completed teeth.

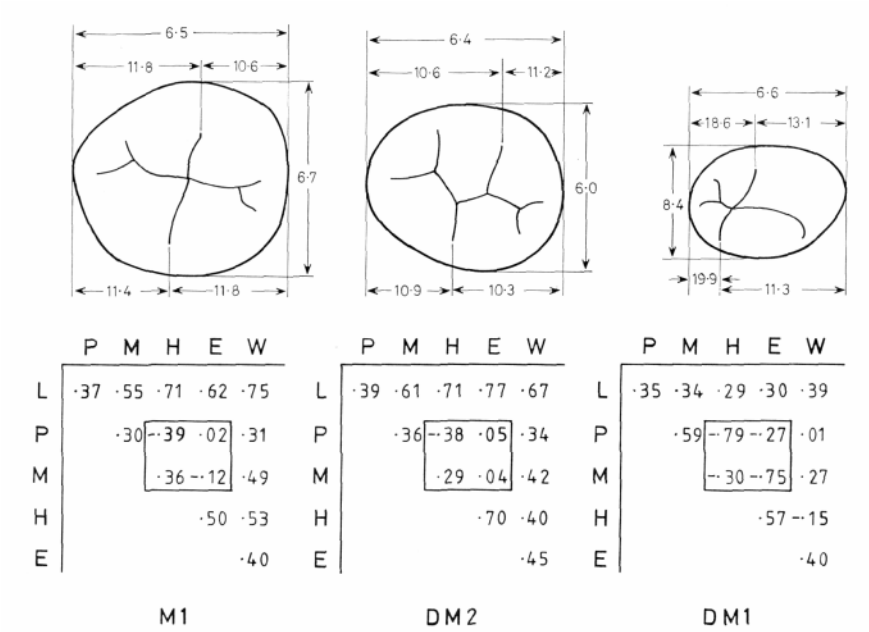


Fig. 2. Above, coefficients of variation. n= 40 (M1); 35 (DM2); 38 (DM1). Below, correlation matrix of the measurements. Trigonid-talonid correlations in box.

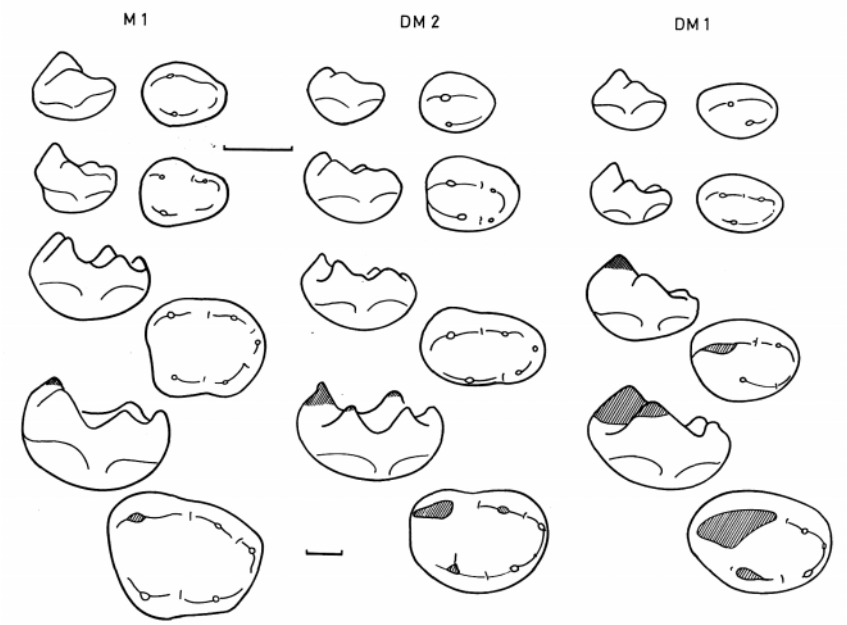


Fig. 3. Comparable stages of development of right Ml, DM2 and DM1. Scale bar = 1 mm. The top row is at twice the magnification of the rest.

During a visit in 1965-6 to Kraus's laboratory in Pittsburgh I made camera lucida drawings of numerous tooth germs, dissected from foetuses, which form the basis of Kraus and Jordan's book (1965). Using these drawings I measured trigonid and talonid lengths on DM1, DM2 and M1, corresponding to the measurements on the Poundbury teeth. The lengths were plotted against protoconid height. This was chosen as abscissa because its growth continues throughout crown development, and it has a rectilinear regression against the crown-rump length of the foetus (Butler, 1992).

The graph (Fig. 4) shows that there are two phases of growth. Trigonid and talonid lengths grow at constant relative rates until the crown height reaches about half of its final value, after which growth in length is slowed. The transition takes place, not at the beginning of calcification, but at about the time when the calcified cusps begin to unite. Growth is then restricted to the remaining intercuspal valleys and the crown margins.

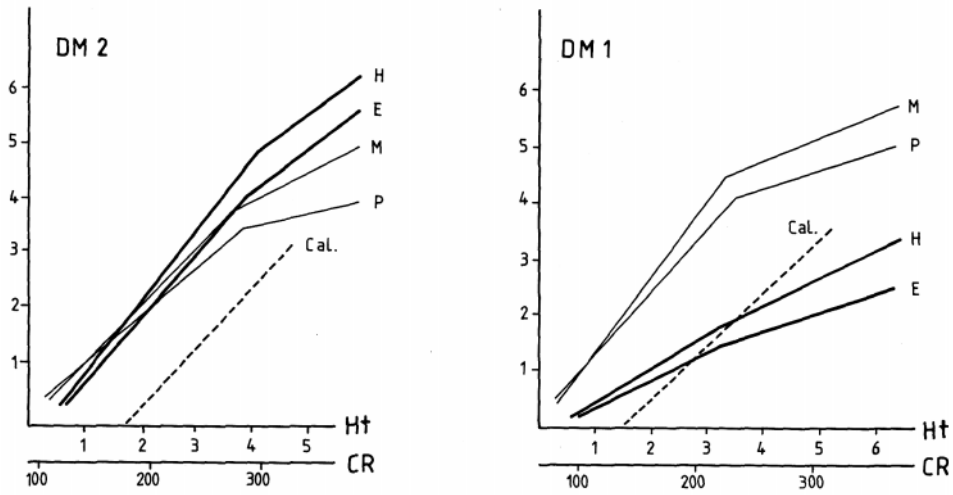


Fig. 4. Relation of lengths of trigonid (P, M) and talonid (H, E) to protoconid height (Ht). Regression lines calculated from the means of grouped specimens. CR, crown-rump length of foetus. Cal, height of calcification on protoconid.

The retardation does not affect all measurements equally. Growth in length of the trigonid, especially the protoconid, is reduced in the late phase much more than that of the talonid. As a result, the talonid increases in length relatively to the trigonid. On DM1 the trigonid grows faster than the talonid in the early phase, more slowly than the talonid in the late phase.

In figure 5 buccal talonid length is plotted against buccal trigonid length for DM1. There is much scatter even in the early phase, due to variable molarisation, but the increase of relative talonid length in the late phase is clear. On the same graph are plotted the mean final values of the great apes and the australopithecines (from data in White et al., 1994). The apes, in which the talonid of DM1 is undeveloped, are in line with the early phase of development in man. The australopithecines show various degrees of talonid enlargement: A. *ramidus* resembles the chimpanzee, A. *africanus* and A. *afarensis* resemble man, and the robust species bridge the gap between DM1 and DM2.

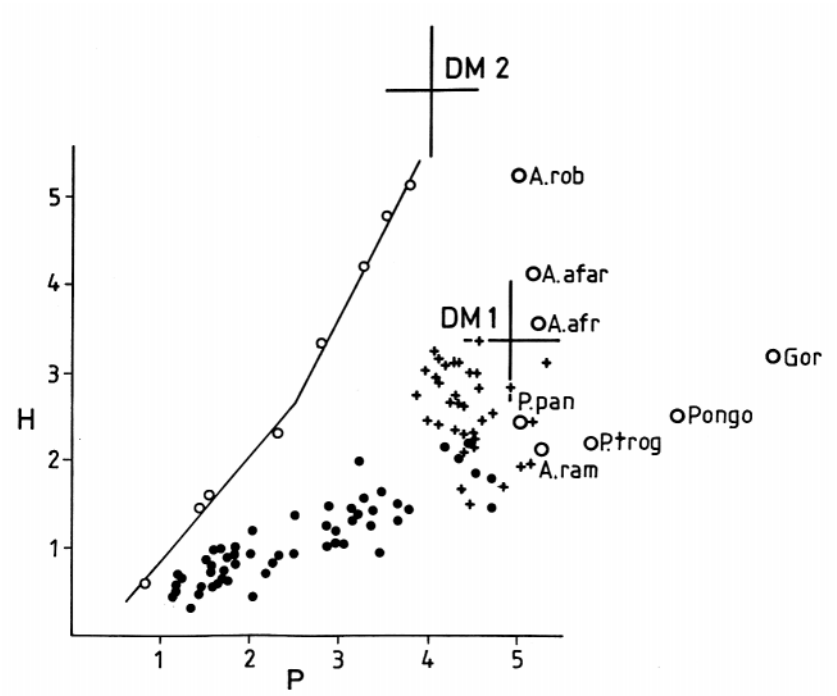


Fig. 5. Buccal talonid length (H) plotted against protoconid length (P), individual data from DM1, early phase and late phase(+). Also shown are the means of grouped specimens of DM2 (o) and the means ±l s.d. of completed DM1 and DM2. The means of DM1 of apes and australopithecines have been added: *Pan troglodytes, P. pan, Pongo, Gorilla, Australopithecus ramidus, A. africanus, A. afarensis,* and *A. robustus.*

The relatively faster growth of the talonid in the late phase reflects the fact that talonid calcification is delayed in comparison with that of the trigonid. Calcification appears first on the protoconid, and it spreads along the mesial and distal ridges of that cusp, so that when the hypoconid begins to calcify only about one-quarter of the length of the protoconid remains epithelial (Fig. 6). Later the hypoconid joins the protoconid, but the talonid can continue to grow in length in the valley between hypoconid and hypoconulid. The entoconid is the last cusp to remain independent, and talonid growth on the lingual side takes place in the valley between entoconid and metaconid.

The spread of calcification on the crown is a result, not a cause, of crown topography, which is due to epithelial growth. The calcification front can be regarded as the line of intersection of the epithelium with a surface, approximately parallel to the tooth base (Fig. 7). As the tooth grows in height, areas in the epithelium reach the level of the calcification front in order of height, so that the margins of calcified areas resemble contours on a map. Calcification spreads more rapidly over the more horizontal areas than over more vertical ones. Minor elevations calcify separately only if they rise above the horizontal. The union of cusps depends upon the depth of the valley between them. Thus

on DM1 the protoconid and metaconid are typically connected by a ridge, along which calcification joins the cusps; on DM2 they are usually separated by a deep valley, and they join via the mesial marginal ridge (Fig. 6).

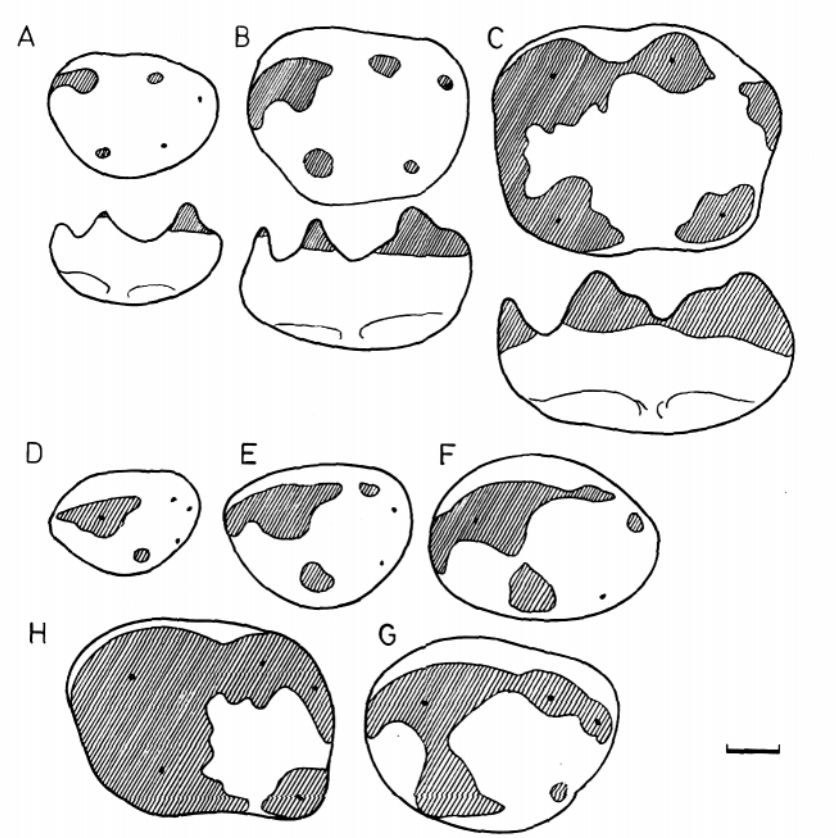


Fig. 6. The commonest mode of calcification spread on DM2 (A to C) and DM1 (D to H). Scale bar = 1 mm.

The uncalcified zone below the calcification front contains a growth zone, and above it a postmitotic zone where ameloblasts and odontoblasts differentiate (Butler, 1971). The total height of the uncalcified zone differs from tooth to tooth, being greater in M1 than in the deciduous molars. Perhaps the number of mitotic cycles varies from tooth to tooth. As cusps begin to calcify when they reach the top of the uncalcified zone, a greater zone height, by delaying calcification, results in greater epithelial growth and a larger tooth.

Growth of the inner enamel epithelium is of course only part of the story of the development of tooth form. There is also expansion of the crown base and the growth of Hertwig's sheath below the papilla, determining the position and size of the future roots. The shape of the tooth is further modified by unequal thickness of enamel on different areas. That teeth can develop normally when explanted shows that the developmental

processes are intrinsic to the tooth germ, even at the cap stage. The explanation of tooth morphology in terms of physicochemical processes at the cellular level is a task for the future.

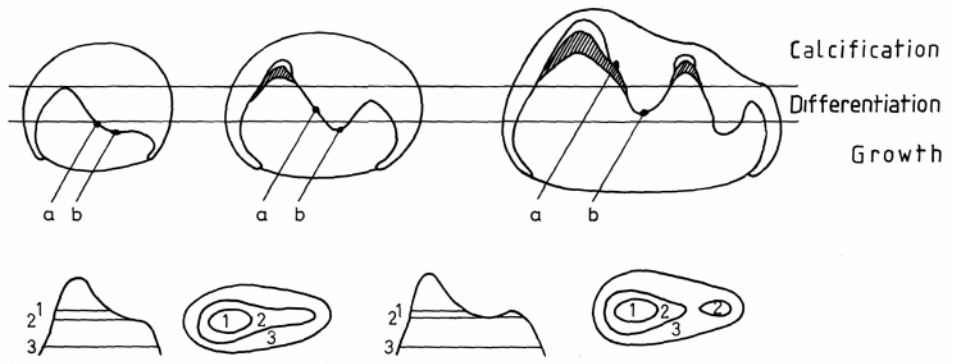


Fig. 7. To illustrate the relation between calcification and height. Above, three stages of development of a tooth. Area a calcifies before b because of its different position in relation to epithelial folding.
Below, a rudimentary cusp calcifies in conjunction with an adjacent larger cusp (left), or it calcifies independently (right). 1,2,3, successive levels of the calcification front.

ACKNOWLEDGEMENTS

I am much indebted to the late Bertram S. Kraus for the opportunity to study tooth germs in his laboratory, and to Dr T.l. Mollison for facilitating access to the Poundbury collection.

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Morphogenesis and Positioning of the Dentition in the Mouse

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INTRODUCTION

Teeth are serially repeated anatomical units whose initial positions are determined during patterning. The unreduced tooth formula in placental mammals is 3/3, 1/1, 4/4, 3/3. In the mouse, the functional dental formula is very reduced (1/1 0/0 0/0 3/3) and consists, in each dental quadrant, of one continuously-growing incisor separated from three molars by a long diastema. During ontogeny, four complementary aspects lead to occlusion within the dentition: 1) initiation and reduction in the number of tooth primordia; 2) growth and morphogenesis of the individual tooth germs including cusp patterning; 3) growth and eruption of a tooth crown including deposition of mineralized matrix and root formation; 4) jaw growth in the context of complex craniofacial developmental events. During development, the relative positions of dental elements change considerably, ultimately leading to functional dental occlusion. Whilst the spatial changes related to tooth morphogenesis can be considered as resulting mostly from autonomous tooth developmental processes, the growth of the jaws allows integrated movement of the developing tooth elements in the maxillar and mandibular dentitions. Although these processes occur at different levels of organization and thus should be under independent control mechanisms, they have to be coordinated (i.e co-regulated) since they result in dental occlusion. Precise morphological data (Peterková et al., 1995; Radlanski et al., 1995; Ruch et al., 1997) is a prerequisite to identify and understand the molecular mechanisms which control odontogenesis (Sharpe, 1995; Slavkin, 1990; Thesleff et al., 1995). We have started a systematic morphological analysis of odontogenesis in the mouse using histology and histomorphometry, combined with computer-aided 3 dimensional (3D) reconstructions. Recent results on morphogenesis of teeth and their progressive positioning in the prenatal mouse dentition are discussed in this paper.

TOOTH MORPHOGENESIS

Dental lamina and bud formation

Before the primordia of functional teeth became distinct in the mouse upper jaw at embryonic day (ED) 13.5, rudimental primordia underwent early morphogenesis at ED12.5-13.5. They consisted in six epithelial placode-like structures in the incisor region (Peterková et al., 1993), and seven antemolar rudiments exhibiting lamina to bud shape (Peterková et al., 1995; 1996; Lesot et al., 1998). At ED13.5, primordia of functional teeth reached the bud stage : the incisor bud arose by integration of the former placode-like structures whilst the upper first molar (M^1) bud emerged behind the antemolar rudiments. The epithelium of these rudiments gradually regressed by apoptosis (Lesot et al., 1996; Peterková et al., 1996; Turecková et al., 1996), possibly under regulatory control of bone morphogenetic proteins BMP-2 and BMP-4 (Peterková et al., 1998). The rudimental tooth primordia in the mouse embryonic maxilla might be related to a more complete ancestral dental formula. The interesting problem of identification of their homology could be elucidated by further comparative morphological and paleontological studies. During this developmental period, only an epithelial lamina was observed in front of the bud-shaped putative molar epithelium in the cheek region of the lower jaw.

During the lamina, bud and cap stages, an area exhibiting a high density of apoptotic bodies was observed in a region of the epithelium anterior to the prospective lower first molar (M_1) (Viriot et al., 1997; Lesot et al., 1998). Apoptosis was involved in the early tooth morphogenesis in the mandibular cheek region and in the remodelling of the epithelium anterior to the M_1 bud and cap (Viriot et al., 1997; Lesot et al., 1998). However, it is not clear yet whether or not these processes reflect the regression of a premolar rudiment in the mandible, as illustrated for the maxilla (Peterková et al., 1996; 1998).

Dental cap and bell formation

Molar

The transition from the bud to the cap stage resulted in an extension of the medial and lateral margins (prospective cervical loop) of the dental epithelium. The completion of the cervical loop was found to progress antero-posteriorly in the M_1 (Viriot et al., 1997) and postero-anteriorly in the M^1 (Lesot et al., 1996).

At ED14, a slight asymmetry was observed on frontal views of the M_1, resulting mainly from slightly delayed development and elevation of the lateral margin compared with the medial one. This asymmetrical latero-medial development of the cervical loop was accompanied by an asymmetric distribution of the laminin-5 subunits (α3, β3 and γ2) (Yoshiba et al., 1998), and of γ catenin (Fausser et al., 1998). Further differences in cell-matrix interactions have also been documented. These relate to the β4 integrin (Salmivirta et al., 1996) and to the BP-230 antigen associated with hemidesmosomes (Fausser et al., 1998). All of these modifications, which started at the bud stage, suggest a reduced adhesion/cohesion of the medial outer dental epithelial cells when compared to the lateral ones (Fausser et al., 1998). The conspicuous medial enlargement of the early bell in M^1 and M^2 led to an asymmetry of the enamel organ on histological sections. The latero-

medial asymmetry was more pronounced in the upper molar primordia (Lesot et al., 1996) than in the lower ones (Viriot et al., 1997), where only two longitudinal rows of cusps form (Gaunt, 1955). The asymmetrical enlargement observed in the M^1 might thus be implicated in the formation of the third medial row of cusps arranged in an antero-posterior direction.

At the early cap stage, epithelial cells uniformly divided in all molars investigated: the existence of specific sites where cells divided (Cohn, 1957), could not be confirmed by 3D representations of metaphases distributions. A striking observation was the clear cut separation of a region where cells divided, to mediate M1 growth, from a region where cells were subjected to apoptosis, mediating the anterior delimitation of M1. At ED16, neither the 3D distribution of metaphases nor the measurement of the mitotic index in the inner dental epithelium (IDE) demonstrated specific changes that could be correlated with the initial cusps formation. The modulation of cell-cell and cell-matrix interactions appeared to play a more critical role.

During the cap stage, the length and dynamics of the enamel knot (EK) (Butler, 1956) varied depending on the molar being considered (M1 vs M2 or upper vs lower). In the M^1, the antero-posterior extension of the EK ranged from 160 µm at the initial cap stage to 185 µm in the well formed cap. The EK extended antero-posteriorly over 44-46% of the antero-posterior length of the forming cap cavity (Lesot et al., 1996). At the early cap stage in the second upper molar (M^2), the presence of an EK was detected in only 25% of sections exhibiting a cap-shaped configuration of the enamel organ. In the M_1 at ED14, the EK was localized in the posterior half of the molar cap and extended over 100 µm along the antero-posterior axis of the tooth. At ED15, the EK had elongated both in anterior and posterior directions to a length of approximatively 250 µm (82% of the whole cavity length). Despite this obvious elongation of the EK, the M_1 maintained a constant length between ED14 and ED15. Compared to the anterior part of the M_1, the histological characteristics of the EK were observed also in the posterior part of the molar although no apoptosis was found there. The opposite picture was observed in the M^1: the anterior part of the EK did not show apoptotic cells. At the early bell stage, the characteristic cellular arrangement of the EK as well as apoptoses disappeared.

Morphologically different developmental stages co-existed along the antero-posterior axis of molars in both jaws; these were maintained at the late cap and early bell stages. These observations were confirmed at the molecular level by immunostaining of sections whose position in the organ was known (Lesot et al., 1997).

Lower incisor

At ED13.5, a slight posterior extension of the former bud (Fig. 1A) was observed, giving rise to early patterning for the development of the cervical loop, which extended only over 10 µm in the labial region (Fig. 1B). At ED14 the incisor reached the cap stage exhibiting a well formed cervical loop (Fig. 1C). At this stage, the labial part of the cervical loop was more developed than the lingual part (Fig. 1C, D, E). This situation was still maintained at the bell stage, at ED16 (Fig. 1F). The bell stage was reached when the labial and lingual parts of the cervical loop were progressively interconnected medially and laterally at ED15.5 (not shown). These observations showed that the initial development of the incisor cap (i.e posterior extension of the cervical loop) appeared to

start in two distinct areas (labial and lingual) which maintained an asymmetrical growth until the bell stage. Later, at postnatal stages, only a single bulbous structure remained which has been suggested to function as a stem cell compartment for the incisor epithelium (Smith, 1975; Smith and Warshawsky, 1975; 1976). The absence of functional ameloblasts on the lingual side of the mouse incisor was suggested to result fom a lack of competence of the cells of the IDE which might withdraw from the cell cycle earlier than on the labial side (Ruch, 1990; Nso et al., 1992). Constituents that might control the differentiation pathway of IDE cells include those of the basement membrane which showed temporo-spatial changes. The existence of single (on the lingual side) or multiple superimposed (on the labial side) basal laminae in the region of the cervical loop reflected either qualitative differences in IDE cells-basal laminae interactions or differences in the metabolic activities of these cells (Meyer et al., 1995). It is not clear yet whether these changes at the epithelio-mesenchymal junction might later influence the mitotic activity or the functional differentiation of cells on each aspect of the incisor. The multiple basal laminae might simply reflect increased cellular mobility in the labial aspect of the cervical loop.

Relative position of tooth germs

Long before root formation has been initiated, important changes in the relative position of teeth were observed. During M^1 development, a clear boundary delimited the developing enamel organ, where cells divided, from an antemolar area, where apoptosis was predominant. This boundary progressively moved from ED14 to 16, suggesting a postero-anterior growth of the anterior part of the M^1 (Lesot et al., 1996). The changes in the relative positions of teeth result from the growth of individual teeth themselves and from jaw development. The evolution of mouse molars size in both jaws has previously been investigated (Gaunt 1963).

Histology and 3D reconstructions demonstrated important changes in the relative positions of molars germs from bud to bell stage (Figs. 2 and 3). These included:

1. The angle between M_1 enamel organs and the sagittal plane through the head changed (compare Fig. 2A with 2E and Fig. 2D with 2H). From ED13.5 to 15.5, the M_1 progressively took on a more vertical position (Figs. 2D, H). This angle appeared more constant for the M^1.
2. The repositioning of opposing molar germs progressed but had not been achieved at ED15.5 (Fig. 2). This was particularly obvious when considering the anterior part of the first molars (compare Fig. 2B with 2F; see also Figs. 3F and 3I).
3. The angle between the two antero-posterior axes of opposing molars progressively decreased during development (Figs. 2C, G). This observation was confirmed by morphometry (Fig. 3).

Morphometry and 3D representations were used to follow the antero-posterior growth and changes in the relative positions of molars and incisors during development. For this purpose, measurements were performed on serial histological sections. Lateral views (Figs. 3A, D, G) illustrated elongation of both the molars and incisors in the two jaws from ED14 to ED18. They demonstrated the change in the orientation of the upper incisors from ED14 to ED16 (compare Fig. 3A with 3D), also visible from frontal views

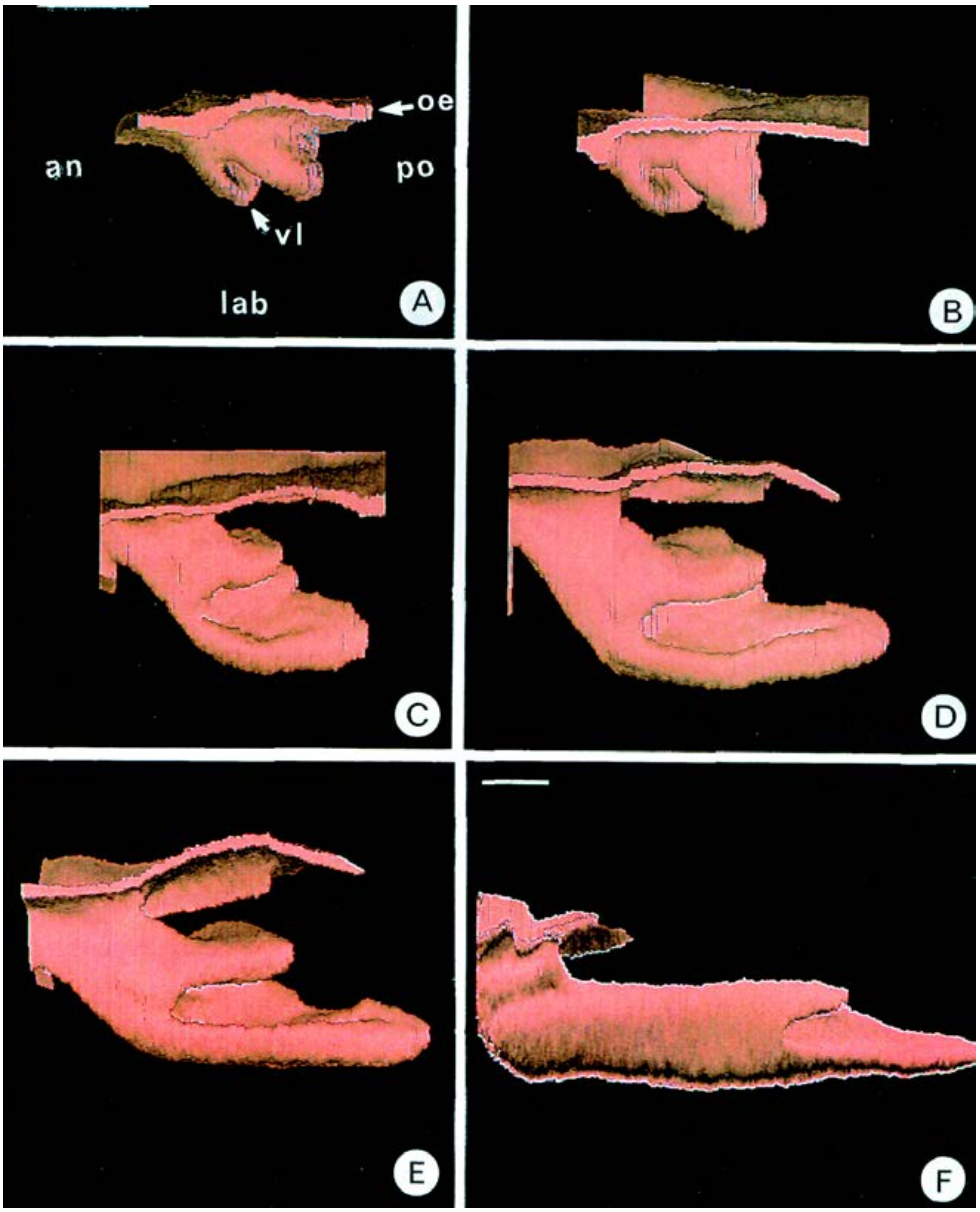


Fig. 1. 3D reconstructions of the lower incisor at ED12.5 (A), 13.5 (B), 14 (C), 14.5 (D), 15 (E) and 16 (F). The medial views show the development of the incisor along its antero-posterior axis and the more pronounced development of the labial (lab) part of the cervical loop. During the early cap stage (C) the closure of the cervical loop was initiated on the lateral side of the incisor. an: anterior; lab: labial; oe: oral epithelium; po: posterior; vl: vestibular lamina. Bars = 200 μm.

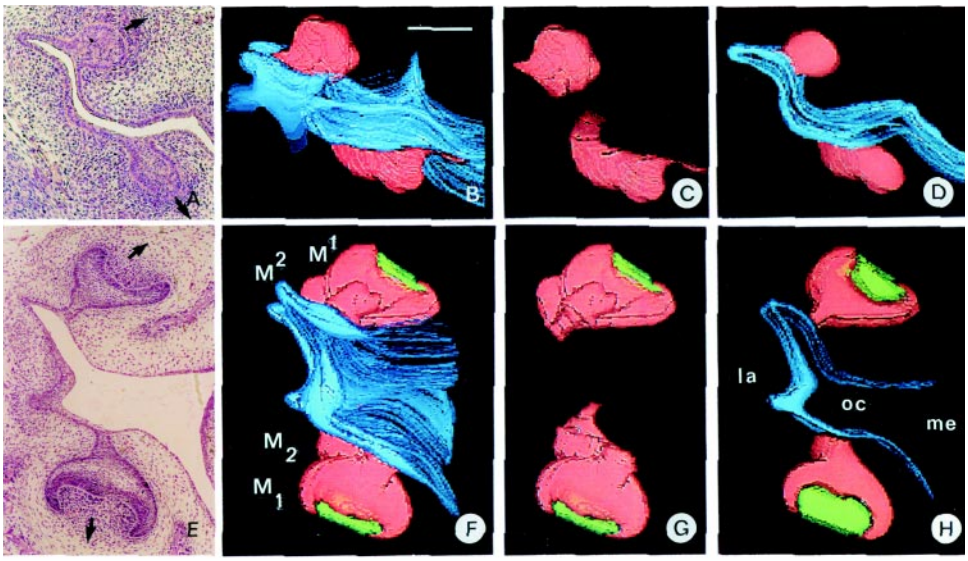


Fig. 2. Frontal histological sections (A, E) and 3D reconstructions (B-D, F-H) showing the upper and lower molars and adjacent part of the oral cavity at ED13.5 (A-D) and 15.5 (E-H). The dental epithelium (brown), oral epithelium (blue) and dental papilla (green) in 3D reconstructions are viewed from the anterior side. Arrows (A, E) indicate the change in the orientation of the developing first M_1 from ED13.5 (A-D) to 15.5 (E-H). Virtual 200 μm thick sections taken in the middle part of M1 (D, H) documented the relative positions of these tooth germs in both jaws. oc: oral cavity; la: lateral; me: medial. Bar = 100 μm.

(Figs. 3B, E). The vertical distance which separated the anterior part of the upper and lower incisors remained almost constant from ED14 to ED18. The growth of upper and lower M1 was similar. In both cases the growth of M1 was slightly faster than that of M2. The antero-posterior growth of incisors was twice as fast as that of the first or second molars. From ED14 to 18, the antero-posterior growth of molars was accompanied by a change in the vertical positioning of opposing first molars (Figs. 3C, F, I). At ED14, the M^1 had a more external position when compared to the M_1 (Fig. 3C). At ED16 there was good adjustment in the posterior part of the first upper and lower molars (Fig. 3F) and at ED18, even their anterior parts were better positioned (Fig. 3I). The Meckel's cartilage, from the posterior part up to the symphysis was twice as long at ED18 as at ED14. At the same time, the common anterior part of the contra-lateral Meckel's cartilages extended from 150 to 610 μm.

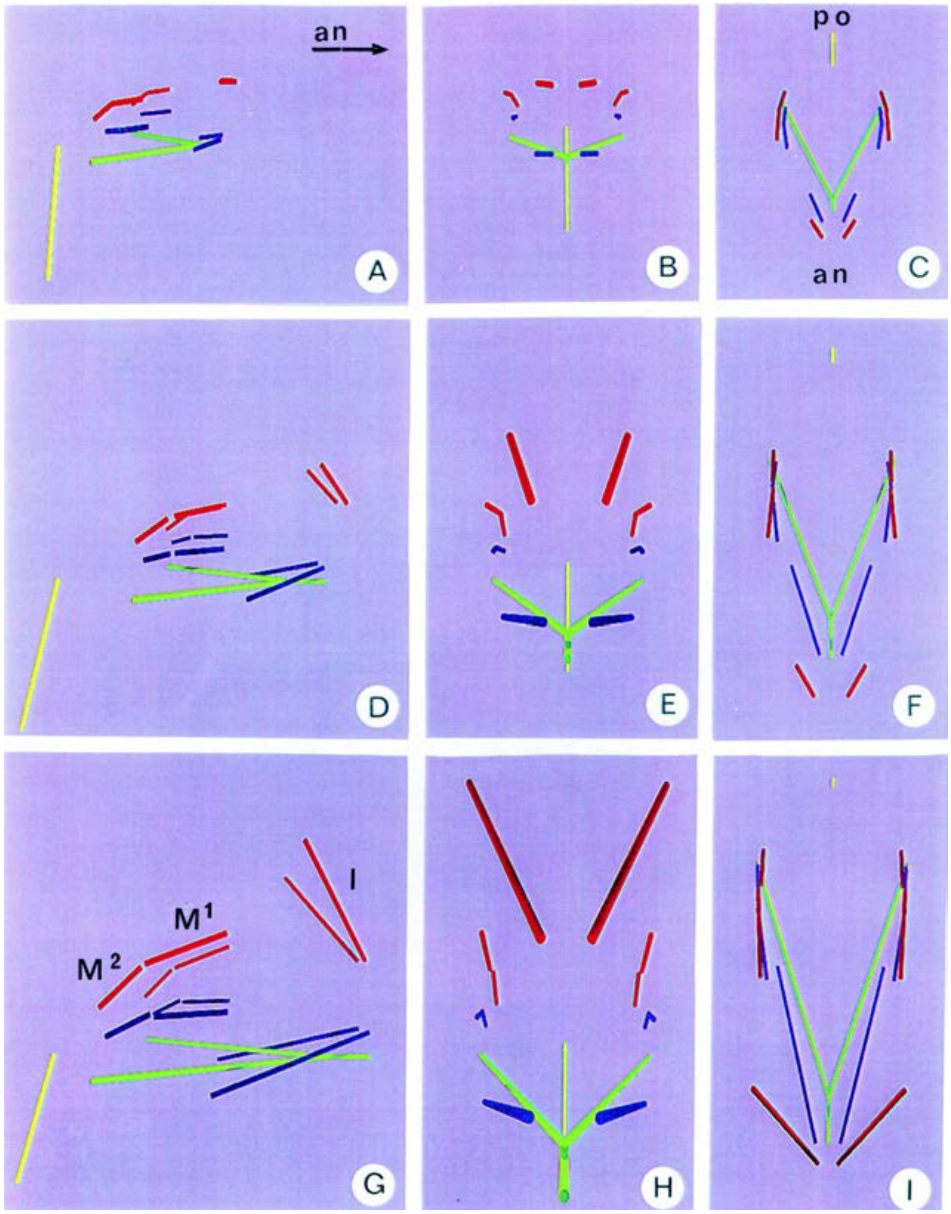


Fig. 3. The relative positions of upper (red) and lower (blue) molars (M1, M2) and incisors (I) at ED 14 (A-C), 16 (D-F) and 18 (G-I) were visualized using lateral views (A, D, G), frontal views (B, E, H) and upper views (C, F, I). Meckel's cartilage was represented in green and the hypophysis-atlas axis in yellow. The data were represented as 3D visualization of virtual cylinders connecting the anterior to posterior extremities of the molars, incisors and part of the Meckel's cartilage. In the 3D representations, the diameter of the cylinders did not take into account changes in the widening of teeth. an: anterior; po: posterior.

CONCLUSIONS

Mouse odontogenesis results from complex mechanisms involving contemporary progressive and regressive processes. In particular, the early phases of tooth development and the determination of the functional dental pattern appear more complex and dynamic than previously expected. Our results were complementary to the classical data by Pourtois (1961) and Gaunt (1955) in that they demonstrated:

- the composite origin of the upper incisor.
- the presence of a dental lamina in the diastema of the lower jaw instead of transitory rudimental tooth primordia in the mouse embryonic maxilla. This has been suggested to reflect various temporo-spatial degrees of dental involution in the two jaws.
- the remodelling of the antemolar region in both jaws, involving apoptosis.
- the difficulty in correlating the evolution of the EK with other aspects of tooth morphogenesis.
- the complex and asymmetrical development and completion of the cervical loops in molars and incisors.

The integration of these morphological data with complementary studies including comparative morphology (Jernvall et al., 1996), molecular investigations (e.g. Maas and Bei, 1997; Schilling,, 1997; Thesleff and Sharpe, 1997) and paleontological approaches (Butler, 1995; Stock et al., 1997) should allow better understanding of the regulatory factors and mechanisms participating in the coordinated morphogenesis and positioning of teeth.

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Dichotomy in Human Dental Morphology: A Plea for Complexity

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INTRODUCTION

There is an increasing trend by those studying dental morphology to report their results as dichotomous entities. The categories of “presence” or “absence” of a particular morphological trait appear to be the norm in the recent literature (Turner, 1985, 1987; Haeussler et al., 1989; Irish, 1997; Hanihara, 1998). Many of these reports indicate that a particular trait is “present” when several categories of a, presumably, quasi-continuous trait are lumped together. The “absent” appellation may also consist of more than one category of expression on the lower end of the continuous trait. For several years writers have warned of the difficulties of the use of only two categories to characterize morphological traits that have identifiable interim points. Dahlberg (n.d.) and Hanihara (1961), and later Turner and his colleagues (1991) produced three-dimensional models to indicate the possible identifiable steps of a trait from the lack of an indication of a trait to the largest manifestation of it, presumably to assist the researcher in his/her description of the continuous nature of the expressions of the trait.

Several studies have indicated the percentages of expressions for each trait in rather large, complicated tables (Hershey, 1979; Mayhall, 1979; Turner, 1990). These have allowed the reader to use the results to draw his/her own conclusions about the discussion and conclusions of the author. On the other hand, recent studies, particularly those that are attempting to draw population comparisons, have reported their results simply as either present or absent. The reader may be informed as to what categories of expression constitute these categories but one is not privy to the variation found within the categories of (non-)expression.

EXAMPLES OF MORPHOLOGICAL TRAITS

Three examples will illustrate the concerns that arise from the use of a dichotomous method of reporting. If we examine the Carabelli’s trait (Table 1) we can take two sets of results and make quite different suggestions about the comparisons of the trait presence in Mongoloid and Caucasoid populations. If we were to include all expressions of the trait in a single “present” category there is very little difference between the percentage of those with the trait in Caucasoids and those that are Mongoloid. If, however, we limit the “present” category to the cusp expressions alone, relegating the grooves, furrows, and pits to the “absent” category we reach a quite different conclusion: the Caucasoids are clearly separated from the Mongoloids.

Table 1. Carabelli's Trait (M^1).

All Expressions	
Mongoloids	56%
Caucasoids	60%
Cusp Only	
North American Indians	5%
Canadian Inuit	14%
North American Whites	61%

(Figures are not from the same studies)

The second set of expressions of traits is from various sources but categorizes Carabelli's trait into 3 cells: smooth (no indication of any expression), intermediate structures (grooves, Y, etc.) or cusps.

Table 2. Carabelli's Trait Expressions[*]

Population	Source	Smooth (%)	Intermediate (%)	Cusp (%)
Wainwright Eskimos	Hershey, 1979	8	66	26
Pima Indians	Dahlberg, 1963	17	59	24
American Whites	Keene, 1968	40	38	22
British Whites	Goose & Lee, 1971	21	58	19
Easter Islanders	Turner & Scott, 1977	67	23	10

[*]After Hershey, 1979

If we were to regard the smooth category as "absent" we would note that there is a major difference between the Eskimos, Indians and British Whites on one hand and the American Whites and Easter Islanders on the other. If, however, we decide that only the cusp expressions constitute "presence" we note that only the Easter Islanders are unique. The other populations have about 20-25 percent of their expressions in the "present" or cusp category while the Easter Islanders have less than one-half the incidence of cusps. I would doubt that the observer of these data would intuitively place Eskimos, Indians and Whites in the same general population. If, on the other hand, we examine the intermediate category we see that the trend that Hershey (1979) identifies, that is that the high frequency of intermediate expressions of Carabelli's structures, rather than their absence should be considered a characteristic of Mongoloids doesn't hold when the British Whites are included in the equation. The differences noted above may be due to differing methods of observation of the expressions but the point here is not these differences but rather that one can constitute categories of expression based on dichotomies that allow for seemingly spurious conclusions.

One further example using another trait, shovel-shaped incisors may further illustrate my concerns. These materials are taken from Turner's description of the major features of sundadonty and sinodonty (1990). I have excerpted only a small section of one table that deals with the Jomon culture of Japan.

Table 3. Maxillary Central Incisor Shoveling (Frequencies in %)[*]

Population	0	1	2	3	4	5
Jomon	0.0	22.2	41.7	27.8	8.3	0.0
SW Jomon	0.0	30.8	46.2	7.7	7.7	7.7
Jomon Tsukumo	0.0	40.0	40.0	10.0	10.0	0.0
Jomon Yoshiko	0.0	14.3	75.0	10.7	0.0	0.0
Jomon Hokkaido	3.3	33.3	33.3	26.7	0.0	3.3

After Turner, 1990; [*]Categories are those of Turner *et al*. 1991

Turner (1990) provides us with the complete range of variation in expressions of the shovel-shaped central incisors for numerous populations. These data illustrate how by changing the definition of "present" we can obtain differing results. If we were to assume that categories 0 and 1 constitute an absence of shoveling then we might conclude that there is a significant proportion of the Jomon groups that does not demonstrate shoveling; the SW Jomon, Tsukumo and Hokkaido groups have up to 40 percent of their central incisors with no evidence of shoveling. But if we had suggested that only the 0 category indicates an absence, all categories show a presence of the trait. In other words, in the five groups illustrated here if "present" is categories 1 and above there is almost a 100 percent occurrence of the trait but if categories 2 and above constitute "present" there are wide disparities in the presence of the trait. The Jomon Yoshiko group now has a much higher presence of the trait than the other Jomon groups. But Turner (1990) states that grades 3 and above constitute shoveling. This completely changes the picture; the Jomon Yoshiko now has the lowest percentage of shovel-shaped incisors.

DISCUSSION

As indicated earlier, previous authors have indicated their disapproval of the use of only present and absent categories of expression. Balakrishnan (1985) noted that,

> The question now arises whether a [quasi-continuous] character is to be scored only as present or absent or in a more detailed manner. The underlying genetic mechanism, if any, for such characters is likely to be polygenic or based on a large number of alleles at one or more loci. In either case, an yes-or-no classification will lead to the sacrifice of information on the variability in frequencies of the alleles over different populations.

Recently, Stephen Jay Gould has written on the use of dichotomies in a very different sense than dental morphology. In *Questioning the Millennium*, (1997), Gould discusses the meaning and importance, or lack of it, in Western society of the coming millennium. He notes that one of the devices we use to bring order to a complicated world is classification, or the division of items into categories that include perceived similarities.

> No strategy cuts deeper- while providing such an even balance of benefits and difficulties - than our propensity for division by two, or dichotomy. …we might argue that dichotomization amounts to little more than good observation of the external world. **But far more often than not, dichotomization leads to misleading or even dangerous oversimplification**. [Emphasis mine] (Gould, 1997).

Gould writes that we are driven to division by two even where it is clearly inappropriate and concludes by observing that dichotomization is more an inherent mechanism of the brain than a valid perception of external reality.

While I can not think of a dangerous situation due to dichotomization in dental morphology there are certainly situations where the use of dichotomies may have lead to misleading conclusions. We must guard against the inappropriate use and reporting of the "present" or "absent" categories. Even when they are clearly defined, their use does not allow the reader to judge the results in a unbiased way. We are given only part of the information that we require to make an informed judgement. This leads to one other development that reduces our ability to judge published results, the author's note in a paper that the detailed data are available on request from the author. I can understand that the author, and the journal editor desire to conserve valuable space, but the use of this addendum presumes that the author will live for a very long time, not move to a new location and that the journal paper will be only valuable for a very short time. The last point is one that we must consider. What if the works of Dahlberg, Kraus, Pederson or any of the other giants of dental morphology were published as dichotomous tables and the reader is asked to consult the author for further data? We could be in a situation where the data might be no longer available and therefore lost to present day researchers; certainly a potentially disastrous situation.

My recommendations for the reporting of dental morphological traits include:

1. Give complete frequencies for each identifiable variation
2. Use recognized standards for recording trait variations
3. Do not use presence/absence unless the trait is truly dichotomous
4. Indicate the ranges of variation
5. Indicate the size of each category of variation
6. If results must contain combined data:
   a)indicate the variability within the group and sub-groups
   b) indicate the sources of variation
   c) indicate the provenance of the sub-groups
   d) indicate the sub-group size.

While these "rules" may seem obvious, one cannot help but notice that they are not necessarily observed in many of the reports of today. Present day researchers must provide readers with complete information even if it means a somewhat longer and more involved presentation. Instead of simplicity, the reporting of dental morphological traits must continue to be complex. In this way we can share valuable information with each other and allow our results to be tested by others. Scientific thought requires the ability to

test results and then test hypotheses finding them false when appropriate. We must allow our data to withstand the scrutiny of others; we are not in the business of sales but careful scientific reporting.

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The Periodicity of Incremental Structures in Dental Enamel Based on the Developing Dentition of Post-Medieval Known-age Children

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INTRODUCTION

The periodicity of incremental structures in dental enamel is increasingly being used to reconstruct crown growth and dental development in children, the regularity of which has important implications for the study of human remains and hominid evolution. Incremental structures offer an independent way of calibrating the chronology of dental development in archaeological and fossil material and, have been used to determine age-at-death (Boyde, 1963, 1990) and dental development in fossil hominids (Beynon, 1992; Beynon and Dean, 1987, 1988; Beynon and Wood, 1987; Bromage and Dean, 1985; Dean, 1987a, 1987b; Dean and Beynon, 1991; Dean et al., 1986; Dean et al., 1992; Dean et al., 1993). The method relies on the regular periodicity of the incremental growth, in which prism cross-striations are believed to represent a circadian rhythm in enamel matrix secretion (Boyde, 1989; Dean, 1987a; FitzGerald, 1995; Hillson, 1996; Risnes, 1986). Some researchers have argued against the regularity of the incremental structures, and the methodology used to reconstruct dental development (Mann et al., 1990a, 1990b, 1991; Warshawsky and Bai, 1983; Warshawsky et al., 1984). The approach, and its accuracy, were therefore tested using the developing permanent dentition of children whose age-at-death was known.

MATERIAL

This paper presents the preliminary results of a broader histological study of dental development, in which the dentitions of approximately eight individuals from the London site of Spitalfields are being analysed. The material was excavated from the crypt of Christ Church in Spitalfields during the 1980's. The church was mostly used to inter parishioners of Huguenot descent between AD 1729 and 1859 (Cox, 1996; Molleson and Cox, 1993; Reeves and Adams, 1993). The age-at-death of many individuals was established from the coffins' plates and parish records. This offered a way of calibrating the periodicity of incremental features in enamel, through the analysis of the permanent dentition of young individuals whose crown formation had been interrupted by death. In this preliminary study, four teeth from one male individual, designated specimen 2365, are discussed. He was born in June 1824 and died on the 7th of November 1827 at the age of 3 years and 4 months (approximately 1216 days).

The upper and lower permanent central and lateral incisors, canines, first premolars and first molars were present in the jaws. One specimen of each tooth type was selected, recorded and sectioned. The results for the incisors (upper left first and second incisor; lower right first incisor) and a molar (upper left first) are given here. Future research will include the analysis of each tooth type present. Apart from the first molars, whose root had started forming, all tooth crowns of specimen 2365 were incomplete, with enamel matrix secretion still active at death.

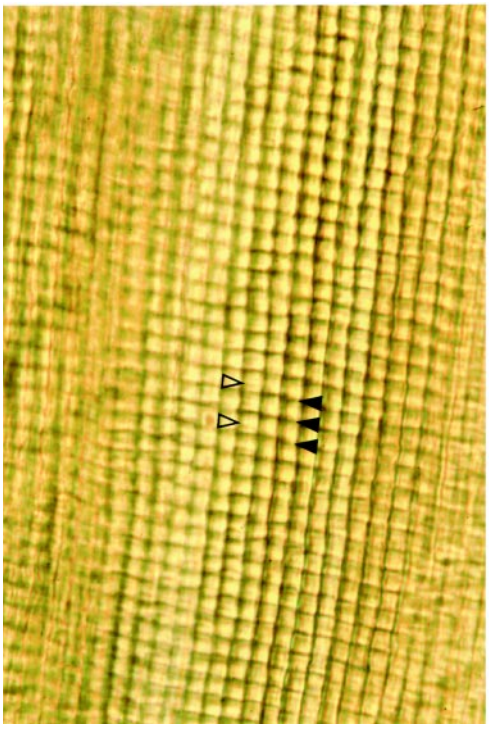
METHODS

Due to the partial destruction caused by sectioning, each tooth was fully recorded using, as discussed in Hillson et al. (this volume), photographs, drawing and casts. The cleaned specimens were then embedded in methylmethacrylate (Boyde, 1984; Hillson, 1996). This prevented any loss of enamel during sectioning, and was particularly important in preventing the loss of the friable immature enamel formed just prior to death. The plane of section was marked on the teeth using a lead pencil before embedding. In the absence of roots, the radial sections were centred through the cusp(s) and the dentine horn(s) so as to cut the earliest enamel increments. These were taken with the kind help of Don Reid and Pam Walton (Dental School, Newcastle upon Tyne), using an annular saw (Malvern Instruments Ltd: Microslice 2) and lapped down to approximately 100 μm thickness (Logitech PM2 A with a 3 μm aluminium oxide abrasive slurry). Slides were observed and photographed using polarising transmitted light microscopy (Olympus BH-2 microscope; Olympus OM-2 camera). Cross-striation counts were recorded on photomontages taken at approximately X300, and calibrated using photographs of a graticule slide.

In order to ascertain if cross-striations represent a circadian rhythm in enamel matrix secretion, counts were taken from the neonatal line (representing birth), to the last surviving layer of enamel matrix formed before death. In the permanent dentition, the first molar is the only tooth to start its crown development prior to birth, in which the neonatal line is recognised as the first accentuated stria found in cuspal enamel (Schour 1936). The location of this stria corresponds to the expected state of molar development at birth as observed in dissected tooth germs (Christensen and Kraus, 1965; Kraus and Jordan, 1965). The upper and lower molar mesiobuccal cusp is the first to be initiated, at around 30 weeks *in utero* (Christensen and Kraus, 1965). Based on an average gestation of 38 weeks (Hillson, 1996), one should expect approximately 8 weeks of prenatal crown formation in the mesiobuccal cusps. The mesiolingual (upper and lower molar) and distobuccal (lower) cusps can also begin to form prior to birth and may exhibit neonatal lines. Cross-striation counts were recorded up to the last surviving layer of enamel matrix to have been formed. The last layers often appeared very cracked and friable, which is most probably due to its higher organic content of partially mineralised immature enamel matrix formed just prior to death.

In transmitted light microscopy, cross-striations can be recognised as dark zones which divide the prisms into regular segments (Fig. 1). These tend to be closer to each other and more rectangular near the enamel-dentine junction, getting progressively further apart and squarer towards the crown surface. The change in cross-striation size is progressive and

areas showing irregular spacing were avoided. In some areas, finer banding could be seen between cross-striations (Fig. 1), most probably the result of interference between adjacent layers of cross-striations (Boyde, 1989; FitzGerald, 1995).



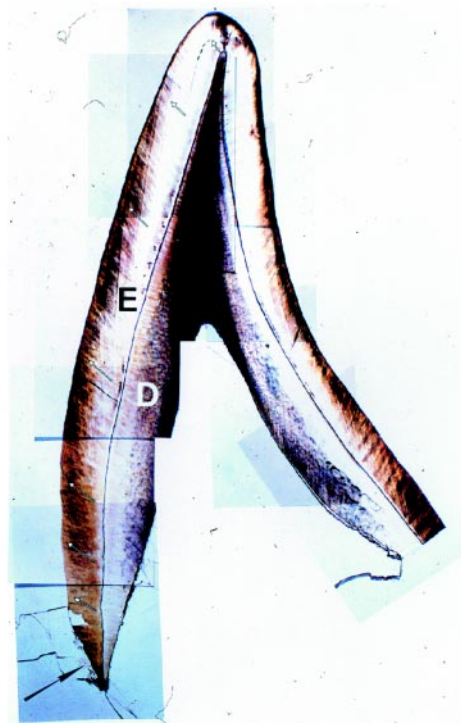


Fig. 1. Prism cross-striations seen in polarised transmitted light microscopy. Examples of clear cross-striations are shown by full arrows. Examples of fine banding between cross-striations are indicated by hollow arrows. Brown striae can be seen running from the lower right to the upper left (100 μm thick ground section of cervical enamel; Olympus BH-2 microscope with polariser; Field width approximately 140 μm).

Fig. 2. Montage of the full ground section of a lower central incisor. Labial to left, lingual to right. Tip of the central mamelon is at the top. Enamel labelled E. Dentine labelled D. Full arrow indicates the developing edge of the crown. The open arrows show the location of cross-striation counts between accentuated brown striae.

Counts were taken along the prisms, but the ease with which cross-striations were observed varied along their length, and the clearest sections were used. The different prism sections were then connected with each other using accentuated brown striae of Retzius, which mark the forming enamel front at one moment in time. Accentuated striae were also used to continue counts from cuspal into cervical enamel by matching photomontages, which had been carefully chosen so that the first striae present in one montage matched with the last striae present in the next montage. Using this method, counts between striae can be followed down the crown until the last layer formed (Fig. 2).

RESULTS

A deliberately cautious approach was used. Several montages were prepared for each tooth crown, including accentuated striae which could be consistently matched between them. In the cuspal enamel for example, three montages were made for the first molar and second incisor, and nine for the first incisors. Counts between accentuated striae were made independently for each montage, based upon the average of several prism paths. As an example, Table 1 shows the cuspal enamel of the mesiobuccal cusp of the upper first molar. The rest of the molar tooth was not used as it was damaged.

Table 1. Variation in cross-striation counts between matched accentuated striae for the mesiobuccal cuspal area of the upper first molar.

	Prenatal	A-B	B-C	C-D	D-E	E-F	F-G
Montage 1*	38	134	109	14	158	6	12
Montage 2			102	14	164	6	
Montage 3			112	14	165	6	12
Average	38	134	108	14	162	6	12

*Three montages from different parts of the cusp were used to produce an average count for the cuspal area. The enamel formed before birth is labelled as prenatal, with the neonatal line as stria A. Accentuated stria were then lettered from B to G. The last row shows the average count between each striae for the three montages.

Overall, there was little variation in cross-striation counts within any one tooth. In the molar example, counts varied between 102 and 112 between striae B and C, with an average of 108. In areas where counts were difficult, prism length measurements (taking account of prism decussation) were divided by the average cross-striation spacing for that region of enamel, to produce count estimates. These compared well with direct counts but, as the average cross-striation spacing progressively changed from the enamel-dentine junction to the crown surface, estimates of this type were only used over short prism lengths (less than 300 μm).

The developmental sequence of different crowns from a single individual can be determined by matching the accentuated striae within enamel (Fujita, 1939; Gustafson, 1955). This method was used to match stria sequences for the four teeth (Fig. 3), and cross-striation counts between matched striae were then compared as shown in Table 2.

Table 2. Cross-striation count between matched accentuated striae from different teeth of specimen 2365.

	Prenatal*	A-B	B-H	H-I	I-last enamel formed
Upper left first molar mesiobuccal cusp	38	134			
Upper left first incisor			470	356	201+
Lower right first incisor			514	360	170+
Upper left second incisor				345	210+

*The enamel formed before birth is labelled as prenatal, with the neonatal line as stria A. Other accentuated stria were then lettered from B to I. The table does not include a complete cross-striation counts for the crown development of each tooth, but simply compares counts between the matched striae of different teeth. The + sign indicates that a variable amount of enamel has survived in the distal portion of the different teeth, with the second incisor having the greatest amount of surviving immature enamel.

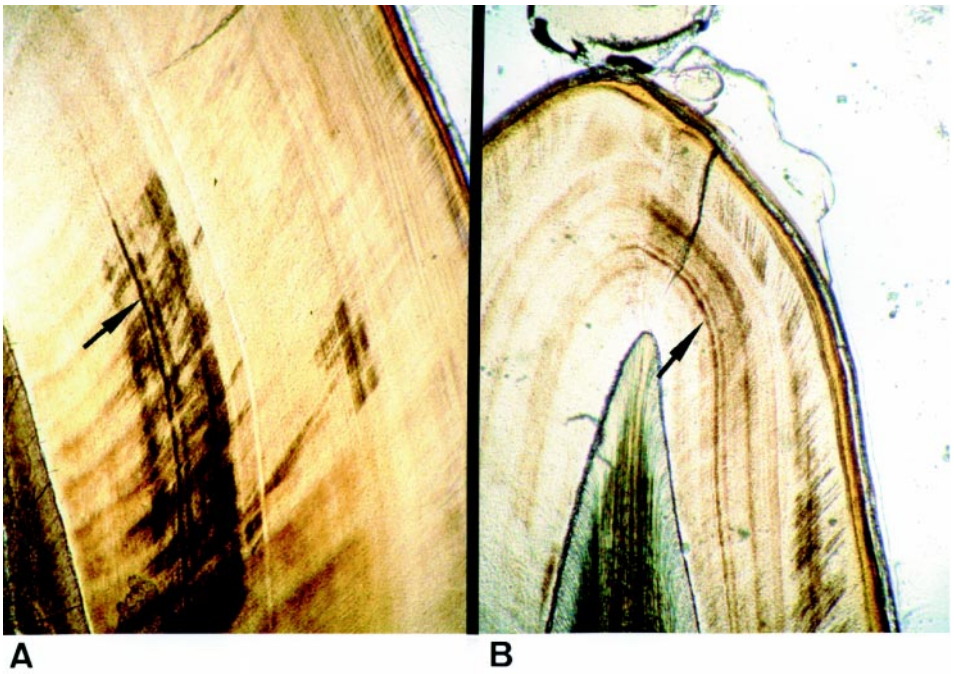


Fig. 3. Section of cuspal enamel in molar and incisor from specimen 2365. A represents the upper left first molar. B represents the upper left first incisor. The arrows point to a prominent patch of brown striae which matches clearly between the teeth.

The counts from the different teeth matched well. Counts between striae B and H in the first incisors presented the greatest difference, with a 470 count in the upper and a 514 count in the lower. The counts between H and I in the incisors showed less variation, ranging between 345 and 360. This is due to counts between striae H and I having been taken in the cervical part of the tooth, where cross-striations are much easier to see. Counts in the last formed enamel (D onwards) were dependent on the preservation of the last few layers of immature enamel. It was noticeable on the section that a variable amount of enamel had been lost. The second incisor showed the best preservation.

The cross-striation counts between the accentuated striae of each tooth (Table 2) were averaged to produce a total count, as seen in Table 3.

Table 3. Total cross-striation count.

Average Count			Highest Count*	Total
A - B	B - H	H - I	I – Last Enamel Formed	A - Last Enamel Formed
134	492	354	210	1190

The average counts between matched accentuated striae of the different teeth analysed were averaged to produce a total count.

* The highest count was used for the last formed enamel (D to last enamel formed) since it is dependent upon, and reflects, the amount of immature enamel preserved.

The total cross-striation count of 1190 from the neonatal line to last surviving enamel matrix compared well with the independently known age of 1216 days.

DISCUSSION

This preliminary study showed that multiple, independently recorded cross-striation counts produce comparable results, within a single montage, and between different montages of a same tooth (Table 1). There was also a strong similarity in counts between the matched accentuated striae from different teeth in this individual (Table 2). Cross-striation counts were therefore consistent across the whole dentition. The greatest difficulty lay in finding sequences of striae that could be matched between teeth with confidence. They need to be defined with great care as their appearance (size, colour, sharpness and outline of the edges) can vary down the crown and, in this research, the matching within and between montages was carefully checked by direct microscope observation of the sections.

The total cross-striation count of 1190, from neonatal line to the last surviving increment of enamel matrix, was close to the independently known age-at-death of 1216 days. If used to predict age-at-death by assuming a circadian rhythm, the cross-striation counts would have predicted an age 26 days short of the known age (or just 0.02% of the age). These preliminary results thus appear to confirm that the cross striations indeed represent a regular circadian rhythm of enamel matrix secretion. A slight shortfall was expected due to the erosion of the poorly mineralised immature enamel at the growing edge, and this was observed in the three incisor teeth (Table 2). Using cross-striations as circadian markers, 38 days of enamel was formed before birth, which was not far from the expected eight weeks as discussed above. Cross-striations were sometimes difficult to observe, but the accurate match within different parts of a single tooth, and between different teeth from one individual, indicates that these features can be counted with a good degree of consistency.

ACKNOWLEDGEMENTS

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Ukraine Mesolithic Cemeteries: Dental Morphological Analysis

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INTRODUCTION

Dnieper Rapids Region Mesolithic cemeteries contained the earliest skeletal evidence for large-scale human habitation of Ukraine (Telegin, 1982, 1989). These burials incorporated a variety of physical features, which resulted from a complex regional interaction of Mesolithic peoples (Gokhman, 1966; Konduktorova, 1973). Voloshskoe contained two groups: Mediterraneans, who were narrow faced and very gracile, and Australoids (two skulls) (Debets, 1955). Vasil'evka I burials were Ancient Mediterraneans and Protoeuropeans, who were broad faced and massive North Europeans descended from a mixture of late Paleolithic peoples, such as those from Brno and Predmosti (Konduktorova, 1957; Gokhman, 1966). Vasil'evka III flexed burials were Protoeuropeans and Vasil'evka III extended burials were Mediterraneans (Gokhman, 1966).

MATERIALS AND METHODS

Thirty-two dental morphological traits and three pathologies of burials from the Ukraine cemeteries and nine comparative geo-cultural groups (Appendix I) were studied to evaluate these proposals. The order of the Ukraine Mesolithic cemeteries follows the classification of Telegin (1982, 1989): Voloshskoe, Vasil'evka I, Vasil'evka III flexed, and Vasil'evka III extended burials. *Near East* means the lands around the eastern shores of the Mediterranean Sea, including northeastern Africa and southwestern Asia. *Mediterranean* includes the Near East.

The plaques and definitions of the Arizona State University and Dahlberg dental anthropology system were the standards for dental morphological trait evaluation. Following the protocol of the system, frequencies of the features, including pathologies, are based on specimen counts (Dahlberg, 1956; Turner et al., 1991). Of the pathologies hypoplasia means two or more teeth with hypoplastic pits and/or lines; caries, one or more carious teeth. The Coefficient of Similarity (C^S) and Indicator of Similarity (I^S) for comparing the morphological dental trait frequencies in small samples (Haeussler, 1998) were used in the mathematical analysis. If two samples are very similar to one another, the C^S value is close to 1.0. The higher the I^S value, the greater the likelihood that the C^S is meaningful.

RESULTS

The dental morphological trait analysis supports the concept that Ukraine Mesolithic peoples were biologically heterogeneous. Table 1 shows that Vasil'evka I, Voloshskoe, and the two Vasil'evka III sub-samples are, indeed, different from one another. The C^S values for the four sets of pair-wise comparisons range from 0.727 to 0.455 (Table 1). Had the samples represented a homogeneous people, the C^S values would be close to 1.000. Voloshskoe and Vasil'evka I, the early samples, are dentally heterogeneous, as are the two Vasil'evka III sub-samples. The Vasil'evka III C^S values indicate that the two types of burials within the cemetery represent different peoples, regardless of chronology or archaeological typology (Telegin, 1982, 1989; Jacobs, 1994).

Evaluation of a Mediterranean (Near East) affinity for Voloshskoe indicates a European dental resemblance in addition to the Mediterranean similarity suggested by Debets (1955). According to the C^S values in Table 1, Voloshskoe is the most closely related to the Crimea Mesolithic, the Caucasus Paleolithic and Mesolithic, and the Czech Republic Paleolithic samples. Of the extra-regional comparisons, Voloshskoe is the least like the Mediterranean and Near East samples. Conversely, Voloshskoe is more like the Russian Upper Paleolithic and Mesolithic samples than the Near East Paleolithic and Neolithic samples.

Examination of the Protoeuropean and Near Eastern similarities of Vasil'evka I gives results that parallel the suggestions of Konduktorova (1957). Vasil'evka I displays a mixed sequence of relationships with both the European and the Near Eastern samples. The most similar to Vasil'evka I are Crimean Mesolithic and Caucasus Paleolithic and Mesolithic and Czech Republic Paleolithic samples, followed by the Sicilian Upper Paleolithic sample (Table 1). Meanwhile, among the least like Vasil'evka I are the Russian Paleolithic and Mesolithic and Near East Paleolithic and Neolithic samples.

Investigation of a Protoeuropean affinity for the Vasil'evka III flexed burial subsample supports a close dental relationship with the European samples, as exemplified by the Russian Upper Paleolithic and Mesolithic samples. These results parallel the outcome of Gokhman's (1966) osteological analysis. As in the previous two comparisons, the Crimea Mesolithic and Caucasus Paleolithic samples are more like the Vasil'evka flexed burial subsample than are all of the others. Yet, the Czech Republic Paleolithic sample is among those least like the Vasil'evka III flexed burial sample (Table 1). In contrast to the close affinity it displayed to Voloshskoe and Vasil'evka I, the Caucasus Mesolithic sample is the least like the Vasil'evka III flexed burial subsample.

Assessment of Mediterranean relationships of the Vasil'evka III extended burial subsample suggests European as well as the Mediterranean affinities of Gokhman (1966). As was the case with the previous comparisons, among the most like the Vasil'evka III extended burial subsample are the Caucasus Paleolithic and Crimean Mesolithic samples (Table 1). Contributing to the picture of dual affinities are the identical C^S values between the Vasil'evka III extended burial subsample and both the Near East Paleolithic and Russian Upper Paleolithic, followed closely by the Russian Mesolithic samples. In contrast, the least like the Vasil'evka III extended burial subsample is the Near East Neolithic sample.

Table 1. Coefficients of Similarity (C^S) for Ukraine Mesolithic Comparisons in Decreasing Order of Magnitude.

Voloshskoe				
Sample	D	T	I^S	C^S
Crimea Mesolithic	3	11	0.963	0.818
Caucasus Paleolithic	12	13	0.988	0.769
Caucasus Mesolithic	2	4	0.858	0.750
Czech Republic Paleolithic	6	11	0.979	0.727
Vasil'evka III Extended	9	22	0.989	0.727
Sicily Upper Paleolithic	2	20	0.972	0.700
Vasil'evka III Flexed	11	22	0.993	0.682
Russia Upper Paleolithic	8	22	0.991	0.682
Russia Mesolithic	41	22	0.996	0.636
Near East Paleolithic	7	22	0.990	0.591
Vasil'evka I	15	22	0.994	0.500
Near East Neolithic	16	17	0.993	0.471
Crimea Paleolithic	1	4	0.733	0.250

Vasil'evka I				
Sample	D	T	I^S	C^S
Crimea Mesolithic	3	11	0.964	0.818
Caucasus Mesolithic	2	4	0.858	0.750
Caucasus Paleolithic	12	14	0.989	0.643
Czech Republic Paleolithic	6	11	0.979	0.545
Voloshskoe	15	22	0.994	0.500
Vasil'evka III Extended	9	22	0.992	0.500
Sicily Upper Paleolithic	2	20	0.972	0.500
Near East Paleolithic	7	22	0.990	0.455
Vasil'evka III Flexed	11	22	0.993	0.455
Russia Upper Paleolithic	8	22	0.991	0.409
Russia Mesolithic	41	22	0.996	0.409
Near East Neolithic	16	17	0.992	0.353
Crimea Paleolithic	1	4	0.733	0.250

Vasil'evka III Flexed				
Sample	D	T	I^S	C^S
Crimea Mesolithic	3	11	0.961	0.727
Caucasus Paleolithic	12	13	0.987	0.692
Voloshskoe	15	22	0.993	0.682
Russia Upper Paleolithic	8	22	0.990	0.636
Vasil'evka III Extended	9	22	0.991	0.636
Russia Mesolithic	41	22	0.995	0.591
Sicily Upper Paleolithic	2	20	0.970	0.550
Near East Paleolithic	7	22	0.989	0.500
Crimea Paleolithic	1	4	0.727	0.500
Czech Republic Paleolithic	6	11	0.977	0.455
Vasil'evka I	15	22	0.993	0.455
Near East Neolithic	16	17	0.991	0.294
Caucasus Mesolithic	2	4	0.852	0.250

Vasil'evka III Extended				
Sample	D	T	I^S	C^S
Caucasus Paleolithic	12	13	0.985	0.923
Voloshskoe	15	22	0.992	0.727
Crimea Mesolithic	3	11	0.960	0.727
Sicily Upper Paleolithic	2	20	0.969	0.650
Vasil'evka III Flexed	11	22	0.991	0.636
Czech Republic Paleolithic	6	11	0.975	0.636
Near East Paleolithic	7	22	0.988	0.591
Russia Upper Paleolithic	8	22	0.989	0.591
Russia Mesolithic	41	22	0.994	0.545
Vasil'evka I	15	22	0.992	0.500
Caucasus Mesolithic	2	4	0.852	0.500
Near East Neolithic	16	17	0.990	0.294
Crimea Paleolithic	1	4	0.722	0.000

D is the number of dentitions in the sample being compared. T is number of traits being compared. I^S is the index of similarity. C^S is the coefficient of similarity. The closer the C^S to 1.0, the more similar are the morphological dental trait frequencies of the two samples being compared. The greater the value of I^S, the more meaningful the C^S. The formulae for calculating C^S and I^S are given in Haeussler (1998). Trait frequencies are in Haeussler (1996 and 1998). Data for the Near East Neolithic are from Roler (1992).

In sum, two of the samples, Voloshskoe and the Vasil'evka III extended burial subsample, have a mixture of dental and skeletal affinities. Voloshskoe has dental morphological trait frequencies more similar to the European samples than to the Near East samples compared herein, but is skeletally like Mediterraneans (Debets, 1955). The Vasil'evka III extended subsample has an alternating sequence (Caucasus Paleolithic, Near East Paleolithic, Russia Upper Paleolithic and Mesolithic, and Near East Neolithic) of dental relatedness to both Near Eastern and European samples, but has Mediterranean skeletal features (Gokhman, 1966). The dental anthropological comparisons parallel the osteological data in two out of the four samples. Vasil'evka I has an alternating sequence

(Caucasus Paleolithic, Near East Paleolithic, Russia Upper Paleolithic, Russia Mesolithic, and Near East Neolithic) of similarities to Near East and European skeletal traits (Konduktorova, 1957). The Vasil'evka III flexed burial subsample is more like the European (Caucasus Paleolithic, Russia Upper Paleolithic) than the Near East Paleolithic and Neolithic samples, and also has skeletal (Gokhman, 1966) traits similar to Europeans.

DISCUSSION

Paleopathology, archaeology, and paleoecology provide additional evidence that the Mesolithic population of the Dnieper Rapids region was indeed the result of a complex interaction of peoples, as suggested by Konduktorova (1957) and Gokhman (1955). This complexity required more than a single linear peopling event, be it of a short or long duration.

First, frequencies of dental hypoplasia corroborate the dental morphological evidence for the heterogeneity of the four Dnieper River samples. Percentages range from 0.0% in Voloshskoe to 20.0% in Vasil'evka I to 37.5% in the Vasil'evka III flexed burial subsample (Haeussler, 1996). The frequency of hypoplasia in the Vasil'evka III flexed burial subsample (37.5%) differs from that in the Vasil'evka III extended burial subsample (9.1%). These variations indicate that the samples may represent people who were members of different contemporaneous groups, who lived under various cultural and subsistence-related stresses, such as those which might have been associated with the many cases of violent deaths (Konduktorova, 1974; Nuzhnyi, 1990; Balakan and Nuzhnyi, 1995; Gokhman, 1997, personal communication). Alternatively, the people may have existed at different times and under dissimilar ecological stresses that affected nutrition and eventually dental enamel formation (Hillson, 1986). In contrast to the broad range of frequencies of hypoplasia, the four Ukraine Mesolithic samples are alike in their mutual lack of caries, abscesses, and periodontal disease. The healthy status in these pathogen-related diseases in all four of the Mesolithic samples indicates a dependence on foods common to a hunter-gatherer subsistence, and a lack of habitual consumption of processed foods associated with a subsistence based on agriculture or transition to it (Turner, 1979, 1982; Clarke et al., 1986; Meiklejohn, et al., 1988).

Second, archaeological features point to the cultural complexity suggested by the dental and skeletal features of Ukraine Mesolithic burials. Examples include the presence of an associated habitation site at Vasil'evka I and III and its absence at Voloshskoe, extended burials in all three cemeteries with flexed burials in Voloshskoe and Vasil'evka III, and red ocher in Vasil'evka I and Vasil'evka III extended burials but not in Voloshskoe (Telegin, 1982, 1989). Extra-regionally, the Ukraine cemeteries are more like the Russian Mesolithic (Oleneostrovski' Mogil'nik and Popova) and Czech Paleolithic (Predmosti) burial grounds than like all of the other sites compared here (Haeussler, 1998; in press). The salient differentiating feature is the presence of a cemetery in the Ukraine Mesolithic and the Russian Mesolithic and Czech Republic Paleolithic sites and the absence of a cemetery in the Near East Paleolithic and Neolithic, Caucasus Paleolithic and Mesolithic, and Russian and Sicilian Upper Paleolithic sites (Haeussler, in press). In addition, the Ukraine burials have relatively less grave goods than do those in Upper Paleolithic and Mesolithic Russia and the Paleolithic Czech Republic. Whether the existence of conflict

within the Ukraine Mesolithic Era contributed to the relative paucity of grave goods is presently unknown. Unfortunately, nearly all of the Czech Republic Predmosti skeletons have been destroyed.

Third, paleoecology of the Dnieper River supplements the understanding of the biological and cultural diversity of the Mesolithic samples. By the Boreal Era, which coincided with the Mesolithic era, forests extended southward from the zone of tundra that bordered the Arctic Ocean. Most of the zone of discontinuous permafrost and deep seasonal freezing of the previous Valdai Glacial Era had become a mixed grass and xerophytic steppe. During the years which encompassed the development of the cultures represented by the Mesolithic Dnieper Rapids cemetery samples, the forest zone moved south to the region of Kiev. From Kiev to the Black Sea, the land remained a steppe (Baulin and Danilova, 1984; Dolukhanov and Khotinskiy, 1984), which gradually transformed into a steppic corridor (Pavel Dolukhanov, pers. comm., 1994). Zones, such as the land around the Dnieper Rapids were ecologically abundant, attracting the animals and fish on which the Mesolithic peoples depended for their subsistence (Nuzhnyi, personal communication, 1997). By 9,000 BP the megafauna, which the Upper Paleolithic peoples utilized, had become extinct. Ecological conditions permitted domestication of animals and later, albeit sporadically, plants (Dolukhanov and Khotinskiy, 1984). Demographically, the Boreal Era Dnieper Rapids region was accessible by the Dnieper River from the north and from the south. The area could also be reached from the west via the tributaries of the Dnieper River and from the east via eastern tributaries and the plains, as the open southern Dnieper River region land supported increasing numbers of peoples. From the south in Crimea, the Caucasus, and the Near East peoples could have moved northward at different times and with varying degrees of successful occupation. However, any movement of people from the Near East and the Mediterranean Sea region had to involve circumventing part of the Mediterranean and arriving at the Black Sea by some route that involved either the Caucasus Mountains and possibly the western Caspian region or Turkey and Bulgaria.

Therefore, the variations in dental trait frequencies, osteological affinities, and archaeological remains discussed above indicate that we may be looking at the result of microevolutionary events caused by complex movements of peoples and their cultures, as suggested by Gokhman (1966) and Konduktorova (1973). This would have occurred when the Boreal Era steppe landscape of Ukraine could support larger numbers of peoples than it did during the Middle and Upper Paleolithic eras, when the land was a zone of deep seasonal freezing.

SUMMARY AND CONCLUSIONS

Examination of morphological and pathological traits and material culture evidence from burials in three Ukraine Mesolithic cemeteries suggests agreement with Gokhman (1966) and Konduktorova (1973) that the physical features of the Ukraine Mesolithic people were the result of a complex interaction of peoples during or preceding the 2,000 carbon-dated years spanned by these samples. Voloshskoe, Vasil’evka I, and Vasil’evka III flexed and extended burial subsamples are dentally and archaeologically heterogeneous on a regional scale. Inter-regionally, the Voloshskoe and Vasil’evka III flexed burial samples

are dentally more like the Russian Upper Paleolithic and Mesolithic samples than those from the Near East studied herein. However, Voloshskoe skeletons are similar to those from the Near East (Debets, 1955), while Vasil'evka III flexed burials are skeletally like Europeans (Gokhman, 1966). In contrast, Vasil'evka I and the Vasil'evka III subsample have an alternating sequence of dental relatedness to European and Near East samples examined during this study. Yet, the Vasil'evka I skeletons resemble Europeans and Near Easterners (Konduktorova, 1957) and the Vasil'evka III extended burials, Mediterranean people (Gokhman, 1966). Archaeologically, Voloshskoe, Vasil'evka I, and Vasil'evka III are cemeteries that have features that differ on the level of intra-regional cultural variation. Inter-regionally, the three cemeteries are more like European burial grounds than like the Near East graves. Interpolation of paleoecological information into these results suggests that the Mesolithic peopling of the Dnieper River occurred when the Boreal Era steppeland of Ukraine was capable of supporting larger numbers of peoples than it did during the Upper Paleolithic Era, when the land was a zone of deep seasonal freezing. The dental and skeletal traits in these samples are the result of numerous microevolutionary events as people moved with varying degrees of success and permanency into the Dnieper Rapids region from the south (Near East, Caucasus, Crimea), west (Czech Republic), and north (Russia).

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Appendix 1. Samples: UKRAINE: Ukraine Mesolithic (Voloshskoe, Vasil'evka 1, Vasil'evka III flexed burials, and Vasil'evka III extended burials), Crimea Paleolithic (Starosel'e), Crimea Mesolithic (Fat'ma Koba, Murzak Koba), EUROPE: Czech Republic Paleolithic (Brno, Predmosti), Russian Upper Paleolithic (Kostenki, Sungir), Russian Mesolithic (Oleneostrovski' Mogil'nik, Popova), CAUCASUS: Caucasus Paleolithic (Akhshtyr', Barakaevskaya Cave, Dzhruchula Cave, Ortvala Cave, Sakazhia Cave), Caucasus Mesolithic (Kvchara), NEAR EAST: Near East Paleolithic (Amud, Qafzeh 9 and 11, Skuhl I and IV, Tabun II), Near East Prepottery Neolithic ('Ain Ghazal), MEDITERRANEAN: Sicily Upper Paleolithic (San Teodoro). All observations except 'Ain Ghazal were done by the author. 'Ain Ghazal data are from Roler (1992). Provenance of the samples is given in Haeussler (1998).

Comparison of Molar Crown Size of Precontact Easter Islanders With Other Pacific Groups

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INTRODUCTION

It is now generally believed that the Polynesian islands were populated by an eastern division of the Lapita Cultural Complex (Kirch, 1984, 1997; Van Tilburgh, 1994). Easter Island is the farthest east of all of the Polynesian islands and was not inhabited until near the end of the first millenium AD, probably sometime between AD 600 and 800 (Bahn and Flenley, 1992; Van Tilburgh, 1994; Green, 1998). It is not known how many people, perhaps a hundred or so, were in the first migration or, if there were more than a single settlement; however Easter Island traditions allude to two early migrations (Van Tilburgh, 1994). On the other hand, Houghton (1996) believes that Easter Island was more likely a chance discovery by a group *in extremis* with no return trip. Also, there is the strong possibility that there may have been occasional contacts with people coming into the Pacific from lands to the east of the Polynesian Islands (Heyerdahl, 1952; Terrell, 1990; Irwin, 1994). The most recent proposal is that of Green (1998) who has suggested that the Pitcairn-Mangarevan region is the likely area from which the earliest migration occured to Easter Island.

This paper will focus on molar tooth size, i.e., crown and root dimensions, in precontact and living Easter Islanders and compare them with the molars of other Pacific populations.

The crown diameters of teeth have been shown to have high heritability in living human populations (Goose, 1971; Alvesalo and Tigerstedt, 1974; Townsend and Brown, 1978). Also, twin studies of crown diameters have demonstrated the high heritability component in identical twins compared to fraternal twins (Osborne, et al., 1958). Since crown diameters can take any value within a given range for one population, they are "known as continuous variants" (Hillson, 1996). In addition, tooth size has an adaptive significance in extant populations related to mastication and various manipulative functions (Wolpoff, 1971; Lavelle, 1978) as well as being relatively independent of the environment (Osborne, 1967). The Pacific islands, with their long history of migrations, should afford an excellent arena for investigating the effects of various microevolutionary processes such as genetic drift, genetic bottlenecks, and selection on tooth size.

MATERIALS AND METHODS

The precontact molars studied in this paper came from Ahu Tongariki located on the south-eastern coast of Easter Island. The excavation was directed by C. Cristino and G. Orefici in 1993. The skeletal material was broken and scattered throughout the excavation; while the teeth were mostly in good condition, they were isolated from the skulls. There were 57 maxillary first molars, 75 second molars and 56 third molars while the mandibular molars consisted of 70 first molars, 56 second molars and 32 third molars. For comparison, the molars of contemporary Easter Islanders were measured on dental casts obtained in 1964-1965 and now housed at Arizona State University (See Turner and Scott, 1977 for a complete discussion of this material).

The molar tooth dimensions used are defined as follows:

Mesiodistal crown diameter: the greatest mesiodistal crown diameter measured parallel to the occlusal surface.

Buccolingual crown diameter: the greatest buccolingual crown diameter measured perpendicular to the mesiodistal diameter.

Crown height: the distance between the tip of the mesiobuccal cusp and the crest of the curvature at the cementoenamel junction on the buccal side parallel to the long axis of the molar.

Root length: the distance between the apex of the mesiodistal root and the deepest curvature of the cementoenamel junction on the buccal side parallel to the long axis of the root.

For consistency, the mesiodistal dimensions were taken on the isolated molars in a manner that matches, as nearly as possible, those taken on the molars on the dental casts. Also, one investigator (DRS) made all measurements to 0.01 millimeter using a Mitutoyo caliper. Molars showing enough wear or pathology to decrease their original dimensions were not measured.

In order to compare molar size among the several Pacific groups standardized deviations were calculated by the formula:

$$X - M/SD$$

where X is the mean of the comparative samples, and M and SD the mean and standard deviation of the Precontact Easter Island molars (Yamada, et al., 1988). The Precontact Easter Island molars were used to represent the base line (0.0 level) since they represent, at least to some degree, the early dental (genetic) characteristics of Polynesia.

RESULTS

Table 1. Easter Island molar crown diameters (mm)

	MANDIBLE					MAXILLA				
	N	M	SD	T-VALUE	P	N	M	SD	T-VALUE	P
M1					PRECONTACT					
M-D	67	12.0	0.54	9.0	<0.001	52	10.8	0.50	3.6	NS
B-L	68	10.8	0.55	7.0		52	11.9	0.45	7.9	<0.0001
					LIVING					
M-D	73	11.1	0.71			60	10.4	0.48		
B-L	71	10.1	0.64			60	11.1	0.57		
M2					PRECONTACT					
M-D	55	11.5	0.60	4.1	NS	70	10.2	0.51	3.6	NS
B-L	55	10.4	0.60	1.6	NS	70	11.8	0.60	2.7	NS
					LIVING					
M-D	65	10.9	0.78			70	9.9	0.42		
B-L	65	10.2	0.62			70	11.5	0.71		
M3					PRECONTACT					
M-D	29	11.2	0.65	2.43	NS	53	9.4	0.66	3.8	NS
B-L	29	10.4	0.58	3.10	NS	53	11.7	0.82	3.3	NS
					LIVING					
M-D	27	10.6	0.78			29	8.8	0.84		
B-L	26	9,8	0.78			29	10.9	0.82		

Table 1 presents the means, standard deviations, T-values, and confidence levels (P) of the mesiodistal and buccolingual crown diameters of the upper and lower molars of the precontact and living Easter Islanders.

The mean values of molar dimensions were slightly larger in the precontact population than in the living Easter Islanders. Of the 24 dimensions, however, only three were statistically significant: the buccolingual diameter of the upper M1 and both dimensions of the lower M1. The maxillary molars of both groups showed a gradual mesiodistal size reduction from M1> M2> M3 while the buccolingual diameter expressed less dimensional reduction from M1 to M3. In fact, the buccolingual dimension of M2 is wider than in either M1 or M3 in the contemporary population. The mandibular molars had a mesiodistal reduction from M1> M2> M3 while the breadths were slightly more variable from M1 to M3 in both groups.

Table 2 presents the means, ranges, standard deviations, and coefficients of variation of the crown heights of the upper and lower molars in precontact Easter Islanders. There were few differences in crown height in either the upper or lower molars from M1 to M3.

In Table 3, means, ranges, standard deviations, and coefficients of variation are presented for the root lengths of the upper and lower molars of the precontact Easter Islanders. As with crown height, there were only slight differences in root length from M1 to M3.

Table 2. Crown height in Precontact Easter Islanders (mm).

	N	M	RANGE	SD	CV
			MANDIBLE		
M1	49	7.5	9.8-6.0	0.99	13.2
M2	43	7.5	9.3-6.2	0.87	11.6
M3	25	6.9	8.1-4.8	0.83	12.0
			MAXILLA		
M1	44	7.7	9.5-6.5	0.66	8.6
M2	62	7.7	9.2-6.1	0.68	8.8
M3	44	7.4	9.6-5.8	0.82	11.1

Table 3. Root length in Precontact Easter Islanders (mm).

	N	M	RANGE	SD	CV
			MANDIBLE		
M1	41	13.7	16.5-10.3	1.92	13.9
M2	41	13.8	17.7-11.0	1.45	10.5
M3	21	12.9	15.9-8.0	2.08	16.1
			MAXILLA		
M1	34	12.5	15.1-9.4	1.60	12.8
M2	57	12.9	17.7-8.0	1.68	13.0
M3	41	12.7	15.7-9.6	1.39	10.9

DISCUSSION

The molars of precontact Easter Islanders are somewhat larger than those of the living population on Easter Island, although of 24 dimensions only three are statistically significant (Table 1). There has been a varying amount of admixture with Europeans since the first contact in 1722, so it is likely that tooth reduction, as represented by molars, is due at least in part to contact with Europeans. Polynesian tooth size is somewhat larger than Europeans. Also, population size has fluctuated several times since 1722, when it was probably somewhere between 3,000 to 2,000 individuals (Metraux, 1940). There were fewer than 200 individuals during the 1860's due to slavers, famine, disease, and internecine conflict (Metraux, 1940). There has been a gradual increaase in population size to where it is today at slightly over 1,000 persons (a census taken in 1965 showed a total of 958 natives, Taylor, 1966).

The essential foods before European contact were plant starches (sweet potatoes, taro, and bananas), fish, and chicken as in other Pacific island societies (Pollock, 1993; Stevenson, 1998). According to Pollock (1993), the preferred food of Easter Islanders was the *kumara*, sweet potato. This diet has probably not changed much for thousands of years, and if there was a major change in diet after European contact, it has been in effect less than three hundred years, a rather short time for evolutionary modifications.

There are no data on crown height for Pacific populations that we are aware of at this time. Wheeler (1956) presents crown height data from Black (1902) which are based on white populations and Kraus, et. al., (1969) has information on crown height. These data are presented in Table 4. Except for the crown height of upper M3, these data compare quite favorably with the crown heights of the Precontact Easter Islanders. There is no root

length data for the Pacific populations except for the measurements presented by Campbell (1925) for Australian aboriginals and the Japanese. This information is presented as root length and total tooth length measurements from which is derived the relative root length index presented in Table 5. The Australian aborigines were found to have relatively longer roots, especially on the third molars, than the other three groups, a feature which is the major variation among the data in the literature. It is also interesting to find that Kovacs (1971) found a relative root length index of 63.59 for the mandibular first permanent molars in humans which is very similar to those presented in Table 5. Indeed, he suggests that more study of such relationships are likely to show "significant differences between the various zoological groups" since he found indices of 73.69 and 77.19 for the mandibular first permanent molars of chimpanzees and gorillas respectively (Kovacs, 1971. p.221).

Table 4. Crown Height (Means) in Three Human Groups.

	Precon E.I.[1]	Wheeler [2]	Kraus [3]
MANDIBLEM1			
	7.5	7.7	7.5
M2	7.5	6.9	7.0
M3	6.9	6.7	7.0
MAXILLA			
M1	7.7	7.7	7.5
M2	7.7	7.2	7.0
M3	7.4	6.3	6.5

[1] Present study [2] Wheeler (1956) [3] Kraus, et al. (1969)

Table 5. Relative Root Length Index: Root lg/total Tooth lg (%).

	Precon E.I.[1]	Australia[2]	Japan[2]	Am. White[3]
MANDIBULAR				
M1	64.6	64.8	62.7	62.9
M2	64.8	67.6	61.4	65.2
M3	65.3	73.7	61.1	64.5
MAXILLARY				
M1	61.9	69.4	65.4	63.5
M2	62.6	69.4	66.7	65.0
M3	63.2	70.6	62.1	66.7

[1]Present study [2]Campbell (1925) [3]Wheeler (1956)

Although hominid tooth size has been studied by odontologists with various interests and goals through the years, it is unfortunate that, except for odontometric studies of Australian aboriginal populations, the island peoples of the Pacific have received only meager attention. For example, we were unable to find any metric investigations of the teeth of Micronesians, although the situation was better for the occupants of the Melanesian and Polynesian islands. It is generally accepted that the populations of the Pacific are separated into two groups, a large tooth-sized group and a small tooth-sized group (Harris, et al., 1975; Harris and Smith, 1983; Yamada, et al., 1988). The former

occupy Australia and Melanesia while the latter are found in Micronesia(?) and Polynesia. We query Micronesia since the only material we know of is presented by Brace, et al., (1989) where the summary tooth size (TS) is given as 1,311 mm for the Guamese which the authors state is larger than the TS (1,172 mm) for Polynesia (no geographic designation). Indeed, in that paper the TS for Guam is the largest presented for several Asian populations albeit no data are presented for Melanesia or Australia.

Table 6. Oceania maxillary molar crown dimensions (mm).

		First Molar				Second Molar				Third Molar			
GROUP	SEX	N	M	SD	CV	N	M	SD	CV	N	M	SD	CV
PRECON. EI[1] (Polynesia)													
M-D	M+F	52	10.8	0.50	4.6	70	10.2	0.51	5.0	53	9.4	0.66	7.0
B-L	M+F	52	11.9	0.45	3.8	70	11.8	0.60	5.1	53	11.8	0.82	7.0
LIVING EI[1] (Polynesia)													
M-D	M+F	60	10.4	0.48	4.6	70	9.9	0.42	4.2	20	8.8	0.84	9.6
B-L	M+F	60	11.1	0.57	5.1	70	11.5	0.71	6.2	29	10.9	0.82	7.5
MOKAPU[2] (Polynesia)													
M-D	M	26	11.1	0.67	6.0	25	10.8	0.93	8.7	7	10.0	0.61	6.1
B-L	M	26	11.9	0.65	5.5	25	12.1	0.74	6.2	-	-	-	-
TONGA[3] (Polynesia)													
M-D	M	7	10.9	0.44	4.0	7	9.9	0.34	3.4	35	9.4	0.80	8.5
B-L	-	-	-	-	-	-	-	-	-	-	-	-	-
NAMU[3] (Polynesia)													
M-D	M	62	11.1	0.48	4.3	25	10.5	0.76	7.2	17	8.7	0.70	8.1
B-L	-	-	-	-	-	-	-	-	-	-	-	-	-
COOK I[4] (Polynesia													
M-D	M	36	10.7	0.48	4.5	35	10.1	0.54	5.4	38	9.1	1.01	11.1
B-L	M	36	12.0	0.53	4.4	35	12.2	0.69	5.7	40	11.1	0.94	8.5
MAORI[3] (Polynesia)													
M-D	M	36	10.2	0.82	8.0	35	10.0	0.59	5.9	33	9.0	0.93	10.3
B-L	-	-	-	-	-	-	-	-	-	39	11.7	1.03	8.8
W. NAKANAI[5] (Melanesia)													
M-D	M	98	11.1	0.61	5.5	85	9.7	0.73	7.5	20	9.5	0.76	8.0
B-L	M	98	11.5	0.67	5.8	85	11.4	0.79	6.9	21	11.5	0.88	7.6
NASIOI[6] (Melanesia)													
M-D	M	85	11.1	0.62	5.6	61	10.1	0.74	7.3	12	9.6	0.95	10.0
B-L	M	82	12.3	0.73	6.0	63	12.5	0.77	6.2	12	11.5	0.88	7.7
GOROKA N.G.[7] (Melanesia)													
M-D	M	30	11.4	0.56	4.9	30	10.6	0.75	7.1	29	10.2	1.0	9.8
B-L	M	31	12.7	0.53	4.2	31	12.5	0.77	6.2	29	12.5	1.0	8.0
LUFA N.G.[7] (Melanesia)													
M-D	M	24	11.4	0.71	6.3	23	11.0	0.94	8.6	12	9.6	0.95	10.0
B-L	M	24	12.6	0.69	5.5	23	12.8	0.75	5.9	12	11.5	0.88	7.7
AUSTRALIAN[8]													
M-D	M+F	34	11.7	0.80	5.5	34	11.4	.80	7.0	29	10.2	1.0	9.8
B-L	M+F	33	12.8	0.70	5.5	33	13.3	.80	6.0	29	12.5	1.0	8.0

[1]Present study; [2]Snow (1974); [3]Kean and Houghton (1990); [4]Yamada, et al. (1988); [5]Turner and Swindler (1978); [6]Bailit, et al. (1968); [7]Doran and Freedman (1974); [8]Smith, et al. (1981).

Table 7. Oceania mandibular molar crown dimensions (mm).

		First Molar				Second Molar				Third Molar			
GROUP	SEX	N	M	SD	CV	N	M	SD	CV	N	M	SD	CV
PRECON. EI[1] (Polynesia)													
M-D	M+F	67	12.0	0.54	4.5	55	11.5	0.60	5.2	29	11.2	0.65	5.9
B-L	M+F	68	10.8	0.55	5.1	55	10.4	0.60	5.8	29	10.4	0.58	5.6
LIVING EI[1] (Polynesia)													
M-D	M+F	73	11.1	0.71	6.4	65	10.9	0.78	7.2	27	10.6	0.78	7.3
B-L	M+F	71	10.1	0.64	6.3	65	10.2	0.62	6.1	26	9.8	0.78	8.0
MOKAPU[2] (Polynesia)													
M-D	M	25	11.5	0.65	5.6	25	11.1	0.86	7.8	32	10.8	0.76	7.0
B-L	M	25	11.1	0.40	3.6	25	10.9	0.54	5.0	-	-	-	-
TONGA[3] (Polynesia)													
M-D	M	7	12.1	0.57	4.7	7	11.4	0.17	1.5	7	12.4	0.82	6.6
B-L	-	-	-	-	-	-	-	-	-	-	-	-	-
NAMU[3] (Polynesia)													
M-D	M	90	12.2	0.61	5.0	81	11.8	0.81	6.9	48	12.0	0.76	7.2
B-L	-	-	-	-	-	-	-	-	-	-	-	-	-
COOK I[4] (Polynesia)													
M-D	M	35	11.9	0.49	4.2	35	11.4	0.51	4.5	19	11.9	0.69	5.8
B-L	M	35	11.1	0.40	3.6	35	11.0	0.43	3.9	-	-	-	-
MAORI[3] (Polynesia)													
M-D	M	36	10.8	0.75	6.9	44	10.9	0.71	6.5	57	11.0	0.92	8.4
B-L	-	-	-	-	-	-	-	-	-	57	10.3	0.82	8.0
W. NAKANAI[5] (Melanesia)													
M-D	M	98	11.5	0.57	5.1	89	10.5	0.74	7.1	38	10.7	1.19	11.1
B-L	M	98	10.5	0.48	4.6	89	10.0	0.73	7.3	35	10.5	0.86	8.2
NASIOI[6] (Melanesia)													
M-D	M	84	11.8	0.63	5.4	69	11.0	0.74	6.7	21	11.4	0.99	8.7
B-L	M	86	11.1	0.59	5.2	66	11.0	0.68	6.2	22	11.1	0.77	6.9
GOROKA N.G.[7] (Melanesia)													
M-D	M	25	12.3	0.65	5.3	28	11.5	0.70	6.1	18	11.2	0.56	5.0
B-L	M	26	11.4	0.41	3.6	29	11.4	0.55	4.8	18	10.8	0.52	4.8
LUFA N.G.[7] (Melanesia)													
M-D	M	22	12.1	0.63	5.2	22	11.6	0.57	4.9	24	12.0	0.80	6.7
B-L	M	22	11.6	0.60	5.2	22	11.2	0.67	6.0	23	11.4	0.70	6.1
AUSTRALIAN[8]													
M-D	M+F	32	12.6	0.90	7.1	32	12.8	0.80	6.3	24	12.0	0.80	6.7
B-L	M+F	32	11.6	0.70	6.0	32	11.7	0.60	5.1	23	11.4	0.70	6.1

[1]Present study; [2]Snow (1974); [3]Kean and Houghton (1990); [4]Yamada, et al. (1988); [5]Turner and Swindler (1978); [6]Bailit, et al. (1968); [7]Doran and Freedman (1974); [8]Smith, et al. (1981).

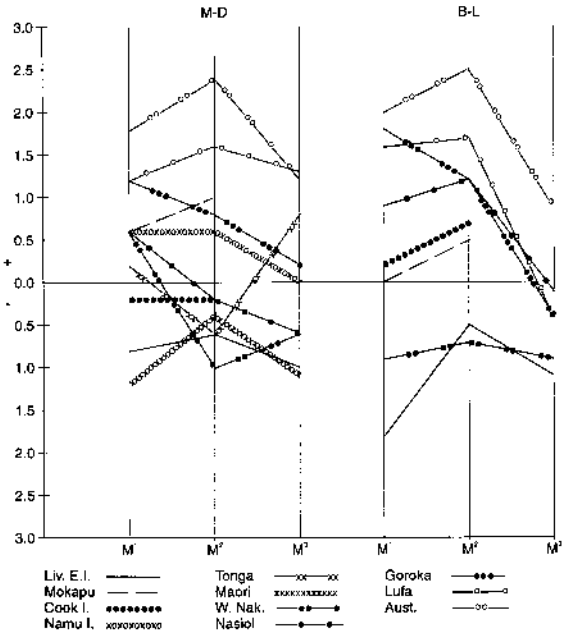


Fig. 1. Standardized deviations in the crown dimensions of the upper molars of several oceanic groups from precontact Easter Islanders shown at the 0.0 level.

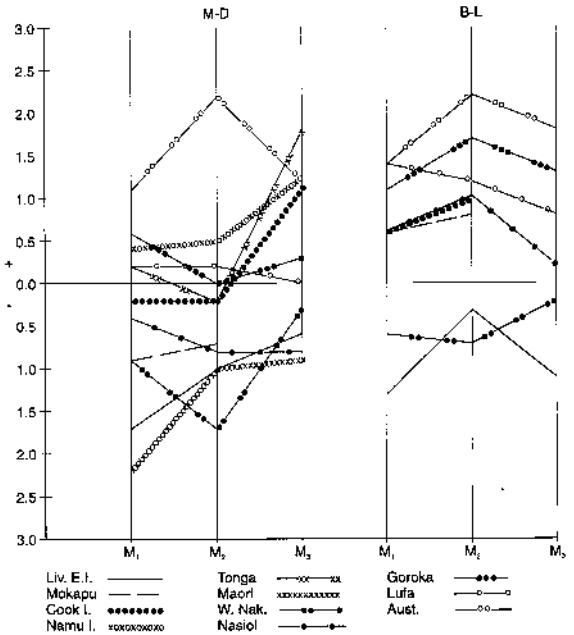


Fig. 2. Standardized deviations in the crown dimensions of the lower molars of several oceanic groups from precontact Easter Islanders shown at the 0.0 level.

Studies of the teeth of Australian aborigines over the last several decades have demonstrated that tooth size is more irregular regionally than originally thought and that there are both larger and smaller teeth present among the different populations (Brace, 1980; Smith, et al., 1981). Even so, these teeth are generally larger than the teeth from other geographical regions of the Pacific (Tables 6 and 7; Figs. 1 and 2). In Melanesia, there are also large and medium-to- small sized teeth as shown by the molar dimensions in Tables 6 and 7. Turner and Swindler (1978, p.369) noted "the relatively small to medium-sized teeth of the West Nakanai." In fact, the West Nakanai, who live in New Britain, also have somewhat smaller molars than the New Britain population studied by Janzer (1927). The Nasioi people from the Solomon islands have slightly larger molars than the West Nakanai while the Goroka and Lufa from the Eastern Highlands of New Guinea have larger molars than either the West Nakanai or Nasioi (Tables 6 and 7). Thus, the information on molar sizes in Tables 6 and 7, and Figs. 1 and 2, demonstrates rather clearly the regional variability of molar sizes in the Pacific. There appears to be large and small tooth-size groups (at least regarding molars) in all of the major geographic regions in the Pacific with the possible exception of Micronesia where odontometric data are lacking. There are no obvious indications of clines, i.e., no clear-cut geographic size clines such as demonstrated by the west-east reduction in frequency of shovel-shaped incisors (Riesenfeld, 1956). For example, the mesiodistal diameter of the lower M1 (Table 7) is 12.0(mm) in precontact Easter Islanders, 12.1 in Tonga, 12.2 in Namu, 12.3 in Goroka, 12.1 in Lufa and 12.6 in Australia. In fact, the Polynesians as represented by Namu, Cook, and Tonga have lower M3 mesiodistal dimensions that are as long or longer than the Australian (Table 7). Unfortunately, Snow (1974) did not measure M3 in the early Hawaiians from Mokapu.

Of the six Polynesian groups depicted in Figures 1 and 2, only the Mokapu from Oahu represent another precontact sample (Snow, 1974). Only M1 and M2 were measured, and of these, only the mesiodistal dimensions of the lower molars are smaller than the Precontact Easter Islanders. The molars of contemporary Easter Islanders are smaller than their ancestors who lived before European contact (see Table 1). The mean values of the other Polynesian groups are rather randomly distributed for both the upper and lower molars with the Maori having among the smallest molars reported in Polynesia (Kean and Houghton, 1990). It is interesting to note that Yamada, et al., (1988) found tooth size differences between the northern and southern groups of the Cook Islands. The Melanesian populations are variously dispersed, and of these groups, the West Nakanai have the smallest molars while the Goroka and Lufa possess the largest molars with the Nasioi falling in between. The Australian populations are represented by the Broadbeach aborigines from S. E. Queensland and have the largest molars of all groups. In fact, it should be mentioned that this group has the largest dentition, especially molars, of all recent Aboriginal populations (Smith, et al., 1981).

In view of the comparative information presented here on molar dimensions (Tables 6 and 7; Figs. 1 and 2) from Australia, Melanesia, and Polynesia it is clear that molar crown size varies geographically from island to island across the Pacific, and in our opinion, these data suggest that the "boundries" between those regions of the Pacific where large teeth are found and those areas where smaller teeth are present are quite vague or nonexistent

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Dentition Pecularities in Human Isolates - South Sinai Bedouin Tribes

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INTRODUCTION

The objectives of this study were to (a) compare the frequency of dental morphological traits among the Bedouin tribes in South Sinai and populations from other geographical areas; (b) to determine the main discrete morphological traits typical in the dentition of South Sinai Bedouins; (c) to assess the extent of symmetry and/or asymmetry in the dental morphological traits of the Bedouins; and (d) to explore the feasibility of using the frequencies of morphological traits of the teeth among Bedouin tribes to estimate biological distances between them.

MATERIALS AND METHODS

Subjects: The study was carried out on children from Bedouin tribes inhabiting the South Sinai desert. These tribes are geographically and culturally isolated from neighboring populations. They are small in size, numbering between 400 and 3500 individuals per tribe. The social customs strongly encourage consanguineous marriages, hence, the coefficient of inbreeding in these groups is among the highest known for human populations (F=0.0908; Kobyliansky and Hershkovitz, 1997).

Material from 352 children, aged 5-14 years from the following four Bedouin groups of South Sinai tribes were examined: 1) Muzeina; 2) Gebeliya; 3) Aleigat (including Hamada); and 4) a mixed group, designated as "other tribes", and including members of the Sawalcha, Beni-Wassal, Haweitat, Gararsha and Awlad Said tribes. The sex ratio in the studied sample did not reflect the true child population structure, because for the most part Bedouin girls do not attend school, and our dental casts were taken in schools (Tables 1 and 2).

Materials: The discrete traits of the permanent (PD) and deciduous (DD) teeth in both jaws were recorded from plaster casts. Microfine alginate was used as an impression material (Algertral-Bayer, Leverkursen, Germany). Casts were made from special hard yellow plaster (Moldaroc, Algetral-Bayer, Leverkusen, Germany) in accordance with recent practice (e.g. Kaul and Prakash, 1981; Hershkovitz et al., 1993; Moscona et al., 1997; Moscona et al., 1998). A total of 352 casts were made, focusing on 31 maxillary traits (29PD and 2DD) and 26 mandibular traits (24PD and 2DD). Incidences of traits in relation to sex, age, and ethnic group were recorded and differences in incidence were tested by "chi-square" test.

Table 1. Examined individuals.

Group	Tribe	No. Males	No. Females	Total[#]	Dental Age[*]±S.D.
1	Gebeliya	27	5	32	9.0±2.1
2	Muzeina	170	18	188	8.1±2.3
3	Aliquat & Hamada	43	2	45	8.6±2.7
4	Bnei Wasal, Suwalha, Huweitat, Gararsha and Walad-Said	81	6	87	8.4±2.6
Total		321	31	352	8.4±2.6

*Dental age (yrs.) estimated by D. Moskona.
#One individual omitted because of absence of gender registration in recording form.

Table 2. Dental age of examined individuals.

Dental Age (yrs.)	5	6	7	8	9	10	11	12	13	14	Total
Number	16	74	64	73	40	15	11	22	30	7	352

Statistical Methods: Frequencies of discrete dental traits were compared by sex, age and tribal affiliation, and assessed by χ^2 test in reference to the following questions: 1. Is there sexual dimorphism of the discrete dental traits? 2. Are there differences in the discrete dental traits among the tribes? 3. What is the association between frequency of traits and age when the age of the individual is an indirect measure of the degree of attrition and/or caries in the teeth?

Biological distances between the tribes were estimated by the “Cluster Analysis Method", based on all the relevant traits. The significance of bilateral asymmetry was also estimated by Chi^2 tests, that is, the right-left asymmetry of the discrete traits for the same teeth from both sides of both jaws. All statistical procedures were carried out at Tel Aviv University's computer center based on the BMDP program (Dixon, 1992).

RESULTS

The distribution of dental morphological traits was compared by sex, age and tribal extraction, then evaluated statistically by the chi-square (χ^2) test. The results showed: 1. No significant differences in distribution of the morphological traits in the two sexes except for 3 traits (shovel-shaped maxillary right incisors, maxillary left central incisor lingual tubercles and mandibular left mesial lobes on the distolingual first molars). 2. No significant effect of age on the discrete dental traits owing to attrition of the occlusal surface. 3. Significant differences between the tribes in trait frequency with respect to 13 of the paired traits on both sides, and another 12 on one side only (Table 3). 4. Five of the studied traits were symmetrical (three in the upper and two in the lower dentition (Figure 1): a. The labial curvature - slight convexity - of the upper central incisors; b. The distance between the crests of the labial and lingual cusps - intercuspal width - in the first upper premolars; c. Grooves and pits in the occlusal plane of the upper second molars; d. Labial curvature - slight inclination - of the lower lateral incisors; e. Lingual convexity - lingual cingulum - in lower canines.

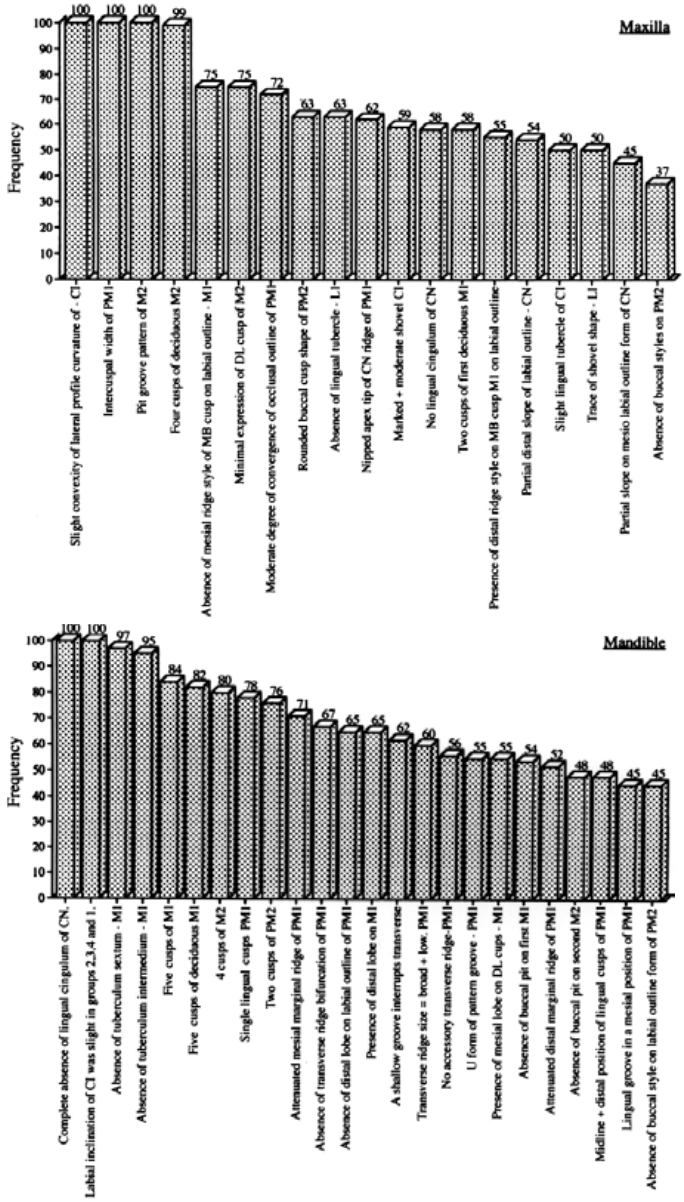


Fig. 1. Frequency of some morphological dental traits in the Bedouins of South Sinai (Total sample).

There were only 3 traits with any significant degree of heterogeneity between the sexes and consequently the data on the sexes were pooled in further analyses.

Table 3. Morphologic differences in the dentition of four Bedouin groups from South Sinai.

Teeth	Trait	Chi-square		Teeth	Trait	Chi-square	
		R	L			R	L
CI	Shovel shape incisor	25.96*	11.46	M_1	Carabelli Cusp	32.86*	29.84*
CI	Lingual Pit/Groove	23.61*	21.5*0	C1	Labial Surface Inclination	38.41*	11.50*
CI	Lingual Tubercle	2.04	9.92	L1	Labial Surface Inclination	8.77*	7.97*
CI	Labial Outline	14.99*	24.42*	CN	Lingual Cingulum	0.00	5.69
LI	Shovel Shape Incisor	11.13	17.68*	PM_1	Transverse Ridge	13.21*	11.43
LI	Peg Shape	8.81*	10.45*	PM_1	Lingual Cusp	11.51	7.38
LI	Lingual Tubercle	4.37	7.81	PM_1	Position of Lingual Cusps	5.40	19.39
LI	Labial Outline	12.47*	14.20*	PM_1	Lingual Groove	22.47	19.10
CN	Cusp Tip	7.24	7.08	PM_1	Accessory Transverse Ridge	10.91	17.66*
CN	Degree of M Slope	11.53	16.32*	PM_1	Transverse Ridge Bifurcation	3.80	5.59
CN	Degree of D Slope	21.79*	5.00	PM_1	Size and Prominence of Transverse Ridge	8.13	10.06
CN	Styles	42.69*	29.74*	PM_1	Mesial Marginal Ridge	6.12	7.80*
CN	Lingual Cingulum	10.41	8.42*	PM_1	Distal Marginal Ridge	5.43	2.01
PM_1	Degree of Converg.	10.70	22.32*	PM_1	Distal Lobe	6.59	6.00
PM_1	Inclination of Cusp Ridges	13.70*	8.21	PM_2	Number of Cusps	1.66	1.35
PM_2	Styles	6.83	6.70**	PM_2	Pit Groove Pattern	5.72	3.18
PM_2	Buccal Cusp	4.62	2.63	PM_2	Buccal Style	11.02	9.32
M_1	Ant. Transverse Ridge	35.06*	35.68*	M_1	Number of Cusps	1.07	0.58
M_1	Oblique Ridge	37.59*	15.42*	M_1	Buccal Pit	13.35	11.35
M_1	Distal Marginal Pit	10.40	7.37	M_1	Tuberculum Sextum	2.83	5.62
M_1	Mesial Ridge on MB Cusp	3.02	4.39	M_1	Tuberculum Intermedium	3.33	4.68
M_1	Distal Ridge on MB Cusp	23.56*	5.68	M_2	Number of Cusps	0.44	6.57
M_1	Buccal Groove	46.55*	18.03*	M_2	Buccal Pit	6.46	5.98
M_1	Lingual Groove	26.45*	30.97*	M_2	Number of Cusps 1st Deciduous Molar	3.72*	35.99*
M_2	Disto-Lingual Cusp	4.99	2.41	M_2	Number of Cusps 2nd Deciduous Molar	20.10*	8.93
M_2	1st Deciduous Number of Cusps	4.05	3.58	M_1	Distal Lob on ML Cusp	6.05	5.10
M_2	2nd Deciduous Number of Cusps	3.07	3.56	M_1	Mesial Lobe on DL Cusp	7.21	22.38*

*=p<0.05

Incisors

Maxillary central incisors: The ‘shovel shape’ of the central incisors occurs at a frequency of 59% (Figure 1) in the first two gradations, namely, moderate and marked. This is undoubtedly a high frequency in terms of Caucasoid populations and probably attests to a Mongoloid influence (Kobyliansky and Hershkovitz, 1997).

In frontal view, the outlines of the central incisors in the Bedouin population are frequently characterized by a ‘square’ appearance (94.5%), by the absence of pits or lingual grooves (56.2%), and by the presence of a slight lingual tubercle (50.0%, Figure 1).

Maxillary lateral incisors: These incisors have recently received much attention because they are deemed to be teeth of a variable and unstable nature in comparison with centrals (Hershkovitz et al., 1993; Moscona et al., 1997; Moscona et al., 1998). In the Bedouin, the "shovel" feature occurs at a frequency of 47.7% in the ‘trace’ gradation and about 20% in the moderate to marked gradations. This is in contrast to the other populations in which the frequency of the feature is similar to that in the central upper incisors (Figure 2).

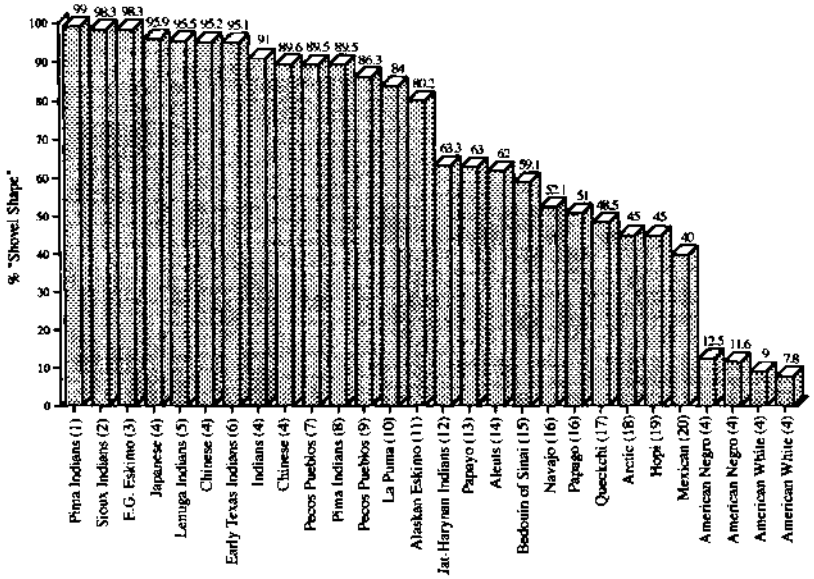


Fig. 2. Prevalence of the “Shovel Shape” trait in the marked + moderate stages in central incisors of various populations.
Authors: 1. Dahlberg, 1949; 2. Hrdlicka, 1921; 3. Pedersen, 1949; 4. Hrdlicka, 1920; 5. Kieser and Preston, 1981; 6. Goldstein, 1948; 7. Nelson, 1938; 8. Dahlberg, 1949; 9. Hooton, 1930; 10. Devoto et al., 1968; 11. Bang and Hasund, 1971; 12. Kaul and Prakash, 1981; 13. Morris, 1965; 14. Moorrees, 1957; 15. Present study; 16. Scott et al., 1983; 17. Escobar et al., 1977; 18. Turner, 1967a,b; 19. Scott, 1980; 20. Wissler, 1931.

Canines

Maxillary canines are characterized by a ‘nippled apex’, at a frequency of 63.1%. The extreme slope gradient is characterized by a partial slope at a frequency of 50.7%, with a slight tendency for a vertical slope, at a frequency of 28.1%.

Premolars

Maxillary first premolars: The configuration lines of the mesial and distal margins (in occlusal view) conjoin to a moderate angle at a mean frequency of 71.9%. The distance

between the apices of the labial and lingual tubercles invariably exceeds 3mm in 100% of the cases (Figure 1). In 50.6% of cases, the sagittal ends of the labial tubercles are only partially sharpened. A tendency toward excess sharpness leading to a highly conical appearance was observed in 34.2% of the cases.

Maxillary second premolars: When viewed from the labial direction, 37.6% of the cases do not show any presence of "buccal styles" (Figure 1). In 63.3% of the cases, the sagittal edge in the labial tubercle is rounded at its end.

Mandibular first and second premolars: These are characterized in 78.4% of the cases by a single lingual tubercle. The edges of the masticatory platform in these teeth appear on the mesial side in 'attenuated' style at a frequency of 70.7%, and at the distal side at a frequency of 51.9%. In 67.5% of the cases, there is no evidence of a split in the tubercle which traverses the occlusal surface from the labial to the lingual direction.

Molars

Maxillary first molars: When viewing the masticatory plane of these molars, one notices an oblique ridge that is partially interrupted by a groove. Such "disruption" occurs in 84.2% of cases. The anterior transverse ridge is usually of minuscule dimensions and its frequency at the 'slightly developed' gradation is 61.8%. As for the mesio-labial tubercle, in 75.5% of the cases there is no mesial ridge on the mesial slope compared to a 55.6% occurrence of a tubercle on the distal slope.

Maxillary second molars: These teeth resemble the first upper molars, with a minimal distolingual cusp.

Mandibular first molars: The frequency of five cusps is 85.0%. The presence of additional grooves in the lingual tubercles is characteristic of the crown.

Mandibular second molars: These are characterized by four tubercles in 80% of the molars. Observations on these molars attest to changes over time in the direction of smaller dimensions and fewer tubercles, compared to the first molars.

Deciduous maxillary and mandibular molars: The deciduous lower molars had four cusps in the first molar in 49.4% of cases, and five in the second molar in 81.5% of the cases.

Inter-tribal morphological differences:

Cluster Analysis: Summarizing all the morphologic dental differences among the four Bedouin groups, a cluster analysis yielded a graphic presentation of the inter-tribal morphologic differences. The final outcome is shown in Figure 3.

It is evident that the dental morphology of the Gebeliya tribe is different from that of the other Bedouin groups in Southern Sinai. Kobyliansky and Hershkovitz (1997) who used the same statistical method but applied it to anthropometric traits of the body, obtained a cluster which is practically identical to ours. In both his and our results, the distance between members of the Gebeliya tribe and members of all the other tribes is almost double that in any pair comparison of the three other tribes.

The traits characterizing the dentition in children of the Gebeliya tribe include the 'shovel-shape' trait in the central incisors, which occurs at a frequency of 69.2% in the marked and moderate gradations (on the left and right side of the maxilla). These values are higher than the mean value for the Bedouin children in the Sinai (Table 3). Another

trait revealing high prevalence in the Gebeliya children is the Carabelli cusp which had a mean frequency of 58.2-62.9% on the left and right sides of the maxilla. Interestingly, the 'shovel-shape' trait is considered to be typical for the Mongoloid race whereas the trait named after Carabelli is deemed rather typical of the Caucasoid race. Our present finding thus provides an important lesson, namely, that in small isolated groups with a high rate of inbreeding, one cannot rely on single morphologic traits to identify the ethnic derivation or country of origin of a given population. Rather, in small groups, certain genes may disappear, while other genes may show an increased frequency, regardless of the situation in the mother population, often through genetic drift.

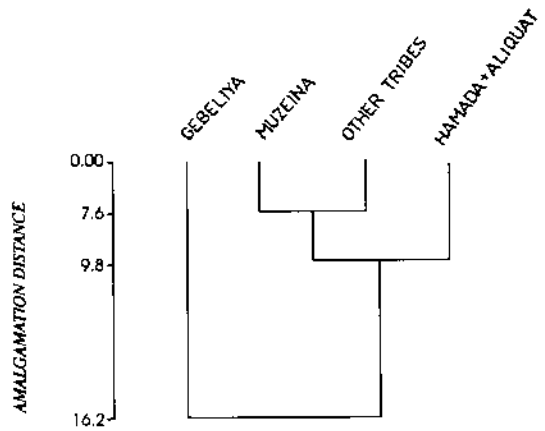


Fig. 3. Cluster analysis of dental distances between four Bedouin tribal groups from South Sinai. Clustering by average distance method.

Comparison of South Sinai Bedouins with groups from other geographic regions:

The comparison was made on the basis of four morphologic traits: a. Shovel-shape incisors - entailing the morphology of the central and lateral upper incisors; b. Carabelli cusp - frequency of occurrence in the first upper molars; c. Number of cusps - in the second upper molars; d. Number of cusps - in the first and second lower molars.

Shovel-shape incisors - On average the frequency of the shovel- shape trait in the mesial incisors is 59.1% for all the Bedouin tribes combined. In other populations the frequency ranges from a high of 80-99% in Mongoloid populations (e.g. Indians, Chinese, Japanese) to a low of 7.8 and 12.5% in American whites and blacks respectively (Figure 2). Accordingly, it would seem that our Bedouin group fits in somewhere in the middle of this range.

Carabelli cusp - The frequency of the Carabelli cusp trait among the Bedouin population is 26.4% (Figure 4). Data from 21 ethnically different populations, show the frequency of this trait to range from 0% in Eskimos to 72.3% in white soldiers of the United States armed forces. As can be seen from Figure 3, a frequency of the Carabelli cusp trait very much similar to that in the Bedouin population was encountered in several Eskimo groups, which is in accord also with the findings for the 'shovel-shape' trait in the central incisors (Figure 2). This is not so, however, for the data on the 'shovel-shape' trait

in the lateral incisors, albeit in both groups there is a marked frequency in favor of the central incisors.

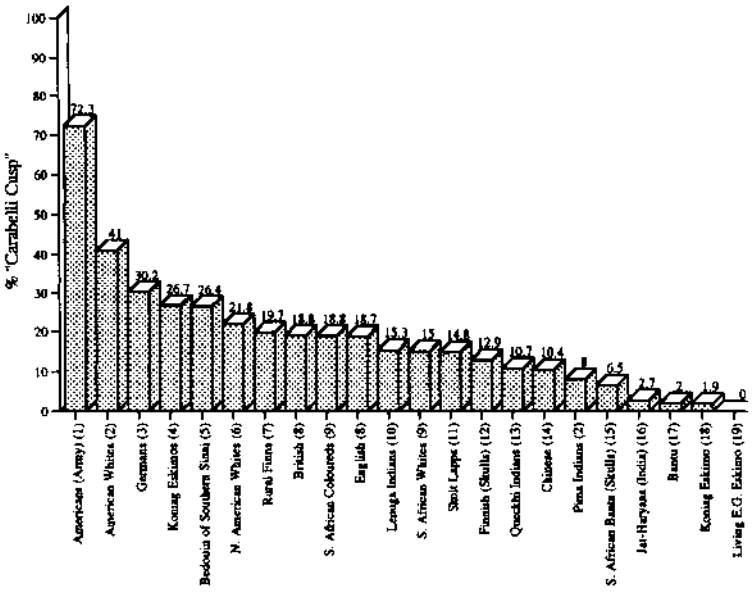


Fig. 4. Frequency of the "Carabelli cusp" trait at the small and large cusp stages in the first molars of different populations.
Authors: 1. Dietz, 1944; 2. Dahlberg, 1949; 3. Zubov, 1973; 4. Dahlberg, 1963; 5. Present study; 6. Keene, 1968; 7. Alvesalo et al., 1975; 8. Goose and Lee, 1971; 9. Kieser, 1978; 10. Kieser and Preston, 1981; 11. Kirveskari, 1974; 12. Hjelman, 1928; 13. Escobar et al., 1977; 14. Lee and Goose, 1972; 15. Shapiro, 1949; 16. Kaul and Prakash, 1981; 17. Shaw 1931; 18. Turner, 1967b; 19. Pedersen, 1949.

Cusp number on upper first and second molars - In modern man, the upper first molars usually bear four cusps, while the second and third molars display a diminution of the distolingual cusps. The frequency of the trait among the Bedouin population is as follows: About ¼ of all the upper second molars possess three cusps, while the remaining ¾ have an additional disto-lingual cusp albeit minuscule in its dimensions. In no teeth were there four cusps of identical size. Despite the relatively small size of our sample, one should not ignore the fact that in the Bedouin population we found no second molar with four equal-sized cusps. Our findings thus resemble those of Smith et al. (1981) on an isolated tribe in Australia. One likely explanation for this resemblance would probably be genetic drift which, as previously pointed out, results from the small size of the population group and especially from a high proportion of intermarriages (Kobyliansky and Hershkovitz, 1997).

Cusp number on the mandibular first and second molars. The findings pertaining to number of cusps in the first mandibular molars in Bedouin and other groups are shown in Figure 5. As noted, in Bedouins about 15% of individuals have lost the fifth cusp, which is a fairly high percentage compared to that in most of the other groups cited. As for the second lower molar (Figure 6), in a fifth of all our Bedouins this molar had five

cusps. Many anthropologists regard the decrease in the number of cusps in molars as proof of the evolutionary progress of modern man (Hrdlicka, 1921a,b; Dahlberg, 1945).

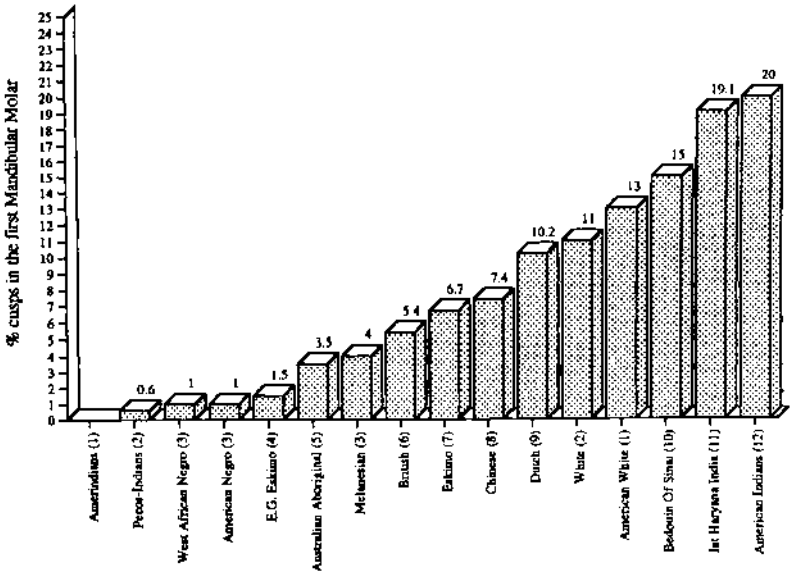


Fig. 5. Distribution of the cusps in the first mandibular molars of different populations. Authors: 1. Hellman, 1928; 2. Nelson, 1938; 3. Dahlberg, 1945; Pedersen, 1949; 5. Campbell, 1925; 6. Lavelle et al., 1970; 7. Goldstein, 1931; 8. Lee and Goose, 1972; 9. Jorgensen, 1955; 10. Present study; 11. Kaul and Prakash, 1981; 12. Hooton, 1930.

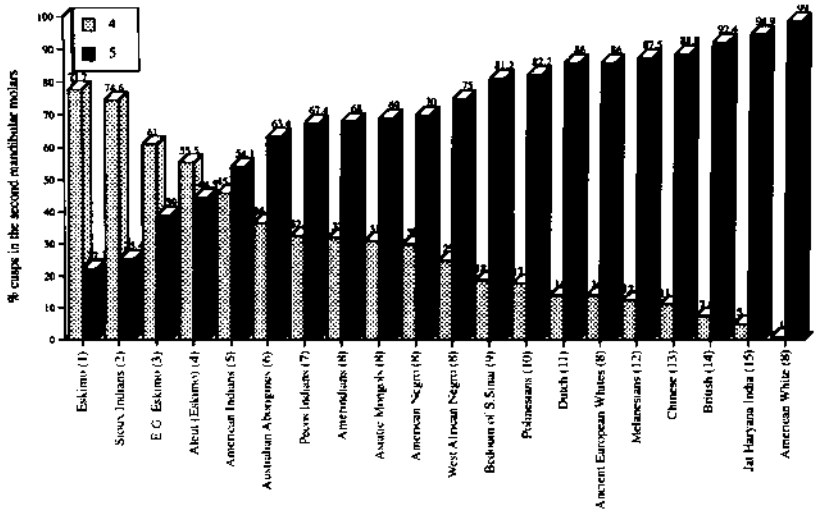


Fig. 6. Distribution of the cusps in the second mandibular molars in different populations. Authors: 1. Goldstein, 1931; 2. Hrdlicka, 1920; Pedersen, 1949; 4. Moorrees, 1957; 5. Hooton, 1930; 6. Campbell, 1925; 7. Nelson, 1937; Hellman, 1928; 9. Present study; 10. Chappel, 1927; 11. Jorgensen, 1955; 12. Dahlberg, 1945; 13. Lee and Goose, 1972; 14. Lavelle et al., 1970; 15. Kaul and Prakash, 1981.

What shows up fairly clearly is that in our Bedouins there is a tendency toward diminution of the size of the molars.

Summary

No significant differences were noted between boys and girls nor any significant interaction between age and sex and the frequency of traits. The dental morphological traits in the total sample of Bedouins divided into three categories:

1. **Stable traits**, occurring in 100% of the entire sample whose bilateral presence provided good evidence of full genotype penetrance, probably due to their functional importance, or perhaps as a result of simple genetic control (not multifactorial).
2. **Less stable traits,** occurring at high frequency (76%-99%) and relating mainly to cusp number, whose considerable bilateral presence was probably due to their functional importance.
3. **Unstable traits,** occurring at frequencies ranging from 10% to 75%.

The results for 13 out of 57 traits on both sides, and for 12 out of 57 traits on one side only, indicated statistically significant differences between the four studied groups. The biological distances determine using 57 dental morphological traits, and through cluster analysis calculated the average distance between the tribes. The Gebeliya tribe differed significantly from the other tribes. These dental morphological differences of the Gebeliya tribe may be attributed to its ethnic composition, comprising a mixture of European, Mongoloid, and Negroid components.

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Reduction in Human Premolar Crowns

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INTRODUCTION

Maxillary premolars are generally regarded as having two cusps and mandibular premolars as having two or three cusps. Our studies (van Reenen, Reid and Butler, this volume) have shown that premolars share a number of features with molars. The buccal cusp of upper premolars consists of a fused paracone and metacone. Most of the lingual cusp is protocone but remnants of the hypocone can be seen distolingually and distally behind the distal fovea. Reduction in upper premolars, therefore seems to have had a constraining effect on the development of the metacone and hypocone. The buccal cusp of lower premolars is formed by fusion of a larger protoconid and smaller hypoconid and the lingual cusp is made up of the metaconid to which a greatly reduced entoconid is fused. A furrow sometimes divides the lingual cusp into two separate cuspules, a larger metaconid and a smaller entoconid. Most of the reduction in premolars therefore has taken place in the talon and talonid. The metacone which is part of the trigon has also been reduced in association with the talon.

Butler (1998) recently compared the talonid sizes of human deciduous molars and first permanent molars. He points out that the trigonid and talonid evolved in different ways. In premolars the talonid is poorly developed because a gradient exists and the trigonid develops first and then the talonid is formed. Both parts grow at different rates (Butler, 1968) and there is evidence that competition for space exists during the formative period. This may be responsible for the great variation in surface morphology observed on premolar crowns (Butler, 1998; Swindler, 1976) and for reduction of the talonid.

Reduction in premolars has not been quantified. In the present study we attempt to do this. A problem we could not resolve on premolars is the difficulty in defining the boundaries between the trigon/talon and trigonid/talonid. We have taken the central fovea of molars, or its equivalent, the distal fovea of premolars as our marker. This is clearly visible on premolars and molars. The fovea divides the crown into a mesial and distal segment. In this study we have measured the mesiodistal length of the two segments.

MATERIALS AND METHODS

The investigation was undertaken on skull material housed in the Raymond Dart Collection of the Department of Anatomical Sciences, University of the Witwatersrand, Johannesburg. A total of 29 skulls were selected for this purpose. Dentitions used in the study had to have at least one complete premolar-molar row, the teeth had to be free of caries and had to show good or fair furrow detail. Photographs were taken of each tooth

with the occlusal surface as near right angles to the camera lens as possible. A millimeter scale was placed next to the tooth. The photographs were enlarged and measurements taken on the prints. Four reference points were determined on each premolar and molar. These were defined by Biggerstaff (1969) and allocated numbers by him which are not used in this study.

1. **Point A** - the central fovea of molars or its equivalent in premolars, the distal fovea, which is the point where the buccal developmental groove intersects the central development groove
2. **Point B**- the most mesial point on the occlusal surface of the mesial marginal ridge opposite the mesial fovea
3. **Point C**- the most distal point on the occlusal surface of the distal marginal ridge located opposite the distal fovea
4. – the distal fovea of maxillary molars is located at the intersection of the central developmental groove, the disto-occlusal developmental groove, the disto-lingual foveal groove, and the disto-buccal foveal groove.

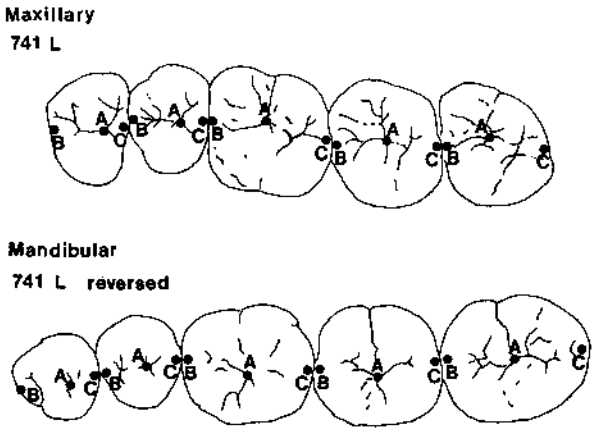


Fig. 1. Points of measurement on maxillary and mandibular teeth.

The distance between points A and B were taken to represent the mesial segment of the tooth, the distance between A and C as the length of the distal segment, and the distance between B and C as the shorter mesiodistal length (Fig. 1). The maximum mesiodistal length of the tooth was also measured. The calipers were held parallel to the mesiodistal axis of the tooth with the beaks touching the most mesial and most distal points on the tooth. Values obtained for measurements made on photographs were converted to real values by reference to the millimeter scale on the photograph and calculation of the enlargement factor. The data were subjected to statistical analysis by a qualified statistician on the staff of the Department of Statistics, University of the Witwatersrand, Johannesburg.

RESULTS

The mean crown lengths of maxillary and mandibular teeth are shown in Tables 1 and 2. In the maxilla the first molar has the greatest and the second premolar the shortest crown length (Table 1). These differences amount to 3.91 and 3.90 mm for MD and BC respectively. The longest mesial segment length is found in the second molar and the shortest in the second premolar. The difference, however, amounts to only 0.83 mm. The mesial segment length in premolars and molars is surprisingly similar but the distal segment length of premolars is considerably less than it is in molars. The first premolar has the shortest and the first molar the longest distal segment length. The difference is 3.51 mm which is slightly less than the difference in range of MD and BC values. Reduction in maxillary premolars, therefore, has had a major impact on the distal segment.

Table 1. Mean lengths (mm) of maxillary teeth (n=29).

TOOTH	MESIODISTAL (MD)	MESIODISTAL (BC)	MESIAL SEGMENT (AB)	DISTAL SEGMENT (AC)
P1	7.17	6.50	4.50	1.99
P2	6.76	6.45	4.12	2.33
M1	10.67	10.35	4.82	5.50
M2	10.31	9.91	4.95	4.99
M3	8.98	8.35	4.38	3.98

Table 2. Mean lengths (mm) of mandibular teeth (n=29).

TOOTH	MESIODISTAL (MD)	MESIODISTAL (BC)	MESIAL SEGMENT (AB)	DISTAL SEGMENT (AC)
P1	7.39	6.95	4.81	2.13
P2	7.46	7.13	4.74	2.38
M1	11.42	10.91	5.12	5.76
M2	10.84	10.47	5.10	5.37
M3	11.06	10.61	5.37	5.24

Mandibular first premolars have the shortest and first molars the longest MD, BC and AC lengths (Table 2). The difference in range of values is 4.03, 3.96 and 3.63 mm respectively. The second premolar has the shortest and the third molar the longest mesial segment lengths but the difference is only 0.63 mm. The mean mesiodistal length of the mesial segment, therefore, varies within narrow limits in all post-canine teeth.

The distal segment length of mandibular premolars is considerably less than it is in molars. The range in values between the first premolar and first molar amounts to 3.63 mm. The reduction in crown size of lower and upper premolars, therefore, is mainly due to a decrease in distal segment length.

The paired t-test showed that differences in mesial segment lengths (AB) between adjacent teeth is significant for maxillary but not for mandibular teeth, with one exception, namely, the difference between the mandibular second premolar and first molar which is significant (Table 3). The range in mesial segment mean values is greater in the maxillary than in the mandibular teeth and this variability probably accounts for the

finding. Mesiodistal (BC) and distal segment (AC) lengths in mandibular teeth are also significantly different ($p<0.05$) with two exceptions, namely, the BC lengths in a comparison between first and second premolars of both jaws and AC lengths in a comparison between mandibular second and third molars are not significantly different. In these exceptions the means of the two adjacent teeth vary by small amounts. The paired t-test therefore shows that a comparison between means of adjacent teeth are significantly different for the majority of measurements undertaken. This is to be expected in teeth of different size.

Table 3. Comparison of differences between means of adjacent teeth (Paired t-test).

COMPARISON	MESIODISTAL	MESIAL SEGMENT	DISTAL SEGMENT
MAXILLA			
P1 - P2	NS	S	S
P2 - M1	S	S	S
M1 - M2	S	S	S
M2 - M3	S	S	S
MANDIBLE			
P1 - P2	NS	NS	S
P2 - M1	S	S	S
M1 - M2	S	NS	S
M2 - M3	S	NS	NS

S= significant at 5% level; NS= not significant

In order to evaluate the influence of tooth size as estimated by the mesiodistal dimension (MD) of the tooth on mesial (AB) and distal (AC) segment lengths, Pearson's correlation coefficients were determined for the data. Values obtained in a comparison of MD with BC, AB and AC lengths are shown in Table 4. As expected, the two mesiodistal lengths, MD and BC, correlate well with each other. Mesial segment values also correlate well with MD lengths with one exception, the mesial segment length of the first mandibular molar. No explanation can be offered for this observation. Only half the distal segment lengths (maxillary P2, M2 and mandibular M1, M2, M3) correlate with the MD lengths. The distal segment therefore, shows greater size variability than the mesial segment. Figure 2 is a representation of mean mesiodistal (BC), mesial segment, distal segment and half BC (in broken lines) lengths for maxillary and mandibular premolars and molars, and illustrates the greater variability of distal segment values.

Table 4. Comparison of maximum mesiodistal length (MD) with mesiodistal (BC), mesial segment (AB) and distal segment (AC) lengths.

	MESIODISTAL (BC)	MESIAL SEGMENT (AB)	DISTAL SEGMENT (AC)
MAXILLA			
P1	0.7289	0.4119	0.3471
	(0.0001)	(0.0264)	(0.0650)
P2	0.9242	0.6179	0.4391
	(0.0001)	(0.0004)	(0.0172)
M1	0.9531	0.7292	0.0991
	(0.0001)	(0.0001)	(0.6088)
M2	0.9101	0.6886	0.538
	(0.0001)	(0.0001)	(0.0026)
M3	0.4355	0.4375	0.1692
	(0.0182)	(0.0176)	(0.3800)
MANDIBLE			
P1	0.7681	0.6617	0.1388
	(0.0001)	(0.0001)	(0.4725)
P2	0.8815	0.7547	0.1651
	(0.0001)	(0.0001)	(0.3920)
M1	0.7437	0.2766	0.6175
	(0.0001)	(0.1464)	(0.0004)
M2	0.9353	0.5343	0.6811
	(0.0001)	(0.0028)	(0.0001)
M3	0.8898	0.5897	0.6614
	(0.0001)	(0.0008)	(0.0001)

Pearson’s correlation coefficients; P values in parentheses.

When the three points of measurement, A, B, C, fall in a straight line, the sum of the mesial (AB) and distal segment lengths (AC) for the particular tooth equals the mesiodistal length (BC). By plotting half the BC values on the graph, variations in length of AC and BC can be visualized. Figure 2 shows clearly that mesial segment lengths vary within a narrow range of less than 1 mm. On the other hand, distal segment lengths and BC values vary considerably between the premolars and molars. The figure also shows that mesial segment lengths of both premolars and third molars to a lesser extent, form the major component of the mesiodistal length (BC) of these particular teeth. On the other hand, the distal segment forms the major component of BC for first and second molars.

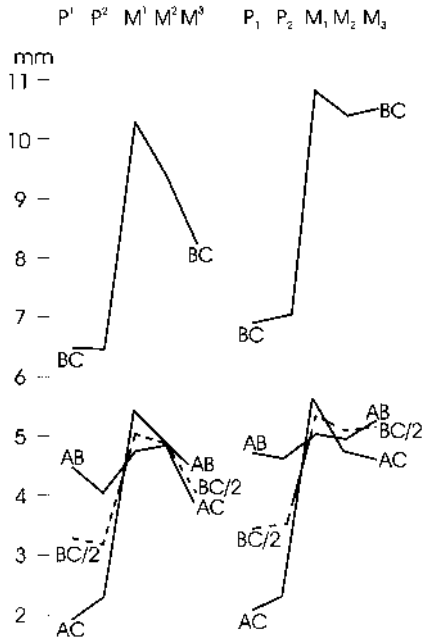


Fig. 2. Graphic representation of mesiodistal (BC), mesial segment (AB), distal segment (AC), and half BC (in broken line) mean lengths for maxillary and mandibular premolars and molars.

DISCUSSION

This study shows that the mesial segment length is less variable than the distal segment length. This is likely to be related to the presence of a gradient in the teeth during the formative process. The mesial segment which includes the paracone in upper and the protoconid in lower premolars/molars develops before the distal segment and is subjected to less competition for space than the distal segment in smaller teeth such as premolars and some third molars (Butler, 1979).

Butler (1998) was able to measure trigonid/talonid lengths on deciduous molars and first permanent molars using the buccal and lingual grooves as markers. On premolars unfortunately, these furrows seldom extend onto the buccal and lingual surfaces of the teeth and their development is often rudimentary. Similar measurements could not be made on premolars for comparative purposes.

A comparison of differences between means of adjacent teeth (Table 3) shows that cusps in the mesial segment of premolars and molars have the opportunity of fuller expression of their size potential, particularly in mandibular teeth, than cusps developing in the distal segment. Butler (1968, 1998) has shown that a gradient exists in the tooth during the formative period. The cusps which develop first in the mesial segment have less competition for space than the cusps which develop later in the distal segment of the tooth. Tables 1 and 2 show that in the case of premolars the mesial segment is approximately two thirds the length of the tooth. In molars it is nearly half way along the

mesiodistal length of the tooth. This shows that the distal segment of premolars which includes the talon/talonid, is greatly reduced resulting in rudimentary development of the cusps in that region.

The influence of tooth size as estimated by the MD length of the tooth on the mesial and distal segments is shown in Table 4. Pearson's correlation coefficients suggest that the length of the mesial segment is determined by tooth size. This again is probably related to the presence of a development gradient in the tooth which makes more space available to the cusps that develop first namely, the paracone and protoconid. These cusps are in the mesial segment (Butler, 1968, 1998). The finding that only half of the premolar and molar teeth of both jaws show a good correlation between MD and the length of the distal segment suggests that cusps in the distal segment, particularly the talon/talonid cusps, are constrained by a space limitation.

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Large Scale Histological Assessment of Deciduous Crown Formation

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INTRODUCTION

A feature that seems to characterise modern human dental development is high levels of inter-and intra-population variation. However, tooth formation as traditionally measured by the appearance of growing teeth on radiographs is fraught with methodological problems (eg. Risnes, 1986; Aiello and Dean, 1990; Beynon et al., 1991). In addition, studies have used idiosyncratic approaches in the way that they collect data and assess tooth maturity; some have been cross-sectional, others semi-longitudinal, and some have used as little as 3 fractional stages to gauge tooth development, others as many as twenty. Not only does this make cross-study comparison difficult, but it raises a more fundamental question: is high ontogenetic intra- and inter-population variability really a feature of modern human dental development, or is some or all of it an artefact of the methodology used to determine it?

Histological techniques that rely on the interpretation of microstructural growth markers, and which therefore provide an accurate endogenous record of tooth development, can overcome these difficulties. Not only do they obviate the need to use radiographs, but they also furnish a continuous history of both rate and total time taken for dental development. And for any dentition with at least one peri-natally developing tooth, timing can be registered precisely with the time of parturition thus also allowing accurate chronological (cf. biological) development to be established. However, conventional approaches to histological analysis tend to be time consuming and therefore impractical to apply on a large scale.

This paper reports on standardised techniques, relying on digital imaging technology, that have been developed to make assessment of large samples feasible. The discussion of methodology will be illustrated with early results from a project whose objective is to determine the true extent of dental development variability among several modern human populations. The results being used as illustrative examples in this paper are from the preliminary investigation of the first population being analysed, deciduous teeth excavated from the Imperial Roman necropolis of Isola Sacra, interred during the 2^{nd}–3^{rd} century A.D. This phase of the project is being jointly undertaken with colleagues from the National Prehistoric Ethnographic "L. Pigorini" Museum in Rome. The entire sample of deciduous teeth from Isola Sacra amounts to more than 200 teeth (Rossi et al., 1998).

Since enamel growth is easier to interpret, the project will concentrate on crown development. It should be noted that although many histological analyses of the type proposed have been carried out on the enamel of permanent teeth, none have been on deciduous teeth. Therefore, there is an implicit assumption that the incremental growth markers, cross striations and striae of Retzius, share similar characteristics in both dentitions.

MATERIALS AND METHODS

Equipment

Most observations were made using an Olympus BH-2 TLM under polarised light. Because large numbers of teeth are being assessed, the authors believe that the use of digital imaging technology is mandatory. Images were captured using a Polaroid™ Digital Microscope Video Camera and displayed on a 21" computer monitor. Both devices are able to visualise colour images with a resolution of 1200 by 1600 pixels. Earlier attempts using an analogue video camera coupled to a frame grabber that provided a resolution of 480 x 640 pixels proved unsuccessful since the microstructures of deciduous teeth are faint and particularly difficult to discern. Photomosaics were created in Adobe Photoshop® 4.0, and analysis and further filtering (when required) carried out in Image Tool 1.28 (University of Texas, San Antonio Health Science Centre, USA). A Pentium 233 MMX computer drove both hardware and software. Rapid response times were achieved by equipping it with 128 MB of RAM, a powerful video accelerator card, and a large hard drive. Sometimes microstructures were too faint to be clearly discerned under the light microscope and it was necessary to use a Zeiss laser confocal microscope system.

Methods

To prepare teeth for analysis, longitudinal sections were taken through the central axis of single cusped teeth and the mesio-buccal cusp of molars, lapped to a thickness of approximately 100 µm, and then mounted under a coverslip.

Enamel formation can be divided into two descriptive stages. The first is a cuspal (sometimes called appositional) stage in which secretion occurs over the cusp tip; in this stage growth increments remain buried below the tooth surface. The second is a cervical (sometimes called imbricational) stage involving the deposition of enamel on the sides of the crown (see Fig. 1). Since the methodology involves evaluating the two stages separately, each will be dealt with in its own subsection; cervical enamel will be covered first, followed by cuspal enamel.

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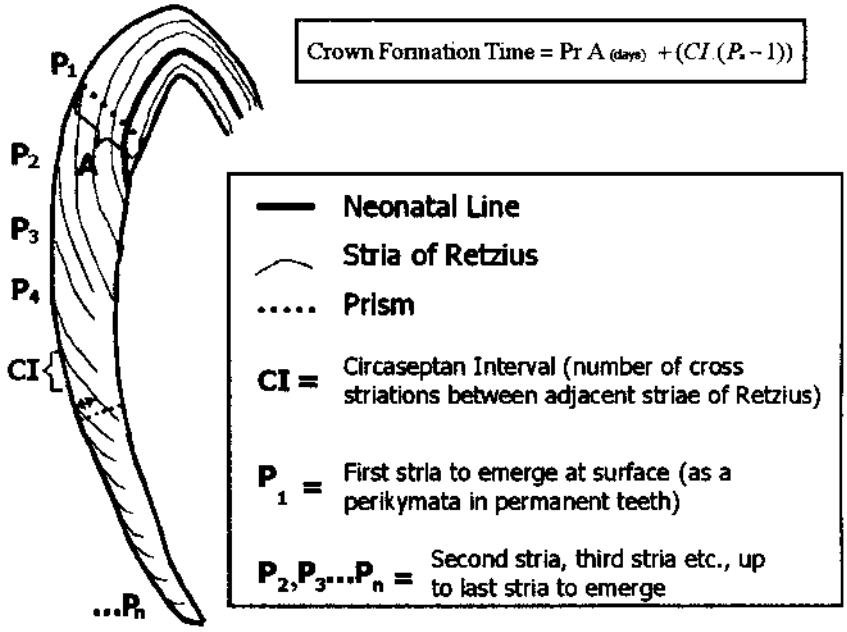


Fig. 1. Schematic diagram of a longitudinally sectioned tooth showing derivation of formula to calculate total crown formation time.

a) Cervical Enamel

Cervical growth is calculated by multiplying the total number of striae of Retzius to emerge at the surface by the 'Circaseptan Interval', the number of cross striations between adjacent striae of Retzius (see Fig. 1). Since the Circaseptan Interval may differ from individual to individual, but is identical within all the teeth of one individual (Fitz-Gerald, 1998), it is therefore only necessary to determine it once within one tooth from any dentition. This is done in whichever tooth cross striations and striae are most clearly discernible in one field of view. In deciduous teeth these structures are sometimes difficult to see clearly under the light microscope, even with images digitally enhanced, and it has been found beneficial to use a laser confocal microscope to help determine this important count of cross striations.

Once the Circaseptan Interval has been established, the first stria of Retzius to terminate at the surface is identified (labelled P_1 on Fig. 1). In a permanent tooth this stria would emerge as a distinct perikymata, but this is not the case with deciduous teeth, in which perikymata are not well defined. All of the striae terminating at the surface are counted down the face of the crown to the cervix (labelled P_2 to P_n on Fig. 1). Particular care should be exercised to ensure that the enamel at the cement/enamel junction, which is fragile and may have broken away post mortem, is present.

Cervical growth is then determined according to the following formula:

$$(CI.(P_n\text{-}1))$$

(where CI is Circaseptan Interval in days, and P_n is the total number of striae to terminate at the crown surface).

b) Cuspal Enamel

A prism at P_1 (the upper dotted line in Fig 1) represents the time elapsed since the initiation of crown growth. However, even if cross striations were easily discernible along the full length of this prism then simply counting all of them would not yield the full growth time for this prism in days because of geometric considerations, discussed below. In any event, counting all cross striations would be a time consuming process and additionally, cross striations are almost never visualised along the full length of prisms, particularly in deciduous teeth. Therefore, the time taken to form the prism P_1 (in Fig. 1) is estimated. This is done by capturing digital images at high magnification along the prism's length, and pasting them together so that the whole prism is imaged. Fig. 2 is an example of such a montage, with the *apparent* path taken by the prism traced across it. The term "apparent" is used because of the impossibility of tracing three-dimensional movement in a two dimensional image. Even if a prism was somehow constrained to move only in two dimensions, common sense alone dictates the unlikelihood of being able to precisely split it along its full length (which in the case of the prism in Fig. 2 is some 700 μm). There is not adequate space here to fully explore the problems presented by, and the investigations into, this three-dimensional movement of prisms (see FitzGerald, 1996). However, the issue is discussed briefly below.

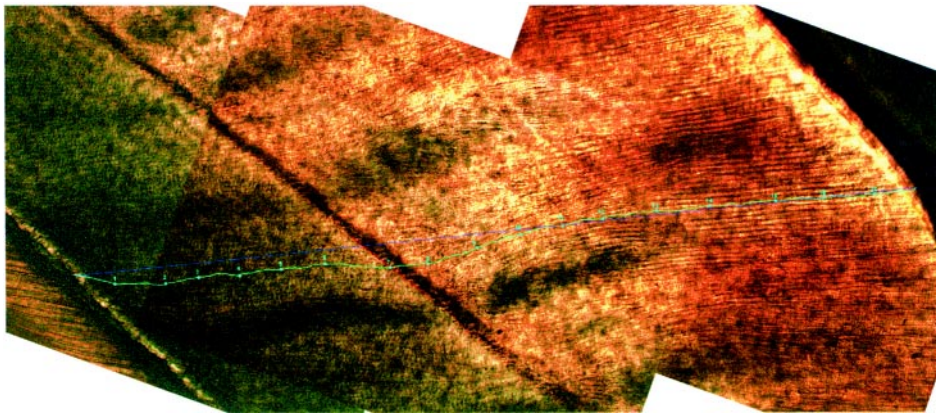


Fig. 2. Montage of prisms in cuspal area of a deciduous mandibular canine from Isola Sacra (taken at 400x magnification). The apparent path taken by the prism that divides cuspal from cervical crown formation is traced on the montage (the light blue line with numbers extending from the EDJ at the left of the photo to the crown surface at the right). Note the neonatal line which is clearly evident as the dark brown line crossing-cutting the prism about 1/3 of its way out from the EDJ.

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Fig. 3 illustrates a cuspal prism visualised in three dimensions, at least the illusion of depth is provided by the wire frames that form a cube through which the prism moves. To obtain these diagrams a tooth was sectioned in two blocks perpendicular to each other along the prism axis. The movement in longitudinal and transverse planes has been scaled up in order to visualise more easily its somewhat helicoidal or sinuous movement. As a result of this investigation it was concluded that, at least at this position in tooth geometry, prisms are equally likely to move as freely in one dimension as another. This means that the amount of deviation needs to be measured, and apparent prism length in the longitudinal plane incremented by the observed amount of deviation.

To estimate the time taken to grow the cuspal prism, it is first measured along its full length using image analysis software. Then the path of the prism is closely scrutinised and wherever cross striations are discernible, either on the prism itself, or more commonly, on adjacent prisms, their positions are recorded. This methodology is better illustrated in Fig. 4, which zooms in on the prism seen in Fig. 2. (Points of discernibility are the numbered dots on Fig. 4). Next, a straight line connecting the origin of the prism and the point of its termination at the surface is drawn and measured (the dark blue line in Fig. 4). Deviations between the straight line and the apparent path suggest the degree of decussation occurring in the longitudinal plane. Then a cross striation repeat interval, representing the average daily appositional growth rate of as large a group of cross striations as can be clearly identified, is calculated at each of the numbered points on the apparent prism path. These rates are then interpolated in 5% increments of length along the prism. Enough information has now been gathered to calculate the total time taken to form the cuspal prism. Table 1 illustrates one of these calculations. Note that this approach yields prism growth rates in relative lengths along the prism. This means that growth rates for prisms of different length can be readily compared.

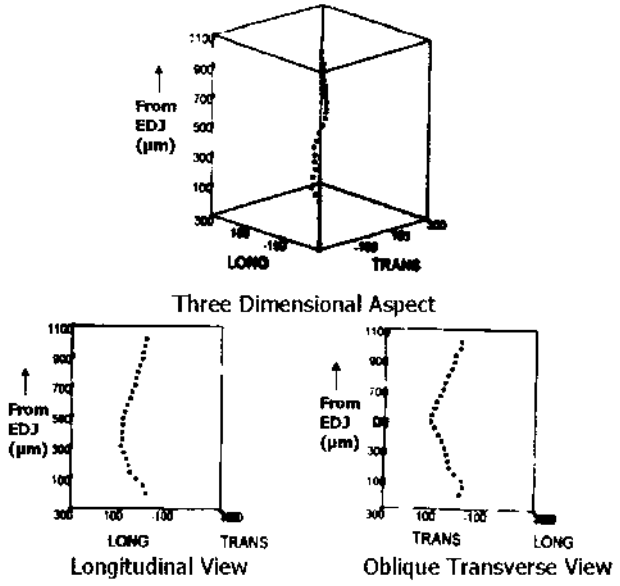


Fig. 3. Wire frame diagrams of the cuspal prism of a permanent mandibular lateral incisor illustrating its three dimensional helicoidal movement. Longitudinal and transverse axis are double-scaled to the distance from the EDJ to better demonstrate decussation. The upper diagram is rotated upward until the 'wire frames' overlap and it is then rotated sideways to achieve the longitudinal view. Further rotation achieves a transverse oblique view.

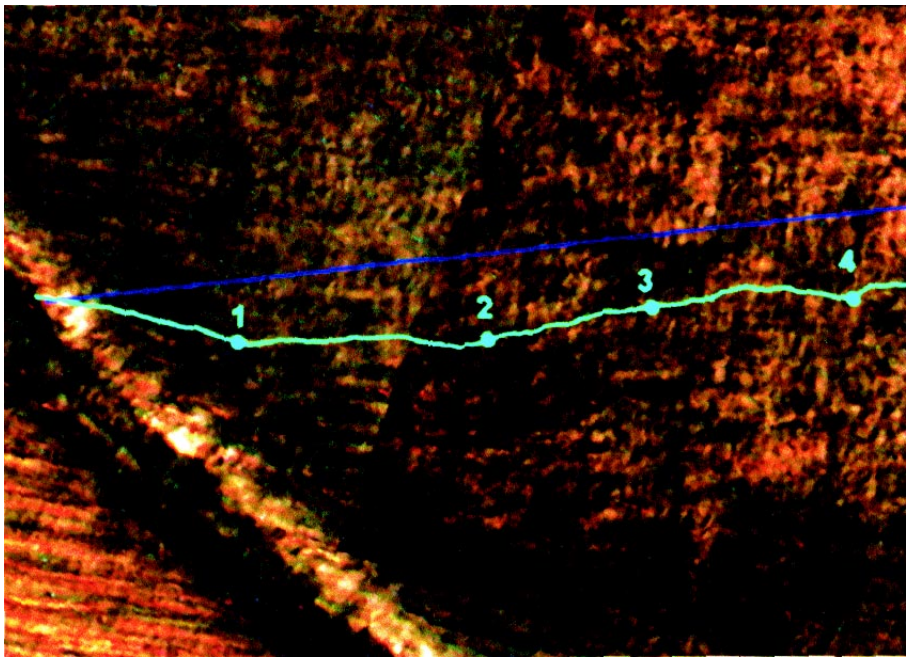


Fig. 4. Detailed view of a portion of the montage in Fig 2 showing the prism close to the EDJ.

RESULTS

The percentile plot of prism rates has been called a ‘prism growth profile’, and Fig 5 illustrates the profiles of four teeth from Isola Sacra, all deciduous mandibular canines. Note that although there are some differences in individual behaviour, there is a general congruency to the patterns. This is reflected in the overall means of the teeth which are also shown in Fig. 5. These preliminary results are surprising in two ways. The first is that t-tests showed no significant difference between pre-natal and post-natal growth rates. Secondly, growth rates are lower than expected. This can be more clearly seen in Fig. 6, which shows a comparison of the averaged deciduous growth profiles (seen in Fig. 5) versus the averaged rates for permanent mandibular and maxillary canines calculated in an earlier study (FitzGerald, 1996). The mandibular profiles are slower than the maxillary profiles, the case for all tooth types tested so far, however, the deciduous mandibular canine is slower than both permanent teeth. Note also that the permanent teeth show an increasing rate of daily growth from the EDJ to the surface, but that the deciduous profile is rather flat.

Table 1. Example of calculation of cuspal enamel formation time for one deciduous mandibular canine from Isola Sacra.

		% Travel	3.2 %	
		Actual µm	811	
		mm Travlld	837	
		Specimen A0032		
Buckets		XSRI (µm/day)	Time (days)	
0	to 5%	3.28	13	
5%	to 10%	3.42	12	
10%	to 15%	3.57	12	
15%	to 20%	3.56	12	
20%	to 25%	3.38	12	
25%	to 30%	3.56	12	
30%	to 35%	3.41	12	
35%	to 40%	3.27	13	<Neo-natal line at 37 %
40%	to 45%	3.11	13	
45%	to 50%	3.20	13	
50%	to 55%	3.45	13	
55%	to 60%	3.56	12	
60%	to 65%	3.26	12	
65%	to 70%	3.51	13	
70%	to 75%	3.75	12	
75%	to 80%	3.31	11	
80%	to 85%	3.27	13	
85%	to 90%	2.54	13	
90%	to 95%	2.54	17	
95%	to 100%		16	

Total	Days	255	Pre vs Post T-test
	Months	8.4	P = 0.083
Pre-Natal	Days	90	Pre-Natal Mean XSRI 3.43
	Months	3.0	
Post-Natal	Days	165	Post-Natal Mean XSRI 3.23
	Months	5.4	
			Diff –6%

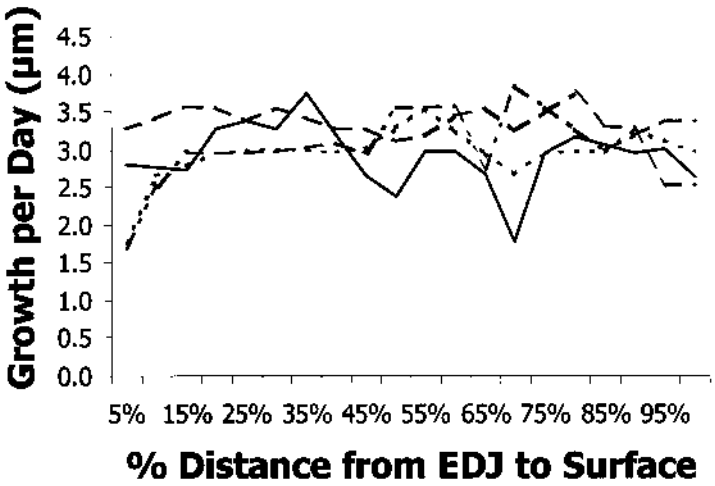


Fig. 5. Plot of Prism Growth Profiles for four deciduous mandibular canines from Isola Sacra

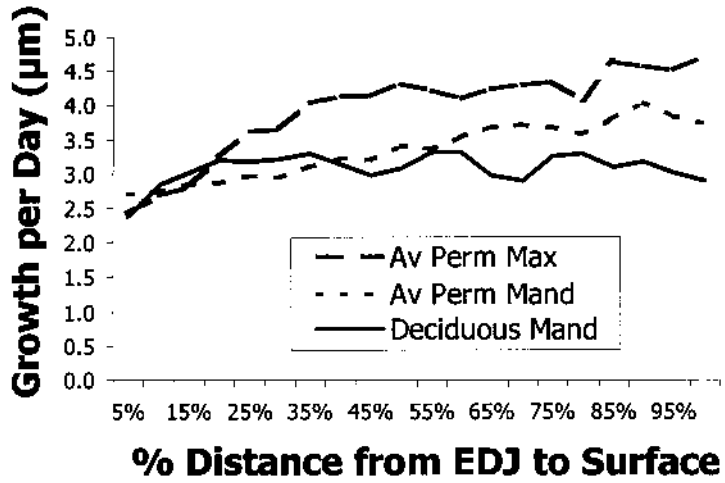


Fig. 6. Prism Growth Profiles for the average of four deciduous mandibular canines from Isola Sacra plotted against averaged permanent maxillary and averaged mandibular canines calculated in FitzGerald (1996).

Cuspal and cervical enamel growth times are added together to yield total crown formation time. The results for the four deciduous canines analysed are summarised in Tables 2 and 3. As can be seen in Table 2, which summarises crown formation in days, there is a consistency among the four teeth, both in total time and in the breakdown between cuspal and cervical enamel times. Table 3, which compares total time in months to the Moorrees, Fanning and Hunt (1963) ranges, shows that all four crown formation times fell somewhat above these standards.

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Table 2. Summary calculation of total crown formation times for four deciduous mandibular canines from Isola Sacra.

Specimen	Crown Formation Time (Days)		
	Cuspal	Cervical	Total
A032	255	272	527
A077	244	264	508
B011	260	256	516
B047	284	259	543

Table 3. Comparison crown formation times of teeth from Table 2 with ranges from Moorrees et al. (1963).

Specimen	Crown Formation Time (Months)		
	This Study	Moorrees *et al.*	Difference
A032	17.3	9.7 - 16.2	1.1
A077	16.7	9.7 - 16.2	0.5
B011	17.0	9.7 - 16.2	0.8
B047	17.9	9.7 - 16.2	1.7

DISCUSSION AND CONCLUSIONS

The methodological problems associated with radiographic studies (referred to in the Introduction) tend to understate crown completion times. The early stages of mineralization are not very radio-dense and are often “burned out” on X-rays of developing teeth. Radiographs also fail to record much of the buccal and lingual aspects of crowns of teeth in the jaws and the image is usually a dense superimposition of mesial and distal aspects. Dean (Aiello and Dean, 1990) has said that as result of this, radiographic definitions of crown completion are based upon observations in these aspects of the tooth, and since enamel formation often continues for very much longer on the buccal surface, radiographic estimates of crown formation will be shorter than true times. This factor, taken together with the initial lag that Risnes (1986) has estimated at 2-6 months in permanent teeth, suggest that it should not be surprising to find that deciduous histological crown formation times exceed those obtained from radiographic data. The times for the teeth reported in this paper exceeded the Moorrees *et al.* (1963) standards by from about two weeks, to about 2 months. In this sense, preliminary results are encouraging and they indicate that applying histological techniques to large-scale studies is a practical and worthwhile undertaking.

It is hoped that large-scale histological assessments of crown development will in the future help to resolve the question of whether and to what extent inter- and intra-population variability is indeed really a characteristic of modern human dental development. It is also a longer term objective of this project to develop general mathematical models of tooth growth, and good data obtained from information intrinsic to teeth themselves should help to further that goal. And ultimately, of course, the ability to accurately assess

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deciduous (and permanent) crown formation patterns and timing on a large-scale is crucially necessary to reconstructing with confidence the palaeodemographic profiles and age-of-death of archaeological and fossil populations (eg. see Smith, 1994).

ACKNOWLEDGEMENTS

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A Detailed Developmental Study of the Defects of Dental Enamel in a Group of Post-Medieval Children from London

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INTRODUCTION

Episodes of fever or dietary deficiency (Pindborg, 1982), experienced by a child during the period of dental development, disrupt the secretion of enamel matrix to create defects which can be seen on the surface of the tooth crowns. The defects take the form of furrows, rows of pits, or prominent steps which follow the circumferential arrangement of perikymata which mark out the sequence of matrix secretion down the crown sides. The overall name for such defects is enamel hypoplasia (Zsigmondy, 1893) but, as this also includes inherited defects, the forms which are investigated in this paper are usually distinguished (Commission on Oral Health, 1982; Hillson, 1996) as Developmental Defects of Enamel (DDE). They are very common in jaws from museum collections and archaeological assemblages around the world, and have been the subject of much anthropological interest (Goodman and Capasso, 1992; Goodman and Rose, 1990; Larsen, 1997). There have also been a number of clinical studies (Goodman et al., 1991; Infante and Gillespie, 1974; Sweeney et al., 1969; 1971), and laboratory experiments (Klein, 1945; Kreshover, 1960; Mellanby, 1929; 1930; 1934; 1941) which clearly show their relationship with childhood infections, protein and vitamin deficiency and a range of other forms of systemic disruptions. In spite of all this work, however, the mechanism by which the defects are created during enamel matrix secretion is little understood (Hillson, 1992a; 1992c; 1998; Hillson and Bond, 1997; Hillson et al., 1998). This paper concentrates on the most common type of enamel defect; a furrow which runs around the circumference of the crown. Such furrows may be several millimetres across, and easily visible with the naked eye in oblique lighting but, under the microscope, they are seen to be the result of variations in the spacing and prominence of perikymata which range from just one pair of neighbouring perikyma grooves (a fraction of a millimetre apart), up to 20 or more (making a band millimetres wide). Furrow defects of all scales can be matched between tooth crowns formed during the same period of development, which demonstrates that they all have an origin in a general disruption to growth. The number of perikymata which diverge from their neighbours in this way presumably reflects the length of period over which the disruption lasted. What is not known is the way in which the surface defect relates to the internal incremental structure of the enamel - the timing of events, the extent to which divergent perikymata are matched by variation in prominence and spacing of the brown striae of Retzius, the part of the surface defect which actually represents growth disruption (the apparent size of the defect is very

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dependent on the view point and angle of oblique lighting), and the mechanism by which the variation in perikyma groove spacing is generated.

MATERIALS

This research is part of a broader histological analysis of dental development based on the permanent dentition of children from the Spitalfields collection. Excavated in the 1980's, the crypt of Christ Church in Spitalfields, London, was mostly used to inter parishioners of Huguenot descent between AD 1729 and 1859 (Cox, 1996; Molleson and Cox, 1993; Reeves and Adams, 1993). The age-at-death of many individuals was established from the coffin plates and the parish records. This can be used to calibrate the periodicity of incremental features in enamel, which was still being secreted at the point of death, so as to determine the precise sequence and timing of crown growth (see detailed discussion in Antoine et al, this volume). For reasons that are not properly understood, histological structures are particularly clear in sections of teeth from Spitalfields, and this provides a good opportunity to investigate in detail the timing, duration and mechanisms of growth disruption.

In this preliminary study, the teeth of a single male child were examined. This individual, designated specimen 2431, was born in May 1820 and died on 14th December 1822 at the age of 2 years 6 months and 3 weeks (2.56 years). The permanent incisors, canines and first molar exhibited a distinct double furrow-form defect, which is clearly visible to the naked eye and can be matched between them all. In addition, the sections of the teeth showed particularly clear prism cross striations and brown striae of Retzius.

The upper and lower permanent central and lateral incisors, canines, first premolars and first molars were all present in the jaws, and one specimen of each was selected and sectioned. The present paper focuses on the lower central incisor and lower first molar, in which the crowns were almost complete, but enamel matrix secretion was still active at the point of death.

METHODS

The teeth were fully recorded prior to sectioning using colour and black and white photography as well as drawings of surface features. Coltène President light body impression material was utilised to make replicas of the teeth for scanning electron microscopy. The replicas were cast from the impressions using epoxy resin (Epotech 301) and then sputter coated prior to being examined in a Hitachi S 570 SEM. These replicas offer a resolution which is better than 1 μm and provide a good record of perikymata and enamel hypoplasia (Beynon, 1987; Hillson, 1992b).

The cleaned specimens were then embedded in methylmethacrylate (Boyde, 1984; Hillson, 1996). This consolidated the friable immature enamel formed just before death and prevented any loss of material during the sectioning. Radial sections centred through the cusp were taken with the kind help of Don Reid and Pam Walton (Dental School,

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Newcastle upon Tyne), using an annular saw (Malvern Instruments Ltd.: Microslice 2). These were then lapped down to approximately 100 μm thickness using a Logitech (PM2 A) lapping machine with a 3 μm aluminium oxide abrasive slurry.

Slides were observed and photographed using polarising transmitted light microscopy (Olympus BH-2 microscope; Olympus OM-2 camera). Photomontages (X300) of the hypoplastic areas were assembled to study the defects (Figs. 4 and 5). Measurements were taken on the photomontages with a clear plastic ruler and calibrated using a graticule slide.

OBSERVATIONS

The furrow-form defect consists of two prominent steps, indicated in Figs. 1 and 2 of the lower first incisor. The steps clearly comprise two increments each (seen as particularly broad ridges of the perikymata), with three ‘normal’ increments in between them. Fig. 3 shows the same defect on the lower first molar. The count of perikymata within and between the two steps is the same as in the incisor, and no other defect of this precise form can be seen on the crown surface of either tooth, although other furrow-form defects are visible (Fig. 1).



Fig. 1. Labial surface of lower right central incisor. Incisal edge at top; not-fully-mineralised immature enamel matrix of the last formed increment at the bottom. The two steps of the double furrow-form defect are marked with arrows. Epoxy replica, examined in a Hitachi S570 scanning electron microscope, operated at 20 kV, using an Everhart-Thornley detector configuration. Field width 3.6 mm.



Fig. 2. Higher magnification view of the hypoplastic area shown in figure 1. This shows the increased size of the hypoplastic perikymata. Field width 225 μm.

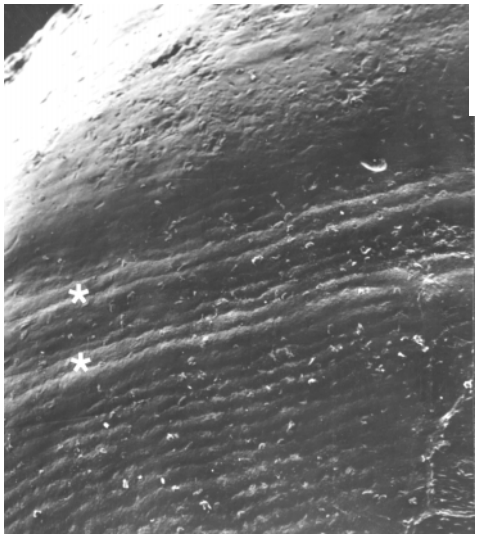


Fig. 3. Lingual surface of lower left first molar. Cervical crown margin to top; occlusal surface below the bottom of the photograph. The double furrow-form defect is marked with white stars. Microscope and replica as for figure 1. Field width 1.5 mm.

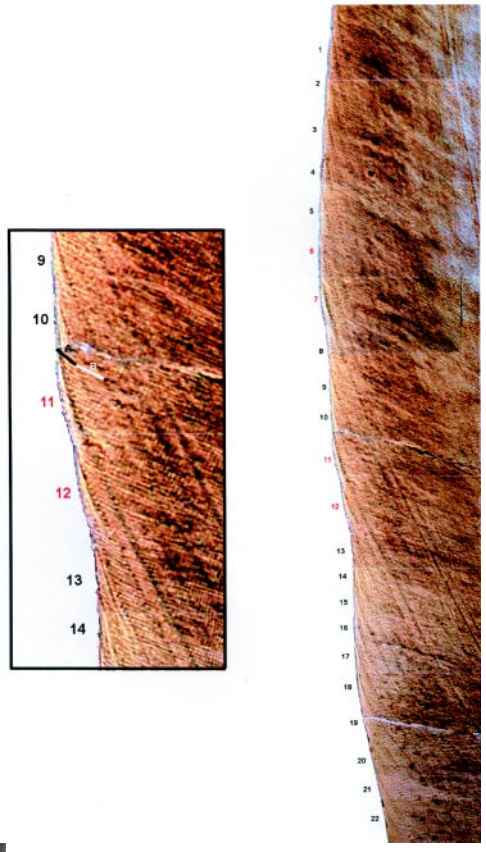


Fig. 4. Section of lower right first incisor including the 22 pairs of brown striae studied, covering 1.79 mm of the crown side. The increments between the pairs of striae are numbered as in figure 6. An example of the brown stria spacing measurements "A" and "B" for figure 7 is also marked.

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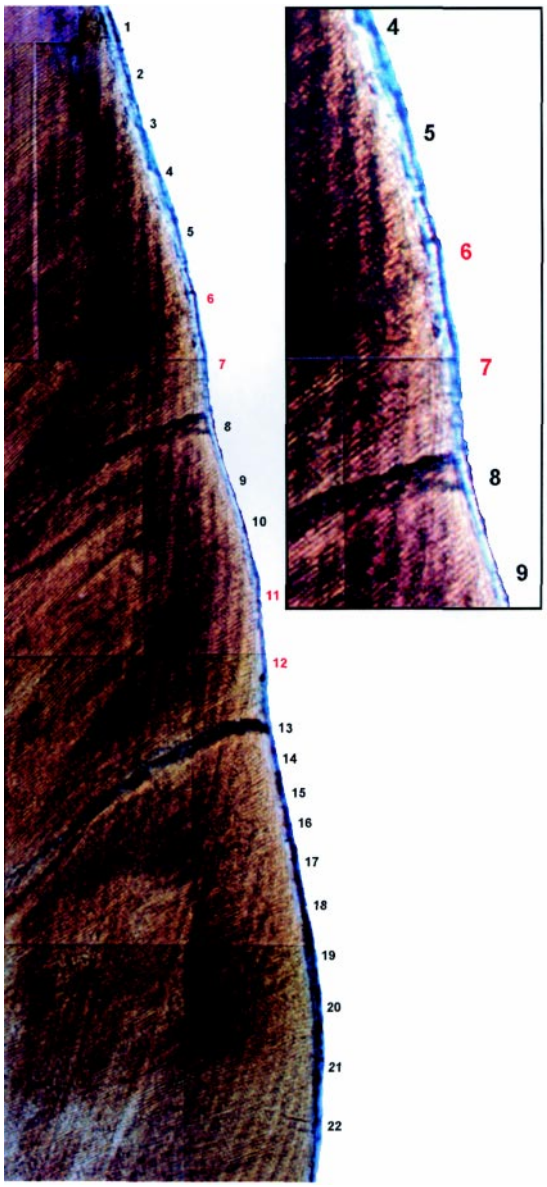


Fig. 5. Section of lower left first molar including the equivalent 22 pairs of brown striae to figure 4, covering 1.58 mm of the crown side.

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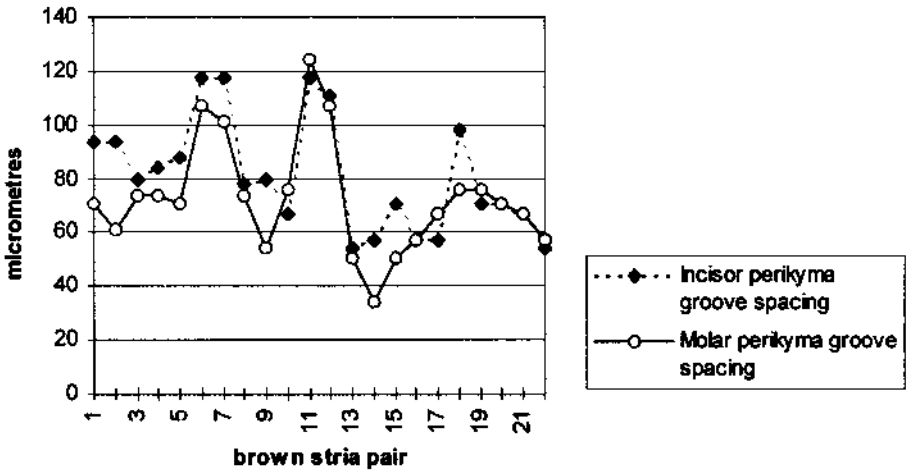


Fig. 6. Plot of perikyma groove spacing (in micrometres) for lower right first incisor and lower left first molar (see figure 4 and 5).

The section of the incisor (Fig. 4) also shows the two steps of the defect clearly, each represented by two pairs of brown striae (increments), and three brown striae between. The same can be seen in the molar section (Fig. 5). Measurements are plotted in Figs. 6 and 7. The following points were observed:

1. The cross striation repeat interval between neighbouring pairs of brown striae is constant throughout the section at 9 in both teeth, even in the hypoplastic zone.
2. In the incisor, the perikyma groove spacing averages 88 μm (80-94 μm) in the highest 5 pairs of brown striae, and 66 μm (54-98 μm) in the lowest 10. In the molar, the average was 70 μm (61-74 μm) for the top 5, and 61 μm (34-76 μm) for the lowest 10. There is thus a gradual trend to decrease spacing down the crown side, as would be expected.
3. In the region of the furrow-form defect, the perikyma groove spacing along the crown surface is considerably greater than expected at the two steps (increment numbers 6 and 7, 11 and 12), at 111-118 μm in the incisor and 101-124 μm in the molar. This is about one third again as much as the average spacing. In between the two steps (increments 8, 9 and 10), the perikyma groove spacing is around what would be expected, taking account of the gradual decrease down the crown. The increments just below (the number is variable) the lower step tend to have slightly closer perikyma groove spacing than would be expected, and this may emphasise the step.
4. thickness of increments below the surface zone, measured along the line of prism boundaries, between pairs of brown striae, shows no clear trend in relation to the pattern of perikyma groove spacing (Fig. 7).
5. The number of prism boundaries, going from one perikyma groove to another, varies between 10 and 21 in the incisor (Fig. 7). This may represent variation in the size of the group of ameloblasts which ceases matrix secretion at each increment, but it may also be affected by the plane of section in which the prisms are sectioned. Nonethe-

less, the spacing of prism boundaries does not vary greatly. The counts are higher by more than 25% in the increments for the two steps (numbers 6 and 7, 11 and 12) than they are elsewhere in the section in both the incisor and the molar.

6. The brown striae before increments 6 and 11 are both particularly prominent, and stand out strongly against the background of other striae.

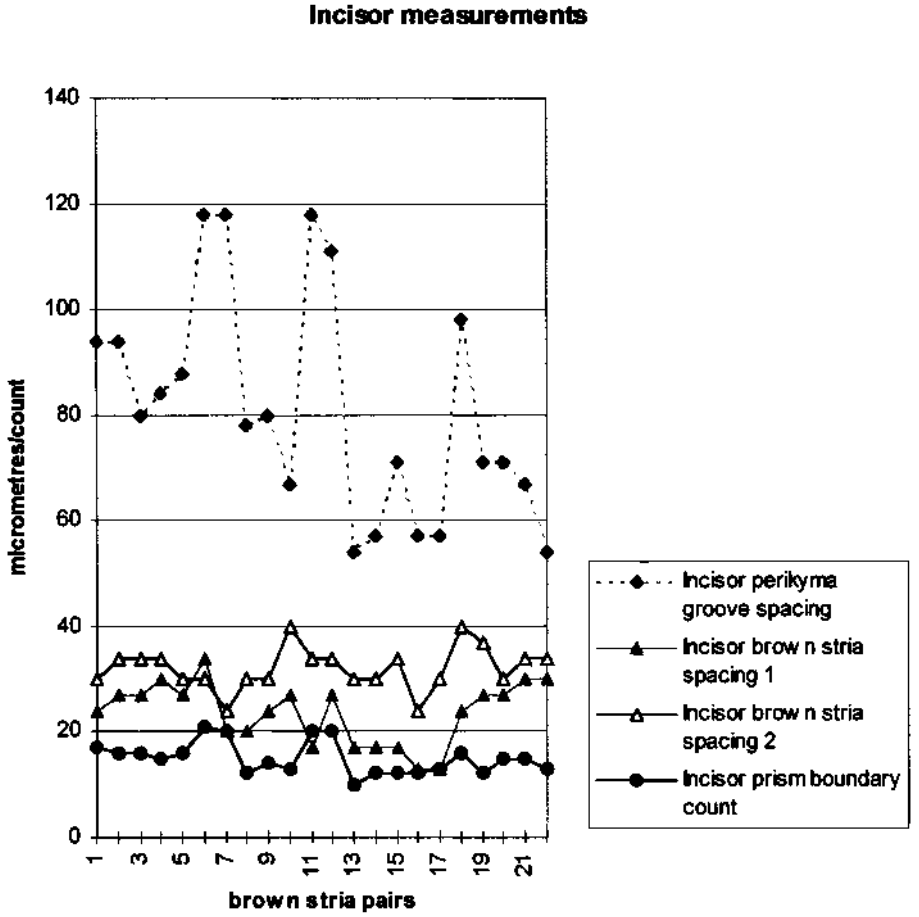


Fig. 7. Plot of perikyma groove spacing, spacing of brown striae along the line of the prism boundaries and the prism counts in the incisor (see figure 4).

DISCUSSION

From the neonatal line, point of interruption of enamel matrix secretion, and known age at death for this specimen (Antoine et al., this volume), it is confirmed that the prism cross striations do represent a 24 hourly growth rhythm. Using the repeat interval of 9 cross striations, this implies that the 22 pairs of brown stria in this study were secreted over a period of 198 days, or approximately 6 and a half months, of the child's life. There

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is an exact match between the incisor and molar for the perikyma groove counts within and between the steps in the defects, and the surface appearance is very similar. The position of the defect also matches the delay in development timing between the two teeth, so it is lower in the earlier forming first molar (Dean and Beynon, 1991). We are therefore convinced that the defects represent the same growth disturbance. This is confirmed by the counts of prism cross striations back from the last increment of enamel matrix, formed at the time of death. Using the 9 cross striation interval between brown striae, with the methods outlined in Antoine et al. (this volume), it can be established that the brown stria at the top of the first step was formed at approximately 1 year and 7 months of age, during the month of December, 1821 (one year before his death). The lowest brown stria of the second step was formed 9 weeks later.

In this form of hypoplastic defect, it seems that the underlying features of enamel histology vary little, as the cross striation interval between brown striae stays the same, together with the spacing of the brown striae. The overall chronology of crown formation, therefore, does not seem to have been affected by the disturbance that caused the defect. Two of the brown striae involved in the steps of the defect are, however, more prominent than their neighbours. This supports the idea, suggested by Rose (Goodman and Rose, 1990; Rose, 1977), that particularly prominent brown striae (called by him “Wilson bands”) are associated with hypoplastic defects and represent a similar type of growth disturbance. The variation in perikyma groove spacing itself, however, seems to be best explained by a variation in the number of ameloblasts ending their period of active matrix secretion at each increment. Perikymata are formed by successive bands of ameloblasts ceasing matrix secretion and, if the prism boundary counts can be taken as an index of the size of these bands, then the number of ameloblasts in a band increases during the period of growth disturbance, to cause the steps of the defect. In effect, there is an inward shift of the starting point for the next increment, lower down along the plane of a brown stria, to make the crown slightly narrower at this point and, creating the step defect (Hillson, 1998). The slightly narrower-than-expected perikyma groove spacings below the lower step are less easily explained in this way, because there is no consistent decrease in the number of prism boundaries associated with them. There is also no consistent change in brown stria spacing.

This specimen is the first in which precise chronology, in terms of age and calendar date, of a hypoplastic defect has been determined. It is also the first in which the surface appearance of a defect has been linked to the internal histological structure of the enamel. It establishes clearly a number of key points which, although they have been postulated for some time, have lacked well documented proof.

ACKNOWLEDGEMENTS

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Tyne) for their help in sectioning the material, and generally providing help and advice. The authors would finally like to thank Stuart Laidlaw, Mike Halliwell and Ken Walton (Institute of Archaeology, University College London) for their help with the illustrations.

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A Record of Hibernation in the Incisor Teeth of Recent and Fossil Marmots (*Marmota flaviventris*)

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INTRODUCTION

The ability to determine life history data from dental tissues, including such information as age of maturation (e.g. Fox, 1995), age at death (e.g. Huda and Bowman, 1995), rates of ontogenetic development (e.g. Beynon and Dean, 1988), parturition (e.g. Klevezal and Myrick, 1984), nutritional stress caused by El Niño (Manzanilla, 1989), and hibernation events (Klevezal and Mina, 1990) is based on a knowledge of the periodicity of dental tissue increment formation. A circadian (daily) periodicity of dentin development has been demonstrated experimentally for a variety of organisms including laboratory rats (e.g. Schour and Steadman, 1935), rabbits (Rosenberg and Simmons, 1980), dogs (Miani and Miani, 1972), pigs (Yilmaz et al., 1977), and alligators (Erickson, 1996). Schour and Steadman (1935) determined that a single dentin growth increment is composed of a hypercalcified and a hypocalcified layer and represents a single cycle in the circadian rhythm of rat incisor development. One approach all the aforementioned studies have shared is the utilization of incremental growth lines of dentin and enamel as seen in histological sections. However, on the external sides of the tooth not covered by enamel, these growth increments can be seen between periradicular bands in rodent teeth, and, therefore, may provide information on rates of incisor growth not available from sectioned specimens (Rinaldi, 1995). Schour and Medak (1951) demonstrated that incisal stress and function regulate and depress the rate of eruption in ever-growing incisors by clipping the incisors of rats and eliminating occlusion, resulting in a near doubling of eruption rate. Similarly, Michaeli and Weinreb (1968) demonstrated that eruption and attrition (wear) follow a daily rhythm in which the rates of both are directly related to diurnal fluctuations in rat activity. The direct relationship between decreased incisal stress resulting from wear and increased rate of eruption makes it possible to compare rates of incisor eruption and to infer variations in rates of attrition in rodent teeth. Variation in the width of dentin growth increments seen on the sides of rodent incisors provides a record of the longitudinal growth of the incisor, and should therefore approximate eruption rate. The objective of this paper is to demonstrate that decreased incisor growth during hibernation in marmots results in a pattern of dentin increment formation that can be identified on the exposed dentin surfaces of the incisor teeth. This hibernation record consists of small scale dentin increments that are separated by deeper, more accentuated periradicular bands (these increments will be abbreviated as HI-hibernation increments-throughout the paper). Commonly associated with this region of small increments is a thickening, or “buckling” of the enamel that can be easily seen at the dentinoenamel

junction. With a knowledge of the periodicity of the increments and the time of hibernation in a population of marmots, precise estimates of season of death can be obtained.

MATERIALS AND METHODS

Fourteen marmot (*Marmota flaviventris*) specimens with known dates of death were used to establish the existence of a record of hibernation in the incremental growth structure of incisor dentin. Ten of the specimens belong to the University of Kansas Natural History Museum Recent mammal collection (KU19960, 25300, 45918, 100898, 112537, 112539, 112957, 112956, 116273, 141288), and four of the specimens were killed by predators or cars while behavioral studies were being conducted by K. B. Armitage and his graduate students at the University of Kansas (field nos. 2, 308, 204/205, 855/859). One lower incisor from each specimen was removed and examined under a dissecting microscope. Narrowly situated periradicular bands with an accentuated relief and associated with a buckling of the enamel (HI), as seen in Figure 1, were identified when present. The number of periradicular bands basal to the HI was counted and checked against the estimated number of days the animals survived after emergence from hibernation.

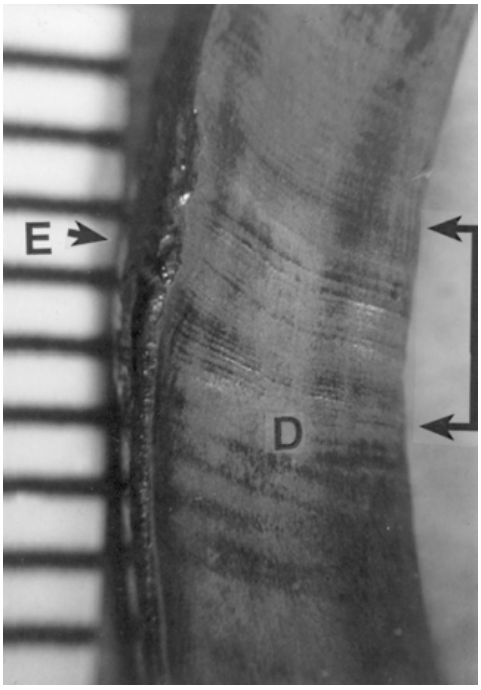


Fig. 1. Medial surface of a marmot incisor stained with silver nitrate. The bracketed arrows show the area of small scale, accentuated dentin increments. The single arrow points to "buckled" enamel. E, enamel; D, dentin. Scale in mm.

Two juvenile, wild caught marmots (field nos. 2038, 2194) were collected to be studied by K. B. Armitage as part of a separate hibernation physiology experiment. Following the physiology experiment, the animals were utilized for this study to demonstrate the periodicity of periradicular bands and determine the relationship between incisor incremental structure and hibernation events.

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Radio transmitters were surgically implanted inside the abdominal cavities of the two marmots before placing them into a dark environmental chamber maintained over winter (16 October to 1 June) at 10ºC. The behavior of the marmots was recorded daily to determine whether the animals were in a state of hibernation or a state of arousal. During the experiment each marmot was removed from the environmental chamber once and placed in a smaller chamber where O_2 consumption and body temperature were recorded for one cycle of torpor. Following the physiology experiment and final arousal of the animals, the temperature in the chamber was raised to 19ºC, the light/dark cycle was set to 12 hours, and pelleted food and water were provided *ad libitum*. Following Klevezal (1996), both animals were given an intramuscular dose of approximately 10 mg oxytetracycline/kg body weight in order to produce a fluorescent label in the dentin developing directly following the time of injection. Because of the reduced renal function in hibernating marmots (Zatzman and South, 1972) and the possibility of excessive accumulation of the drug resulting in liver toxicity in the presence of renal impairment (Physicians Desk Reference), the drug was administered to the animals only after evidence of food and water consumption was observed for both animals. Food and water consumption were assumed to be indicative of the return of digestive and kidney function. Both marmots were given a second dose of oxytetracycline 72 hours following the first dose. The animals were sacrificed by administering lethal doses of ketamine and xylazine, followed by pentobarbital 24 hours after the second dose of tetracycline. The teeth were removed from the skulls and examined under a microscope fitted with a UV light source to fluoresce the tetracycline labels. The distance of the fluorescent labels to the basal end of the incisor was measured for each tooth. The teeth were then stained in a 2% solution of silver nitrate for 24 hours, rinsed with deionized water, and placed in a sunlit room for several days until the surfaces of the teeth completely darkened. This staining technique enhances the appearance of the dentin increments allowing them to be counted and measured, but obscures the tetracycline labels. Therefore, the dentin increments found between the previously measured locations of the labels were counted and measured with an ocular micrometer after staining the teeth. Measurements of the dentin increments were plotted as growth rate histories similar to that in Figure 2. The HI were counted and compared with the number of periodic arousals recorded during hibernation.

Silver nitrate reacts with calcium phosphate to form silver phosphate (Preece, 1965). Use of this stain in undemineralized specimens reveals variations in degree of mineralization through differential staining and provides histological evidence that the dentin increments between periradicular bands in rodent incisors are composed of an alternating hyper- and hypocalcified layer (seen as dark and light increments, respectively) (Rinaldi, 1995). This technique of staining rodent incisors to reveal circadian increments on the surface of dentin was initially developed using beaver (*Castor canadensis*) incisors (Rinaldi, 1995). The contrast between alternating light and dark stained layers that correspond to hypo- and hypercalcified layers is marked in beaver dentin and has allowed the relationship between stain intensity and topography of the increments to be established (Rinaldi, in press). Grooves on the dentin surface between increments (periradicular bands) correspond to the light-staining layers and the elevated portion of each increment corresponds to the dark-staining layer (Fig. 3). In instances where the mineralization of the alternating layers was more homogeneous and staining

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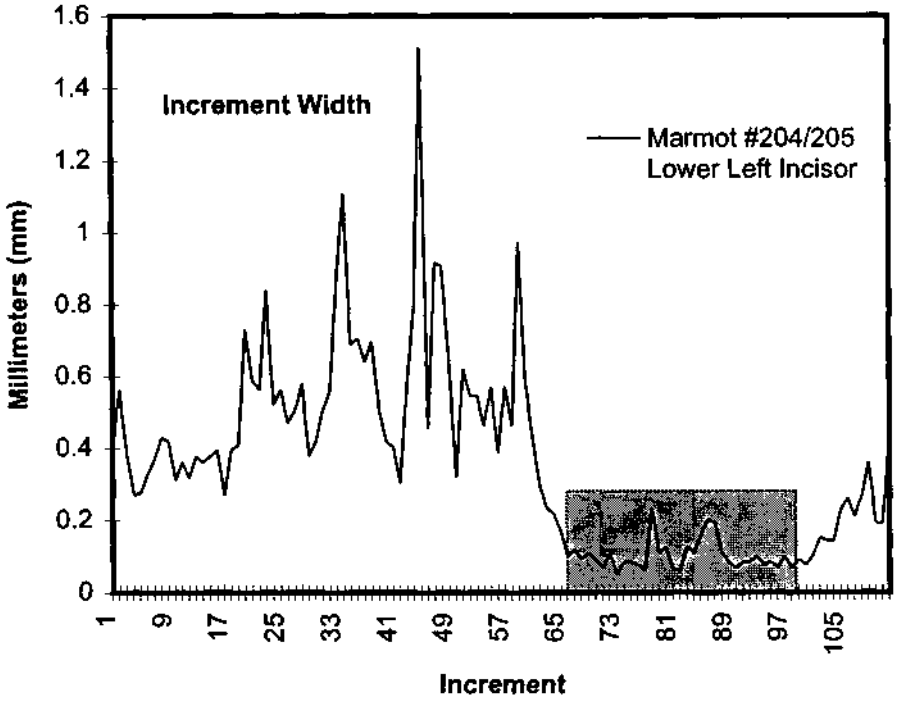


Fig. 2. Growth rate history of a marmot lower incisor. The widths of all the increments found on the medial surface of the incisor are plotted in the graph. The width of the last increment formed prior to death is increment 112, and lower numbers correspond to increments formed earlier. The increment widths that fall inside the gray box were associated with a "buckling" of enamel, and were formed during a period of hibernation. The animal died approximately two weeks following emergence from hibernation.

suboptimal, measurements were made topographically measuring from one groove to the next. Suboptimal staining with this technique is more common in smaller rodent species and among older animals within a species (personal observation), which seem to have a more homogeneous pattern of incremental mineralization.

RESULTS

Thirteen of the fourteen marmot specimens from the KU Recent mammal collections and behavioral studies had incisor eruption rate patterns consistent with the assumption that the HI correspond to hibernation events. In other words, when HI were present on the tooth surface, the number of "normal" increments formed following the affected increments was approximately equal to the number of days the animal survived past emergence from hibernation. Five of the specimens were juveniles (KU 112539, 112537, 112957, 112956, and field no. 2) and died before the first hibernation season. The increments on the incisors of these animals were generally wide and evenly spaced. No indication of a hibernation record was observed. Nine of the specimens were either

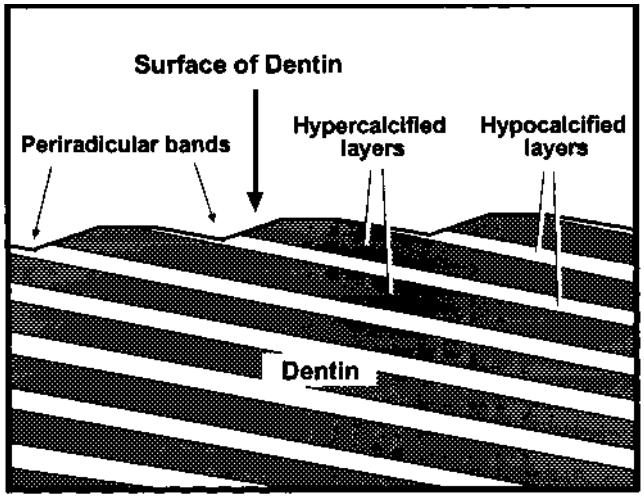


Fig. 3. Schematic representation of a longitudinal section through a rodent incisor stained with silver nitrate demonstrating the relationship between the hyper- and hypocalcified layers of dentin with respect to the tooth's surface topography.

yearlings or adults, and HI were present on the incisors of all but one of these animals (field no. 308). However, the animal was from a population of marmots that are known to emerge from hibernation in mid-April (Hopkinson, pers. comm.) and the animal died on 7 July. The incisor had a total of 56 increments suggesting that the hibernation record would have already worn off at the occlusal end. Only one of the fourteen specimens surveyed had too many "normal" increments formed after the disrupted dentin and before death to figure an emergence date that corresponds with knowledge of the population's behavior. The specimen (field no. 855/859) had approximately 30 increments more than expected for a known emergence of early May. In view of the results observed in the other thirteen specimens that consistently support a record of hibernation in the pattern of incremental development, it seems likely that an error has been made in recording the date of death or perhaps in determining the area of affected dentin.

The results from the experimental part of this study support this assertion and establish the circadian periodicity of the periradicular bands in marmot incisors. The marmots were each given two injections of tetracycline three days apart after arousing from a hibernation state. Two fluorescent labels were produced in all the incisors of one of the marmots (field no. 2038). Staining the incisors revealed the presence of three increments located between the positions of the two labels, confirming the existence of a circadian rhythm of increment formation. Four fluorescent labels were produced in the lower left incisor of the other marmot (field no. 2194), and three fluorescent labels could be identified in two other incisors from the same animal. Staining the incisors demonstrated that the first fluorescent marker and last fluorescent marker were separated by three increments. The experiment was initiated directly following a period of hibernation, and a difference in the degree of alertness between the two animals was observed. Marmot 2038 was first observed alert eight days prior to the arousal of marmot 2194. Marmot 2038 sat upright and produced an alarm call when his cage was approached. Marmot 2194 was not

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in the curled posture of hibernation, but he remained quiet with his eyes partially open for the duration of the experiment. The difference in behavior undoubtedly reflects different stages in the process of arousal from hibernation, and the series of fluorescent labels produced by the two injections of tetracycline in marmot 2194 probably resulted from an inability of the animal to completely metabolize the drug.

The dentin increments of one incisor from each animal were measured and plotted. The number of HI did not match the number of periodic arousals recorded during hibernation for both marmots. Seventy HI were found on a lower incisor of marmot 2038, but the marmot was only observed alert 21 times during hibernation when evidence of shallow torpor is excluded (evidence of shallow torpor exists when the animal does not appear alert, but is not in the curled posture characteristic during a hibernation state) and 25 times when evidence for shallow torpor is included. However, fifteen HI were found on the lower incisor of marmot 2194, and it was observed alert 11 days during hibernation excluding evidence for shallow torpor and 39 times including evidence of shallow torpor.

The number of dentin increments of “normal” morphology between the HI and the basal end of the incisors was counted. These increments were formed after the affected dentin increments. Because the circadian rhythm of increment formation had been established with the tetracycline labels, the utility of counting these increments to predict the number of days the animal survived following emergence from hibernation could be tested. The counts of the increments were made before knowledge of the total number of days the animals were awake was made available to the author to insure that no bias in judgment would occur. Nineteen increments basal to the disrupted dentin and enamel were found on the lower incisor of marmot 2038. The marmot had survived 19 days following hibernation before being sacrificed. Eight increments were found basal to the disrupted dentin and enamel on the lower incisor of marmot 2194. This marmot had survived 12 days following emergence from a hibernation state, making the error in estimating the date of emergence for these two animals zero to four days.

Finally the presence of hibernation records was identified in several specimens from the Pleistocene fossil locality, Natural Trap Cave, located in Wyoming. The oldest *Marmota* specimen examined from this locality (KUVP 36785) is approximately 18,000 years old - occurring during the glacial maximum - based on radiometric dating.

DISCUSSION

The experiment demonstrated that the metabolic changes occurring during hibernation affect the development of the ever-growing incisor teeth of yellow-bellied marmots. The tetracycline labels confirmed the circadian periodicity of periradicular bands in the dentin formed after the animals emerged from a hibernation state. The small scale, accentuated increments are not numerous enough to represent circadian increments for the entire eight-month period of hibernation characteristic of yellow-bellied marmots. The author’s original hypothesis was that the HI represent dentin development occurring during periodic arousals in hibernation. However, the results from the hibernation experiment suggest that the relationship between observed number of periodic arousals and number of small scale increments is complex. Labeling the teeth prior to the onset of hibernation would probably provide more information regarding the formation of these increments,

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but the opportunity to conduct this experiment was made possible after a separate physiology experiment was begun. Labeling the teeth during hibernation would be ideal; however, success labeling teeth of hibernating animals may be difficult due to reduced dental development. Previous researchers have documented deficient dentinogenesis (Mayer and Bernick, 1963) and decreased rate of eruption (Sarnat and Hook, 1942) resulting from decreased metabolism during hibernation, but until now, the presence and causes of buckled enamel and HI have not been examined. It is suggested here that the accentuation of the dentin increments and the buckling of enamel are the result of a decrease in eruption rate that does not equally match the decrease in dental tissue development. Perhaps dentinogenesis and amelogenesis resume when increases in metabolism (i.e., arousal) occur during hibernation, but lack of eruption causes the tissues to compress and buckle in the alveolus during development. Results from the separate physiology experiment conducted on the animals showed that 2038, with seventy HI, lost 1.58mg/day per gram immergence mass, whereas 2194, with 15 HI, lost 1.25mg/day per gram immergence mass suggesting that a relationship does exist between metabolism and number of HI. Finally, because the rhythm of increment formation is daily, the HI provide a method for using increments to estimate season of death that is more precise than use of cementum increments when time and duration of hibernation are known for the species.

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Tooth Development Relative to the First Permanent Molar in a Mediaeval Population from South-West Scotland

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INTRODUCTION

In the course of age estimation of the juveniles among the mediaeval skeletons excavated at Whithorn in south-west Scotland, it became evident that there were considerable discrepancies between the age estimates derived from different teeth, irrespective of which method of ageing was used. The discrepancies were not random, but tended to be consistently in the same direction, suggesting that the relative stages of development of the teeth may not be the same in the mediaeval skeletons from Whithorn as in the modern American populations on which the ageing criteria are based. As the real ages of the Whithorn juveniles cannot be known, we decided to analyse the Whithorn data to ascertain the relative stages of development of different tooth types, using the first permanent molar as the reference tooth, and to compare these with the American groups.

MATERIALS AND METHODS

The extensive cemetery at Whithorn in the south-western part of Scotland had been used between the 6th and 15th centuries A.D. Over 1700 articulated burials were excavated, but only 972 yielded dentitions or partial dentitions, of which 332, that is 34%, were juveniles. Their ages were assessed using the charts of Schour and Massler (1941), Ubelaker (1989) and Hillson (1986) and the systems of Demirjian et al. (1973, 1976) and Smith (1991). Tooth development was recorded according to the system of Moorrees and colleagues (1963) rather than that of Demirjian because age estimates were to be calculated using the tables published by Smith (1991) based on the Moorrees data and because the Moorrees system allowed the stages of development to be more finely graded. At the time the study commenced, the elaboration of the Demirjian system devised by Tompkins (1996) had not yet been published. To compare relative stages of tooth development of the mediaeval Scots in Whithorn, modern French Canadians and native Americans (Tompkins, 1996) and modern white Americans (Moorrees, 1963) a concordance between the systems of Moorrees, Demirjian and Tompkins was prepared (Table 1).

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Table 1. Concordance of the systems for recording stages of tooth development.

Moorrees[1]	Demirjian[2]	Tompkins[3]	Tompkins
Stage		ICPM	M
Ci	A	1	1
Cco	B	2	2
Coc	B	2	2
Cr½	C	3	3
Cr¾	C	4	4
Crc	D	5	5
Ri	D	6	6
Cli	E		7
R¼	E	7	7
R½	F	8	8
R¾	G	9	9
R¾	G	10	10
Rc	G	11	11
A½	H	12	12
Ac	H	13	13

[1]Moorrees et al. (1963); [2]Demirjian et al. (1973,1976); [3]Tompkins (1996)

Blind assessment of the stages of tooth development of 137 jaw specimens using the Demirjian and Tompkins scales and comparison of the results with those achieved using the concordance table indicated an overall accuracy of 80% for the concordance table, though for some stages the table gave very high accuracy and for others the results were poorer. For comparison of the Whithorn data with Moorrees' white Americans, figures had to be derived from his plots, since no indication was given of the numbers of individuals on which the plots were based. To do this, it was necessary to estimate the likelihood that a certain tooth, X say, exhibited a stage, A say, at the same age as tooth Y exhibited stage B. This could be calculated if we had a probability distribution that X exhibited A at age G: P(X, A, G) and similarly for Y at B: P(Y, B, G). The probability that X exhibited A and Y exhibited B at age G was then P(X, A, G) x P(Y, B, G). The problem then reduced to obtaining the functions P(X, A, G) from Moorrees' data. The bar charts in the original paper were measured to give the mean age at which a stage was attained, and also the +/- 1 standard deviation and +/- 2 standard deviation ages. The data on male and female subjects were averaged. For I1, there were no data for male R¼, so only female data were used. In general, the differences between the sexes were very small, but the largest were in the canine root development stages. The data were then used to generate a matrix of stage against age frequencies. For each stage, the mean age, +/- 1SD and +/- 2SD were fixed at 50%, 16% and 3% respectively, and frequencies at intermediate ages were calculated by interpolating between these five fixed points. The percentage of simultaneous presence of different stages in different teeth was then calculated by taking the age distributions of the two stages and adding the products of the frequencies of different stages. Because actual numbers were unknown, the significance of the differences from the white Americans could not be measured, but for the other population groups significance was assessed using the Mann-Whitney test.

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RESULTS

Throughout this study the stages of development were expressed relative to only one reference tooth, the first permanent molar. Table 2 presents the percentage figures for second permanent molars produced using our concordance. At each stage of development the Whithorn second permanent molars are at least one stage further advanced than those of the white Americans. From R½ to Rc they are one stage ahead of the French Canadians, the differences being significant at R½ ($p < 0.0005$) and R¾ ($p < 0.0001$). Very little difference was evident between Whithorn and the native Americans, except at stage Rc, where Whithorn is one stage behind ($p = 0.001$). The statistical significance of comparisons with Moorrees data cannot be calculated because the number of individuals is not known.

Table 2. Percentages for stages of development of M_2 relative to M_1 using concordance (Table 1).

M_1 stages			M_2 stages									
			Ci	Cco/ Coc	Cr½	Cr¾	Crc	Ri	Cli/ R¼	R½	R¾	n[1]
			1	2	3	4	5	6	7	8	9/10	
Crc	5	Moorrees/white Americans	100									
Ri	6	Whithorn		71.5	14.3	14.3						7
		Moorrees/white Americans	98	2								
Cli/R¼	7	Tompkins/French Canadians	8.3	8.3	83.4							12
		Tompkins/native Americans	4.8	19	50	26.2						42
		Whithorn		28.6	57.1	14.3						14
		Moorrees/white Americans	44	37	14	4	1					
R½	8	Tompkins/French Canadians		4.8	61.9	28.6	2.4	0	2.4			42
		Tompkins/native Americans			15.4	76.9	7.7					13
		Whithorn			17.6	58.8	11.8	11.8				17
		Moorrees/white Americans	1	16	41	31	10	2				
R¾	9/10	Tompkins/French Canadians		1.7	20.3	38.1	35.6	2.5	1.7			118
		Tompkins/native Americans			0	24.3	57.6	6.1	12.1			33
		Whithorn			8.7	4.3	39.1	47.8				23
		Moorrees/white Americans		3	22	35	28	10	1			
Rc	11	Tompkins/French Canadians			1.5	4.7	53.1	14.1	26.6			64
		Tompkins/native Americans					6.5	22.5	71			31
		Whithorn					20	70	10			10
		Moorrees/white Americans			9	25	39	23	3			
A½	12	Tompkins/French Canadians					16	10	62	12		50
		Tompkins/native Americans						100				20
		Whithorn						53.3	40	6.7		15
		Moorrees/white Americans				3	22	47	27	1		

[1]Number of dentitions used.

Table 3. Percentages for stages of development of C relative to M_1 using concordance (Table 1).

M_1 stages			C stages									
			Cco/Coc	Cr½	Cr¾	Crc	Ri	Cli/R¼	R½	R¾	Rc	n[1]
			2	3	4	5	6	7	8	9/10	11	
Cr½	3	Whithorn	100									2
		white Americans[2]	100									
Cr¾	4	Whithorn	64.3	28.6	7.1							14
		white Americans[2]	21	79								
Crc	5	Whithorn	9.1	81.8	9.1							11
		white Americans[2]		84	16							
Ri	6	Whithorn		4.8	71.4	23.8						21
		white Americans[2]		11	84	5						
Cli/R¼	7	Whithorn			72.2	27.8						18
		white Americans[2]			18	54	23	5				
R½	8	French Canadians[3]			5.8	25.7	11.5	57.1				35
		native Americans[3]				33.3	58.4	8.3				12
		Whithorn			6.7	40	40	13.3				15
		white Americans[2]				11	45	43	1			
R¾	9/10	French Canadians[3]				9.4	6.5	75.7	7.5	0.9		107
		native Americans[3]				15.7	34.3	50				32
		Whithorn				8.7	43.5	34.8	8.7	4.3		23
		white Americans[2]				2	21	66	11			
Rc	11	French Canadians[3]				1.9	1.9	63	21.3	11.1	0.9	108
		native Americans[3]					3.6	57.1	28.6	10.7		28
		Whithorn					20	50	20	10		10
		white Americans[2]					7	63	30			
A½	12	French Canadians[3]						22.3	34.1	42.4	1.1	85
		native Americans[3]						21	15.8	57.9	5.3	19
		Whithorn						50	25	25		12
		white Americans[2]						13	74	10	3	

[1]Number of dentitions used; [2]Moorrees et al. (1963) [3]Tompkins (1996).

In the canine (Table 3) Whithorn tended to be one stage behind the other populations, with significant differences at R½ for French Canadians (p=0.03) and A½ for native Americans (p=0.04). Table 4 presents the data for I1. At Cli/R¼ and R½ Whithorn tends

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to be a stage behind the white Americans, but at R¾, Rc and A½ the pattern changes, and Whithorn is ahead. Similarly Whithorn is a stage behind the French Canadians and native Americans at Cli/R¼ ($p<0.001$ for native Americans), but by R½ there is little difference, and no other significant differences were identified. The pattern in P1 was very variable (Table 5), but for P2 it was much more consistent (Table 6), with Whithorn ahead of the white Americans and significantly ahead of the French Canadians ($p<0.0001$) and also the native Americans ($p<0.0005$) at stage R¾.

Table 4. Percentages for stages of development of I_1 relative to M_1 using concordance (Table 1).

M_1 stages			I_1 stages										
			Cr½	Cr¾	Crc	Ri	Cli/R¼	R½	R¾	Rc	A½	Ac	n[1]
			3	4	5	6	7	8	9/10	11	12	13	
Cco	2	Whithorn	100										1
Cr½	3	Whithorn	100										3
Cr¾	4	Whithorn	33.3	58.3	8.3								12
Crc	5	Whithorn		70	30								10
Ri	6	Whithorn		5.3	47.4	47.4							19
Cli/R¼	7	French Canadians[3]			10	20	70						10
		native Americans[3]				21.3	78.7						47
		Whithorn			5.9	64.7	23.5	5.9					17
		white Americans[2]					88	11	1				
R½	8	French Canadians[3]			3.5		44.8	31	10.3	10.4			29
		native Americans[3]					92.3		7.7				13
		Whithorn				16.7	58.3	16.7	8.3				12
		white Americans[2]					45	43	10	3			
R¾	9/10	French Canadians[3]					4.4	12	31.5	45.6	3.3	3.3	92
		native Americans[3]					40	15	35	10			20
		Whithorn					6.2	12.5	50	25	6.2		16
		white Americans[2]					17	38	28	13	2	1	
Rc	11	French Canadians[3]							7.8	45.1	9.8	37.3	51
		native Americans[3]							4.3	21.7	52.2	21.7	23
		Whithorn							22.2	44.4		33.3	9
		white Americans[2]					5	23	38	24	7	3	
A½	12	French Canadians[3]								3.8	3.8	92.4	53
		native Americans[3]								13.3	46.7	40	15
		Whithorn									25	75	8
		white Americans[2]						2	18	28	29	24	

[1]Number of dentitions used; [2]Moorrees et al. (1963) [3]Tompkins (1996).

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Table 5. Percentages for stages of development of P_1 relative to M_1 using concordance (Table 1).

M_1 stages			P_1 stages											
			Ci	Cco/Coc	Cr½	Cr¾	Crc	Ri	Cli/R¼	R½	R¾	Rc	A½	n[1]
			1	2	3	4	5	6	7	8	9/10			
Cr½	3	white Americans[2]	100											
Cr¾	4	Whithorn	100											1
		white Americans[2]	100											
Crc	5	Whithorn	75	12.5	12.5									8
		white Americans[2]	53	47										
Ri	6	Whithorn	10	15	50	25								20
		white Americans[2]	1	73	26									
Cli/R¼	7	Whithorn			44.4	44.4	11.1							18
		white Americans[2]		5	49	35	10	2						
R½	8	French Canadians[3]			3	42.4	27.3	9.1	18.2					33
		native Americans[3]				38.5	53.8	7.7						13
		Whithorn				52.9	17.6	29.4						17
		white Americans[2]			2	29	44	22	4					
R¾	9/10	French Canadians[3]				2.8	37.8	6.6	50	2.8				106
		native Americans[3]				8.8	23.5	35.3	32.4					34
		Whithorn					4.5	63.6	27.3	4.5				22
		white Americans[2]				9	31	39	21					
Rc	11	French Canadians[3]					7.8	3.1	76.6	7.8	4.7			64
		native Americans[3]						6.9	75.9	6.9	10.3			29
		Whithorn						20	50	20	10			10
		white Americans[2]				2	16	40	40	3				
A½	12	French Canadians[3]							34	42	22	2		50
		native Americans[3]							31.6	47.4	21			19
		Whithorn						15.4	46.2	38.5				13
		white Americans[2]					1	11	51	33	3			

[1]Number of dentitions used; [2]Moorrees et al. (1963) [3]Tompkins (1996).

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Table 6. Percentages for stages of development of P_2 relative to M_1 using concordance (Table 1).

M_1 stages			P_2 stages											
				Ci	Cco/Coc	Cr½	Cr¾	Crc	Ri	Cli/R¼	R½	R¾	Rc	n[1]
			0	1	2	3	4	5	6	7	8	9/10	11	
Crc	5	white Americans[2]		100										
Ri	6	Whithorn		26.7	40	33.3								15
		white Americans[2]		93	7									
Cli/R¼	7	Whithorn		6.2	31.2	43.7	18.7							16
		white Americans[2]		18	53	23	6	1						
R½	8	French Canadians[3]	3		6.1	33.3	48.5	9.1						33
		native Americans[3]		7.7		23.1	61.5	7.7						13
		Whithorn				31.2	50	12.5	6.2					16
		white Americans[2]			7	42	35	13	3					
R¾	9/10	French Canadians[3]		2.9	2.9	2.9	31.4	43.1	4.9	11.7				102
		native Americans[3]				16.7	33.3	26.6	16.7	6.7				30
		Whithorn						42.9	38.1	19				21
		white Americans[2]			1	19	34	30	15	2				
Rc	11	French Canadians[3]				1.6	3.2	20.6	7.9	65.1	1.6			63
		native Americans[3]				3.3	0	3.3	30	56.7	6.7			30
		Whithorn							70	20	10			10
		white Americans[2]				6	21	38	27	8				
A½	12	French Canadians[3]		2		0	2	4	2	62	16	12		50
		native Americans[3]							57.1	28.6	14.3			21
		Whithorn							28.6	64.3	7.1			14
		white Americans[2]					2	16	35	39	8			

[1]Number of dentitions used; [2]Moorrees et al. (1963) [3]Tompkins (1996).

DISCUSSION

It has been well established that tooth eruption is influenced by various factors including sex, race and socio-economic levels (see review in Tompkins, 1996). Less work has been done on variations in tooth development. In her review of methods of recording dental development and their applications to age assessment, Smith (1991) commented that valid comparisons between studies can only be made when similar types of assessment have been used. She recommended, amongst others, the methods of Moorrees et al. (1963) and Demirjian et al. (1973, 1976), which were used to assess development of the Whithorn dentitions, but cautioned that too often European standards derived from one population have been applied indiscriminately to other populations.

Only Tompkins (1996) has previously compared the relative development of different teeth. Using a modification of Demirjian's method, he found the biggest differences in the development of M3, with black southern Africans being well ahead of French Canadians and somewhat ahead of native Americans. Perhaps the most unexpected result of the

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present study was that the mediaeval Scots so often showed advanced dental development in comparison with Caucasians from North America. This supports the observation of Harris and McKee (1990), based on their age assessment results, that regional differences in environmental as well as genetic factors are concerned in tooth development. It must be remembered, however, that Sapoka and Demirjian (1971) felt that the French-Canadian group examined by Tompkins had slower dental maturation than other North Americans.

Since age-referenced standards are unobtainable for most archaeological material, consideration of relative development of tooth types is very useful for population comparisons and it is to be hoped that further studies of this kind will be undertaken.

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A Unifying Model for the Aetiology of Enamel Defects

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INTRODUCTION

Over many years a substantial body of information has accumulated in the literature concerning the causes of developmental defects of enamel. These causes may be environmental or genetic. There are prenatal, neonatal and postnatal environmental influences on enamel development and these include trauma, infection, hypoxia and toxins (Brook et al., 1997). Recognised genetic causes are the group of conditions determined by single genes of major effect, termed Amelogenesis Imperfecta and the hereditary generalised syndromes of which enamel abnormalities are one manifestation (Winter and Brook, 1975).

The clinical features of developmental defects of enamel vary considerably. They range from a small circumscribed area of one surface of a tooth to widespread defects affecting all surfaces throughout the full thickness of enamel. Similarly the defects may be localised to one or two teeth or they may be generalised, involving many teeth or even the whole dentition. Across the midline the distribution of the defects may be symmetrical or asymmetrical. The clinical appearance may be of an opacity or of hypoplasia. In opacities there may be a full thickness of enamel but the appearance and texture of the surface is altered due to deficient mineralisation. In hypoplasia pitting, grooving or an area of absent enamel represent deficiencies in the amount of enamel formed.

However, rather than a particular appearance being related to a specific cause, the nature and extent of the enamel defects are influenced by factors such as the severity and duration of the insult, the development stage and the host's repose to the injury (Brook and Winter, 1975). Disturbances in enamel formation which can be confused with the effects of excess fluoride include those caused by chronic acidosis, hypoxia and some type of amelogenesis imperfecta (Winter, 1996; Whitford, 1997). Thus, in considering the aetiology of these defects not only the environmental and genetic factors have to be considered but also their interaction with the developmental process, tooth morphogenesis.

The aim of this paper is to construct a unifying aetiological model for developmental defects of enamel. This is based on an evaluation of the evidence for a multifactorial aetiology and whether the available data conform with the expectations for a quasi-continuous statistical model.

TOOTH MORPHOGENESIS

The tooth develops from epithelial and mesenchymal tissues. The former is derived from the ectoderm of the facial processes and the mesenchymal cells arise from neural crest. Sequential epithelial mesenchymal interactions are thought to be the most important

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single mechanism in the regulation of morphogenesis, associated with the various stages of differentiation of the tooth germ (Thesleff and Åberg, 1997). Associated with these various stages must be the interventions which lead to the dental anomalies of tooth number, size, form and structure.

A multifactorial model based on a quasicontinuous distribution related to tooth size and with thresholds determining hypodontia, microdontia, megadontia and supernumerary teeth has been proposed (Brook, 1984). Evidence has been advanced that this model applies not only to modern populations but that it also explains findings in Romano-Britons (Brook 1995). It is probable that the higher frequency of hypodontia and microdontia, and lower frequency of supernumeraries, in the Romano-Britons are explained by major environmental insults.

Thus, if there is evidence that there is multifactorial influence on the earlier stages of tooth morphogenesis it is reasonable to consider whether this also applies in the later stages, specifically in amelogenesis.

THE PROPOSED MODEL

Fig. 1 shows the model with a continuous distribution at the lower end of which are two thresholds. These determine opacities and hypoplasia respectively.

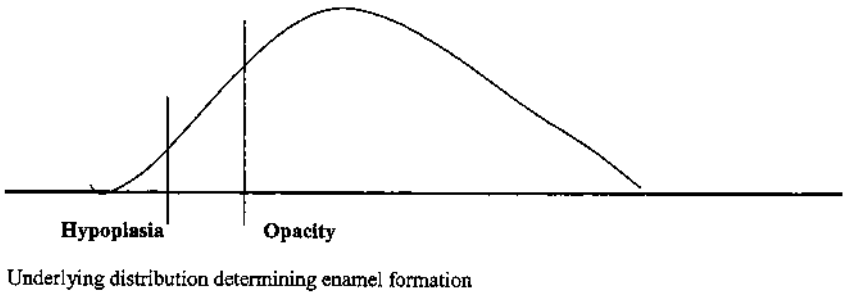


Fig. 1. The aetiology of enamel defects.

POPULATION AND FAMILY STUDIES

There is evidence from epidemiological and family studies to support this model. In a large study of white Caucasian London schoolchildren the prevalence in individuals of opacities was 68% and of hypoplasia was 14.6%; the tooth prevalence was opacity 13.5% and hypoplasia 1.7%.

A relevant finding was that in the large majority of cases of hypoplasia there was also an opacity clinically apparent; it is probable that in all instances of hypoplasia some degree of hypomineralisation (opacity) would be detectable on laboratory investigation. That the above prevalences are reasonable for the population is demonstrated by the similar prevalences found using the same F.D.I. D.D.E. Index for white Caucasian children in New Zealand and Ireland (Brook et al., 1997).

Based on the prevalence study in London a family study was undertaken. The first degree relatives of 101 index cases in the prevalence study who had hypoplasia of two or more teeth were examined and a detailed medical history questionnaire completed. The same number, age and sex controls without enamel defects and their first degree relatives were similarly examined.

The findings for the Index Cases were that 3 had Amelogenesis Imperfecta and 18 had Chronological hypoplasia related to systemic illness. For those with hypoplasia of incisors and premolars there was a significantly higher frequency in the relatives of index cases than of controls, the first expectation in the multifactorial hypothesis.

A further expectation of the hypothesis is that the frequency in first degree relatives should be the square root of the population frequency when the figures are expressed as proportions. For hypoplasia of the lower incisors it is shown in Table 1 that the observed and expected proportions are indeed close. Using Falconer's (1965) method for 'heritability' the contribution of genetic factors to the aetiology was calculated as 70 per cent (SE ± 38 per cent).

Table 1. The observed and expected frequency of hypoplasia in lower incisors in relation to the population prevalence.

	Frequencies expressed as proportions	
Population	1st degree relatives	
Observed (q)	Observed	Expected ($\sqrt{q}$)
0.014	0.10	0.12

Hypoplastic defects of enamel have also been studied in a Romano-British sample. This sample represents some five generations of a homogeneous population living in the third to fifth centuries AD, excavated from the Christian Cemetery at Poundbury, Dorset, UK. The data in Table 2 shows that there was a higher frequency in the Romano-Britons than in the modern London population, and that the defects were more widespread. The pattern of the hypoplasia in the Romano-Britons suggested that it might be related to repeated illnesses between two and six years of age. The teeth and bones of the Poundbury sample have a high lead content (Waldron et al, 1979; Stack and Whittaker, 1982). Although this may not be a direct cause of the enamel hypoplasia, it may have had an indirect effect by increasing susceptibility to other diseases.

Table 2. Overall prevalence and distribution of hypoplastic defects in two populations examined by the same observers.

	Romano-British	Modern
Prevalence	37%	15%
4 or more teeth affected	25%	1%
Horizontal grooves	30%	2%
Buccal surface	82%	73%

Table 3 shows the comparison of a population prevalence study of Chinese children in Hong Kong carried out by King and Brook (1984) using the same diagnostic criteria as Brook and Smith (1998) for the London sample. The Hong Kong sample had higher

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overall frequencies for opacities and for hypoplasia as well as having an increased number of individuals with more widespread defects. Some of the known environmental influences in Hong Kong during tooth formation for the children examined were a concentration of fluoride in the water supply above the optimum for the climate, poor housing conditions and a high frequency of infections during childhood.

Table 3. Overall prevalence and distribution of developmental defects of enamel in two modern populations.

	Hong Kong (King and Brook, 1985)	UK (Brook and Smith, 1998)
Opacity	94%	68%
Hypoplasia	64%	15%
4 or more affected teeth	80%	16%

FURTHER EVIDENCE OF MULTIFACTORIAL AETIOLOGY

Table 4 summarises some of the major genetic and environmental factors influencing enamel formation. There are general background factors which may be involved in the susceptibility to developmental defects. Superimposed are the specific occasional factors which will lead to formation of defects in susceptible individuals depending on the nature, severity, duration and timing of the insult. What is remarkable is the variation of expression often seen even in affected members of a given family with Amelogenesis Imperfecta. Considerable variation is seen between affected family members, and in individuals between the dentitions, between teeth in the same dentition and even in different areas on the same tooth.

Table 4. Major factors influencing enamel formation.

	General	Specific
Genetic	Polygenic	Single genes
		Amelogenesis Imperfecta Syndromes
Environmental	Nutrition	Excess Fluoride
	Mineral Metabolism	Trauma
		Infection
		Severe hypoxia

Evidence for the polygenic background has increased. Different genes coding for proteins related to enamel formation have been located on Chromosomes 1 (tuftelin/ enamelin), 4 (ameloblastin), X and Y (Amelogenins) (Aldred and Crawford, 1997). Within the X-chromosomal amelogenin gene several mutations have been described and there is heterogeneity of amelogenin mRNA with alternative splicing (Gibson et al., 1997).

Nutrition and mineral metabolism are important for normal enamel formation. Fluoride absorption is inversely related to dietary calcium. Fluoride balance and tissue concentrations and the risk of fluorosis are increased by such factors as high protein diets, residence at high altitude, and certain metabolic and respiratory disorders that decrease pH. Factors that increase urinary pH and decrease the balance of fluoride include

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vegetarian diets, certain drugs and some medical conditions (Whitford, 1997). Thus there is an interaction between the general background factors and the documented specific genetic (Bäckman, 1997) and environmental factors (Brook et al. 1997).

CONCLUSION

A multifactorial model has been proposed for developmental defects of enamel. The model has a quasi-continuous distribution with those being delineated by thresholds. The underlying distribution is related to the multiple genetic and environmental influences involved in normal enamel formation. It also incorporates the many specific major genetic and environmental agents recognised as leading to enamel defects in susceptible individuals. The purpose of the model is both to unify the many existing findings and to suggest further testable hypotheses.

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Dental and Skeletal Maturation in American Children

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INTRODUCTION

Timing and treatment planning in pediatric dentistry is often dependent on the stage of eruption and maturation of the teeth involved. Individual variations from the norm are frequent and can be large. Predictions for any individual can be difficult and many scientists have tried to correlate chronological age, dental development and skeletal development in an effort to better estimate dental maturation and apply appropriate treatment planning. These methods include correlation among tooth formation, tooth calcification stages, tooth emergence time, number of teeth present in the arch and crown and root development, as well as skeletal development of the hand and wrist. Some of these studies were cross-sectional, some longitudinal and some were cross-sectional analysis of longitudinal data. Some investigators found good correlation in their studies (Haag and Matsson, 1985), some only sometimes found an useful correlation, and others found no correlation (Gron, 1962). If treatment relies on the eruption of a particular group of teeth this treatment must be delayed or accelerated according to the pattern of maturation. Care must be taken to evaluate velocity curves against standard conventional norms for different individuals.

REVIEW OF THE LITERATURE

Studies correlating tooth eruption, tooth maturation and chronological age indicate that environmental factors such as premature loss or dental treatment of antecedents as well as genetic factors have an effect on dental maturation and eruption of permanent teeth (Loevy, 1983). The study of dental maturation evaluation is more reproducible than is use of eruption timing since different researchers interpret eruption differently. One group considers it as the time of piercing of the gingiva (a very difficult moment to pin point); others consider it the time of occlusal alignment.

While different methods studying the radiographic appearance of teeth during development have been proposed and are in use, it is important that the method used shows both precision and accuracy. Several investigators have compared the method used by Demirjian and co-workers (1973) and the method described by Moorrees' group (1963) and concluded that they afford a high degree of reliability and reproducibility. Different methods for skeletal age have been described (Roche et al., 1971) with varying degrees of correlation with chronological age. Some investigators prefer the Gruelich and Pyle atlas (1959) while others prefer the different Tanner-Whitehouse standards (Leinonen et al., 1972). Nystrom et al. (1986) examined semilongitudinally 248 healthy

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Finnish children and concluded that their dental maturation was more advanced than in the French-Canadian children. According to these findings, Finnish boys had a difference of 4.5 months at age 5-10 years and 7 months at the age of 11-12 years. In girls the difference started at age 4. They were on the average 3.5 months ahead of French-Canadian girls at age 4-9 years, and 9 months ahead at age 10-14 years. The same group (Nystrom et al., 1988) also demonstrated that even with a fairly homogeneous population, as in Finland, differences were still significant according to the region. Children in Kuhmo (Northwest Finland) had advanced dental maturity when compared to children of Helsinki (Southern Finland) indicating that some adjustment of the maturation curves must be made depending on the population being studied. Leinonen et al., (1972) found good correlation between dental age and chronological age. Liebgott (1978) in a serial study in males concluded that skeletal age as determined by the hand-wrist radiographs showed a high correlation with circumpubertal mandibular growth, but no significant correlation between circumpubertal mandibular growth and dental age. Lee and co-workers (1965) found good correlation between skeletal age and total number of erupted teeth in a cross-sectional study of 5500 Southern Chinese children while Lamons and Gray (1958) and Liebgott (1978) reported low correlation between dental and hand wrist maturation in their samples.

Racial composition and socioeconomic levels have been found to play roles in dental emergence and maturation studies (Koyoumdjisky-Kaye et al., 1977). Cahn and Roche (1981) reported that in their sample of 120 normal Australian children there was no evidence of illness which affected skeletal maturation.

MATERIALS AND METHODS

The data for this study were collected from duplicates of serial cephalometric radiographs from the files of the College of Dentistry, University of Illinois. The original longitudinal study was started in 1930 and continued until 1960. Our use of this material was approved by the Institutional Review Board for Human Studies. Caucasian-Americans were examined soon after birth, and annually thereafter with cephalometric radiographs taken at each examination. Most individuals had no dental pathology but a few small amalgam restorations were present in some patients. In some instances, the subjects had teeth extracted at the time orthodontic treatment was initiated. Cases with dental agenesis were excluded in the present study.

Six months after the cephalometric radiograph was taken a wrist radiograph of each patient was also taken and was available for many of the patients with cephalometric records. Since medical histories of the children were not available there was no possibility of excluding patients with systemic disease. Also, no information was available on height and weight for these children so it is not possible to determine if the physical development in general was within normal limits. Since photographs were also taken at yearly intervals (in some cases over 25 years), and showed no facial abnormalities, it can be concluded that they developed within normal parameters. In our study, attempts were made to use as the first record those radiographs taken before the third birthday of each child.

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Archival cephalometric radiographs of 157 Caucasian-American children (879 individual cephalometric films) were evaluated. These represented 56 girls (462 films) and 101 boys (417 films). The children ranged in age from 2.7 to 12+ years. Tooth mineralization and maturation of the permanent teeth as seen on the radiographs on the mandibular left side were recorded according to the methods described. These results were then tabulated for each child, and plotted against chronological and skeletal ages. Chronological age, date of the radiograph and gender were recorded. As each age group was examined, the number of cases in which dental age and chronological age were the same (±0.5 years) was plotted as a percentage of total sample size. According to Demirjian and colleagues (1973), eight stages of development of each tooth were defined based on the degree of calcification and root formation; stage A defined as the start of calcification and stage H being defined as the stage in which the apical end of the root is complete and the periodontal membrane has an uniform width around the root and its apex. The intermediate stages form a continuum. Demirjian and his group established maturation percentiles and developed graphs using the chronological age as the abscissa and the quadrant score as the ordinate. The numerical scores are derived from standard tables and are the sum of the scores of evaluated teeth for each quadrant. The data obtained in our study were plotted using these graphs to obtain a separate growth pattern evaluation for each patient studied.

Moorrees et al.'s method (1963) is also based on the evaluation of different stages of the dental maturation process. The process is divided into 13 stages and the evaluation of the teeth is computed to provide dental age.

Girls and boys were evaluated using separate curves. Extensive hand-wrist series were available for 23 boys and 36 girls. Only patients with six or more radiographs in this series were evaluated in this study. According to the method described by Roche and co-workers (1971) the stages of bone mineralization of the hand and wrist were compared to the standards in Greulich and Pyle's atlas (1959) and then scored. The scores were then computed separately for each age of each patient and the skeletal age determined. This method provides a numerical score which is later translated into an age score. The data obtained from skeletal evaluation were compared to the dental ages in order to evaluate the correlation among dental, chronological and skeletal ages.

RESULTS

Comparison of the three methods of calculated development age used showed that each method had better agreement with chronological age than with each other (Table 1). Demirjian's method has a better fit at the lower end of the plot but the range is greater. In the under 3-6 year group, 50% of the children had an agreement with the use of Demirjian's method. Using Moorrees' method this agreement was smaller (Figure 1). In the 6-8.9 year group, while Moorrees' method has a higher correlation coefficient, Demirjian's method is closer to chronological age. Moorrees is about 1½ years too high in this part of the age range. In the 9+ year group Demirjian's method provides data that are closer to chronological age at the low end of group but there is yet another change about age 10.

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Table 1. Percentage of sample of calculated age agreeing (±0.5 year) with chronological age.

AGES		GIRLS			BOYS	
	C/S %	C/D%	C/M%	C/S%	C/D%	C/M%
3-4	30	35	45	14	30	28
4-5	36	45	57	34	44	81
5-6	30	52	57	18	21	49
6-7	8	47	49	14	35	33
7-8	5	53	33	12	37	20
8-9	3	32	27	10	35	16
9-10	0	28	11	5	9	50
10-11	0	30	2	9	26	34
11-12	0	29	0	9	43	26
12-13	0	36	0	2	60	13

C/S = Chronological/Skeletal age.
C/D = Chronological/Dental age by Demirjian's method.
C/M = Chronological/Dental age by Moorrees' method

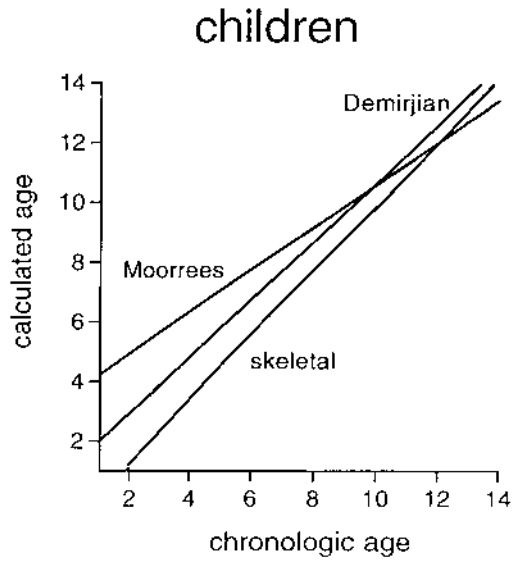


Fig. 1. Comparison of the calculated ages determined using best fit lines. Data were plotted and calculations performed using CA CRICKETGRAPH.

The skeletal curve remains constant and the Demirjian curve shifts further from the skeletal one after the age of nine. Correlation coefficients are not much different. In the older age group, there is a convergence of the three curves of the methods used. When we follow each person longitudinally through the analysis the results are less encouraging. Comparison of the percentage of agreement among the ratings given to each film by the three methods (Moorrees, Demirjian and skeletal) and chronological age (±0.5 year of chronological age) showed a number of individuals in agreement with one method of

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determination. It was unusual to have this agreement with two methods and rare for all three methods. While this was true for all age groups the specific individuals in agreement were different in the various age groups. A good fit at one age did not predict a good fit at all ages. Of the cases showing best agreement by both methods of dental age evaluation used, 11 patients showed this agreement at one age, 10 patients showed agreement at two different ages, 3 patients at three different ages and 2 patients at four different ages.

Of the cases showing best agreement with chronological age by the three methods used, five girls showed this agreement at one age, six girls and one boy showed this agreement at two different ages, three children (2 girls and 1 boy) showed agreement at three different ages, and one boy and one girl agreed at four different ages.

DISCUSSION

With the current emphasis on maximizing the care received while managing costs and time involvement, it is important to focus the timing of treatment to take advantage of developmental and growth changes both during and after the treatment period. There is a need to distinguish between favorable and unfavorable developmental conditions in order to establish treatment planning guidelines in the mixed dentition. Tooth maturation in the patients in our sample varied greatly. While our sample was made up of Caucasian children, it probably was not a population of homogenous background. Since evaluation of both dental and skeletal age are dependent on two dimensional radiographs a bias can be introduced. Some changes may occur during the year that may be insignificant in a morphologic two dimensional evaluation and may decrease reliability. We did not have information on height and weight for these children so it is not possible to determine whether the physical development in general was within normal limits. Since medical histories of the children were not available there was no possibility of excluding patients with systemic disease. We also did not have information on the socio-economic status or the dietary intake of these children, but had to depend on photographs taken at regular intervals to conclude that the children were developing within normal parameters. This lack of information cannot yield an exact assessment, but can indicate an approximate value. It lacks the precision we emotionally attach to it. In spite of its inadequacies at this moment these are the best tools available and application of clinical judgement is necessary. It also demonstrates that follow-up studies are needed in children to find outliers. Morphologic grades are not equally spaced across the age span and this interferes with the accuracy of the methods used. Further studies on intra-individual variations should also demonstrate correlation in dental development which might be useful in treatment planning. Regional variations in the timing of tooth mineralization have been demonstrated in a cross-sectional study comparing maturation in Midwest and Midsouth United States Caucasian-American adolescents. An 18 to 24 month delay in the Midsouth could be demonstrated. On the other hand, no such difference was noticed in the analysis of the hand-wrist development, apparently indicating the independence of development of the dental and osseous tissue systems (Mappes, et al., 1992).While different dental maturation systems have been proposed, the system developed by Demirjian and his colleagues (1973) is convenient to use and reproducible. It is now

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being used by numerous investigators in several countries allowing comparisons among population groups. Our previous studies have demonstrated (Loevy, 1997) that a shift towards maturation acceleration can be demonstrated in our sample as compared to the French-Canadian group. Our findings are similar to those reported in Finnish and Swedish children (Hagg and Matsson, 1985; Nystrom et al., 1986, 1988) indicating advanced dental maturation as compared to French-Canadian children but it is not possible to determine whether a shift accelerating maturation was present in those samples in Finnish and Swedish patients. If there are shifts towards a faster maturation in other populations similar to the one found in the present sample an adjustment of the standards currently used could be made for different populations. Whether the difference of our findings as compared to the maturation curves of the French-Canadian sample is due to a combination of all teeth in the arch or whether rules determining maturation of each tooth act independently for each tooth in the arch creating their own maturation parameters remains to be determined.

CONCLUSION

Our data indicate that there is considerable risk when predicting growth for treatment planning prior to age eight. The shift from below median to above median value is a prominent feature which must be considered in treatment planning of young patients. The risk of missing this shift is highest in children below age six years. These results indicate that various methods can be used predicting patterns of maturation with some expectation of accuracy but differences exist among the results of these methods and require clinical judgement in their interpretation. Longitudinal data are needed in order to minimize inaccurate inference from outlying cases. Longitudinal data and gender differences must be considered if one is to minimize erroneous interpretation. Further studies on intra-individual variations should also demonstrate correlation in dental development which might be useful in treatment planning.

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Size and Shape Modification of Molars in Children with Down Syndrome

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INTRODUCTION

The permanent molars of individuals with Down syndrome (DS) display a significant decrease in intercusp distances (Prahl-Anderson and Oerlemans 1976; Townsend, 1986). Peretz et al. (1996) showed that these differences were chiefly due to the altered position of the disto-lingual cusp and developed a multivariate probability model for DS and normal individuals.

The general decrease in the intercusp distances of maxillary molars in DS individuals suggests a disturbance in the early stage of formation of the permanent teeth. The change in shape was considered to occur in a late stage of crown formation, and is mainly expressed in reduced growth of the distolingual cusp. In the lower permanent molar, Brown and Townsend (1984) found that the lower permanent molar of DS individuals also showed reduced intercuspal distances and shape changes, with the distal cusps most affected.

This study was conducted in order to examine differences between the upper and lower molars of DS individuals with respect to the severity of the changes observed. We measured the intercusp distances and angles of the left mandibular first permanent molars in individuals with DS, and in a control group and developed a multivariate probability model for DS and normal individuals, which was compared to the model previously carried out for the first maxillary molar.

METHODS

All intercuspal distances and angles of 25 permanent mandibular first molar teeth of DS children (14 boys, 11 girls aged 7-14 years) and 30 permanent mandibular first permanent molars of normal children (12 boys, 18 girls aged 10-14) were measured from dental casts, taken in the course of routine treatment.

A video camera, monitor, and a computer with an image analyzer program were used for the measurements. Each tooth was analyzed separately. The casts were put on a wooden plate and adjusted to be parallel to the plate and perpendicular to the camera. The cusp tips, reflected by the highest points, were then marked with a graphite pencil. The images of the occlusal surfaces of the teeth were then transferred to the monitor on which the variables were measured with the image analyzer program. All intercusp distances were significantly smaller in the DS group.

Wilcoxon nonparametric test was used for univariate comparisons of the groups. Significance level was chosen at 0.05.

RESULTS

All teeth in both groups displayed five cusps. All intercusp distances were significantly smaller in the DS group (Table 1). Significant differences between DS and normals were found in three angles: the d-mb-dl angle was smaller than in normals, the mb-d-dl angle was higher in DS, and the mb-dl-d angle was smaller in DS. Stepwise logistic regression, applied to all intercusp distances was used to design a multivariate probability model for DS and normals.

Table 1. Means and SD of variables on the mandibular teeth (mm for intercusp distances).

	Down (n=25)	Normal (n=30)
Distance		
mb-db	4.26 ± 0.44	4.60 ± 0.54 *
mb-d	7.02 ± 0.45	7.69 ± 0.60 *
mb-dl	6.90 ± 0.57	8.00 ± 0.54 *
mb-ml	4.66 ± 0.46	5.04 ± 0.48 *
db-d	3.14 ± 0.45	3.39 ± 0.41 *
db-dl	5.06 ± 0.54	5.69 ± 0.42 *
db-ml	6.43 ± 0.56	6.94 ± 0.62 *
d-dl	3.80 ± 0.56	4.65 ± 0.44 *
d-ml	7.48 ± 0.55	8.36 ± 0.56 *
dl-ml	4.92 ± 0.42	5.68 ± 0.51 *
Angle		
db-mb-ml°	91.86 ± 4.95	92.15 ± 6.12
mb-db-ml°	46.37 ± 3.34	46.47 ± 3.19
mb-ml-db°	41.76 ± 4.79	41.37 ± 4.51
d-mb-db°	31.52 ± 3.98	34.37 ± 3.73*
mb-d-dl°	76.22 ± 6.10	69.00 ± 5.12*
mb-dl-d°	72.26 ± 4.53	76.64 ± 5.55*

* P < 0.05, Wilcoxon test.

A model based on only two intercusp distance (mb-dl and mb-db), proved sufficient to discriminate between the teeth of DS and the normal population (p = 0.0001). Thus, the probability for DS in the lower molar is as follows:

$$p\,(DS) = e^{30.6-5.6(mb\text{-}dl)+25(mb\text{-}db)} / 1+ e^{30.6-5.6(mb\text{-}dl)+25(mb\text{-}db)}.$$

The probability for DS is higher when mb-db is relatively higher in the mb-db/mb-dl ratio. The scaled model shows that for low values of mb-dl (< 7.0 mm), the probability for DS is high. For values of mb-dl (> 7.7 mm), the probability of DS is very low. In the “grey zone” (mb-dl distances between 7.2 and 7.6 mm), the probability for DS is proportional to the mb-db distance (Figure 1a).

DISCUSSION

The opposite signs entered into the model, show that both size and shape are affected. This is reflected in the univariate analyses, that show significant differences in both intercusp distances and angles between the cusps of DS and normal individuals. The scaled model (Figure 1a) demonstrates that these differences are most marked in the disto-lingual cusp of the lower tooth.

Comparison of the formula developed here with that previously reported for the upper molar (Peretz et al., 1996) suggests that the size and shape changes found in lower and upper molars of DS individuals are very similar, with the distal cusps most affected in both teeth. The probability model for the upper molar was as follows:

$$p\,(DS) = e^{20.15-4.53(mb-dl)+2.98(mb-ml)} / 1+ e^{20.15-4.53(mb-dl)+2.98(mb-ml)}$$

The scaled model for the upper molar shows that for low values of mb-dl (< 7.0 mm) in the maxillary molars, the probability for DS is high. For higher values of mb-dl (> 9.2 mm), the probability of DS is very low. In the 'grey zone', (mb-dl distances between 7 and 9.2 mm), the probability for DS is proportional to the mb-ml distance (Figure 1b).

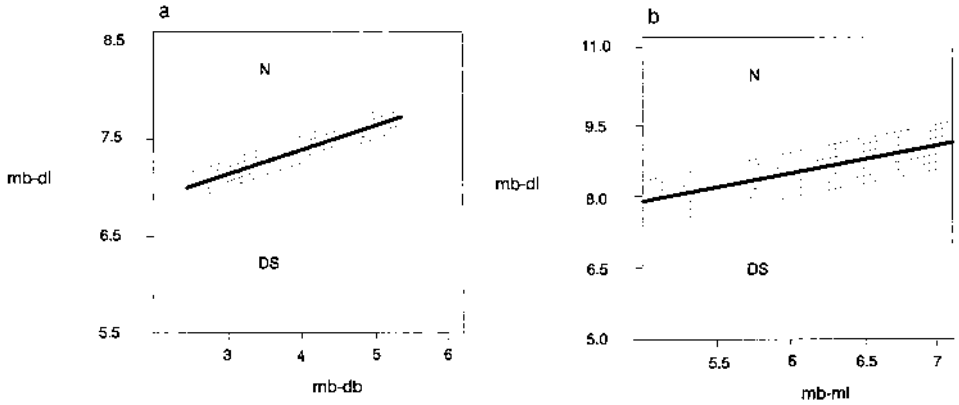


Fig. 1. The scaled models for the upper molar (a), and the lower molar (b). The dotted lines represent the 'grey zones'.

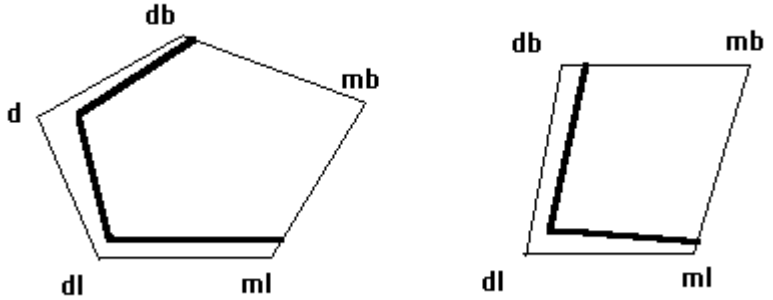


Fig. 2. Superimposition of the cusp tip patterns of a mandibular molar (a) and a maxillary molar (b) of the Down syndrome (DS) and the normal groups using the mb-ml line as an anchor. The heavy lines represent the DS group.

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As for the shape of the crowns, it seems that the expression of the syndrome in the upper and lower molars is similar in both, the decreased intercusp distances and in the distal cusps closer to the mesial cusps (Figure 2).

The growth insult in molar formation can be explained by a general slowing down of the mitotic cycle and rate of cell proliferation, resulting in growth retardation in the cells of the inner enamel epithelium of DS tooth germs (Mitwoch, 1972). The fact that it is mainly expressed in the distal portion of the tooth supports the hypothesis of Brown and Townsend (1984). It seems that in DS individuals, the change in size in both the mandibular and maxillary first molars occurs at an early stage, while the change in shape reflects the accumulated effects of continued growth retardation.

The model provides a reliable tool for predicting the probability for Down syndrome from the morphology of mandibular and maxillary molars that may prove of value in other studies of growth defects.

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The Variability of Root Trunk of Human Molars

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INTRODUCTION

The root trunk of multirooted teeth is located between the cemento-enamel junction (CEJ) and the entrance of furcation (Schumacher, 1983). The height of the root trunk seems to be of clinical importance in cases of marginal periodontitis and their apical progression. The toxic components of periopathogenic bacteria released from bacterial plaque accumulations are found within about 3 mm of the connective tissue (Waerhaugh and Ainamo, 1976). Thus, multirooted teeth with a short root trunk (<3 mm) may have a higher risk for the progression of the inflammatory process in the furcation area. The treatment of teeth with furcation involvement is difficult and often without longtime success (Cobb, 1996: Carnevale et al., 1995).

Enamel projections are often seen at multirooted teeth (Kleber et al., 1993). Such enamel projections are mainly located at the vestibular site of the first lower molar. In the region of enamel projections there is no typical connective attachment between tooth and alveolar bone, since the connective fibers can not be incorporated into the enamel. Thus, this region is designed as a place with greater risk for microbial attack.

The present study examines the hypothesis that teeth with enamel projections show shorter root trunks. Additionally, we examined the possible correlations between the lengths of root trunks and the length of roots or the total length of teeth.

MATERIALS AND METHODS

A total of 916 human extracted teeth from three different tooth collections (Humboldt-University Berlin, University of Rostock, Martin-Luther-University Halle, Germany) were measured with a pair of dividers (Aesculap, Germany), a calliper rule (Siemens, Germany), and a dentimeter (Aesculap, Germany). Other methods for metric measuring (photography and metric measurements, scanning and computerizing with metric grid measurements) checked previously in a pilot study had been classified as unsuitable for this study. The entrance of the furcation region, the length of tooth, and the circumference of the root trunk could not be measured by these methods.

Out of 16 different measurements of upper molars and 14 of lower molars the following measurements were selected:

- length of the tooth
- length of the root trunk
- lengths of the roots, and

– strength of enamel projections (three grades according to Masters and Hoskins, 1964).

The length of root trunk was measured at each site with an entrance to the furcation: in the upper jaw the distal, mesial, and vestibular; in the lower jaw the vestibular and lingual. For determining of the length of the root trunk the distance between CEJ and the roof of the furcation was measured parallel to the axis of tooth. The values of singular measurement were summarized and calculated as the mean length of root trunk per tooth. The distribution of all teeth used for this in-vitro study are noted in Table 1.

Table 1. Distribution of teeth investigated in the in-vitro study.

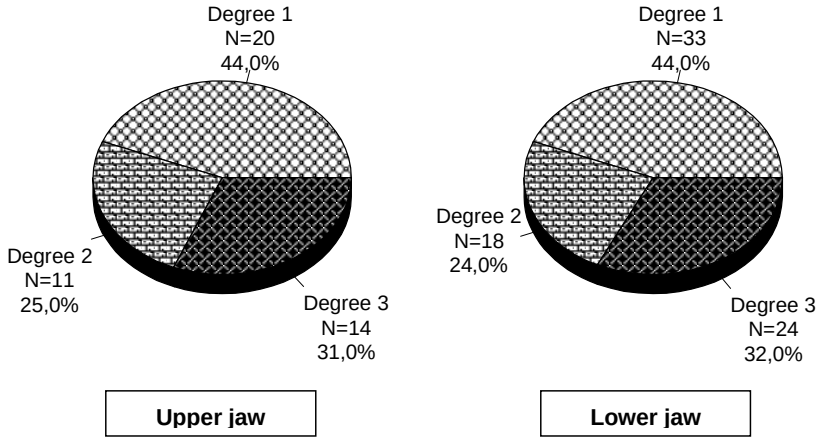
	First Molar	Second Molar	Molars with Enamel Projections	Total
Upper Jaw	179	220	45	444
Lower Jaw	195	202	75	472
Total	374	422	120	916

In the second, clinical part of the study a total of 217 molars (108 upper 1^{st} and 2^{nd} molars, 109 lower 1^{st} and 2^{nd} molars) scheduled for extraction because of marginal periodontitis, caries or pulpopathies were involved. The clinical investigation included measurements of pocket depth at four sites with a periodontal probe (PCPUNC 15, Hu-Friedy) and registration of furcation involvement (three grades). After extraction the teeth were cleaned and measured as mentioned above.

The values of all measurements were managed by computer software SPSS-PC. The mean values, standard deviation, t-test (Student), u-test (Mann/Whitney), and correlation's (Pearson, Spearman) were calculated, and differences were examined at a significance level of at least $p \leq 0.05$.

RESULTS

Out of 916 human molars investigated in the in-vitro study a total of 120 teeth (=13.1%) demonstrated enamel projections (Table 1). The differentiation of enamel projections according to Masters and Hoskins (1964) resulted in 40% for degree 1, 25% for degree 2, and 32% for degree 3 (Fig. 1).



Degree 1 - 3 for enamel projections according to Masters/Hoskins

Fig. 1. Graphic illustration of differentiation of enamel projections

The lengths of teeth from the upper and lower jaws differed significantly. The upper molars were considerably longer than the lower molar teeth (Table 2). The lengths of the root trunk (mean value of site specific measurements) of the upper molars were higher compared with the values of lower molars (Table 3). Teeth with enamel projections showed a shorter root trunk than teeth without such enamel projections. Additionally, the lengths of roots of teeth with enamel projections from the lower jaw were shorter than those from the upper molars (Fig. 2, Table 4). Depending on the length of the enamel projections (according to Masters/Hoskins 1964 from degree 1 up to degree 3) both the lengths of the root and the lengths of the root trunk were different (Fig. 3).

Table 2. Mean value (mean) and standard deviation (s) of tooth length (mm) and significance's between 1st and 2nd molars from upper and lower jaw.

	1st molar			2nd molar			
	Mean	s	N	Mean	s	N	
Upper jaw	23.43	1.2	179	22.25	1.7	220	P<0.0001
Lower jaw	21.96	2.1	195	20.18	1.3	202	P<0.0001
	P<0.05			P<0.05			

Table 3. Mean value (mean) and standard deviation (s) of root trunk (mm) and significance's between 1st and 2nd molar from upper and lower molars.

	1st molar			2nd molar			
	Mean	s	N	Mean	s	N	
Upper jaw	4.47	0.83	179	4.59	1.09	220	P=0.326
Lower jaw	4.15	0.96	195	4.01	0.875	202	P=0.172
	P<0.001			P<0.001			

Table 4. Mean value (mean) and standard deviation (s) of root lengths (mm) from teeth with or without enamel projections and significance's.

	Teeth with enamel projections			Teeth without enamel projections			
	Mean	s	N	Mean	s	N	
Upper jaw	13.71	0.65	19	13.69	0.86	222	n.s.
Lower jaw	12.44	0.97	33	13.37	1.24	193	P<0.002

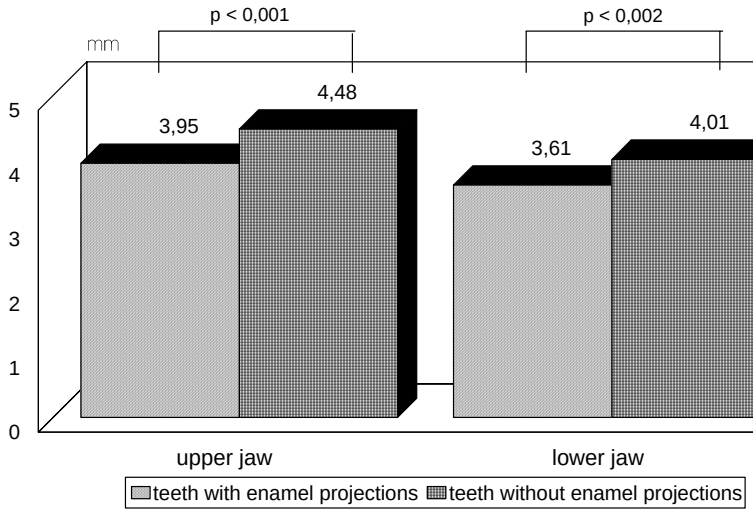
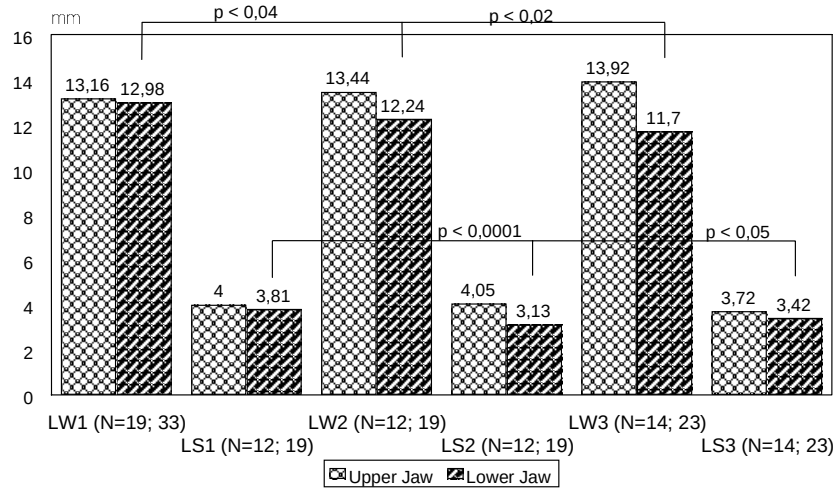


Fig. 2. Illustration of mean values of root trunk from teeth with or without enamel projections.



LW: length of root; LS: length of root trunk; 1, 2, 3 represent the degree of enamel projection (Master/Hoskins).

Fig. 3. Illustration of mean values of root trunk (LS) and length of roots (LW) depending on the degree of enamel projections (Masters and Hoskins 1964).

The results of the clinical part of the study demonstrated a positive correlation between pocket depth and furcation involvement: upper jaw r = 0.81, p≤0.01, lower jaw r = 0.87, p≤0.01. A negative correlation between furcation involvement and the length of root trunk was also registered: upper jaw r = -0.65. p≤0.01, lower jaw r = -0.54, p≤0.01 (Figs. 4 and 5).

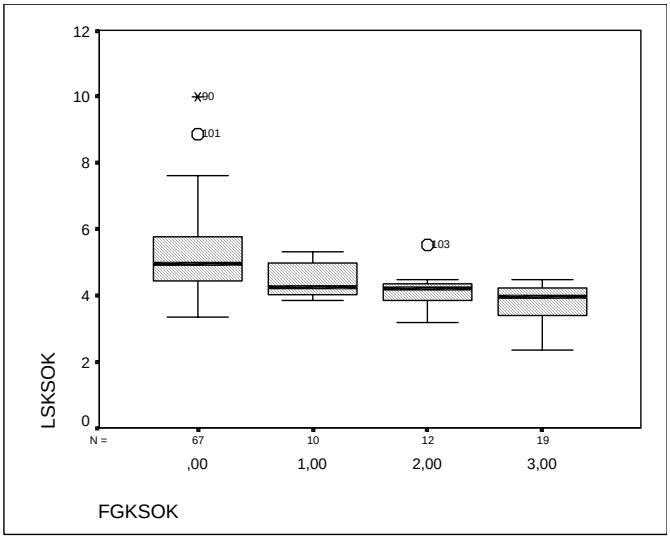


Fig. 4. Boxplot of root trunk length (LSKSOK) in mm depending from furcation involvement (FGKSOK 0, 1, 2, 3) in upper jaw. Black line through plots represent the mean.

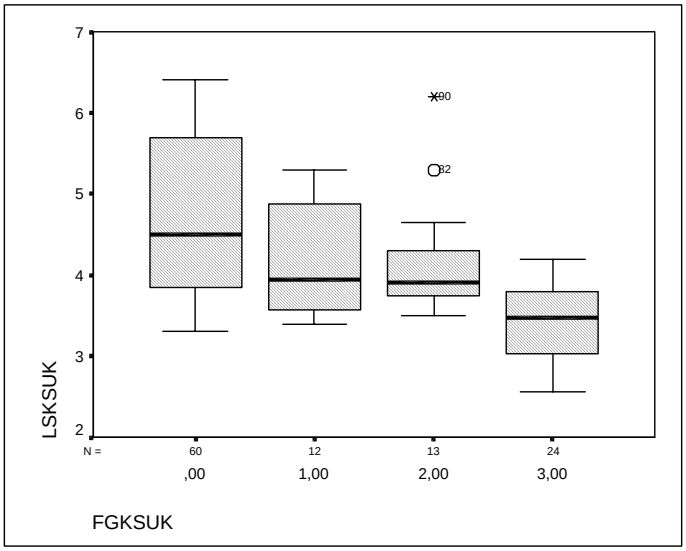


Fig. 5. Boxplot of root trunk length (LSKSUK) in mm depending from furcation involvement (FGKSUK 0, 1, 2, 3) in lower jaw. Black line through plots represent the mean.

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DISCUSSION

Despite the simple metric measurement methods used in the in-vitro investigation the results are representative (low standard deviation). Comparable data for root length, tooth length, and the height of the root trunk have been published (Schumacher, 1983; Mühlreiter et al., 1928; Jepsen, 1963). Teeth from tooth collections were assigned to in the different types of molars without any doubt. Thus, the results of this study confirm the hypothesis that teeth with enamel projections are characterized by a shorter height of the root trunk. These teeth are especially at danger for a progression of the marginal inflammatory process, if plaque control is not regular and careful (agreeing with the study by SimaoKon et al., 1991). The enamel projections can be easily removed by diamond burs before the marginal periodontitis begins. The results of the clinical part of the study indicate that teeth with a short root trunk show a higher degree of furcation involvement. These teeth need increased preventive attention, mainly in the plaque control at all sites by flossing and brushing. Otherwise, we have to search for easy examination methods for such teeth with a short height of the root trunk. Clinical attention must be addressed to detecting the small enamel projections on molars.

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Size of Crown Components of the Mandibular Deciduous and Permanent Molars in Australian Aborigines

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INTRODUCTION

The permanent molars have no deciduous predecessors and are additional to the deciduous molars. The deciduous and permanent molars belong to the first dentition embryologically. Tooth shape is similar in form and size from the first deciduous molar (dm1) to third permanent molar (M3). The mandibular molars are composed of two crown components; the mesial component is the trigonid, and the distal is the talonid. A morphological gradient from the mesial to the distal direction has been reported in the deciduous and permanent molar region (Butler, 1939). The morphological differences among the molar teeth are thought to be quantitative rather than qualitative. This gradient occurs as a result of quantitative changes of the two crown components. Yamada (1992b) and Kondo et al. (1999) reported the metrical variability of these components for the permanent molars, and Kondo et al. (1998) also reported the odontometrical structure of the two crown components in the molariform teeth of dm2, M1 and M2. The overall variability among the deciduous and permanent molars, including dm1, dm2 and from M1 to M3, is not clear. The present study aimed to investigate the metrical variability of the crown components in the deciduous and permanent molars of Australian Aborigines.

Materials and Methods

Sixty-eight dental casts of male Australian Aborigines were measured using a sliding caliper (0.05 mm). These casts were collected during a longitudinal growth study of Australian Aborigines living at Yuendumu, 285 km northwest of Alice Springs in the Northern Territory of Australia (Barrett et al., 1965). The measurements of the tooth crown are shown in Figure 1. They are the mesiodistal and buccolingual diameters (Fujita, 1949), the mesiodistal crown diameters of the trigonid and talonid (Yamada, 1992a), the buccolingual crown diameters of the trigonid and talonid (Kondo et al., 1998). The definition of the border between the trigonid and talonid followed Yamada (1992); the midpoint between the mesial central fossa and the intersection of the buccal groove with protoconid-hypoconid ridge. Crown component areas (trigonid area = trigonid mesiodistal diameter X trigonid buccolingual diameter, talonid area = talonid mesiodistal diameter X talonid buccolingual diameter) were calculated to compare the trigonid with the talonid.

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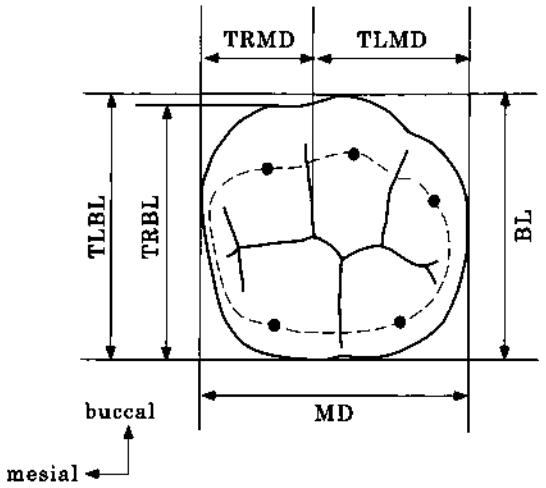


Fig. 1. Measurements of crown diameters for the mandibular deciduous and permanent molars. Abbreviations: BL = buccolingual diameter, MD = mesiodistal diameter, TLBL = buccolingual diameter of the talonid, TRBL = buccolingual diameter of the trigonid, TLMD = mesiodistal diameter of the talonid, TRMD = mesiodistal diameter of the trigonid, TRA = trigonid area = TRMD X TRBL, TLA = talonid area = TLMD X TLBL.

Descriptive statistics including distribution parameters were performed with JMP statistical software (SAS Institute Inc., Ver. 3.2 for Windows) on a personal computer. Differences among the means were analyzed using the multiple comparisons test (Tukey-Kramer HSD). Statistical significance was established at the $P<0.05$ or $P<0.01$ level.

RESULTS

Table 1 shows basic statistics of crown dimensions and indices in the mandibular deciduous and permanent molars, and Table 2 shows the results of multiple comparisons among them. Figure 2 shows standardized deviations of the crown diameters, and Figure 3 shows coefficients of variation.

The two deciduous molars were smaller than the three permanent molars, especially in buccolingual diameters. Although the trigonid mesiodistal diameter was significantly larger in dm1 than in dm2 ($P<0.01$), dm1 was the smallest of the teeth examined here. The mean values of the talonid mesiodistal diameter in dm2 and M1 were almost the same.

With the exception of the trigonid mesiodistal diameter, which was slightly larger in M2 and M3 than in M1, M1 was the largest molar as a whole. M2 was more reduced in size than M1 in the talonid mesiodistal diameter. Although the talonid mesiodistal diameter was significantly smaller in M2 than in M3 ($P<0.05$), M2 and M3 had similar crown dimensions.

Table 1. Crown dimensions of the mandibular molars.

		dm1	dm2	M1	M2	M3
	N	45	50	63	58	35
			Crown Diameter (mm)			
MD	mean	8.54	11.19	12.07	11.36	11.66
	SD	0.54	0.67	0.60	0.69	0.86
BL	mean	7.96	9.91	11.84	11.39	11.37
	SD	0.57	0.54	0.64	0.66	0.70
TRMD	mean	5.12	4.49	5.38	5.61	5.51
	SD	0.50	0.41	0.41	0.48	0.55
TLMD	mean	3.42	6.70	6.69	5.75	6.15
	SD	0.52	0.58	0.46	0.54	0.81
TRBL	mean	7.96	9.37	11.55	11.27	10.91
	SD	0.57	0.46	0.61	0.60	0.75
TLBL	mean	7.05	9.91	11.79	11.21	11.29
	SD	0.68	0.54	0.66	0.70	0.74
			Crown Area (mm^2)			
TRA	mean	40.89	42.15	62.30	63.34	60.38
	SD	5.81	4.85	7.09	7.49	9.25
TLA	mean	24.26	66.61	79.03	64.63	69.64
	SD	4.99	8.69	8.24	8.96	11.93

Table 2. Results of multiple comparisons among the mandibular molars (Tukey-Kramer HSD).

	MD	BL	TRMD	TLMD	TRBL	TLBL	TRA	TLA
dm1-dm2	**	**	**	**	**	**	NS	**
dm1-M1	**	**	*	**	**	**	**	**
dm1-M2	**	**	**	**	**	**	**	**
dm1-M3	**	**	**	**	**	**	**	**
dm2-M1	**	**	**	NS	**	**	**	**
dm2-M2	NS	**	**	**	**	**	**	NS
dm2-M3	*	**	**	**	**	**	**	NS
M1-M2	**	**	NS	**	NS	**	NS	**
M1-M3	*	**	NS	**	**	**	NS	**
M2-M3	NS	NS	NS	*	*	NS	NS	NS

NS: not significant;*: P<0.05; **: P<0,01.

Comparison of coefficients of variation showed that M1 was the most stable in size, and the variation increased in both mesial and distal positioned molars (Fig. 3). The size variability in the trigonid dimensions was nearly the same in all five molars, but the variability in the talonid dimensions was larger in dm1 and M3 than in the other molars. The buccolingual diameters showed less variability than the mesiodistal diameters.

Figure 4 shows plots of mean value of the trigonid area against the talonid area. The trigonid area was significantly smaller in the deciduous molars than in the permanent molars (P<0.01), and there were no differences among deciduous molars or among permanent molars. As for the talonid area, dm1 was the smallest, and M1 was the largest. The 95% confidence region for the bivariate means in M3 almost included those in M1

and M2. The difference of the trigonid area corresponded to the discrepancy between the deciduous and permanent molars, while that of the talonid area corresponded to the discrepancy between mesial and distal molars within the deciduous or permanent dentitions.

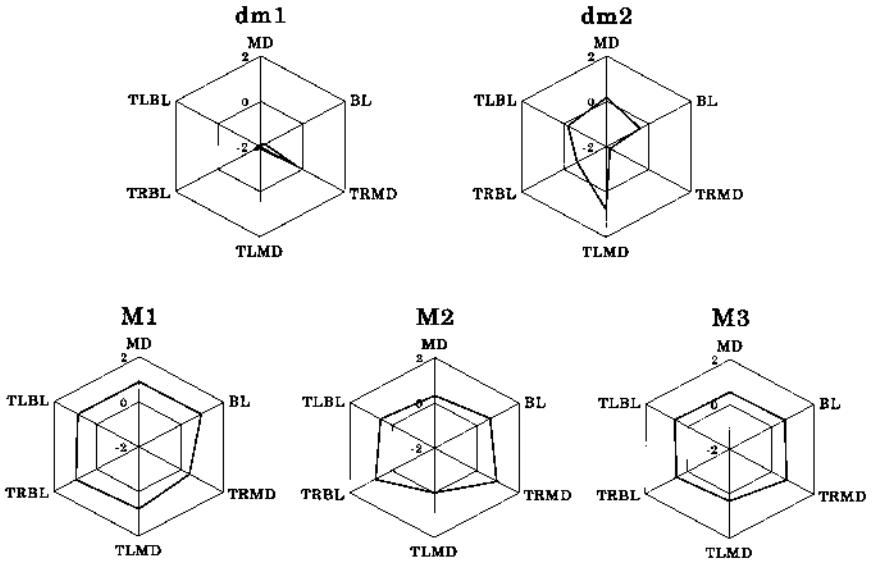


Fig. 2. Standardized deviations of crown diameter (σ). Zero shows the grand mean of the five molars.

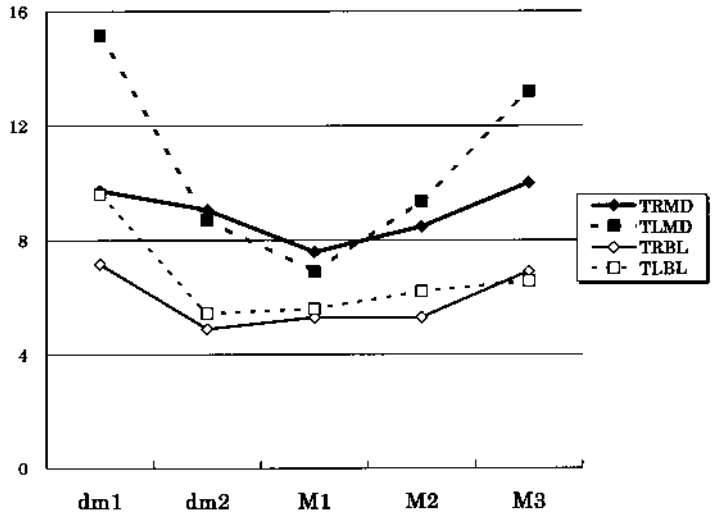


Fig. 3. Coefficients of variation of the crown diameter (%).

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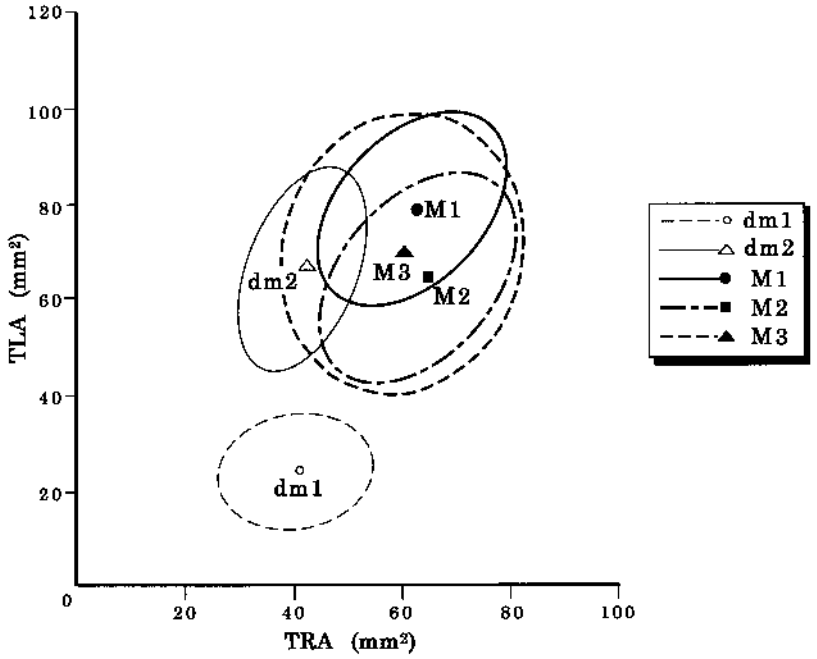


Fig. 4. Plots of mean value of the trigonid area (TRA) against the talonid area (TLA) for the mandibular deciduous and permanent molars (mm^2). The ellipse shows a 95 % confidence region for the bivariate mean.

Discussion

Dahlberg (1945) was the first to apply Butler's field theory (1939) to human dentitions and he described the gradient in the permanent teeth from a non-metrical perspective. The gradient of the dental trait frequency in both the deciduous and permanent molars was investigated by Saunders and Mayhall (1982). Their findings illustrated the gradient in form and expression of various dental morphological traits from deciduous to permanent teeth and provided support for the view that permanent molars were developmentally extensions of the deciduous molar tooth row. Smith et al. (1987) stated that the traits which appear in early stages of tooth development were more frequent in dm2 than in M1, while the traits that appeared later were more frequent in M1. They concluded that the relative frequency of traits in the molariform teeth reflected their ontogenetic history. In the molar region, the gradient is considered to be a result of quantitative changes of the trigonid and talonid components. We analyzed the metrical variability of the two components in order to express the gradient in the deciduous and permanent molars.

The two deciduous molars were smaller than the three permanent molars, and they were more reduced in buccolingual than in mesiodistal diameters. Thus the deciduous molars were buccolingually compressed. Dahlberg (1949) proposed a "compression factor" which affects mandibular deciduous teeth. A buccolingually compressed second

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premolar was found in Amerindians, Inuit (Dahlberg, 1949) and Hawaiians (Sakai, 1974). These cases seem to be caused by a similar "compression factor" which affects deciduous teeth as well. Buccolingual compression in the deciduous molar has also been reported in other primates (Kondo et al., 1996). This condition is considered to be characteristic of the deciduous molar, although why it occurs is not clear.

The size variability of the trigonid was similar in all five molar teeth. This result seems to relate to the fact that the trigonid is the first part of the crown to differentiate in both ontogeny (Kraus and Jordan, 1965) and phylogeny (Osborn, 1907). Gingerich (1974) noted an early-developed tooth was more stable than a later-developed tooth in size. His hypothesis could be extended to the difference between the two components in a molar. The early-formed trigonid was stable in size and there were no differences among deciduous molars or among permanent molars. On the other hand, the later-developed talonid showed a gradient among the molars. In the talonid, M1 was the most stable, and the size variation increased in both mesial and distal positioned molars. As to the variability in size, M1 is considered as a key tooth, and size was reduced in both the mesial and distal positioned teeth.

Crown component area showed that the trigonid was smaller in the deciduous molars than in the permanent molars, and the talonid increased from dm1 to M1 and decreased from M1 to M2 and M3. The size variability of each crown component is controlled by an independent factor, as Yamada (1992a) and Kondo et al. (1999) reported. It appears that genetic rather than the environmental factors influence the size of trigonid. While the talonid is formed later in the developmental stage, more variability is observed (Kondo et al., 1999). The trigonid was a stable crown component, and the talonid may be more influenced by environmental factors; shape and size relate to its position within the molar series.

Although both of the deciduous and permanent molars belong to the first dentition embryologically, their trigonids were significantly different in size. The trigonid of the deciduous molars almost completes calcification before birth, while that of the permanent molars has not begun to calcify except for the protoconid of M1 (Kraus and Jordan, 1965). The size discrepancy between the deciduous molars and permanent molars may relate to differences in the developmental timing of the trigonid.

The morphological gradient along the molar dentition from mesial to distal direction could be expressed primarily by the talonid size, and the variability gradient as mentioned above reflected the gradient of talonid area. This result is consistent with those of the molariform teeth in a Japanese population (Kondo et al., 1998).

Butler (1967) explained the morphological differences between the deciduous and permanent molars from a functional point of view. The deciduous molars have a much shorter period of function than the permanent molars, and they operate in a smaller mouth and shorter jaw. From the chronological growth study of human teeth (Schour and Massler, 1940), the deciduous molars develop more rapidly than the permanent molars. In the deciduous molars calcification starts at a small size of the crown, and the deciduous molars are smaller than the permanent molars when their growth concludes. The form differences between dm2 and M1 are established early in tooth development, by the time the dentine-enamel junction acquires its final form, and increase during the later phases of enamel apposition (Smith et al., 1997). These ontogenetical differences between them would also affect the shape of their completed crowns.

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Trigonid size contributed to the discrepancy between the deciduous and permanent molars, while talonid size contributed to the discrepancy between mesial and distal molars among the deciduous or permanent molars. The trigonid, which forms at an early stage ontogenetically, was a stable crown component.

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Morphological Variations of the Lower Premolars in Asian and Pacific Populations

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INTRODUCTION

The morphology of the premolar is said to be under control of morphogenesis of the canine and molars according to field theory by Butler (1939), while Dahlberg (1945) proposed the independent field for the premolars themselves. These theories were supported by principal component analyses of the mesio-distal diameters of the teeth in modern humans (Hanihara, 1976; Lombardi, 1975). Premolar morphology showed wide variety from its original form due to caninization and molarization. So far, the general morphology and its variation (Kraus and Furr, 1953; Ludwig, 1957; Kamijo, 1962; Konishi, 1988; Carlsen and Alexandersen, 1994), the relative size in comparison to adjacent teeth (Kitai, 1981), phylogenetical and anthropological significance (Yamada, 1964) and genetic significance (Sakai et al., 1969; Wood et al., 1969) of the premolars have been investigated. However, the classification of premolar variation has not been established, because researchers have based the classification on their particular criteria.

Since two dental complex types, i.e., Sundadonty and Sinodonty in Asiatic Mongoloid populations were proposed by Turner (1985, 1987), premolar morphology has been studied and it has been revealed that the premolars of the two types are quite different in size and trait morphology. However, the traits used so far for comparison were limited to tooth diameters and cusp number. Other morphological traits on the occlusal surface have been neglected because of the difficulty of quantification. In this study, we propose new morphological criteria for the lower premolar and compare the differences of their frequency among Asia-Pacific populations.

MATERIALS AND METHODS

Materials for observation are plaster casts of the dentitions of a total of 2,289 individuals from 11 Asia and Pacific populations. Geographical locations of the populations are shown in Figure 1. Almost all the materials were collected in the overseas field research under the title of "Anthropological Study on Food Intake and Oral Morphology of Polynesians in Special Oceanic Environments" with the financial support from an Interdisciplinary General Joint Research Grant for Nihon University. The data were collected from the right side of the dentition. The sample sizes for the lower premolars in each population are given in Table 1. Teeth in which the expression of a particular feature was affected by wear or pathology were not included. Significant sex differences in the frequencies at the 5% level by chi-square tests were only seen in a few morphological

features in the Mongols, probably because of the small sample size. In this study, the data from both sexes were combined as it is usually done in non-metric studies in which little or no sexual dimorphism is expected (Turner et al., 1991).

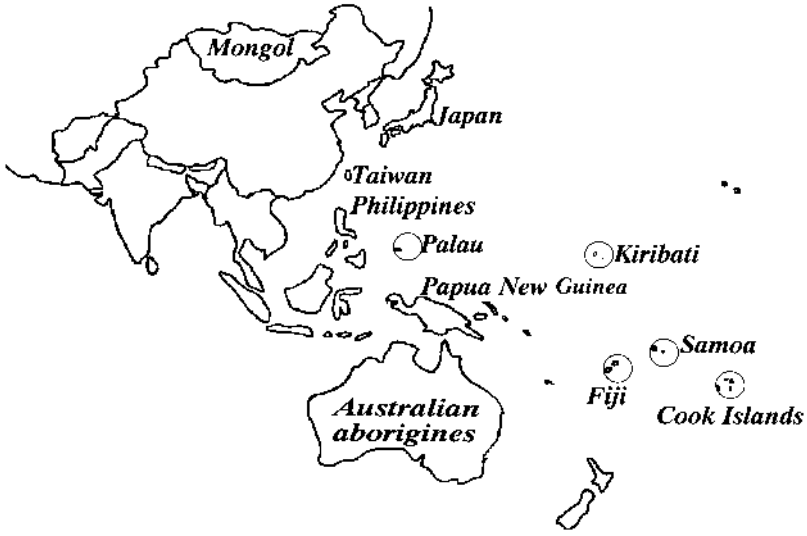


Fig. 1. Geographical presentation of populations investigated.

The biological distances among populations were computed using the B-square distance coefficient (Balakrishnan and Sanghvi, 1968) based on the frequencies of the morphological features, which represented the multi-dimensional scaling method and cluster analysis (group average method).

Table 1. Origin of dental casts used in this study.

Population	Collection	Location	Age	n		
				Male	Female	Total
Mongol	Kyoto University	Ulan Bator, 1993	14-52	19	30	49
Japan	Nihon University at Matsudo	Dental Students, Chiba, 1992-96	18-25	300	146	446
Taiwan	Nagasaki University	Yami tribe, 1980	8-18	87	74	161
Philippines	Showa University	Manila, 1990s	16-35	168	131	299
Palau	Nihon University at Matsudo	Koror, 1996	11-19	48	45	93
Kiribati	Nihon University at Matsudo	Tarawa Is., 1995	17-21	51	57	108
Cook Islands	Dr. Yamada	Pukapuka, Rarotonga & Mangaia Is., 1986-88	8-35	162	155	317
Samoa	Nihon University at Matsudo	Savaiĩ Is., & Apia, 1994-95	4-45	110	103	213
Fiji	Nihon University at Matsudo	Suva, 1994-95	5-45	156	171	327
Papua New Guinea	Nihon University at Matsudo	Enga, 1997	10-69	93	75	168
Australian aborigines	University of Adelaide	Yendum	18-20	56	52	108

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Morphological features

From the samples described above, information on the variation of 11 morphological features of the lower premolars was collected. Both the left and right teeth of individuals were scored by a single investigator (A. N.) for each feature, but data from the right side were used for the present study. The list of the morphological features with the criteria used in scoring variations are shown below. There is little agreement concerning the classification and interpretation of the morphological features of the lower premolars but these explicit definitions should facilitate interobserver comparison.

Lingual cusp number

0. Absent: no lingual cusp.
1. One lingual cusp.
2. Two lingual cusps.
3. Three lingual cusps.
4. Four lingual cusps.

Position of lingual main cusp (LMC)

1. Tip of LMC present in mesial half.
2. Tip of LMC present in central area.
3. Tip of LMC present in distal half.

Height of lingual main cusp (LMC)

1. Tip of LMC is equal to the marginal ridges.
2. Tip of LMC is lower than 1/2 of the buccal cusp.
3. Tip of LMC is 1/2 of the buccal cusp
4. Tip of LMC is 2/3 of the buccal cusp

Position of lingual accessory cusp (LAC)

(Scored for the presence of 1 and 2 lingual accessory cusps.)

1. LAC present mesial to LMC.
2. LAC present distal to LMC.
3. Two LACs present mesial to LMC.
4. Two LACs, mesial and distal to LMC.
5. Two LACs present distal to LMC.

Height of lingual accessory cusp (LAC)

(Scored for the presence of one lingual accessory cusp.)

1. Tip of LAC is a slight elevation of marginal ridges.
2. Tip of LAC is lower than LMC tip.
3. Tips of LMC and LAC are equal in height.
4. Tip of LAC is larger than LMC tip.

Lingual surface groove

0. Absent: lingual surface is smooth.
1. Groove on the mesial marginal ridge.
2. Groove on the central area of the lingual surface.
3. Groove on the distal marginal ridge.
4. Two grooves on both the mesial marginal ridge and the central area.
5. Two grooves on both the mesial and the distal marginal ridges.
6. Two grooves on both the central area and the distal marginal ridges.

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7. Three grooves on the mesial, distal, and central areas of the lingual surface.

Transverse ridge

0. Central groove extends mesiodistally across the occlusal surface and is not interrupted by the buccal and lingual triangular ridges.
1. Buccal and lingual triangular ridges weakly combined
2. Buccal and lingual triangular ridges fuse at their central part. The central groove is interrupted, and these ridges combine forming the transverse ridge.

Buccotriangular ridge

0. Buccotriangular ridge directly extends from the buccal cusp tip to the central groove.
1. Buccotriangular ridge bifurcates at 2/3 of buccal side.
2. Buccotriangular ridge bifurcates distinctly at the buccal cusp tips.
3. Buccotriangular ridge trifurcates.

Occlusal tubercle

0. Absent: normal occlusal surface.
1. Central area of occlusal surface is weakly elevated.
2. Corn-like projection on the occlusal surface.

Mesiolingual groove (P_1).

0. Absent: no groove of lingual surface.
1. Weak groove present on mesial lingual surface. The mesial marginal ridge is connected with the mesial slope of the lingual cusp.
2. Distinct groove separates mesial marginal ridge from the lingual cusp.

Separation of lingual main cusp and lingual accessory cusp (P_2).

0. Contact of the lingual main and accessory cusps.
1. Intermediate area between 2 cusps is more than 1/2 of the distance between 2 cusp tips. Mid-point of 2 cusps is flat.
2. Lingual main and accessory cusps are more separated and mid-point is elevated.

RESULTS

The percentage frequencies in the 11 populations are shown in Table 2. Among the features, the position of the lingual main cusp, the lingual surface groove and the transverse ridge were observed over in 85% in the second premolars, more frequently than in the first premolar. The variation in the second premolar was more stable than the first premolar. Many of the mongolians had single lingual cusps without accessory cusps. On the other hand, Fijians and Australian Aboriginals had many cases of double or triple lingual cusps. Many of Pacific populations had the triangular ridges of buccal and lingual cusps joined in the first premolar and the buccal triangular ridge was bifurcated in the second premolar. Especially Fijians had large tooth size with bifurcations of the lingual main cusp and the lingual accessory cusp compared to the other populations. B-squared distance coefficients applied to the eleven morphological features recorded for 11 populations are given in Table 3. A two-dimensional scattergram derived from the multi-

dimensional scaling of the B-squared values is shown in Figure 2. The first and second principal co-ordinate scores expressed 86.3% of the total variance. As a result, Mongol, Japan, and Taiwan were located in the left side of the X axis while Pacific populations such as the Philippines in east-Asia, Palau and Kiribati in Micronesia, the Cook Islands and Samoa in Polynesia, Fiji and Papua New Guinea in Melanesia, and the Australian aborigines were located on the right side on the X axis. It is worth noting that the scatter pattern of the populations closely resembles the pattern of their geographic relationships. Figure 3 shows the dendrogram based on cluster analysis from the same distance matrix; two major clusters are evident. The first three Asiatic populations fall in one major cluster within which Mongol and Japan are in a sub-cluster, and Taiwan is joined in the same cluster. The second included the Pacific populations, within which the Philippines, Palau, Kiribati, Cook Islands and Papua New Guinea are in a sub-cluster, and Samoa, Fiji and Australian aborigines are in another sub-cluster.

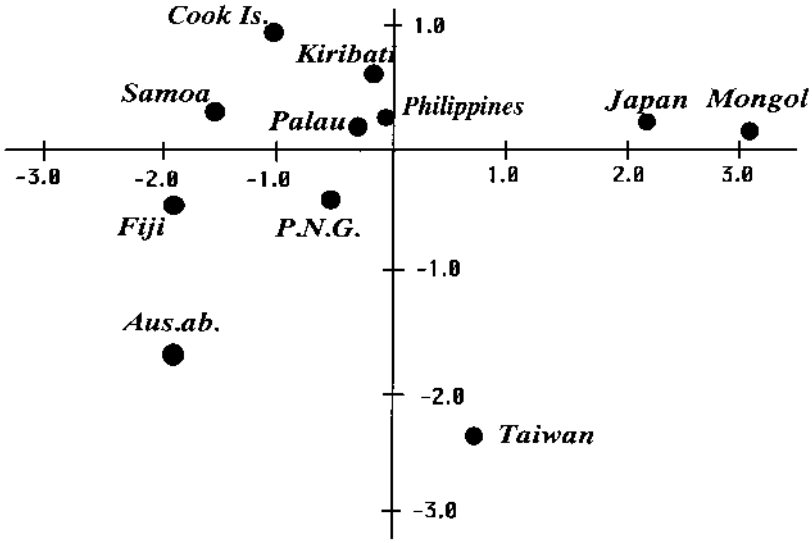


Fig. 2. Multi-dimensional scaling based on B-square distances among 11 populations (P1 and P2).

Table 2. *Frequency distributions of premolar morphological features.*

population	Number of lingual cusp						Position of LMC				Height of LMC					Position of LAC						Height of LAC				
	0	1	2	3	4	n	1	2	3	n	1	2	3	4	n	1	2	3	4	5	n	1	2	3	4	n
Mongol	0.0	68.1	29.8	2.1	0.0	(49)	72.3	27.7	0.0	(49)	21.7	58.7	19.6	0.0	(49)	0.0	93.3	0.0	0.0	6.7	(16)	71.4	28.6	0.0	0.0	(15)
Japan	1.6	54.9	41.5	1.8	0.2	(446)	72.6	19.7	7.7	(439)	20.9	50.2	25.2	3.7	(439)	0.5	95.2	0.5	1.1	2.7	(193)	56.4	30.4	8.2	5.0	(185)
Taiwan	1.9	44.1	47.2	6.8	0.0	(161)	80.1	10.9	9.0	(158)	20.5	54.5	22.4	2.6	(158)	9.1	78.4	0.0	4.5	8.0	(87)	69.8	26.3	3.9	0.0	(76)
Philippines	1.1	45.0	47.6	5.9	0.4	(299)	78.0	16.5	5.5	(296)	27.5	54.3	16.3	1.9	(296)	1.4	87.3	0.0	1.4	9.9	(160)	80.6	11.6	7.0	0.8	(142)
Palau	0.0	49.2	47.1	3.7	0.0	(93)	85.5	11.4	3.1	(93)	23.8	56.1	17.9	2.2	(93)	0.0	93.8	0.0	0.0	6.2	(47)	77.4	17.1	5.5	0.0	(44)
Kiribati	0.9	51.9	43.4	3.8	0.0	(108)	81.6	15.5	2.9	(107)	27.2	55.4	13.5	3.9	(107)	2.0	92.2	0.0	1.9	3.9	(51)	72.4	23.4	2.1	2.1	(47)
Cook Is.	1.6	46.9	43.6	7.5	0.4	(317)	75.7	14.6	9.7	(312)	34.8	44.4	17.7	3.1	(312)	4.0	82.1	0.7	3.3	9.9	(162)	68.3	21.1	8.3	2.3	(138)
Samoa	0.8	35.5	53.9	7.2	2.6	(213)	87.1	10.6	2.3	(212)	34.3	42.6	19.3	3.8	(212)	1.2	88.1	0.0	2.4	8.3	(130)	56.6	32.9	6.6	3.9	(115)
Fiji	1.6	35.8	51.7	6.8	4.1	(327)	80.6	15.4	4.0	(322)	35.3	44.9	17.3	2.5	(322)	3.2	82.9	1.8	2.8	9.3	(191)	69.5	26.3	4.2	0.0	(169)
P. N. G.	0.0	41.3	50.6	7.0	1.1	(168)	83.1	13.6	3.3	(168)	24.0	55.4	18.0	2.6	(168)	0.0	95.7	0.0	0.0	4.3	(97)	60.7	33.9	5.4	0.0	(85)
Aus. Abor.	4.9	33.0	53.4	8.7	0.0	(108)	84.7	14.3	1.0	(105)	29.3	50.0	17.6	3.1	(105)	1.6	84.4	0.0	1.6	12.4	(67)	72.7	25.5	1.8	0.0	(58)
Mongol	0.0	12.2	82.9	4.9	0.0	(49)	85.7	9.5	4.8	(49)	0.0	2.5	35.0	62.5	(49)	5.6	88.9	0.0	0.0	5.5	(43)	38.2	61.8	0.0	0.0	(41)
Japan	0.0	19.2	72.8	7.8	0.2	(446)	88.8	8.6	2.6	(446)	0.2	7.6	36.7	55.5	(446)	3.3	88.0	0.6	3.6	4.5	(358)	25.9	66.6	6.8	0.7	(323)
Taiwan	0.0	10.3	70.6	18.4	0.7	(161)	92.6	5.9	1.5	(161)	0.7	10.3	43.4	45.6	(161)	3.3	76.7	0.0	8.3	11.7	(143)	27.1	68.8	3.1	1.0	(114)
Philippines	0.0	15.0	73.8	10.9	0.3	(299)	96.7	3.3	0.0	(299)	0.4	11.5	60.2	27.9	(299)	2.7	84.4	0.0	1.8	11.1	(253)	33.7	63.3	2.6	0.4	(221)
Palau	0.0	13.6	74.3	12.1	0.0	(93)	95.1	4.2	0.7	(93)	0.0	3.4	45.7	50.9	(93)	1.1	90.2	0.0	1.1	7.6	(80)	27.1	70.2	2.7	0.0	(69)
Kiribati	0.0	18.9	76.4	4.7	0.0	(108)	94.3	5.7	0.0	(108)	0.0	1.9	31.9	66.2	(108)	1.1	93.0	0.0	1.2	4.7	(88)	24.1	70.4	4.3	1.2	(83)
Cook Is.	0.0	9.5	79.9	10.2	0.4	(317)	96.3	3.1	0.6	(317)	0.7	4.4	40.3	54.6	(317)	1.9	86.7	0.4	1.1	9.9	(286)	23.1	74.4	2.5	0.0	(253)
Samoa	0.0	3.7	78.2	18.1	0.0	(213)	98.5	1.5	0.0	(213)	0.0	1.4	34.0	64.6	(213)	0.9	88.5	0.9	0.0	9.7	(205)	37.5	60.8	1.7	0.0	(167)
Fiji	0.0	5.4	73.9	20.2	0.5	(327)	93.7	4.0	2.3	(327)	0.6	4.9	22.4	72.1	(327)	3.6	74.6	1.2	2.4	18.2	(307)	37.8	55.2	7.0	0.0	(242)
P. N. G.	0.0	7.3	80.9	11.8	0.0	(168)	94.8	3.8	1.4	(168)	0.0	3.2	47.1	49.7	(168)	0.0	83.1	0.0	1.1	15.8	(156)	31.4	59.8	8.8	0.0	(136)
Aus. Abor.	0.0	3.9	71.8	23.3	1.0	(108)	94.9	4.1	1.0	(108)	2.9	14.6	25.2	57.3	(108)	4.3	74.2	0.0	5.4	16.1	(101)	41.1	52.1	5.5	1.3	(78)

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Table2.(continued)

Lingual surface groove									Transverse ridge				Buccotriangular ridge					Occlusal tubercle				Mesiolingual groove				
0	1	2	3	4	5	6	7	n	0	1	2	n	0	1	2	3	n	0	1	2	n	0	1	2	n
75.6	11.2	4.4	4.4	0.0	4.4	0.0	0.0	(47)	58.7	28.3	13.0	(42)	51.1	17.8	31.1	0.0	(42)	97.8	2.2	0.0	(42)	79.2	6.3	14.5	(47)
66.3	20.1	4.3	3.2	2.9	2.5	0.5	0.2	(443)	44.0	41.2	14.8	(433)	48.0	28.8	21.1	2.1	(433)	90.1	9.2	0.7	(433)	74.5	12.4	13.1	(443)
68.9	11.3	6.8	6.2	1.9	1.2	0.6	3.1	(161)	50.3	32.9	16.8	(152)	50.9	31.4	15.3	2.4	(152)	100.0	0.0	0.0	(152)	85.1	3.7	11.2	(161)
58.1	12.5	10.4	6.1	5.0	6.1	0.7	1.1	(299)	47.2	34.6	18.2	(290)	21.3	31.8	46.4	0.5	(290)	97.1	2.2	0.7	(290)	75.7	9.7	14.6	(299)
56.6	14.7	11.8	8.0	4.3	3.3	0.0	1.3	(93)	40.8	44.3	14.9	(54)	23.4	30.2	45.4	1.0	(48)	100.0	0.0	0.0	(48)	76.7	12.3	11.0	(93)
57.9	14.3	7.6	7.6	5.9	3.8	1.0	1.9	(106)	40.6	42.6	16.8	(101)	24.2	31.4	41.7	2.7	(101)	97.1	2.8	0.1	(101)	80.9	6.7	12.4	(106)
56.3	20.4	8.6	1.6	5.4	6.3	0.7	0.7	(317)	38.5	43.1	18.4	(304)	23.7	21.7	53.6	1.0	(304)	98.7	1.0	0.3	(304)	66.9	10.5	22.6	(317)
59.1	16.7	13.2	3.7	5.1	1.5	0.0	0.7	(213)	47.9	39.9	12.2	(192)	21.2	35.3	38.1	5.4	(192)	95.7	2.9	1.4	(192)	75.9	7.3	16.8	(213)
56.5	13.0	11.4	4.1	7.3	3.6	1.0	3.1	(327)	40.4	44.6	15.0	(311)	24.8	30.4	38.6	6.2	(311)	96.4	2.6	1.0	(311)	71.8	8.7	19.5	(327)
59.8	17.3	12.9	3.3	5.7	1.0	0.0	0.0	(163)	49.7	40.3	10.0	(142)	28.9	32.1	38.0	1.0	(142)	97.7	2.3	0.0	(142)	76.0	12.6	11.4	(163)
58.4	12.9	14.8	6.9	4.0	1.0	0.0	2.0	(103)	46.6	41.7	11.7	(92)	23.8	37.6	33.0	5.6	(92)	99.0	1.0	0.0	(92)	81.4	10.8	7.8	(103)

Separate type of LCs																									
																						0	1	2	n
97.6	0.0	2.4	0.0	0.0	0.0	0.0	0.0	(41)	100.0	0.0	0.0	(41)	56.1	43.9	0.0	0.0	(41)	97.6	2.4	0.0	(41)	95.1	4.9	0.0	(49)
92.2	0.0	6.4	0.0	0.0	0.0	0.2	1.2	(421)	98.0	2.0	0.0	(433)	54.5	35.3	6.1	4.1	(433)	91.7	7.0	1.3	(433)	93.8	5.2	1.0	(446)
91.9	0.0	7.4	0.0	0.0	0.0	0.0	0.7	(136)	100.0	0.0	0.0	(141)	45.1	52.2	0.0	2.7	(141)	99.3	0.7	0.0	(141)	97.1	0.7	2.2	(161)
91.9	0.0	7.8	0.3	0.0	0.0	0.0	0.0	(283)	99.2	0.8	0.0	(274)	34.8	52.7	11.0	1.5	(274)	96.6	2.7	0.7	(274)	97.0	0.7	2.3	(299)
91.3	0.0	8.7	0.0	0.0	0.0	0.0	0.0	(90)	100.0	0.0	0.0	(58)	31.3	58.9	6.8	3.0	(50)	97.0	3.0	0.0	(49)	88.4	9.6	2.0	(93)
91.4	0.0	8.6	0.0	0.0	0.0	0.0	0.0	(106)	99.0	1.0	0.0	(97)	30.2	55.8	9.6	4.4	(97)	96.2	2.8	1.0	(97)	83.1	11.5	5.4	(108)
91.9	0.3	7.1	0.0	0.3	0.0	0.4	0.0	(305)	98.2	1.8	0.0	(293)	25.7	58.6	7.5	8.2	(293)	98.6	1.1	0.3	(293)	83.3	14.3	2.4	(317)
92.7	0.0	6.6	0.0	0.0	0.0	0.0	0.7	(198)	100.0	0.0	0.0	(204)	23.5	65.0	3.4	8.1	(204)	98.6	0.7	0.7	(204)	78.2	12.5	9.3	(213)
94.5	0.0	3.4	0.0	0.0	0.0	0.5	1.6	(336)	99.4	0.6	0.0	(309)	16.9	71.8	5.1	6.2	(309)	95.5	3.9	0.6	(309)	74.9	13.5	11.6	(327)
95.6	0.0	3.3	0.0	0.0	0.0	0.0	1.1	(155)	100.0	0.0	0.0	(147)	33.5	54.9	8.3	3.3	(147)	96.2	3.8	0.0	(147)	86.1	10.6	3.3	(168)
88.3	1.0	5.8	0.0	0.0	0.0	0.0	4.9	(101)	100.0	0.0	0.0	(94)	26.5	66.4	1.9	5.2	(94)	97.1	2.9	0.0	(94)	78.4	14.7	6.9	(108)

Table 2. B^2 distances among 11 populations (P1 and P2.)

	Mongol	Japan	Taiwan	Phil.	Palau	Kiribati	Cook Is.	Samoa	Fiji	P. N. G.	Aus. Abo.
Mongol	-										
Japan	2.061	-									
Taiwan	3.122	2.979	-								
Phil.	3.735	3.326	3.041	-							
Palau	3.219	2.995	2.960	0.938	-						
Kiribati	2.899	2.413	3.289	1.560	0.512	-					
Cook Is.	4.132	3.594	3.465	1.593	1.145	1.177	-				
Samoa	4.459	3.977	3.518	2.587	1.451	1.592	1.409	-			
Fiji	5.039	4.293	3.597	2.916	2.083	1.973	1.520	1.017	-		
P. N. G.	2.876	2.322	2.829	1.665	0.770	1.029	1.391	0.946	1.825	-	
Aus. Abo.	5.268	4.712	2.962	3.018	2.353	2.669	2.590	1.643	1.096	2.241	-

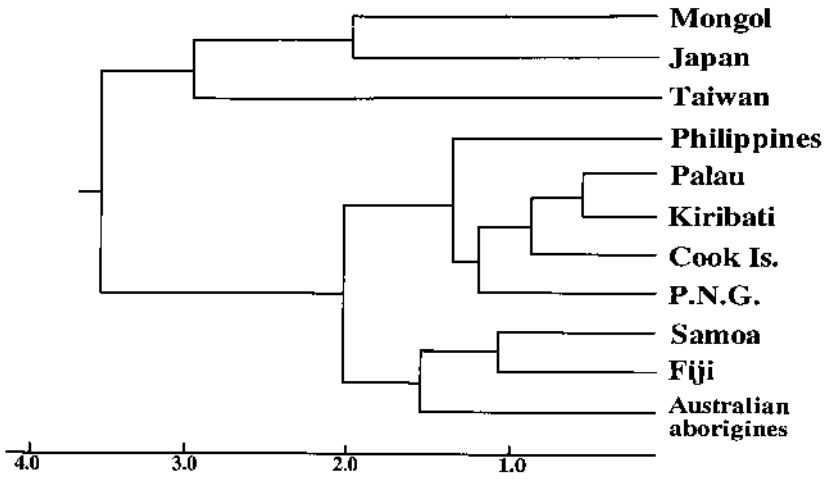


Fig. 3. Dendrogram based on cluster analysis computed from B-square distances among 11 populations (P1 and P2).

DISCUSSION

Turner (1987) showed that there were two morphological patterns within Mongoloid dental variation by using 28 nonmetric tooth crown and root traits. The two patterns, called Sinodont and Sundadont, are characteristic of northeast Asia and southeast Asia populations, respectively. He reported that the incidence of two lingual cusps in the lower second premolar was somewhat higher in Sundadonts, by comparison within two dental patterns. Nagai and her colleagues (1998) set up a classification system for variants of the lingual cusp number in lower premolars, and worked with Pacific populations including Mongol, Japan and Australian aborigines, where found a higher frequency of multiple lingual cusps of both premolars in South-Pacific groups. Their results separated two groups of east Asia populations and Pacific populations. In this study, the cluster analysis (Fig. 3) showed that two major clusters were evident, one was Sinodont-like pattern such

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as Mongol, Japan and Taiwan, and the other was Sundadont-like pattern such as Philippines, Palau, Kiribati, Cook Islands, Papua New guinea, Samoa, Fiji and Australian aborigines. Manabe (1992) compared 20 east Asia populations including 7 Sinodont populations and 11 Sundadont populations derived from Turner's data (1987) and data on Yami, Bunun and Ami tribes of the aborigines of Taiwan based on the ASU system (Turner et al., 1991). The two tribes of Yami and Bunun were classified into the Sinodont cluster (Manabe, 1989; Manabe et al., 1991) but the Ami tribe resembled Sundadont populations more than the Sinodonts (Manabe et al., 1992). There were both Sinodont and Sundadont characteristics in the Ami. The present study classifies Taiwan into a Sinodont-like cluster with Mongol and Japan. Concerning tooth size, the Filipinos have Sundadont characteristics based on the cluster analysis of crown indices (Hamada, 1997), a conclusion also reached in this study. Mastuno (1997) reported on the comparative tooth size on populations of Micronesia, Polynesia and Melanesia, suggesting that the Pacific Islanders showed polymorphic dental characteristics, including a Sundadont pattern. This might indicate that the original people of the South Pacific developed a polymorphism of dental characteristics in the process of migration. Harris and his colleagues (1975) examining the frequency distribution of non-metric tooth variants in the Pacific, suggested that, generally, there was a dichotomous variation; one group consisting of populations in Australia, New Guinea, Tasmania and the neighbouring island area, is characterized by large teeth, and primitive molar crown features, and another group which covered the rest of South Pacific areas (i.e. Micronesia, Polynesia and some outlying Polynesians), where populations exhibited smaller teeth and a reduced molar cusp. Similar arguments emerged for non-metric tooth crown morphology and tooth size in the Cook Islands (Kawamoto, 1989; Yamada et al., 1988). In these studies, two major clusters were suggested; a cluster consisting of Samoa, Fiji and Australian aborigines, and another cluster that included Papua New Guinea with the Philippines, Palau, Kiribati and Cook Islands. Cluster analysis and multi-dimensional scaling based on premolar features gave similar results as the previous reports based on the crown morphology of different teeth. This indicated that the lower premolars had characters of Sundadonty and Sinodonty in themselves.

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Morphological traits on the Dentino-Enamel Junction of Lower Deciduous Molar Series

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INTRODUCTION

The morphologies of the outer enamel surface (OES) of teeth are basically determined by the form of the dentino-enamel junction (DEJ) and the thickness of the enamel substance covering the DEJ. Although several morphological reports on the OES of deciduous molars (Hanihara, 1956a, 1956b; Jørgensen, 1956; Morito, 1977; Ozumi, 1960) and a few reports on the DEJ of permanent teeth (Korenhof, 1960; Sakai et al., 1965, 1967a, 1967b, 1969; Sakai and Hanamura, 1971, 1973a) have been published, little information about the DEJ of deciduous molars has been obtained, except for the upper second deciduous molar (Sasaki, 1997).

Morphological traits on the OES of deciduous molars have been described by dental anatomists and anthropologists (Hanihara, 1956a; Jørgensen, 1956). Although deciduous molars have particular characters derived from the buccal cingulum, they are comparable with characters in permanent molars, because both molars belong to a primary dentition. When the frequencies of enamel surface characters were compared between the second deciduous molar and the first permanent molar, primitive characters were found to be retained more often in the former than in the latter (Hanihara, 1956a; Korenhof, 1960; Sakai and Hanamura, 1973a,b). In another aspect, morphological characters in the DEJ show more primitive conditions than in the OES. One can speculate from these studies that the DEJ of deciduous dentition might reflect the most primitive condition among combinations of the DEJ and OES of deciduous molars and permanent molars. Sasaki (1997) studied the DEJ morphology of upper deciduous molars to throw light on this problem and demonstrated that the primitive characters were most frequently found in the DEJ of deciduous molars.

The purpose of this study is to describe the characters derived from cingulum and the transverse ridge on the OES and DEJ of lower first and second deciduous molars and to compare the frequencies with other members of the molar series.

MATERIALS AND METHODS

Materials were 48 mandibular deciduous first molars (dm_1) and 85 mandibular deciduous second molars (dm_2), with minimum attrition and negligible caries, collected from primary school children and patients in dental clinics. Developmental grades and occurrence of the buccal cingulum, buccal marginal ridges (BMR: mesial and distal), protostylid, middle trigonid crest (MTC) and distal trigonid crest (DTC) on OES of the

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deciduous molars were observed under the microscope at 10 to 15 times magnification. Impressions of the OES were obtained using Silicone rubber impression material (Dow Coning Co. Ltd., Injection Type) and casts were made using Epoxy-resin materials.

Enamel substance of the original tooth was then decalcified by 5.0% formic acid in 2 or 3 days. The exposed DEJ of the tooth was observed directly under the microscope, and the frequency and the grade of development of the four traits were examined. Plastic casts of the DEJ were also obtained to preserve its original form.

The degree of expression of the traits was scored according to three categories. Frequency of appearance was scored with a three-grade scale, (-) not observed, (+) observable but not developed, and (++) observable and developed. The scoring method was based on Sakai and Hanamura (1973a), although an extremely developed (+++) type in their study was included in the (++) type in this study, because it was difficult to distinguish between these two types. The followings are descriptions of observable grades in scoring:

Buccal cingulum:

(++) Protrusion is distinct, showing shelf-like form.

(+) Protrusion is weak, but it is still distinct from the whole buccal surface.

Buccal marginal ridges (BMR: mesial and distal):

(++) The ridge is distinct from the buccal surface over the 2/3 of the crown height.

(+) The ridge is weak and its length is under 2/3 of the crown height.

Protostylid:

(++) Protrusion is distinct, and the tip directing occlusally is demarcated like a tip of a pen.

(+) The tip and its border are not distinct, but the distal border is distinguished from buccal surface.

MTC and DTC:

(++) The crest is distinct, and it is continuous and well-developed.

(+) The crest is observable, but it is interrupted by a groove.

RESULTS

Buccal Surface

The buccal cingulum on the OES and DEJ (Figs. 1, 2 and 3) in the lower deciduous molars was not developed compared with that on the OES of upper deciduous molars in which a shelf-like protrusion was occasionally observed. At the DEJ, the combined frequency of (+) and (++) was 77.1% on dm_1 and 66.6% on dm_2. The frequency of the buccal cingulum on the OES of dm_1 was not significantly different from that on the DEJ. The protrusion of the buccal cingulum in the OES of dm_1 was obscure without clear distinction between cingulum and whole buccal surface. This protrusion was frequently distinct in the OES of dm_1, in which the trait was observed as a continuity of the molar tubercle.

There are two kinds of BMR, i.e., mesial and distal ones (Figs. 1, 2 and 4) . They arise from the mesial and distal angle of the cingulum respectively, and there was a clear distinction between the ridges and the whole buccal surface. The mesial BMR terminated at the mesial occlusal angle of the protoconid. The pattern of the distal one was obscure,

but it extended to the tip of hypoconid in dm_1 and of the hypoconulid in dm_2. For the mesial BMR, the total of (+) and (++) categories was highest in dm_1 (68.7%), followed by dm_2 (60.2%) in DEJ. The frequency in the OES was lower than that of the DEJ, but 50.1% of dm_1 and 18.1% of dm_2 showed a positive expression.

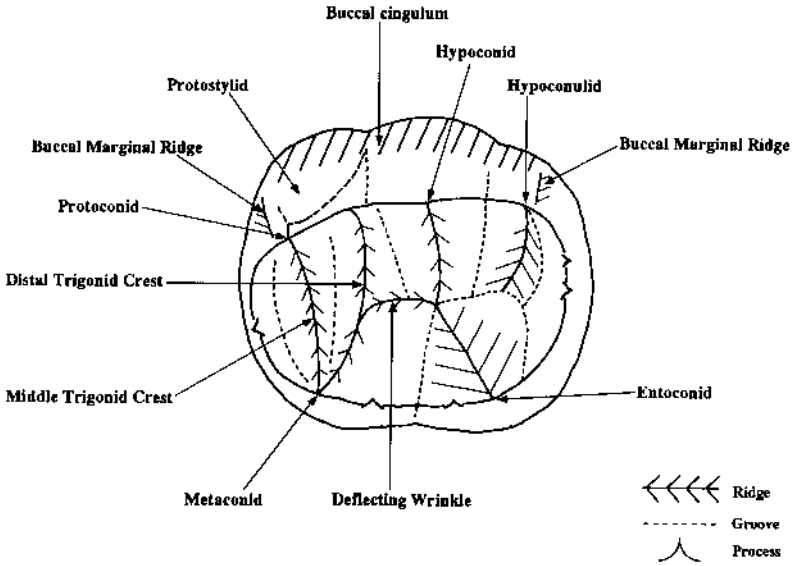


Fig. 1. Schematic representation of morphological characters on the DEJ of the lower right second deciduous molar.

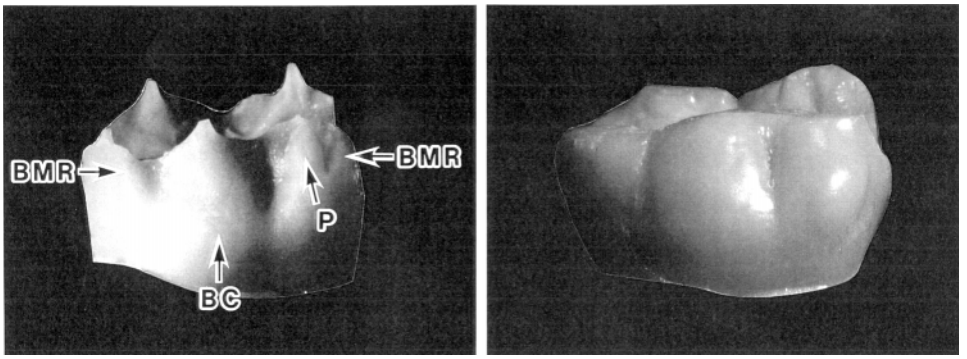


Fig. 2. The arrows point to the buccal cingulum (BC), buccal marginal ridge (BMR), and protostylid (P) on the DEJ of the lower right second deciduous molar. End; Entoconid. The photograph on the right is the OES of the corresponding tooth.

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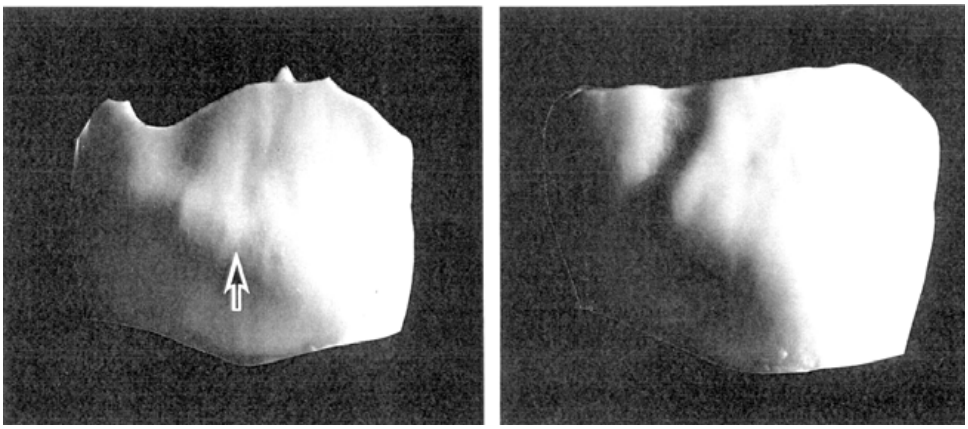


Fig. 3. The arrow points to the buccal cingulum on the DEJ of the lower right first deciduous molar. The photograph on the right is the OES of the corresponding tooth.

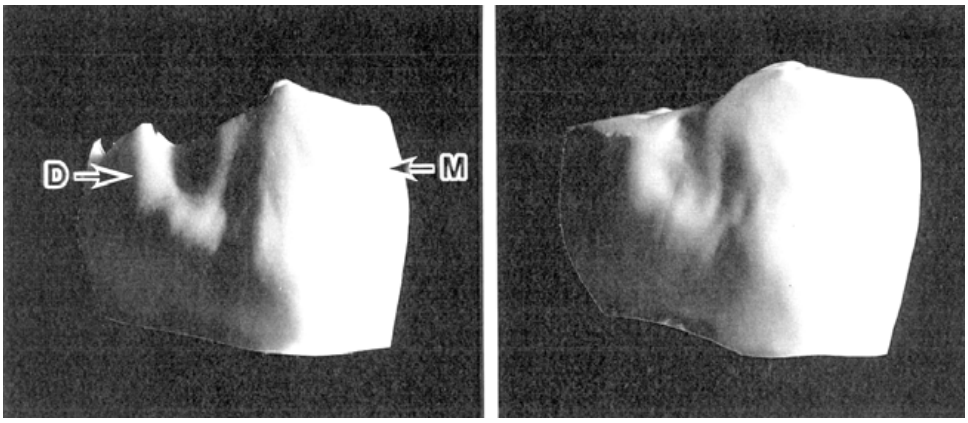


Fig. 4. The arrows point to the mesial (M) and distal (D) buccal marginal ridge on the DEJ of the lower right first deciduous molar. The photograph on the right is the OES of the corresponding tooth.

The development of the distal BMR in the DEJ of dm_1 was weak (20.9%) and of the dm_2 it was more frequent (62.0%), because the tooth itself had an underdeveloped distal portion. On the other hand, it was developed in the DEJ of dm_2 parallel with the development of the talonid of this tooth. This trait almost disappeared in the OES of deciduous molars.

The protostylid (Figs. 1 and 2) was sometimes observed on the mesiobuccal surface of the lower deciduous and permanent molars. It was developed occlusally from the mesial portion of cingulum as a pyramid-like or pen-shaped protrusion on buccal surface of protoconid. The protostylid was distinguished from the whole buccal surface by a groove or pit. The distal border of this trait was demarcated by a groove branched from the buccal groove that lies between the protoconid and hypoconid. The mesial edge was bordered by a groove formed by development of the mesial BMR. The protostylid was

most frequently found in the DEJ of dm_2 (69.5%), while there was almost no trace in dm_1. The frequency of the protostylid in the OES was lower than that of the DEJ, while the total of (+) and (++) categories was 0.0% on dm_1 and 39.0% on dm_2.

Table 1 shows the differences of appearance of characters between the DEJ and OES, the statistically significant ones denoted by asterisks. In dm_1, only one significant difference at the 5% probability level was seen in the distal BMR. In dm_2, however, four characters showed significant differences at the 1% level. The fact that more traits on the DEJ of dm_2 were not observed on the OES suggested that they might be masked by thicker enamel substance compared with dm_1.

Table 1. Frequency of the cingulum derived characters on buccal surface of the DEJ and OES.

	Buccal cingulum		Buccal marginal ridge				Protostylid	
			mesial		distal			
	DEJ	OES	DEJ	OES	DEJ	OES	DEJ	OES
dm_1	%(n=48)	%(n=48)	%(n=48)	%(n=48)	%(n=48)	%(n=48)	%(n=48)	%(n=48)
++	18.8 (9)	20.8(10)	20.8(10)	18.8(9)	4.2(2)	0.0(0)	0.0(0)	0.0(0)
+	58.3(28)	47.9(23)	47.9(23)	31.3(15)	16.7(8)	4.2(2)	0.0(0)	0.0(0)
-	22.9(11)	31.3(15)	31.3(15)	50.0(24)	79.2(38) *	95.8(46)	100(48)	100(48)
dm_2	%(n=84)	%(n=84)	%(n=83)	%(n=83)	%(n=79)	%(n=79)	%(n=82)	%(n=82)
++	20.2(17)	0.0(0)	28.9(24)	1.2(1)	25.3(20)	0.0(0)	18.3(15)	15.8(13)
+	46.4(39)	1.2(1)	31.3(26)	16.9(14)	36.7(29)	0.0(0)	51.2(42)	23.2(19)
-	33.3(28) **	98.8(83)	39.8(33) **	81.9(68)	38.0(30) **	100(79)	30.5(25) **	61.0(50)

Asterisks denote significant difference of the frequency of negatives between the DEJ and OES within a character. ** P<0.01;* P<0.05

Figure 8 is a graphic representation of the frequencies of the buccal traits on the OES and DEJ in the deciduous and permanent molar series, denoted by solid (dm_1), half-toned (dm_2) and open (M_1 and M_2) circles. Comparisons between the OES and the DEJ showed that the frequency of each trait was almost higher in the DEJ. The frequency of appearance of the buccal cingulum and mesial BMR decreased gradually from dm_1 to M_2. The frequencies of the distal BMR and protostylid were significantly higher in dm_2 than in dm_1 except for the OES of distal BMR. On the OES, the frequencies of buccal cingulum and BMR in M_1 and M_2 were relatively low, but the frequency of protostylid on the OES was not different from the DEJ except for dm_2.

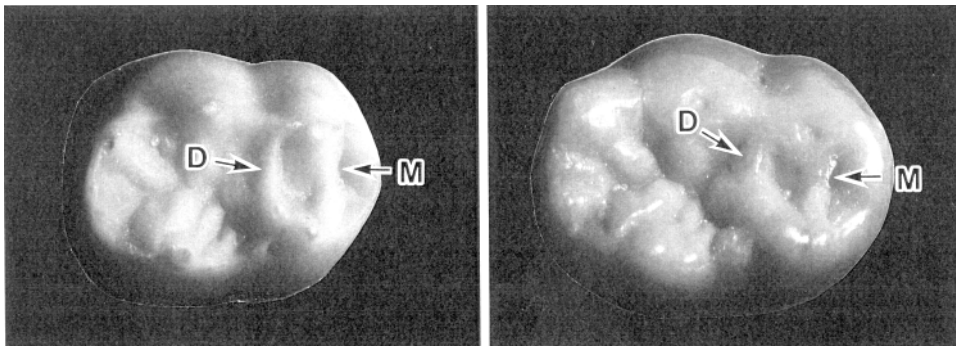


Fig. 5. The arrows point to the middle trigonid crest (M) and distal trigonid crest (D) on DEJ (left) of the lower left second deciduous molar. The photograph on the right is the OES of the corresponding tooth.

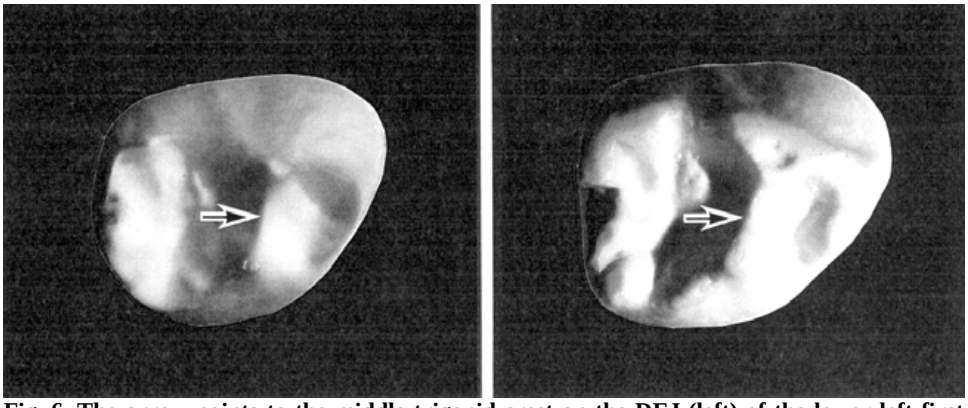


Fig. 6. The arrow points to the middle trigonid crest on the DEJ (left) of the lower left first deciduous molar. The photograph an the right is the OES of the corresponding tooth.

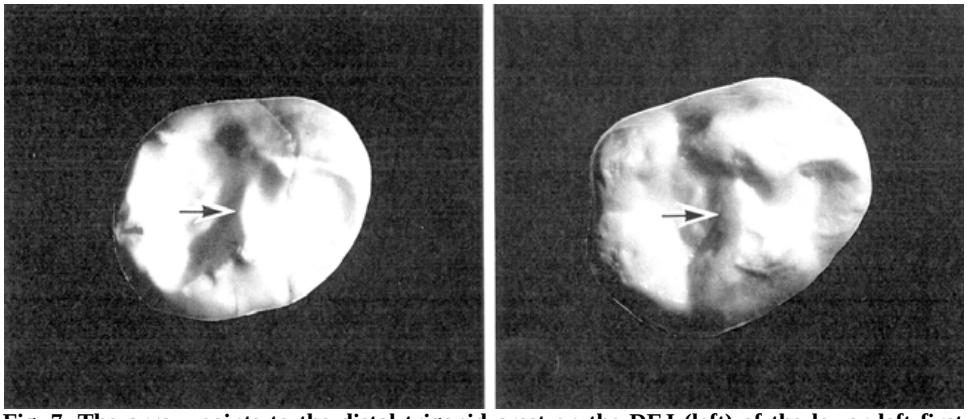


Fig. 7. The arrow points to the distal trigonid crest on the DEJ (left) of the lower left first deciduous molar. The photograph an the right is the OES of the corresponding tooth.

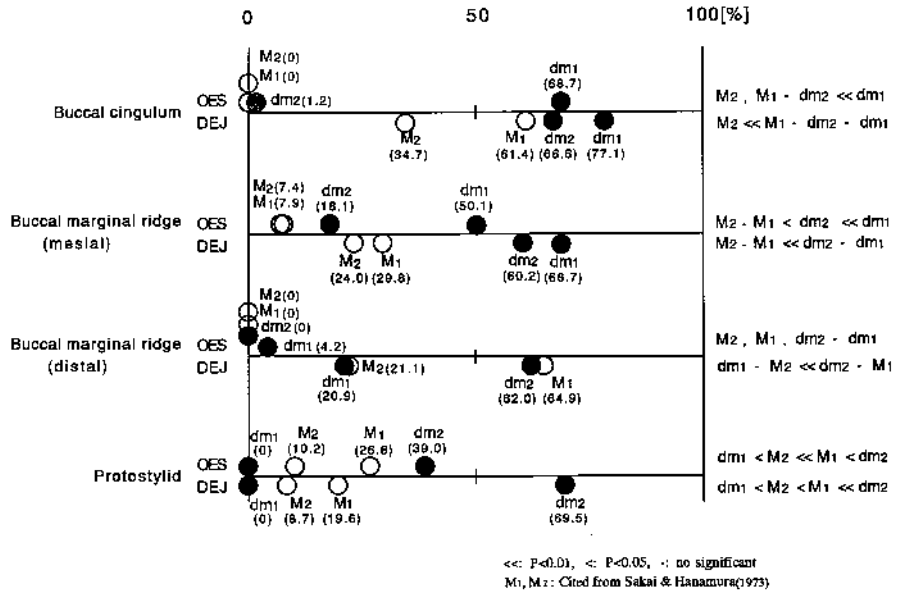


Fig. 8. Schema frequencies of appearance of traits derived from buccal cingulum in deciduous lower molar series.

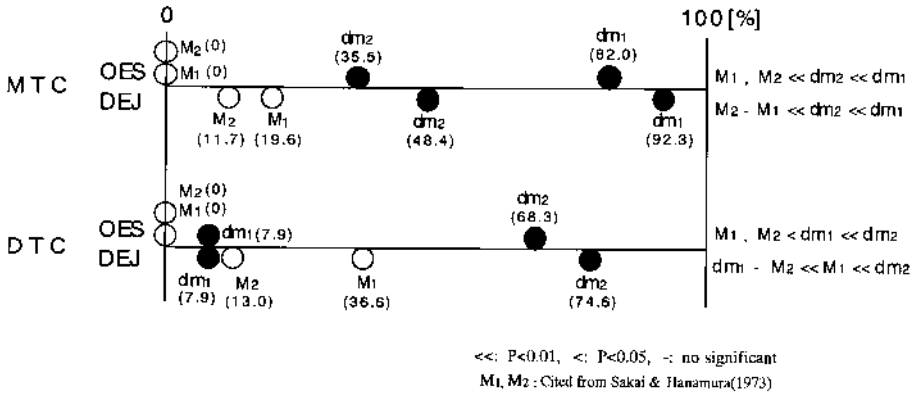


Fig. 9. Schema frequencies of appearance of MTC and DTC in deciduous lower molar series.

Occlusal Surface

Ridges connecting the protoconid (mesiobuccal cusp) and metaconid (mesiolingual cusp) were collectively called transverse ridges, MTC and DTC being two representative types. The MTC connects both cusps directly with a straight line. The portion of the DTC

is just distal to it, from the tip of metaconid to the buccal marginal ridge of the distal portion of the protoconid with a slightly curved line (Figs. 1, 5, 6 and 7). On the DEJ, these crests were observed clearly and sharply but they were narrower than on the OES.

Table 2 shows the frequency of appearance of MTC and DTC in dm_1 and dm_2. In dm_1, a well-developed MTC was most frequently observed in the DEJ, being evident in 71.8% of the surfaces investigated. About half of the samples showed a well-developed MTC on the OES (53.8%). Only 7.7% of dm_1 failed to show a MTC in the DEJ and 18.0% in the OES. On the other hand, a well-developed DTC was not found on either the DEJ or OES of dm_1 and 92.1% of teeth did not show the trait at all.

Table 2. Frequency of two crests on occlusal surface of the DEJ and OES.

	Middle trigonid crest		Distal trigonid crest	
	DEJ	OES	DEJ	OES
dm_1	%(n=39)	%(n=39)	%(n=38)	%(n=38)
++	71.8(28)	53.8(21)	0.0(0)	0.0(0)
+	20.5(8)	28.2(11)	7.9(3)	7.9(3)
-	7.7(3)	18.0(7)	92.1(35)	92.1(35)
dm_2	%(n=62)	%(n=62)	%(n=63)	%(n=63)
++	14.5(9)	3.2(2)	28.6(18)	14.3(9)
+	33.9(21)	32.3(20)	46.0(29)	54.0(34)
-	51.6(32)	64.5(40)	25.4(16)	31.7(20)

In dm_2, the MTC was not found as frequently as in dm_1. Half or more teeth did not have the trait both in the DEJ and OES. A well-developed or observable DTC was found more often than MTC in both DEJ and OES. The frequencies of well-developed expressions of both traits were greater in the DEJ than in the OES. One sample in the OES and two samples in the DEJ had both MTC and DTC.

Figure 9 is a graphic representation of the frequencies of MTC and DTC on the OES and DEJ in the deciduous and permanent molar series. Data for the permanent molar were taken from Sakai and Hanamura (1973a). The MTC was found most frequently in dm_1, secondly in dm_2 and lastly in M_2 both on DEJ and on OES. The differences between dm_1 and dm_2, and between dm_2 and permanent molars, were significant at $p<0.01$. The DTC was found most frequently in dm_2 on the DEJ and OES, followed by DEJ of M_1. On the OES, differences in frequency between dm_2 and dm_1 were significant at $p < 0.01$, and between dm_1 and permanent molars at $p<0.05$. On the DEJ, differences in frequency between dm_2 and M_1, and between M_1 and M_2 or dm_1 were significant at $p<0.01$. Although not statistically significant, frequencies of the MTC and DTC were higher in the DEJ than the OES in the deciduous and permanent molar series.

DISCUSSION

The cingulum is a ledge-like rim, either partially or completely surrounding the base of the crown of a tooth (Szaley and Delson, 1979). Various kinds of cusps and tubercles in fossil primates and extant mammals are considered to be derived from a part of cingulum

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(Sakai, 1974). The buccal cingulum and BMR are developed in the lower molar buccal surface of *Australopithecus* (Weidenreich, 1937), *Theilhardina* (Korenhof, 1960), *Propriopithecus* (Frisch, 1965; Szalay and Delson, 1979), *Limnopithecus* (Frisch, 1965), *Pliopithecus* (Szalay and Delson, 1979), *Sinanthropus* (Weidenreich, 1937), and *Gorilla gorilla* (Korenhof, 1960; Weidenreich, 1937). The existence of the trait was also reported partly on the molars of *Proconsul* and *Dryopithecus* (Sakai and Hanamura, 1973a). In addition, a well-developed cingulum in mesiobuccal portion and protostylid was observed in *Australopithecus* (Dahlberg, 1950; Robinson, 1956), *Meganthropus* (Dahlberg, 1950; Weidenreich, 1945), *Sinanthropus* (Dahlberg, 1950; Weidenreich, 1937) and *Proconsul* (Dahlberg, 1950). These characters were regarded as specialized features of the cingulum. In modern humans, these buccal characters were often observed on enamel surface of permanent and deciduous molars, and more frequently observed on its DEJ. They were reported as phylogenetically primitive characters derived from cingulum characters (Sakai and Hanamura, 1973a,b; Sasaki, 1997). Cingulum derived features were not clearly observed on the OES of the deciduous lower molars because of overall swelling of the buccal surface. After the removal of the enamel substance, however, they were able to be observed as protrusions surrounding the neck of the tooth, as fine ridges upward from the mesial and distal portion of the cingulum and as a triangular protrusion from the part of the cingulum to the tip of protoconid. Sakai and Hanamura (1973a) reported that all of the features of folds, processes and ridges on the buccal surface of the DEJ of the first permanent molar were cingulum derived characters. These features were also observed in deciduous molars in this study. This indicates a morphological continuity between permanent and deciduous molars.

Two kinds of comparisons were possible in the present study. One was between the DEJ and OES, and the other was between DEJs of the deciduous molars and permanent molars. Comparisons between the DEJ and OES of the same tooth indicated that frequencies of characters on the DEJ were higher than on the OES except for the case of the protostylid on permanent molars. This exception is attributed to the fact that the character was exaggerated by enamel thickness in these teeth. It was noted that the significant differences in frequencies of occurrence between the DEJ and OES were greater in dm_2 than in dm_1. There is a report that the enamel substance in dm_2 is thicker than that in dm_1 (Nozaka, 1988a). This suggests that thicker enamel in dm_2 may mask the minor characters on the DEJ.

Comparisons of the frequencies of occurrence of characters between the DEJs of dm_1 and dm_2, indicated no significant differences of occurrence in buccal cingulum and mesial BMR, but there was a tendency for the frequency to decrease gradually from dm_1 to M_2 on the OES and DEJ. On the other hand, distal BMR and protostylid were observed significantly more often in dm_2 than in dm_1 except distal BMR on the OES. The development of distal BMR or protostylid might parallel the development of talonid, but there was no significant at the distal BMR on the OES, suggesting that the enamel substance in dm_2, M_1 and M_2 is thicker than that in dm_1. Protostylid was the trait that appeared particularly in dm_2 followed by M_1. According to Frisch (1965), Carabelli's cusp in *Sivapithecus* and protostylid in *Dryopithecus fontani* are similar structures derived from the cingulum, but the development of the latter is weaker than the former.

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Carabelli's cusp is also observed more often in dm^2 and M^1, but not so frequently in dm^1 of the upper dentition. No frequency of protostylid in dm_1 is a parallel phenomenon with Carabelli's cusp, that may have some relation to a differentiation of the talonid.

On the occlusal surface, there is a transverse ridge that runs between the protoconid and metaconid, and the ridge is classified into MTC and DTC. Remane (1960) described two ridges connecting the protoconid and metaconid in the lower first molar of *Gorilla gorilla*, i.e., *vordere Trigonid-Hauptleiste* mesially and *hintere Trigonid-Hauptleiste* distally. Weidenreich (1937) described in *Sinanthropus*, that the DTC emerges from the buccal edge exactly at the indentation between the end of the distal ridge descending from the tip of the protoconid and that of the corresponding ridge of the hypoconid; it meets the metaconid just at its tip, and the mesial trigonid crest emerges from the end of the mesial ridge descending from the tip of the protoconid and meets the paraconid also on its mesial side. In the lower deciduous molars of *Sangiran*, Korenhof (1982) grouped ridges between the protoconid and metaconid into three, i.e., distal crest most distally, mesial marginal ridge most mesially, and mesial crest in the middle. It seems that the DTC (Weidenreich, 1937; Korefhof, 1982) and distal crest (Korenhof, 1982) correspond to *hintere Trigonid-Hauptleiste* (Remane, 1960), while mesial trigonid crest (Weidenreich, 1937) and mesial marginal ridge (Korenhof, 1982) correspond to *vordere Trigonidrandleiste* (Remane, 1960), and mesial trigonoid crest (Korenhof, 1982) corresponds to *vordere Trigonid-Hauptleiste* (Remane, 1960). We consider that *vordere Trigonid-Hauptleiste* is MTC, and that *hintere Trigonid-Hauptleiste* is DTC. Sakai and Hanamura (1973a) described five patterns of transverse ridge formed by the MTC and DTC. Type I was a singular MTC type, Types II and III were DTC-developed types, Type IV was DTC and trace MTC type, and Type V was developed types of both MTC and DTC.

Although MTC and DTC are frequently observed on the OES of deciduous lower molars, especially dm_2, they are variably interrupted by a central groove. On the other hand, they are found as narrow, sharp and demarcated ridges in the DEJ, and their running or furcation pattern can be easily observed. There was no significant difference in their frequency between the OES and DEJ of dm_2. Although, the well-developed type (++) was more frequently observed in the DEJ. In other words the morphology on DEJ was more primitive than on OES. Moreover, the frequency of appearance of the MTC on the OES and DEJ decreased gradually from dm_1 to M_2, and that of the DTC was highest at dm_2. This indicates that lower deciduous molars retain phylogenetically primitive characters, the MTC and the DTC, more than the lower permanent molars, and that dm_1 was not progressive enough to have developed DTC.

As is shown in the result for dm_1, the development of MTC was more conspicuous than DTC especially in the DEJ. On the other hand, DTC was more developed in dm_2 and M_1 than MTC. This suggested that MTC and DTC are not characters with the same origin, but ontogenetically and phylogenetically different. The dm_1, dm_2, M_1, and M_2 represent a series of molars in the primary dentition. MTC was developed in the anterior portion of this molar series, while DTC was developed in posterior portion. This suggests that MTC basically connected the buccal and lingual cusps of premolars and also the two mesial cusps of molars, whereas DTC was derived distally from MTC accompanied with the development of distal cusps (talonid) in permanent molar series.

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CONCLUSION

The frequencies of the primitive dental crown characters were retained more in the DEJ of the deciduous molars. Among crown traits investigated, buccal cingulum, mesial BMR, and MTC were developed in the anterior portion of the primary molar series, while distal BMR, protostylid, and DTC were developed in posterior portion. It seems that the buccal cingulum, mesial BMR, and MTC were basic characters that are formed at an early stage ontogenetically and phylogenetically, whereas distal BMR, protostylid, and DTC are characters that were formed with development of talonid.

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Amelogenesis Imperfecta on a Deciduous Molar of *Coelodonta antiquitatis* (Blumenbach) (Mammalia, Perissodactyla, Rhinocerotidae) from Grotta di Fumane (Verona, Northern Italy): A Rare Case Report

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INTRODUCTION

In a cave, described as Grotta di Fumane (about 350 m asl) (Lessini Mountains, Verona, Northern Italy), in the aurignacian levels, in addition to the skeletal remains of several mammalian and bird species, a tooth, belonging to a Rhinocerotidae, was discovered. It is the first and only discovery of Rhinocerotidae remains in this site. The deposit, rich in levels - the oldest is referable to the Middle Palaeolithic and the most recent to the Aurignacian - shows evidences of intense human occupation. The remains are mainly the result of hunting (Cassoli and Tagliacozzo, 1994). A series of C^{14} dates place the aurignacian occupation between 37,000-35,000 and 32,000 years B.P. (Bartolomei et al., 1994). According to the authors, the introduction of the tooth into the cave could probably be due to an occasional human action.

Compared to the permanent teeth, the discovery of rhinoceros deciduous teeth in archaeo-palaeontological sites is very rare, moreover, very often they are recovered as single specimens. The tooth is preserved at the Museo Nazionale Preistorico Etnografico “Luigi Pigorini” in Rome (inv. RF 93).

MORPHOLOGICAL DESCRIPTION OF THE SPECIMEN FROM FUMANE

The tooth is a very worn upper deciduous molar - probably a second. On most of its surface there is a good quantity of MnO_2. The tooth was very likely lost during the life of the animal because of the eruption of the second upper premolar. Although it is damaged in the bucco-distal and linguo-distal portion, the dimensions of the specimen are drastically reduced (the length, along the buccal axis, is 27.5 mm), but the profile of the ectoloph shows the characteristic undulation.

The interior valley is connected to the *medifossetta* which is not completely closed. From the *norma pulparis*, the scarce development of the *bulbus pulparis* and the extremely reduced thickness of the enamel of the crown are evident. The morphological features of the specimen (Fig. 1), including not completely closed *medifossetta*, allow its attribution to *Coelodonta antiquitatis* (Blumenbach, 1799), the hypsodont Late Pleistocene “woolly rhino”. (The dental characteristics of the *Coelodonta antiquitatis* have been investigated by several authors [Gaudry, 1876; Pavlov, 1892; Schroeder, 1900; Passemard, 1924; Friant, 1957, 1963; Bouchud, 1966b; Borsuk-Bialynicka, 1973;

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Rensbergen, 1973; Guérin, 1980; Fortelius, 1979, 1981, 1982, 1985]. In the rest of the Italian peninsula, *C. antiquitatis* is recorded at Fadalto nel Polesine [Rovigo, Northern Italy] [Leonardi, 1947a, 1947b], at Opicina [Trieste, Northern Italy] [Leonardi, 1947a, 1947b; Bartolomei, Peretto and Sala, 1977], at Monte Circeo [Lazio, Central Italy] [Palmarelli and Palombo, 1981], in Terra d'Otranto [Apulia, Southern Italy] [Botti, 1890; Vaufrey, 1927], in the Grotta dei Pipistrelli [Matera, Southern Italy] [Flores, 1895; Guérin, 1980], at Ingarano [Gargano, Southern Italy] [Capasso Barbato et al., 1992; Billia et al., 1995; 1996].)



Fig. 1. *Coelodonta antiquitatis* (Blumenbach, 1799); Late Pleistocene; Fumane (Verona, Northern Italy); upper deciduous molar; *norma occluso-labialis* (max length 27.5 mm).

DENTAL ANOMALIES OF RHINOCEROSES

The case under study is particularly interesting because in the literature some anomalies of rhinoceroses have been reported, but they only refer to malpositionings, irregular eruptions, *hyperodontiae*, asymmetries and rotations.

Capellini (1894) reported, in a fossil rhinoceros, a malpositioning of a premolar associated with the retention of the corresponding deciduous and Vialli (1955) identified a case of rotation, malpositioning and morphological anomaly in an fourth upper premolar of a modern African rhinoceros *Rhinoceros simus cottoni* Lyd. (=*Ceratotherium simum cottoni* Lyd.) preserved at National History Museum in Milan. Chow (1961) reports a mandible of *C. antiquitatis* from the site of Siki (China) presenting pathological features and Groiss et al. (1981) describe the malpositioning of second, third and fourth upper premolars always in *C. antiquitatis*; still in a *C. antiquitatis*, Garutt (1990) has described a supernumerary fourth upper premolar and a third lower molar (hyperodontia) as possible atavism, while the same author (1992, 1994) shows a case of malpositioning, again in *C. antiquitatis*, of a fourth upper premolar due to its delayed eruption; such anomaly, according to the author, might be related to a juvenile fracture. Another malpositioning and malformation of a fourth upper premolar was reported for a *Diceros bicornis* (Garutt 1994). Hillman-Smith et al. (1986) describe a supernumerary third lower molar in a *C.*

simum, in a *C. antiquitatis* of Tatarsan and in a *Rhinoceros mercki* (=*Stephanorhinus kirchbergensis*) preserved at the Halle Museum (Germany).

AMELOGENESIS IMPERFECTA IN *COELODONTA ANTIQUITATIS* FROM FUMANE

As is well known, amelogenesis imperfecta is hereditary and affects both arcades of the deciduous and permanent teeth. It may be present in three forms: hypoplastic, hypomineralized (Weinmann, 1945), and hypomature (Witkop and Sauk, 1971); the hypoplastic form - the case specifically of interest here - presents a normal structure of the enamel, but the quantity of tissue is reduced. In this reduced matrix, minerals are deposited in normal quantity, therefore, abrasion reduces the thickness of the crown in a very short time; the crown is smooth and bright.

The hypothesis of a structural anomaly of the enamel produced by genetic factors referable to amelogenesis imperfecta of hypoplastic type is predicated on the macroscopic analysis of the specimen from Fumane that indicates extremely reduced dimensions in comparison with homologous specimens, it is made mainly of dentine, the whole surface is smooth and bright with reduced traces of thin and transparent enamel along the edges, inside the *medifossetta*, the distal *fossetta*, and the interior valley, the color is very close to that of amber and this anomaly affects both dentitions.

HISTOLOGICAL ANALYSES

In order to confirm the macroscopic diagnosis a histological examination of the specimen was undertaken. The specimen from Fumane has been observed under a binocular microscope and photographed from different views. Using fine diamond disks - in order not to damage the remain - two portions of dental tissues, one from the occluso-distal part (distal *fossetta*) in longitudinal section, and the other one from the disto-cervical part, were removed. From these thin sections were made by embedding them in a solid block of epoxy-resin Epo-thin (Buehler) and then sectioning them with a microtome until the desired thickness (110µ-80µ) was obtained. Each specimen was then polished with paper disks and powder (Micropolish B-0.05 µ gamma-alumina, Buehler).

During the operations, about 15% of sections was lost because of the fragility and porosity of the materials themselves. For the same reason, in only 40% of the cases was it possible to obtain samples where enamel and dentine were observable in the same plane.

The resulting thin sections were then examined and photographed through a microscope under transmitted polarized light (Laser-Scan ZEISS, Oberkochen-Germany) at the Faculty of Dentistry of Calvary Hospital in Rome.

RESULTS

On the occluso-distal section of the specimen from Fumane the presence of enamel and dentine can be observed; dentinal tubules are regular, branched, developing anastomosis and finishing with dead ends in the interglobular spaces and in the dentino-enamel

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junction (Fig. 2). The enamel presents a normal qualitative aspect (Fig. 3), but quantitatively it is thinner than in normal cases (Fig. 4). Also, crack on dentine with MnO_2 sediments is observed (Fig. 5).

On the surface of the distal-cervical sample, which is irregular, dentine and MnO_2 sediments are present, but enamel is absent (Fig. 6). It is evident that on the surface of the crown the enamel was worn because of an anomalous development.

The fact that the enamel-dentine junction is undulating and the trend of the dentinal tubules is regular (Figs. 2, 5, 6) excludes the possibility of dentinogenesis imperfecta; if this were the case the enamel-dentine junction would be straight and the trend of the dentinal tubules would be irregular.



Fig. 2. Occlusal-distal section, EDJ (20x).

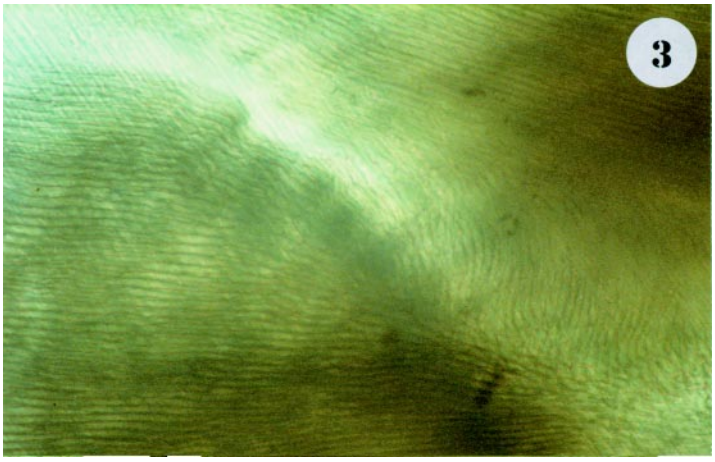


Fig. 3. Occlusal-distal section, enamel (40x).

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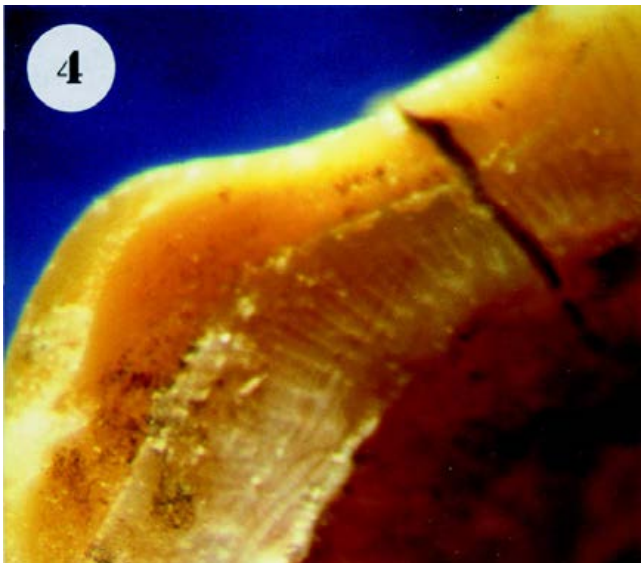


Fig. 4. Thickness of the enamel in the distal *fossetta.*

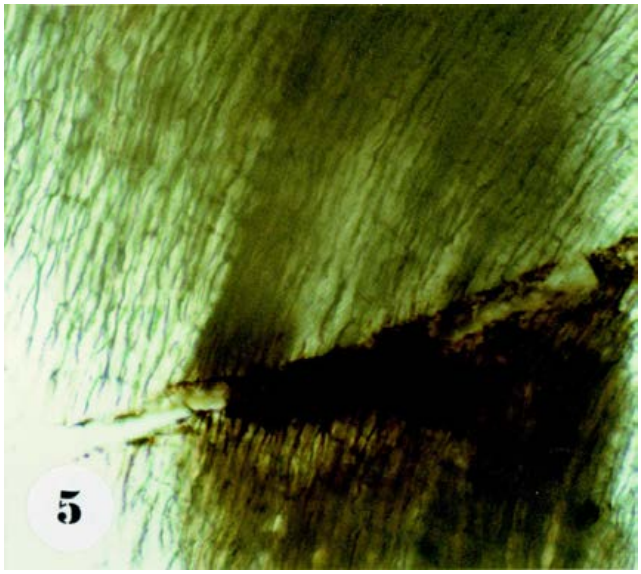


Fig. 5. Occlusal-distal section, dentine-crack (20x).

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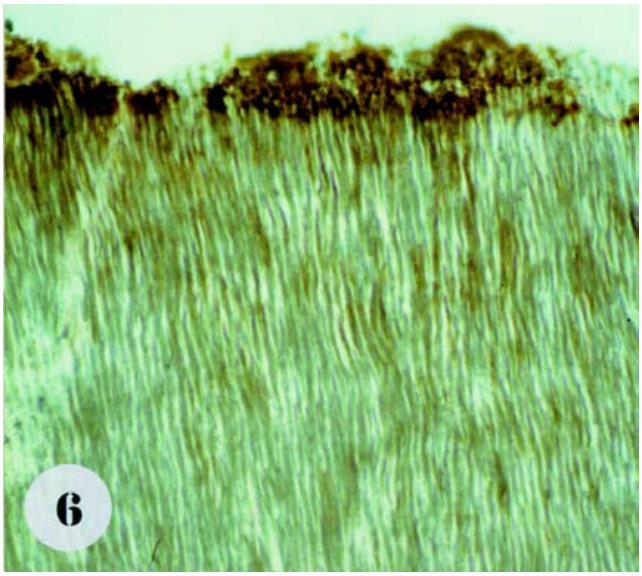


Fig. 6. Distal-cervical section, dentine (40x).

CONCLUSIONS

The condition observed on the tooth from Fumane supports the initial hypothesis of amelogenesis imperfecta of the hypoplastic type. The analyses performed are only quantitative. At the present time histochemical (specific coloration) and immuno-histochemical analyses, to evaluate the organic vs. non-organic components, are not appropriate for providing reliable data in the case of fossil remains. DNA analyses are not possible in this specific case because of the reduced dimensions of the remains and because they have been manipulated possibly contaminated by other DNA; furthermore, in these cases, it would be very difficult to find a whole molecule of DNA. In this case, it would be necessary to pulverize the tooth in order to take away from it a non-contaminated portion, a process that would require the destruction of this rare specimen.

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Timing of Permanent Tooth Eruption in London School Children

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INTRODUCTION

The timing of permanent tooth eruption in different populations provides data for comparison between ethnic groups. Moreover studies in the same ethnic group at different times provides evidence to evaluate the secular trend in growth and development. Eruption data are also important clinically, providing norms for dental development.

There have been no new data in Britain for eruption times since Clements et al., (1953). The aims of this study were to derive modern data from a cross-sectional study of a white caucasian population within the U.K., to consider whether the earlier secular trend in eruption times (Brook, 1973) has continued and to compare the findings with other ethnic groups.

METHOD

White Caucasian schoolchildren aged 5 - 15 years of age consisting of 1,500 boys and 1,423 girls (total = 2,923) participated in this study. The participating schools were chosen at random from all those in the Borough of Havering in East London and timed to cause the least disturbance by including the annual school dental inspections. The sample was socially and geographically representative of the area.

All examinations were undertaken by one investigator (J.M.S). Each child was examined under standard conditions on a medical couch in the supine position with lighting from an anglepoise lamp and an illuminated dental mirror. The criterion for eruption was the presence in the mouth of any part of the crown. Kärber's method of calculating the 50% eruption point, which is a measure of central tendency comparable to the mean, was chosen for the statistical analysis (Hayes and Mantel, 1958, Brook and Barker, 1972).

RESULTS

The results are presented in Tables 1, 2, 3 and 4.

Table 1. 50% eruption points (yrs.) in present study.

Maxillary														
	Right							Left						
	M2	M1	Pm2	Pm1	C	I2	I1	I1	I2	C	Pm1	Pm2	M1	M2
F	12.24	6.38	11.52	10.60	11.10	8.02	7.07	7.06	8.01	11.06	10.94	11.41	6.46	12.20
M	12.51	6.28	11.71	10.82	11.71	8.56	7.19	7.16	8.60	11.67	10.80	11,82	6.37	12.54
Mandibular														
F	11.67	6.31	11.58	10.45	9.94	7.20	6.06	6.15	7.24	9.81	10.45	11.62	6.27	11.58
M	12.07	6.25	11.98	10.95	10.79	7.47	6.17	6.26	7.47	10.71	10.89	11.96	6.32	12.06

Table 2. Standard deviations for the 50% eruption data (Combined sexes; in yrs.)

Maxillary														
	Right							Left						
	M2	M1	Pm2	Pm1	C	I2	I1	I1	I2	C	Pm1	Pm2	M1	M2
Mean	12.37	6.34	11.61	10.70	11.41	8.29	7.14	7.11	8.29	11.42	10.96	11.62	6.41	12.37
S.D.	1.32	0.61	1.50	1.39	1.37	0.83	0.57	0.55	0.81	1.30	1.31	1.48	0.55	1.23
Mandibular														
Mean	11.86	6.28	11.78	10.70	10.37	7.34	6.13	6.21	7.36	10.27	10.67	11.79	6.30	11.83
S.D.	1.27	0.51	1.35	1.30	1.15	0.64	0.55	0.58	0.66	1.17	1.33	1.45	0.56	1.25

As expected the standard deviations of the 50% eruption points were smaller for the earlier erupting teeth.

Table 3. 50% eruption points (yrs.) from this study compared to Clements et al data (1953).

	CURRENT STUDY (Kärber's method)		CLEMENTS et al (1953) (Probit Analysis)	
Maxillary				
Tooth	Boys	Girls	Boys	Girls
I1	7.18	7.07	7.01	6.62
I2	8.58	8.02	8.18	7.82
C	11.69	11.08	11.46	10.67
PM1	10.85	10.55	10.41	9.79
PM2	11.77	11.47	11.52	11.06
M1	6.33	6.42	6.11	5.94
M2	12.53	12.22	11.97	11.50
Mandibular				
I1	6.22	6.11	6.08	5.77
I2	7.47	7.22	7.30	7.01
C	10.75	9.88	10.51	9.41
PM1	10.97	10.45	11.35	10.53
PM2	11.97	11.60	12.32	11.64
M1	6.29	6.29	6.14	5.84
M2	12.07	11.63	11.41	11.18

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Table 4. Comparison of results from different ethnic groups

	Current Study (Kärber's method)		Blankenstein et al. (Probit Analysis) (1990)		Virtanen et al.* (Probit Analysis) (1994)	
Maxillary						
Tooth	Boys	Girls	Boys	Girls	Boys	Girls
I1	7.18	7.07	6.97	6.84	7.4	7.2
I2	8.58	8.02	8.00	7.73	8.5	8.1
M1	6.33	6.42	6.07	6.06	6.9	6.8
Mandibular						
I1	6.22	6.11	5.86	5.80	6.6	6.4
I2	7.47	7.22	6.98	6.74	7.7	7.4
M1	6.29	6.29	5.78	5.71	6.8	6.7

Kärber's method gives estimates up to 0.05 years later than Probit analysis (Heidman 1986). * Based on longitudinal data.

DISCUSSION

To judge any secular trend, the data from the study undertaken by Clements et al. (1953) were used for comparison. For international comparison, data from the research undertaken by Blankenstein and colleagues. (1990) on 1024 South African Black children were used along with data from the study by Virtanen et al. (1994) in Finland of 911 children. These two sets of data have been chosen to give comparison between two separate white Caucasian populations and a comparison with a South African black population, which generally has shown to have earlier dental development than European children (Blankenstein et al. 1989).

Clements et al. (1953), Blankenstein et al. (1990) and Virtanen et al. (1994) all used Probit analysis and are therefore directly comparable. The current data were analysed using the Kärber method and therefore some justification is necessary for the comparison. It was not possible to obtain all the data necessary to transform the current data into Probit form, but Heidman (1986) compared the two methods. It was found that the Kärber method produced slightly later estimates for eruption compared with Probit but none of the results differed more than 0.05 years (less than one month) from the Probit analysed data. Taking this into consideration, the international and national comparisons remain much the same as the differences are large enough not to be affected by altering the Kärber results by 0.05 years.

Compared to Clements et al. (1953) (Table 3), the current data from Havering, London have produced slightly later eruption times for all teeth except the mandibular premolars for both sexes. This could reflect a cessation of the secular trend towards earlier eruption times. It was noted by Bernhard and Glockler (1995) that there was no evidence of a secular trend within their data. However, the greatest difference between the results of the current study and Clements et al. (1953) is seen between the second molars of both jaws and the maxillary premolars. The main reason for the later eruption times is thought to be the reduction in the early loss of deciduous molars and first permanent molars due to caries. A marked decline in dental caries in children occurred in the U.K. between the time when the data of Clements et al. (1953) and the current results were gathered

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(Downer, 1991). Early loss of deciduous teeth accelerates the eruption of the successors. This was shown by Loevy (1989), in a study comparing the eruption time of premolars in patients having one tooth extracted but not its antimere. Out of 33 patients, 26 had their successor on the extracted side erupt earlier by between 4 months and two and a half years.

Comparing the international data (Table 4) it can be seen that the eruption times for black Africans are markedly earlier than for European white caucasians. However, the eruption times from the current sample show earlier eruption times for all teeth compared to the Finnish results. As the Finnish data are based on a longitudinal study with annual examinations their eruption times should be reduced by approximately 0.5 years for comparison with cross-sectional studies such as current work. There is then little difference between the Finnish data and the current study.

For all sets of data the girls permanent teeth erupt significantly earlier than boys (Hagg and Taranger, 1986, Blankenstein et al., 1990, Virtanen et al., 1994 and Larmas et al., 1995). There was an exception in the current study with some first molars erupting first in boys (Table 1).

There were no significant differences at the 95% confidence interval when comparing teeth on the left and right sides of each jaw within each gender. However, there were some possible trends to asymmetry. In the maxilla boys displayed the greatest asymmetry of second premolars and first molars and girls of first and second premolars. In the mandible girls and boys had the greatest asymmetry with the central incisors and the canines. There was a trend for the maxillary teeth to erupt earlier for males on the right and on the left for females. Generally, the mandibular teeth erupted before the maxillary teeth.

The eruption order of the teeth within the current study was the same as that noted by Clements et al., (1953) except that the present data show the mandibular second premolar erupting before the second molar. As with the Finnish and South African data, the mandibular tooth erupted before the corresponding tooth in the maxillary arch (Blankenstein et al., 1990, Pahkala et al., 1991 and Virtanen et al., 1994).

CONCLUSIONS

This study has shown that eruption times have not changed for the white caucasian population in the U.K. since the study of Clements et al., (1953). This may indicate a slowing of the secular trend but a more significant factor may be the greater retention of deciduous teeth and first permanent molars with a decline in caries.

In general the frequently reported earlier eruption of permanent teeth in girls than boys and in the mandible before the maxilla was the norm here. Comparison to the South African sample of Blankenstein et al. (1990) showed continuing evidence that African Black populations have earlier development of the permanent dentition than white Caucasians.

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Morphological Studies on Human Premolar Crowns

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INTRODUCTION

Butler (1937, 1939) in his study of cenozoic mammals applied the field concept of Huxley and De Beer (1934) to the dentition and postulated that a morphogenetic field exists as a progressive gradation from anterior incisor to posterior molar. It is differentiated into regions corresponding to the incisor, canine and molar districts of the dentition. Premolars of these mammals fall within the molarization field. He pointed out that almost every detail on a tooth could be compared with corresponding details on adjacent teeth. Only a common morphogenetic cause acting on more than one tooth germ could create such similarity.

Scott and Turner (1997) recently reviewed the literature on morphogenetic gradients and highlighted problems that have been expressed regarding the field concept of Butler (1937, 1939). They also discuss the clone model of Osborn (1978) which also sets out to explain the development of tooth classes and gradients. They conclude that there are inconsistencies in both models but point out that they provide a useful insight into the nature of gradients in the dentition.

Dahlberg (1945) adapted Butler's field concept to the human dentition and proposed the existence of four and not three dental fields. He introduced the idea that premolars and molars belong to two different tooth districts. Scott and Turner (1997) reviewed evidence in support of this proposal. They conclude that as far as crown form and size are concerned, lower premolars could belong to a molar field/clone, but the case for upper premolars is not so obvious.

Primary and permanent molars have similar crown features and there is little doubt that they belong to the same morphogenetic field/clone. In man and most other mammals premolars differ markedly from their primary molar predecessors and uncertainty has been experienced regarding their inclusion in the molar field. Yet the degree of difference between the two series is subject to evolutionary change, and in some mammals it has been largely eliminated and premolars have become molariform (Butler 1937, 1952 a, b, 1995). Premolars could be regarded as potential molars that have deviated in their development from the molar path to various degrees. Frequently the deviation takes the form of inhibition of the development of the distal part of the tooth.

The earliest placental mammals had four, or even five premolars, but no living primate has more than three, and in the catarrhines (Old World monkeys, apes and man) it is reduced to two. Premolar crowns tend to vary considerably in morphology. The premolars of catarrhines most commonly have two cusps, but the first lower premolar is usually modified for honing the upper canine. Observations on human lower premolars have been

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made by a number of workers including Kraus and Furr (1953), Ludwig (1957), Turner and colleagues (1991) and Scott and Turner (1997). They have described cusp number, ridge variability, and position of buccal to lingual cusp and other characteristics such as frequencies of a trait in various population groups.

A feature of studies on human premolars is the tendency to avoid using the Osborn (1895) terminology in recording observations. This does not seem to be the case in descriptions of primate premolars (Swindler, 1976). Gregory (1922) refers to the two major cusps as buccal and lingual moities. Weidenreich (1937), Robinson (1956) and Tobias (1967, 1991) call them buccal and lingual cusps yet all of them use the Osborn (1895) terminology in describing molars. This practice may reflect an uncertainty in the minds of research workers that the two major premolar cusps are homologues of the paracone and protocone in upper molars, and the protoconid and metaconid in lower molars. There ought to be fairly close agreement between crown features found on premolars and molars but there is not a great deal of evidence in the literature to show that this is the case. The purpose of the present study, therefore, was to use the principles of serial homology (Butler, 1939, 1972, 1978) to identify the crown features of human premolars.

MATERIALS AND METHODS

Skull material housed in the Raymond Dart Collection, Department of Anatomical Sciences, University of the Witwatersrand, Johannesburg, was used for the study. Maxillary and mandibular dentitions were selected which show a complete premolar-molar row on at least one side, minimal wear, no caries and fairly clear topographical markings. A total of 45 Negro skulls were included in the investigation. Photographs were taken of the occlusal surfaces and used to study the cusp, crest and furrow systems.

Attention was paid particularly to the arrangement of furrows. The furrow system of Zoubov (1977) was used as a basis. Cusps and ridges are probably more reliable than furrows in serial homology, but they are more affected by wear (Butler, 1997).

A preliminary study showed that there were a few key features on premolars, which helped to demonstrate the similarities and differences between premolars and molars. These are listed below.

Maxillary premolars

Buccal furrow (Figs. 1 and 2) extends from central fovea onto the buccal surface, as in the case of molars. It separates the paracone from metacone.

Scoring: 1. extends onto buccal surface (not shortened)
2. present but does not extend to buccal crest (shortened)
3. absent

Foveal cusp (Figs. 1 and 2). Described by Butler (1979). Lies distal to mesial fovea and often extends distally on buccal side of central developmental furrow separating paracone from protocone.

Scoring: 1. present
2. absent

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Central and distal foveae (Fig. 2). The central fovea is the point where the buccal developmental furrow intersects the central developmental furrow. The distal fovea is the point where the central developmental furrow intersects the distolingual furrow.

Scoring: 1. two separate entities as in molars
2. fused into one fovea
3. no longer visible.

Mandibular premolars

Buccal furrow (Fig. 3). Extends from central fovea over buccal crest onto buccal surface dividing protoconid from hypoconid.

Scoring: 1. extends over buccal crest (not shortened)
2. present but does not run over buccal crest (shortened)
3. absent

Protoconid-metaconid crest (Fig. 3). The central ridge of the protoconid and metaconid is generally low in molars and separated by the mesiodistal main furrow. In premolars, particularly the first, a prominent uninterrupted ridge can develop.

Scoring: 1. Unbroken ridge dividing premolar crown into mesial and distal occlusal basins
2. central developmental furrow crosses ridge and separates protoconid from metaconid
3. absent

Mesiolingual furrow (Fig. 3). Zoubov (1977) described it as the first metaconid furrow (Med. 1). It is the most mesial of the metaconid furrows and in molars separates the mesial marginal ridge or paraconid (when present) from the metaconid. In molars it seldom extends over the mesiolingual occlusal crest.

Scoring: 1. Extends onto lingual surface (not shortened). It separates the mesial marginal ridge from the metaconid, and is frequently prominent and deep
2. present but does not extend onto lingual surface (shortened)
3. absent

Distolingual furrow (Fig. 3). In molars it runs from the central fovea over the lingual crest separating the metaconid from entoconid.

Scoring: 1. extends over lingual crest onto lingual surface (not shortened). It may present as a prominent and deep furrow particularly on first premolars
2. present but does not extend over lingual crest (shortened)
3. absent.

Statistical Analysis: Frequencies were calculated and Spearman's rank correlation coefficient determined in order to establish whether observations made on first and second premolars and on left and right sides showed close correlation with each other. The Wilcoxon paired sample signed rank test was used to determine whether differences between first and second, and between right and left premolars were significant.

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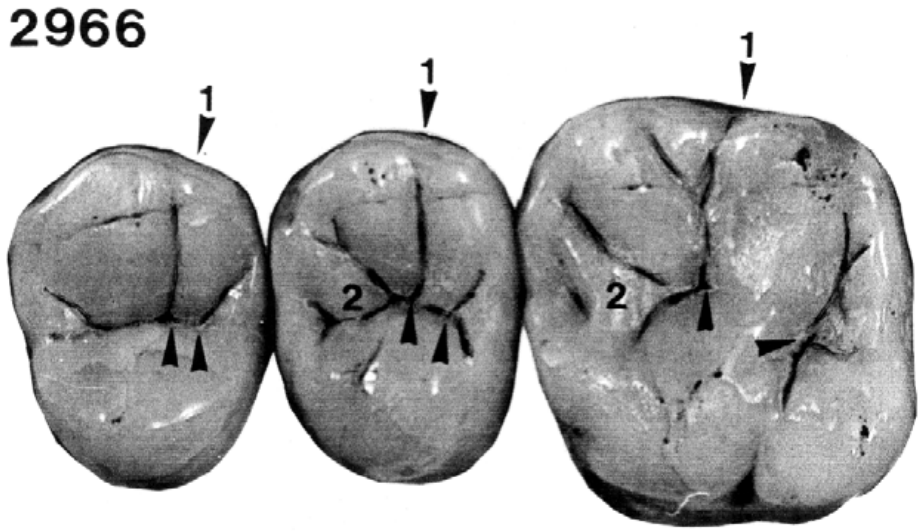


Fig. 1. Photograph of maxillary premolars and first molar on left side (Skull number 2966). A buccal furrow (1) on both premolars extends over buccal crest (not shortened). A foveal cusp (2) is present on second premolar and first molar. The central and distal foveae on premolars and molar are separate entities.

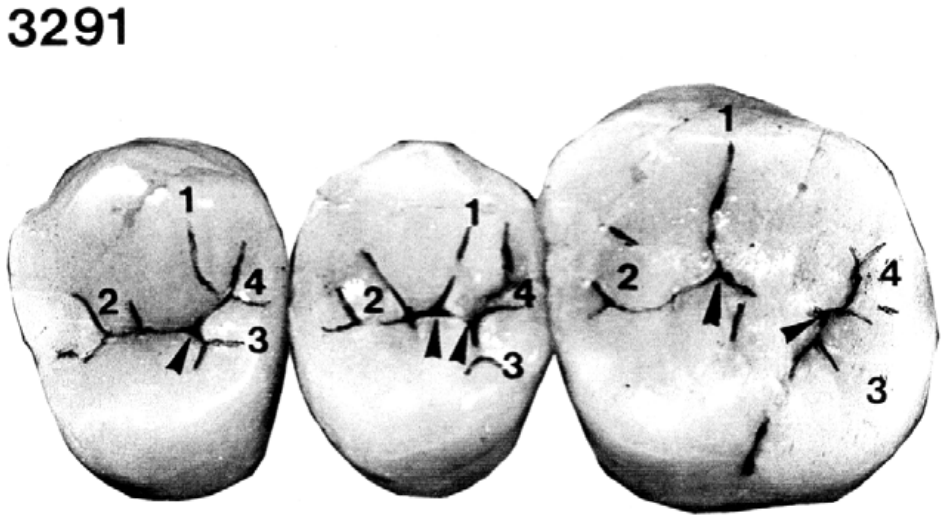


Fig. 2. Photograph of maxillary premolars and first molar on left side (Skull number 3291). Buccal furrows (1) on both premolars do not extend as far as buccal crest (shortened). Foveal cusps (2) are present on both premolars and first molar. The central and distal foveae are separate entities on second premolar and first molar but are fused on first premolar. The hypocone (3) on both premolars is reduced. A hypostyle (4) is present on molar and both premolars.

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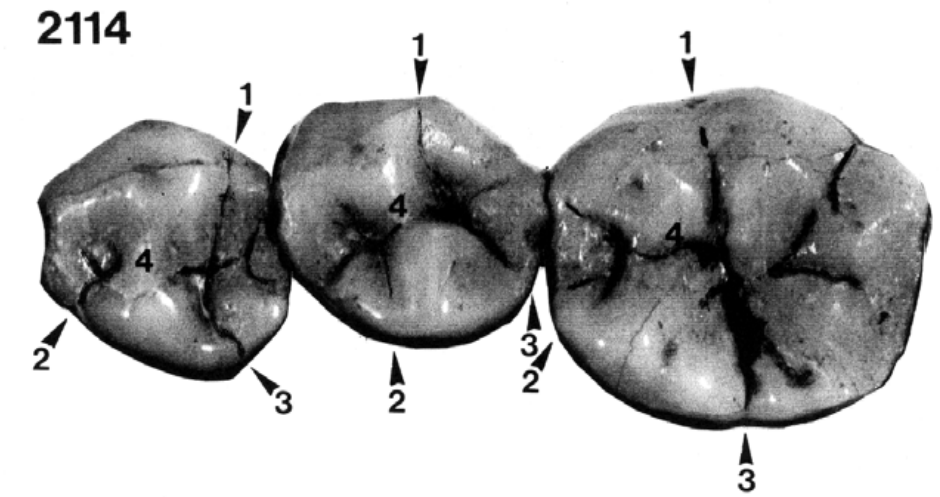


Fig. 3. Photograph of mandibular premolars and first molar on right side (Skull number 2114). The buccal furrow (1) extends over buccal crest (not shortened) on both premolars. The mesiolingual (2) and distolingual (3) furrows on first premolar extend onto lingual surface (not shortened) but on second premolar they are shortened and do not extend over lingual crest. A high and continuous protoconid-metaconid crest (4) is present on first premolar. On the second premolar it is low and interrupted by the mesiodistal furrow.

RESULTS

Maxillary premolars

The Wilcoxon matched pairs signed ranks test showed that there was no significant difference between frequencies of features on right and left sides. The two sets of observations, therefore, were combined.

A well-developed unshortened buccal furrow was observed on approximately 3 and 10 per cent of first and second premolars respectively (Table 1). This furrow separates the larger paracone from the smaller metacone. It usually produces a distinct shoulder on the buccal crest of the tooth. The metacone extends as a low ridge, remnants of the crista oblique, in a lingual direction into the basin of the occlusal surface. A shortened buccal furrow was seen on approximately 49 and 69 per cent of first and second premolars respectively. In this group a flat extension of the metacone spreads onto the lingual slope of the buccal cusp. Almost half (47.6 per cent) of first premolars and 21.9 per cent of second premolars showed no evidence of the buccal furrow. This group, however, usually showed evidence of a small furrow considered to be remnants of the buccal and lingual furrows. On these specimens the metacone seemed to have coalesced with the distal end of the paracone.

The paracone and protocone form the major segments of the buccal and lingual cusps respectively and are separated by the central developmental furrow in the floor of the occlusal basin.

Evidence of a foveal cusp was seen on 30.2 and 58.9 per cent of first and second premolars respectively (Table 2). When present on premolars it was always found on molars in the same row. In only one specimen was the foveal cusp seen on a first but not on a second premolar.

On the basis of the observations made on the central and distal foveae (Table 3), the metacone on premolars may be divided into three categories. In the first, its largest form, it extends as far as the central mesiodistal developmental furrow where it makes broad contact with the protocone from the central to the distal foveae, the situation found in the typical molar. This type of contact was observed on 25.4 and 32.9 per cent of first and second premolars respectively (Table 3).

In the second category, the central fovea has fused with the distal one, the lingual metacone crest is short and separated from the central mesiodistal developmental furrow by a Y-shaped furrow. The two buccal arms of the furrow are formed by remnants of the buccal and distobuccal furrows and define the lingual border of the metacone. The length of the lingual leg of the furrow defines firstly, the distance of separation between the metacone and the central mesiodistal developmental furrow and secondly, the length of contact between the paracone and hypocone, a contact not made in molars. This much reduced form of the metacone was observed on 42.9 and 57.5 per cent of first and second premolars respectively (Table 3).

In the third category, the metacone seems to have coalesced with the distal edge of the paracone and evidence of the Y-shaped furrow and the two foveae are lost. This was seen on 31.7 and 9.6 per cent of first and second premolars respectively (Table 3).

Table 1. Frequencies (%) of buccal furrow on maxillary premolars.

Tooth	Not shortened	Shortened	Absent	N	Correlation*	Significance
P1	3.2	49.2	47.6	63		
					0.6054	0.000
P2	9.6	68.5	21.9	73		

*Spearman's rank correlation coefficient

Table 2. Frequencies (%) of foveal cusp on maxillary premolars.

Tooth	Present	Absent	N	Correlation*	Significance
P1	30.2	69.8	63		
				0.499	0.000
P2	58.9	41.1	73		

*Spearman's rank correlation coefficient

Table 3. Frequencies (%) of separated and fused central (CF) and distal foveae (DF) on maxillary premolars.

Tooth	CF & DF			N	Correlation*	Significance
	separated	fused	not visible			
P1	25.4	42.9	31.7	63		
					0.4774	0.000
P2	32.9	57.5	9.6	73		

*Spearman's rank correlation coefficient

When viewed from the occlusal surface the hypocone is wedge-shaped in molars with its widest edge lingually and the narrow end centrally situated between the distal fovea and the distal surface of the tooth. This part of the hypocone is separated from the protocone by the distolingual furrow. The distobuccal furrow extends from the distal fovea in a distobuccal direction and separates the hypocone from the metacone. On some molars, a cuspule is found in this area called the hypostyle by Osborn (1895), the postentoconule by Hershkovitz (1971) and the metaconule by Harris and Bailit (1980). The hypostyle is situated on and mesial to the distal marginal ridge between the metacone and hypocone and when present on molars is seen on some premolars (Figs. 2 and 4). On premolars, most of the lingual part of the hypocone seems to have disappeared into the distal ridge of the protocone. Remnants of the central part, however, are always present distal to the distal fovea. Minor furrows present in this region on first molars are usually present on adjacent second premolars in the same row (Fig. 4).

Spearman's rank correlation coefficient (Table 4) showed that there was a significant positive correlation ($p<0.01$) between each one of the three features observed on the first and second premolar (buccal furrow, foveal cusp, central and distal foveae), indicating that these features show minimal variability presumably because they fall under the molarization sphere of influence.

Table 4. Spearman's rank correlation coefficients for features observed on maxillary first and second premolars.

Feature	Correlation	Significance	N
Buccal furrow	0.6054	0.000	63
Foveal cusp	0.4992	0.000	63
Central and distal foveae	0.4774	0.000	63

Table 5. Frequencies (%) of buccal furrow on mandibular premolars.

Tooth	Not shortened	Shortened	Absent	N	Correlation*	Significance
P1	0.0	87.0	13.0	46	-	NS
P2	6.4	87.2	6.4	47		

*Spearman's rank correlation coefficient
NS: Not significant

Table 6. Frequencies (%) of protoconid-metaconid ridge on mandibular premolars

Tooth	Ridge unbroken	Ridge crossed by mesiodistal furrow	Absent	N	Correlation*	Significance
P1	65.2	34.8	0.0	46	0.3743	0.010
P2	17.0	80.9	2.1	47		

*Spearman's rank correlation coefficient

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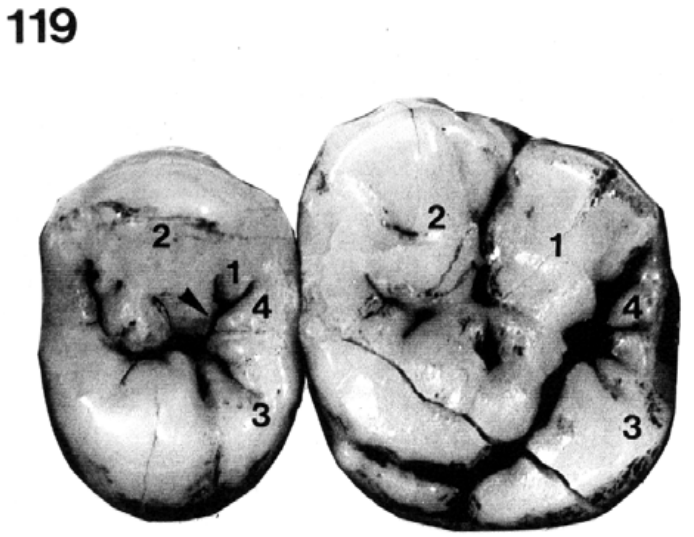


Fig. 4. Photograph of maxillary second premolar and first molar on left side (Skull number 119). Hypocone furrows on first molar are also present on second premolar. The metacone (1) on second premolar has receded distobucally and the paracone (2) makes contact with the hypocone (3) as indicated by the arrow. A hypostyle (4) is present on both teeth.

Mandibular premolars

The buccal furrow in molars runs from the central fovea onto the buccal face separating the protoconid from the hypoconid. An unshortened buccal furrow occurred on 6.4 per cent of second premolars and a shortened one in approximately 87 per cent of both first and second premolars. It was absent in 13 and 6.4 per cent of first and second premolars respectively (Table 5). The majority of lower premolars, therefore, showed evidence of this furrow, which separates the larger protoconid from the smaller hypoconid. The protoconid forms the mesial and central parts of the buccal cusp. A broad continuous buccal crest extends from the mesial to the distal margin of the tooth. On 6.4 per cent of second premolars the buccal furrow interrupts the buccal crest as it crosses onto the buccal surface of the tooth. It is seldom possible to distinguish the hypoconid as a separate cuspule because it seems to merge into the distal ridge of the protoconid. The shortened buccal furrow present on most premolars, however, defines remnants of the hypoconid.

Evidence of a protoconid-metaconid ridge (Table 6) was present on all except one second premolar (2.1 per cent). It formed a high unbroken ridge on 65.2 and 17 per cent of first and second premolars respectively. A lower ridge divided by a central mesiodistal developmental furrow was seen on 34.8 and 80.9 per cent of first and second premolars respectively. This ridge is not a feature of molars.

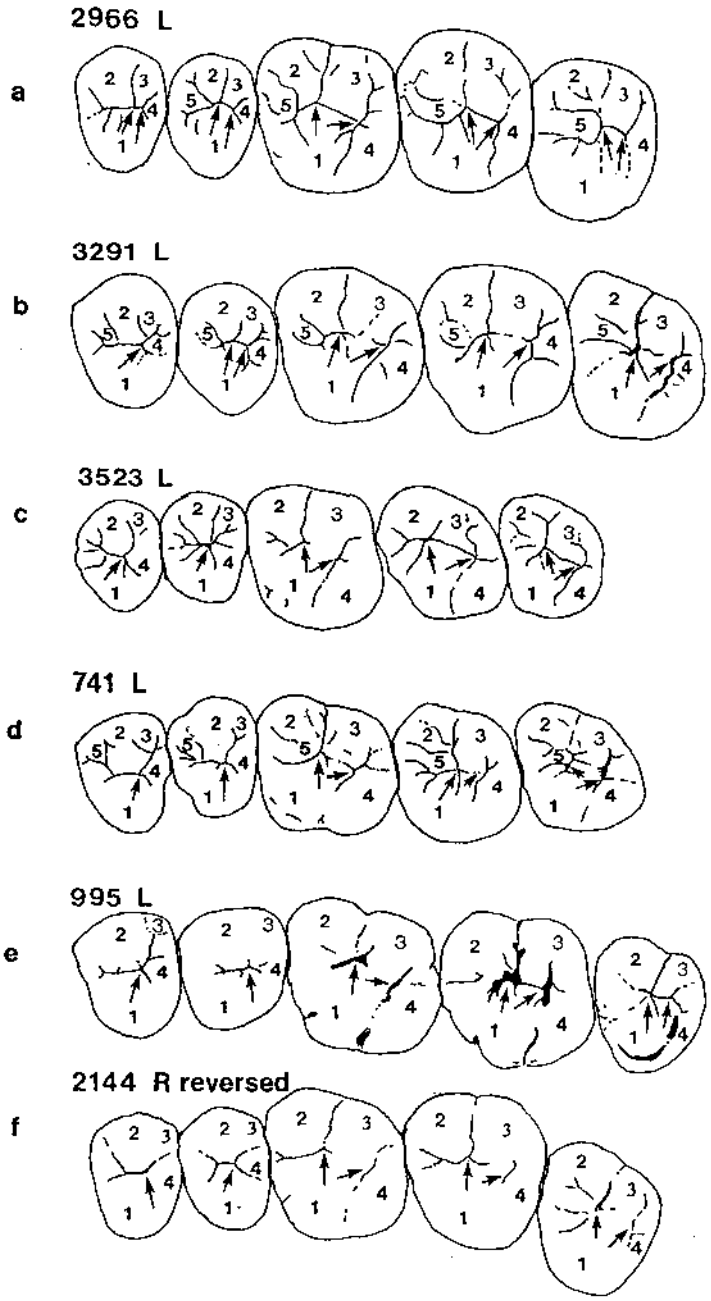


Fig. 5. A series of tracings showing outline and main furrows of maxillary premolars and molars. The cusps are numbered as follows: 1. Protocone, 2. Paracone, 3. Metacone, 4. Hypocone, 5. Foveal cusp. Arrows indicate central and distal foveae. Teeth in the top tracings show more furrow detail than those in the bottom premolar-molar rows. Skull numbers are indicated.

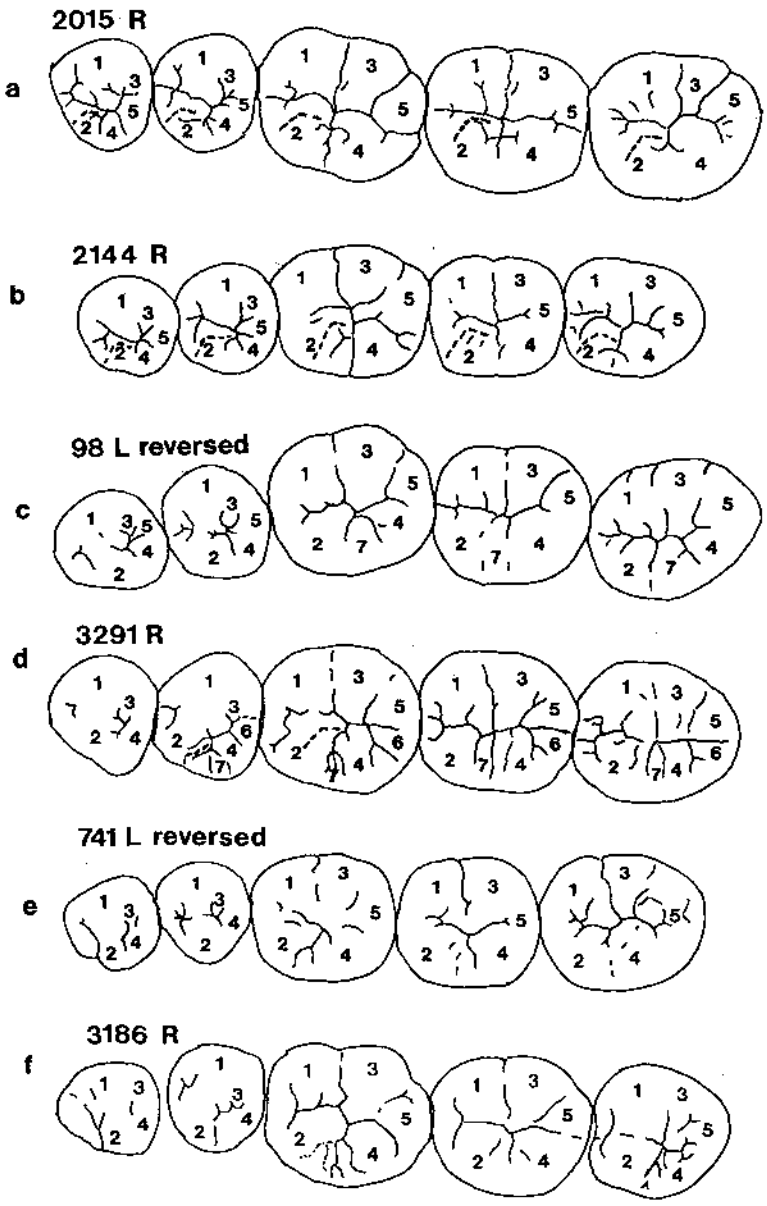


Fig. 6. A series of tracings showing outline and main furrows of mandibular premolars and molars. The cusps are numbered as follows: 1. Protoconid, 2. Metaconid, 3. Hypoconid, 4. Entoconid, 5. Hypoconulid, 6. Tuberculum sextum (entoconulid, C6), 7. Tuberculum intermedium (metaconulid, C7). Skull numbers are indicated. Note the deflecting wrinkle (indicated by broken line) is present on both premolars and molars of skull 2015 and 2144, and on second premolar and first molar of skull 3291. Teeth in top tracings show more furrow detail than those in the bottom premolar-molar rows.

The mesiolingual furrow labeled 1 Med by Zoubov (1977) seldom extends onto the mesiolingual surface of molars. On premolars, however, it often becomes a prominent feature. In this study, 43.5 and 4.3 per cent of first and second premolars respectively had a prominent and not shortened mesiolingual furrow running onto the lingual surface of the tooth, sometimes as far as the cervical margin (Table 7). It was shortened on 52.2 and 82.9 per cent and not visible on 4.3 and 12.8 per cent of first and second premolars respectively. In the latter group the mesial fovea was well-developed and associated with a distinct mesial fossa or basin separated from the distal fossa by the prominent protoconid-metaconid ridge.

In molars a lingual furrow runs from the central fovea onto the lingual surface of the tooth separating the metaconid from the entoconid. This furrow is also present on premolars and in this study is called the distolingual furrow in order to distinguish it from the mesiolingual furrow. As a result of reduction of the talonid in premolars, the central fovea on molars becomes a distal fovea on premolars. The distolingual furrow (Table 8) was not shortened on 30.4 and 17 per cent of first and second premolars respectively and sometimes presented as a feature with a prominence not found on molars. On 69.6 and 83 per cent of first and second premolars respectively it was shortened and did not extend onto the lingual surface of the tooth. The metaconid forms the major portion of the lingual cusp. The entoconid is usually a small cuspule on the side of the distal marginal ridge.

The positive correlation which existed for each one of the three features observed on maxillary first and second premolars, does not occur for features on mandibular premolars – except for the protoconid-metaconid crest. A comparison of the latter data for first and second mandibular premolars gave a Spearman's rank correlation coefficient value of 0.3743 for 46 observations ($p.<0.01$) The Wilcoxon matched pairs signed ranks test produced a similar result. Observations made on the protoconid-metaconid crest, therefore, are less variable than those made on the buccal, mesiolingual and distolingual furrows.

Table 7. Frequencies (%) of mesiolingual furrow on mandibular premolars.

Tooth	Not shortened	Shortened	Absent	N	Correlation*	Significance
P1	43.5	52.2	4.3	46		
					-	NS
P2	4.3	82.9	12.8	47		

*Spearman's rank correlation coefficient

Table 8. Frequencies (%) of distolingual furrow on mandibular premolars.

Tooth	Not shortened	Shortened	Absent	N	Correlation*	Significance
P1	30.4	69.6	0	46		
					-	NS
P2	17.0	83.0	0	47		

*Spearman's rank correlation coefficient

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DISCUSSION

This study confirms the serial homology between premolar and molar crown patterns. Though differing in proportionate development, nearly all the features noted on the premolars can be found again on the molars. The principal difference lies in the reduction on premolars of the distal part of the crown: the mesial cusps (paracone and protocone; protoconid and metaconid) dominate the tooth and are comparably developed on premolars and molars (Figs. 5 and 6).

The foveal cusp, lingual to the paracone, is similarly developed on premolars and molars in the same row (see Fig.5 a, b and d). This cusp was described by Butler (1979) in deciduous molars, and by Van Reenen and Reid (1996) on permanent molars, but it has hitherto not been noted on premolars.

Reduction of the distal cusps varies. On maxillary premolars the metacone shows various degrees of distinctness from the distal ridge of the paracone, and when it is most reduced its lingual ridge (equivalent to the oblique ridge of the molar) loses contact with the protocone, so that the paracone meets the reduced hypocone (see Figure 5b, c, d, e and f). Similarly, on mandibular premolars the hypoconid becomes merged into the distal ridge of the protoconid, and with reduction of the distal cusps the central fovea of the molars is represented by the distal fovea of premolars (see Fig. 6a, b, c and d).

The distal reduction of premolars is probably linked with the fact that during molar ontogeny distal cusps develop later than mesial cusps (Kraus and Jordan 1965). Assuming that this is true also for premolars (of which the cusp development has not been described in detail), it may be that they are undeveloped molars in which the pattern has been fixed at an earlier stage, or in which the distal development has been retarded. The smaller size of premolars, especially in mesiodistal length, would advantage the mesial cusps in the competition for space.

Two features on lower premolars are not found on molars. These are the presence of a well-developed protoconid-metaconid ridge, and a well-defined mesio-lingual furrow (1 Med, described by Zoubov, 1977) separating the mesial marginal ridge from the metaconid. These are features characteristic of the first deciduous molar (Butler 1979). On that tooth the protoconid and metaconid meet at a level higher than the mesial marginal ridge, and they are often connected by a ridge. Also, the mesiolingual furrow is normally present. The second deciduous molar does not show these characters, but resembles the permanent molar. The second premolar differs from the first in that the protoconid-metaconid ridge is on average less high, and the mesio-lingual furrow is less frequent.

First deciduous molars resemble premolars in other respects. In the maxilla, the distal part of the tooth is short, the metacone is small and sometimes absent, the oblique ridge is rudimentary, and the hypocone is undifferentiated from the distal marginal ridge. In the mandible, the hypoconid and entoconid are small, and the central fovea is near the distal end of the tooth. Second deciduous molars are fully molariform, but second premolars are distally reduced like first premolars, though to a somewhat lesser degree: the metacone, hypoconid and entoconid tend to be better developed.

The difference between the first and second deciduous molars might be considered to be a field effect: the first deciduous molars are near the edge of the molar field, and its weakened molarisation results in distal reduction and mesiobuccal enlargement (Butler

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1988). The premolars might be affected in the same way by weak molarising factors. Whatever the morphogenetic factors may be, they operate on a basic potential crown pattern that is common to molars, deciduous molars and premolars.

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Relative Dental Formation in Humans before Emergence of M_1

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INTRODUCTION

Tooth formation is traditionally quantified by fractions of crown and root formation. These stages are subjectively estimated fractions of the unknown, future completed tooth. In order to do this, one has to predict if a child is likely to have a large or small anatomical crown. An alternate approach is to express tooth formation relative to one particular dental growth stage, i.e. crown completion of dm_1 or M_1. The aim of this study was to quantify relative molar formation before clinical emergence of M_1 using the first molar in the deciduous (dm_1) and the permanent dentitions (M_1) as reference teeth. The stages of formation were defined by Demirjian et al. (1973, 1976) with reference stages: crown complete (Demirjian stage D) and part root formed (Demirjian stage E).

MATERIAL

The sample consisted of radiographs of 313 London children. The younger individuals were from a coffin-buried recent archaeological group of skeletal remains from Spitalfields (N=142) who lived in the East End of London around 200 years ago (Molleson et al., 1993). The older individuals (N=171, 88 boys, 71 girls) were patients attending the Paediatric Dental Department (Royal London Hospital) for routine or emergency treatment. Children with M_1 in stages D and E prior to clinical emergence were selected from a larger study (Speechly and Liversidge, 1998). Data for boys and girls were combined.

METHOD

Deciduous molars (dm_1 and dm_2) and the first two permanent molars (M_1 and M_2) were staged from radiographs. Stage assessment was defined by Demirjian, Goldstein and Tanner (1973, updated 1976) for permanent molars and adapted for deciduous molars (Figure 1). This method of stage assessment was chosen for clarity; both radiographic and line illustrations are given, as well as several descriptive criteria for each stage. For some stages, the length of the crown is measured relative to root length thus omitting subjective estimation. Data are presented in order to answer the following question: when dm_1 is at stage D (crown complete), at what stages are the other developing molars? Similarly, when M_1 is at stage D, at what stages are the other developing molars?

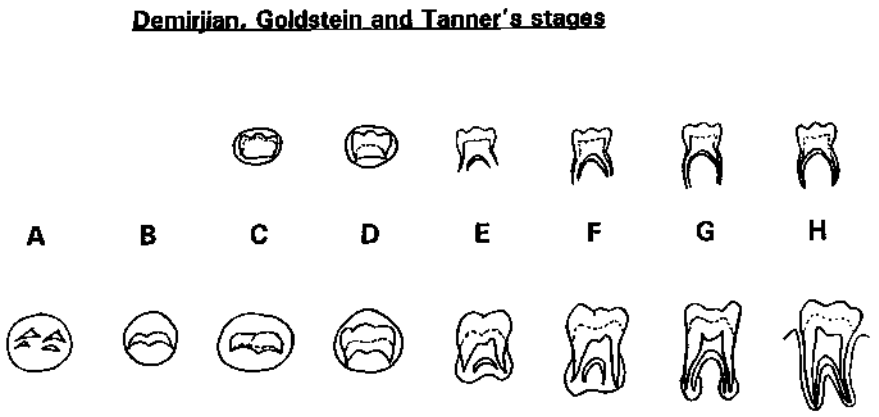


Fig. 1. Stage assessment adapted from Demirjian et al. (1973, 1976). Stage A: cusp tips visible; Stage B: fusion of two or more cusp tips forming the occlusal surface; Stage C: occlusal enamel complete; Stage D: crown complete (=radiographic crown complete) with mesial and distal initial root present; Stage E: initial formation of the radicular bifurcation, root length ≤ crown height; Stage F: funnel shaped roots with root length ≥crown height; Stage G: distal root canal walls parallel with open apex; Stage H: distal root apex closed, uniform periodontal ligament width. (See references for detailed description.)

RESULTS

Results are shown in Tables 1 to 4. Table 1 represents all individuals where dm_1 was at stage D, showing the distribution and range of tooth formation stages of other molars. The relative formation of dm_2 at this developmental stage shows most of the individuals to be stage C; while the modal stage for M_1 was stage B. The next reference stage (Table 2) of dm_1 at stage E, the relative formation of M_1 showed a similar proportion of children in stages B and C. Reference tooth M_1 at stage D (Table 3) the relative formation of M_2 modal stage was stage B. When M_1 is at stage E (Table 4), the development of M_2 is evenly split with a similar proportion of individuals in stage B and C.

Table 1. Reference tooth dm_1 at stage D.

	Number	B	C	D
dm_1				*
dm_2	15		13	2
Percentage			87%	13%
M_1	13	11	2	
Percentage		85%	15%	

Table 2. Reference tooth dm_1 at stage E.

	Number	B	C	D	E
dm_1					*
dm_2	29		9	17	3
Percentage			31%	59%	10%
M_1	28	17	11		
Percentage		61%	39%		

Table 3. Reference tooth M_1 at stage D.

	Number	A	B	C	D	E	F	G	H
dm_1	15							2	13
Percentage								13%	87%
dm_2	16						5	9	2
Percentage							31%	56%	13%
M_1					*				
M_2	30	8	20	2					
Percentage		27%	66%	7%					

Table 4. Reference: M_1 at stage E.

	Number	A	B	C	D	E
M_1						*
M_2	129	4	59	62	4	
Percentage		3%	46%	48%	3%	

DISCUSSION

Difference in tooth formation between individuals or between groups of children is usually quantified using the entire dentition and does not allow for relative differences. Differences in relative molar formation between Australian aborigines and North American children were first quantified by Fanning and Moorrees (1969). Tooth formation timing disparity between American-Indians and recent children was noted by Owsley and Jantz (1983). Recently Tompkins(1996a) investigated and quantified relative tooth formation in Southern African, French Canadian and American Indian children. He noted that African children were advanced in M_2 formation relative to M_1. Comparative data of M_2 formation of several groups with reference tooth M_1 at stage E is depicted in Figure 2. Tompkins describes stage C as the modal stage of M_2 whereas results from the present study show a similar proportion of children in stages B and C when M_1 was at stage E, however, this may be a reflection of sample size. Any growth study requires adequate numbers to truly represent the population including early and late developers. Another possible factor is the sex ratio of boys to girls; any difference in the pattern in relative tooth formation between the sexes remains to be explored.

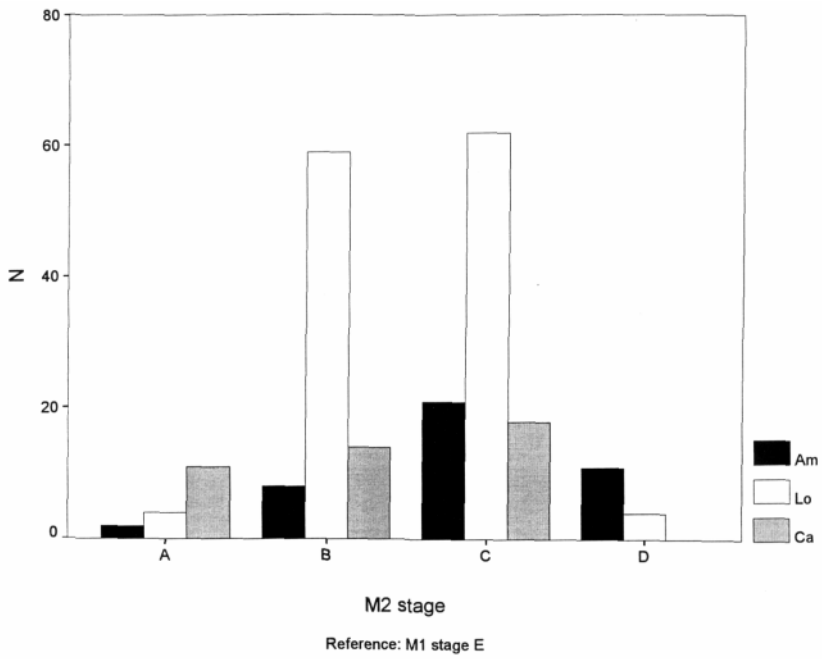


Fig. 2. Relative tooth formation of M_2 of London (Lo), American-Indian (Am) and Caucasian (Ca) children with reference tooth M_1 at stage E. Data from Tompkins (1996a) and Fanning and Moorrees (1969). N= sample size.

Some comparative data are available of non-human primates although sample sizes are very small. Chimpanzee dental development shows a similar pattern to humans of M_2 formation relative to M_1 at stage D (N= 9), but when M_1 is at stage E, the modal stage of M_2 in six individuals was stage C (Conroy and Kuykendal, 1995). Some differences in tooth formation between species have been quantified by relative formation of the permanent central incisor to first molars with the "human" and "ape" pattern (Smith, 1986,1994). Tooth formation of juvenile hominid fossils has been investigated in the light of these patterns (Smith,1994, Tompkins, 1996b). These patterns rely on permanent tooth formation, quantified by crown or root fractions. Despite the large number of radiographic growth studies (reviewed by Smith, 1991, Liversidge et al., 1998) very few growth data are available for fractions of anterior permanent teeth. In addition, no relative data during early growth have been published; this means that the normal "human pattern" is not well described during early childhood and highlights the need for more data for both humans and non-human primates.

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DENTAL EVOLUTION

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Enamel Thickness in the Lower Incisors of Old World Monkeys

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INTRODUCTION

Noble (1969) first reported on the lack of lingual incisor enamel in *Papio*. However, it was Delson (1973, 1975) who first described the significance of this trait in papionins (baboons, mandrills, and gelads). He considered papionins, like rodents, to have eliminated the lingual layer of enamel on mandibular incisors effectively producing a self-sharpening, chisel-like edge on these teeth (Szalay and Delson, 1979). However, Swindler (1976) in a study of the lower incisors of *Papio*, *Cercocebus*, *Macaca* and *Theropithecus*, stated "in our opinion, their lingual surfaces possess a thin layer of enamel, and therefore we cannot agree with Delson's observations" (p. 138). Furthermore, Gantt (1977) in a histological study of enamel thickness in the anterior dentition of nonhuman primates reported the presence of lingual enamel in the lower incisors of two cercopithecines, *Papio* and *Macaca*.

In an attempt to resolve this controversy Shellis and Hiiemae (1986; also see Hiiemae and Shellis, 1984) conducted a histological study of enamel thickness on 20 unworn or slightly worn central lower incisors from five cercopithecine and two colobine genera. They used polarized light microscopy to aid in distinguishing between cementum, plaque and enamel. They reported that the lower incisors of the colobine monkeys had a substantial layer of enamel on both lingual and labial aspects, while the cercopithecines and papionins had little or no enamel on the lingual aspects. Strasser and Delson (1987) interpreted these findings to document that cercopithecines, especially the papionins, have eliminated the lingual layer of enamel on their mandibular incisors. In the present study, we have investigated the mandibular incisors of cercopithecids to determine: 1) if an enamel layer is present on the lingual surfaces in the cercopithecines, and 2) if species of Cercopithecinae have thinner labial incisor enamel than a comparably sized Colobinae species.

MATERIALS AND METHODS

Specimens

This study is based on thin section analysis of 54 unworn or slightly worn mandibular central and lateral incisors from 6 species of Old World monkeys (Table 1). In addition, four unerupted mandibular incisors were viewed with a scanning electron microscope

(SEM) but were not sectioned. The sex of specimens was unknown in most cases, therefore a test for the presence of sexual dimorphism was not possible. Gross measurements were made using a hand held Mitutoyo™ Digimatic caliper calibrated at 0.01mm to record mesiodistal width and labiolingual width (Table 2). Other measurements were made on thin sections. Extracted teeth were stored in vials filled with a solution of thymol, ethyl alcohol and distilled water to prevent drying and cracking of the enamel (Gantt, 1986). The larger incisors of *Papio* were embedded in epoxy resin (Buehler Epo-thin™) to allow sectioning.

Table 1. Species and number of mandibular incisors studied.

Species	Central Incisors	Lateral Incisors	Total
Cercopithecinae (Papionini)			
Papio anubis	4*	5*	9
Macaca nemestrina	9	10	19
Macaca mulatta	5	5	10
Macaca fascicularis	2	4	6
Cercopithecinae (Cercopithecini)			
Cercopithecus aethiops	4	4	8
Colobinae			
Colobus polykomos	5	1	6
Total	29	29	58

*Includes two unerupted central and two unerupted lateral incisors which were obtained for SEM analysis but were not sectioned.

Table 2. Body mass and mandibular incisor dimensions (mm) for each species.

		Mesial-Distal Width						Lingual-Labial Width					
		I_1			I_2			I_1			I_2		
Species	Body Mass*	N	Mean	S.D.	N	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Papio anubis	16.75	2	7.315	0.672	3	6.310	0.497	2	7.775	0.559	3	6.357	0.412
Macaca nemestrina	8.85	9	3.461	0.166	10	3.697	0.278	9	3.122	0.356	10	3.055	0.453
Macaca mulatta	9.90	3	4.497	0.047	5	4.206	0.100	3	4.793	0.268	5	4.410	0.251
Macaca fascicularis	4.48	2	4.880	0.750	4	3.648	0.560	2	4.840	0.537	4	4.295	0.692
Cercopithecus aethiops	3.62	4	3.255	0.139	4	3.070	0.045	4	3.120	0.191	4	2.913	0.054
Colobus polykomos	9.10	2	4.215	0.007	1	3.736	0.000	2	4.535	0.035	1	5.007	0.000

***** Body Mass data represents an average of male and female values taken from Smith and Jungers (1997).

Sectioning

In preparation for sectioning, extracted and embedded teeth were mounted in specially designed holders using dental wax that hardens upon cooling to secure the tooth in position. The incisors were sectioned through the center of the crown in a labiolingual direction. Thin sections of approximately 120µm were cut using a diamond edged blade four inches in diameter and approximately 150µm in thickness. All sections were prepared using a Silverstone-Taylor™ hard tissue microtome per the methods and procedures described by Gantt (1986). After the thin sections were cut, they were polished using 1.0 and 0.3µm aluminum oxide Buehler® fibrmet® discs to remove

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scratches. Thickness of polished specimens was determined using a Fowler™ micrometer. Each specimen was then mounted on a glass slide using Duco® cement with labial and lingual positions identified.

Measurement Technique

Glass mounted thin sections were directly placed into a Drust™ photographic enlarger. Enlarged (30X) high-resolution black and white photographs were produced of each specimen and of a 0.01mm scale. Specimen photographs and the photograph of the millimeter scale were then scanned into the computer via a Hewlett Packard ScanJet Plus™ flatbed scanner using the Deskscan™ software program. Each photograph was scanned in as a black and white photo at 300 dpi and at a 1:1 ratio. The images were then digitally processed using NIH Image™. The digitized images were saved as a bitmap file and opened in Jandel Scientific SigmaScan3™ for image analysis.

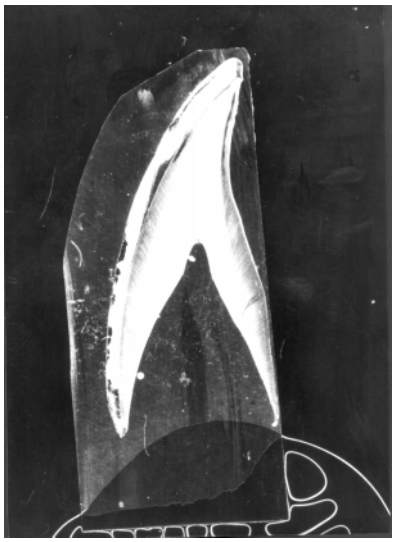


Fig. 1. Thin section from a right central lower incisor of *Macaca mulatta* demonstrating the absence of a lingual enamel layer.



Fig. 2. Thin section from a right central lower incisor of *Colobus polymokos* demonstrating the presence of a lingual enamel layer.

Enamel thickness measurements were made after the image was calibrated with the 0.01 millimeter scale. The measurements of enamel thickness were recorded using a mouse and directly transferred into a spreadsheet for subsequent statistical analysis. The eight measurements in the present analysis are illustrated in Figure 3. They were made at the dentine horn (apex) and perpendicular to the dentine-enamel junction (DEJ) at ¼, ½, and ¾ distance from the apex. The later three locations of measurements were calculated via pixel position using SigmaScan3™by calculating the total distance from the dental horn to the cervical margin using **y**-coordinates and dividing the distance into four regions. The thickness measurements were from the DEJ to the enamel surface.

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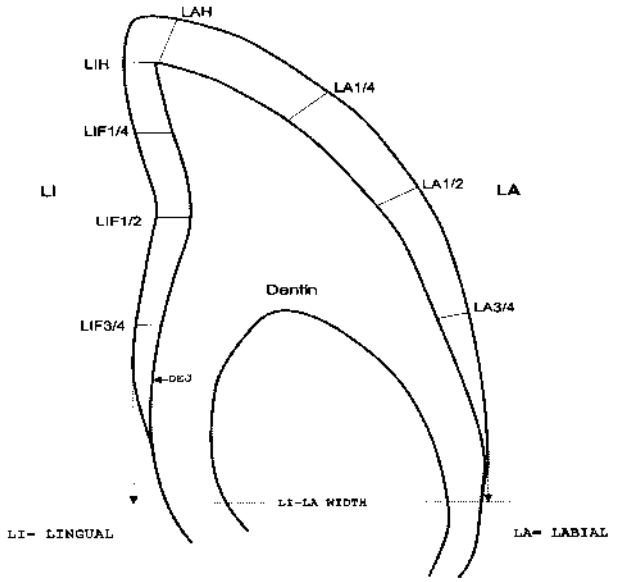


Fig. 3. A diagram of a thin section from a mandibular right central incisor of *Colobus polymokos* illustrating the location and position of each measurement and variable labels.

Statistical Analysis

Student's t-tests were used to test for bilateral symmetry for each of the species used in the study. Because no asymmetry was evident left and right central and left and right lateral incisors were pooled. The means and standard deviations were calculated based on central or lateral position within species (Table 2 and Table 3).

Analysis was conducted to compare species for each of the two tooth dimensions and the three thickness measurements, LA1/4, LA1/2, and LA3/4. Because of wear, relatively few LAH measurements were made, and there are not enough to perform the statistical analysis on this variable. No analysis is necessary to compare lingual measurements which are zero for all species except *Colobus polykomos*. For most of the five analyses which were conducted, Levene's (Neter et. al. 1996) test identifies variance heterogeneity, so care was taken to confirm statistically significant differences among the species using a multiple comparison procedure which is appropriate when variances differ. All of the four such procedures available in the SPSS™ (version 8) software package give the same results. The pairwise multiple comparisons are made at the 5% familywise level of significance.

Because of our interest in differences between both species and tooth position (central versus lateral) a two factor analysis of variance (ANOVA) was conducted as part of each analysis. We are quite satisfied that the robustness of this procedure was sufficient to overcome the variance heterogeneity. This analysis allows the investigation of a possible interaction between the two factors as well as testing for differences between central versus lateral incisors.

Table 3. Descriptive statistics for enamel thickness measurements (mm).

		I_1			I_2	
Variable	N	Mean	S.D.	N	Mean	S.D.
Papio anubis						
LAH	1	0.716	0.0	0	--	--
LA1/4	2	0.659	0.043	3	0.575	0.072
LA1/2	2	0.588	0.024	3	0.483	0.058
LA3/4	2	0.474	0.072	3	0.378	0.045
LIH	0	--	--	1	0.0	0.0
LIF1/4	1	0.0	0.0	2	0.0	0.0
LIF1/2	1	0.0	0.0	2	0.0	0.0
LIF3/4	2	0.0	0.0	2	0.0	0.0
Macaca nemestrina						
LA1/4	9	0.193	0.021	9	0.207	0.022
LA1/2	9	0.182	0.029	10	0.185	0.017
LA3/4	9	0.141	0.029	10	0.134	0.018
LIF1/4	6	0.0	0.0	3	0.0	0.0
LIF1/2	8	0.0	0.0	10	0.0	0.0
LIF3/4	9	0.0	0.0	10	0.0	0.0
Macaca mulatta						
LAH	5	0.476	0.089	2	0.535	0.001
LA1/4	5	0.447	0.053	3	0.413	0.027
LA1/2	5	0.386	0.028	5	0,304	0.017
LA3/4	5	0.324	0.040	5	0.243	0.026
LIH	4	0.0	0.0	2	0.0	0.0
LIF1/4	4	0.0	0.0	2	0.0	0.0
LIF1/2	4	0.0	0.0	2	0.0	0.0
LIF3/4	4	0.0	0.0	2	0.0	0.0
Macaca fascicularis						
LAH	2	0.471	0.024	3	0.507	0.087
LA1/4	2	0.471	0.056	4	0.460	0.028
LA1/2	2	0.412	0.005	4	0.348	0.027
LA3/4	2	0.349	0.043	4	0.274	0.021
LIF1/4	2	0.0	0.0	4	0.0	0.0
LIF1/2	2	0.0	0.0	4	0.0	0.0
LIF3/4	2	0.0	0.0	4	0.0	0.0
Cercopithecus aethiops						
LAH	3	0.403	0.046	3	0.375	0.029
LA1/4	4	0.391	0.026	4	0.346	0.031
LA1/2	4	0.333	0.016	4	0.293	0.039
LA3/4	4	0.266	0.020	4	0.221	0.021
LIH	0	--	--	3	0.0	0.0
LIF1/4	3	0.0	0.0	4	0.0	0.0
LIF1/2	3	0.0	0.0	4	0.0	0.0
LIF3/4	3	0.0	0.0	4	0.0	0.0
Colobus polykomos						
LAH	3	0.426	0.048	1	0.469	0.0
LA1/4	5	0.445	0.103	1	0.421	0.0
LA1/2	5	0.381	0.126	1	0.331	0.0
LA3/4	5	0.307	0.091	1	0.290	0.0
LIH	3	0.110	0.039	1	0.136	0.0
LIF1/4	4	0.127	0.047	1	0.100	0.0
LIF1/2	5	0.120	0.060	1	0.114	0.0
LIF3/4	5	0.111	0.058	1	0.157	0.0

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RESULTS

Average body mass as well as means and standard deviations for the gross measurements, mesiodistal width and labiolingual width, are reported in Table 2. Means and standard deviations for the eight enamel thickness measurements are in Table 3. For all five species in the subfamily, Cercopithecinae, lingual enamel is missing from the mandibular incisors. Lingual enamel is present in *Colobus polykomos*, although it is clearly not as thick as labial enamel. It appears to vary between approximately 20 percent and approximately 50 percent of the thickness of corresponding labial enamel (see Tables 2 and 3).

Multiple Comparisons Among Species

The pairwise multiple comparisons among the species for the gross tooth dimensions, mesiodistal width and labiolingual width, each identified the same three distinct groupings. The species with the smallest mandibular incisors are *C. aethiops* (mass 3.6 kg) and *M. nemestrina* (mass 8.9 kg) (Body mass measurements were obtained from Smith and Jungers (1997)). Those species with larger incisors are *M. fascicularis* (mass 4.5 kg), *C. polykomos* (mass 9.1 kg), and *M. mulatta* (mass 9.9 kg). *P. anubis* (mass 16.8 kg) has significantly larger mandibular incisors than any of the other species in the study.

The results of multiple comparisons among the species for the three labial (LA) enamel thickness measurements, LA1/4, LA1/2, and LA3/4, are consistent but differ slightly from the results for the tooth dimensions. The labial enamel for *M. nemestrina* is significantly thinner than for the other species studied. The next thickest enamel is found in *C. aethiops*. A group of three species, *M. mulatta*, *M. fascicularis*, and *C. polykomos* has the next thickest labial enamel with *P. anubis* having significantly thicker labial enamel than any of the other species in the study.

Comparisons of Central Versus Lateral Incisors

With the exception of *M. nemestrina*, there is a consistent pattern for each of the species studied for tooth dimensions and enamel thickness to be greater for central incisors than for lateral incisors. In four of the five two factor ANOVAs the difference is highly statistically significant. The exception is the analysis of thickness measure LA1/4. There is some suggestion of an interaction between species and tooth position, which is explained by the lack of difference between central and lateral incisors for *M. nemestrina*. The interaction is statistically significant at the 5% level in one of the five ANOVAs. The plot of means for measurement LA1/2 in Figure 5 is representative of the results in each of the analyses.

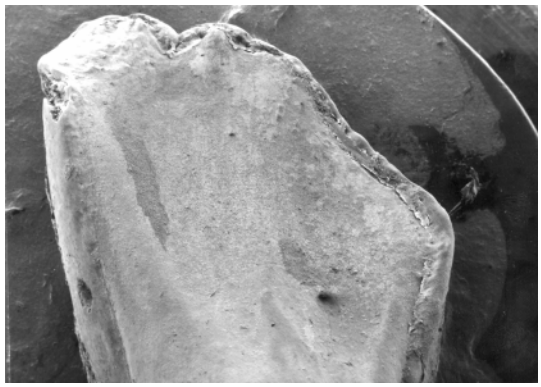


Fig. 4. SEM microphotograph of an unerupted central incisor from *Papio a. cynocephlus* (35X).

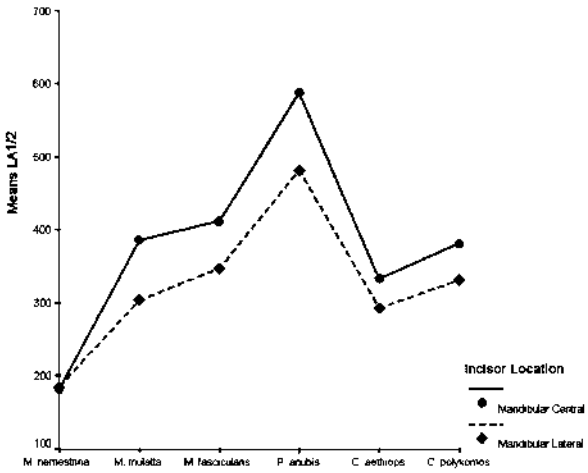


Fig. 5. A plot of the mean labial thickness for central and lateral mandibular incisors in μm, taken ½ the distance between the dental horn to the cervical margin.

DISCUSSION

The objective of this investigation was to evaluate the thickness of enamel in the lower incisors of cercopithecids. Analysis of the data revealed the presence of bilateral symmetry in incisors enamel thickness. Differences in enamel thickness distribution in cercopithecine monkeys may be attributed to differences in body size. The exception being the genus *Macaca*, especially between *M. mulatta* and *M. nemestrina*. An evaluation of these differences is presently under way.

Our results clearly document the absence of enamel on the lingual surface of the lower incisors in the Cercopithecinae, especially in the Papionini. This lack of enamel is a developmental characteristic and can be easily seen in the SEM microphotograph of an unerupted incisor from *Papio* (Figure 4). In contrast, the Colobinae have a substantial

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layer of lingual enamel. A maximum lingual thickness of 0.193 mm was recorded in *Colobus* while the maximum labial enamel thickness was 0.616 mm. Shellis and Hiiemae (1986) reported a maximum lingual enamel thickness of 0.125-0.060 mm and a maximum labial enamel thickness of 0.505-0.400 mm for the lower incisors of *Colobus* and *Presbytis*. The larger central incisors of *Papio* and *Macaca* were reported as having 0.580-0.480 mm of enamel on the labial surface. We recorded a maximum of 0.690 mm in *Papio* and 0.524 mm in *M. mulatta*.

Gantt's (1977) original cercopithecine data were obtained on isolated specimens of *Papio.* These samples were worn teeth which were identified as "mandibular", however from the data obtained in this investigation of known animals we believe that the original material were "maxillary" incisors, which have lingual enamel. Shellis and Hiiemae (1986) also noted that "...apart from the value for *Papio*, Gantt's results agree with ours (p. 111)".

We believe the absence of lingual enamel in the Papionini is explained by dietary adaptation to frugivory and resulting feeding behaviors. A shift in the ancestral cercopithecines towards frugivory would have resulted in a reduction in the use of the incisors of stripping leaves. The anterior dentition is the first to show extensive attrition, especially in the lower incisors. Smith (1969) observed that the incisors are the first teeth to show extensive attrition in *Papio* and *Theropithecus* and suggested that the differences in chipping were due to thinner enamel especially lingual enamel. This dental wear is most often caused either by contact of opposing teeth during mastication (attrition), or by contact between teeth and food (abrasion) (Ungar, 1992). Food and other objects that come into contact with enamel are known to leave characteristic patterns of pitting and scratching on the tooth surface (Ungar, 1990).

Walker (1981) was the first to examine microwear on anthropoid incisors. He showed significant differences between the Cercopithecinae and the Colobinae in respect to the orientation of incisor wear striations. He demonstrated that wear striations in colobines tend to be mesiodistally oriented, whereas the cercopithecines exhibit a more labiolingual orientation of wear striations. Thus colobines produce greater attrition on the labial surfaces. These differences in scratch orientation are associated with reported differences in feeding behaviors between the two subfamilies. Walker (1981) concluded that the low frequency of striated surfaces on the incisors of *Colobus* and *Presbytis* results partially from the fact that a large proportion of their diet requires little incisal preparation. Also, it has been demonstrated that terrestrial Old World monkeys possess more striations than the arboreal species due to the presence of grit on foods eaten on or near the ground and the mechanical demands of food breakdown (Walker, 1976; Ungar, 1992).

Ungar has combined studies of microwear and primate feeding ecology to demonstrate that striation densities relate to degree of use of the incisors in food processing, that scratch orientations relate to the directions that food items are passed across the teeth, and that striations relate to the types of abrasives contacting the front teeth (Ungar, 1992; 1994a,b; 1995a,b; 1996). High incidences of mesiodistally oriented scratches are associated with frequent nipping, or stripping foods laterally across the mouth (Ungar, 1994a). Ungar (1996) looked at the relationship of incisor size to diet and anterior tooth use in *Macaca fascicularis*, *Presbytis thomasi*, *Hylobates lar*, and *Pongo pygmaeus*. He demonstrated that incisor row widths were both absolutely and relative to body size greater in *M. fascicularis* than in *Presbytis*, but found no clear relationship between

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incisor size and the ratio of fruits to leaves eaten. The function of the anterior dentition in cercopithecids is concerned with prehenison of food. In the frugivores cercopithecines this is typically biting fruit and stripping away the outer husks, while the folivorous colobines use their incisors to nip off leaves or to scrape them but not to chew them. The feeding behavior and dietary habits of the cercopithecines results in increased attrition to the lingual surfaces of the lower incisors compared to that found in the colobines. This pattern results in producing chisel-like teeth with enamel on the labial surfaces and the absence of enamel on the lingual surfaces. In comparison, Hylander (1975) has demonstrated that colobines have smaller incisors than cercopithecines. These incisors have thick labial and lingual enamel surfaces, which are well suited for nipping and shredding leaves without further mastication. The developmental differences in the cercopithecines may be attributed to the evolutionary use of the mandibular incisors in the peeling of tough skinned fruits, resulting in thinner enamel and the eventual loss of enamel on the mandibular central and lateral incisors. The colobines do not exhibit this type of feeding behavior.

CONCLUSIONS

In summary, this investigation revealed that the thickness of enamel and its pattern of distribution differ among the subfamilies of Cercopithecidae, Colobinae and Cercopithecinae. Notably, Colobines have lingual enamel while Cercopithecines, especially the Papionini do not. Interspecific differences exist between species of *Macaca*, while differences between the genera are explained as differences in body size. Bilateral symmetry is present in all species studied. Colobines have lingual enamel while Cercopithecines, especially the Papionini do not. The differences in mandibular incisor enamel thickness does provide a diagnostic character state to assign phyletic relationships in the Cercopithecidae as proposed by Strasser and Delson (1987).

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The Origin of the Anatomically Modern Human Face Through Differential Rates of Tooth Size and Facial Size Reduction

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INTRODUCTION

The debate about the origin of anatomically modern *Homo sapiens* is centered on a disagreement as to whether anatomically modern humans descended only from late archaic *Homo sapiens* populations of Africa or from both African and Eurasian populations. I agree with Krantz (1994) and Brace (1995c) that we should focus more on finding out the cause or causes of this last major evolutionary transition in human evolution than arguing for or against the single or multiple areas of origin. After all, anatomically modern humans evolved from archaic humans, wherever this transition first took place. I have evaluated in light of European fossil evidence two behavioral models that have been proposed as explanations for this transition: the teeth-as-tools model and the speech model. I am testing which one of these models provides a better explanation for why the modern configuration replaced the archaic one.

The anatomically modern *Homo sapiens* are differentiated from the archaic *Homo sapiens* by a reduced average degree of skeletal robusticity, smaller tooth sizes, and much shorter faces located more directly beneath the anterior braincase. Because the degree of robusticity and tooth sizes overlap between the late archaic and early anatomically modern *Homo sapiens* specimens, differences in the craniofacial configuration are by far more visible and diagnostic (Lieberman, 1995).

MATERIALS AND DISCUSSION

These craniofacial differences are shown well by Yaroch's (1996) average cranial profiles of four Neandertal males and 44 Norwegian males, which I have standardized for the braincase size (Fig. 1). I superimposed porions (the most superior margins of the external auditory meatuses) and set points glabella and opisthocranion along the horizontal plane. The Frankfurt Horizontal was not used because archaic humans' relatively more superior positions of orbits would have tilted a skull as if the specimen in question is positioned looking slightly downwards.

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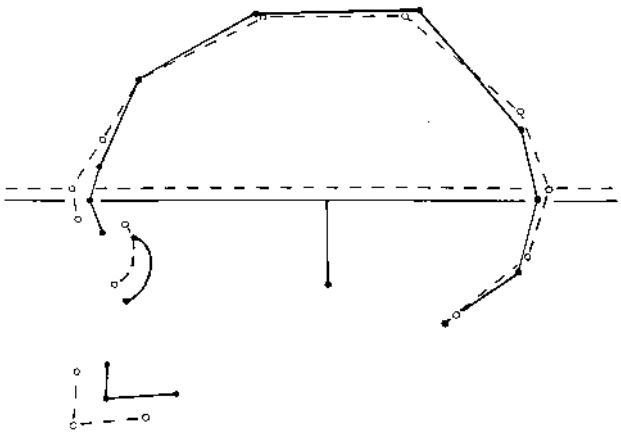


Fig. 1. Superimposed lateral cranial profiles of the average Neandertal (broken line) and the slightly enlarged average Norwegian (solid line).

These profiles exhibit marked differences in facial and browridge projection, as well as in facial height. Differences in facial height are mainly results of differences in facial projection, because faces grow both forward and down (Smith and Paquette, 1989; Lieberman, 1998). Reduction in the facial projection is also responsible for more vertical foreheads, reduced browridge projection, prominent chins, lack of retromolar spaces, and more globular braincases of the anatomically modern humans (Krantz,1980, 1981; Niskanen, 1990a,b; Lieberman, 1995, 1998). Because modern craniofacial configuration resulted from markedly reduced facial projection, I have reviewed the anatomical evidence to evaluate whether the teeth-as-tools model or the speech model explains it better.

The teeth-as-tool models are based on the view that modern anatomy emerged through reduction of the size and robusticity of the facio-dental anatomy. The average tooth sizes reduced as a result of softening food by cooking, which relaxed selection pressure to maintain large teeth. The tooth size reduction started the earliest in Europe about 250 kyr because the glacial climate made cooking obligatory to thaw and soften frozen meat. As a result, Europeans have the smallest average tooth sizes (Brace, 1964, 1979, 1995a,b,c).

A plot of average anterior (I1-C) and posterior (P3-M3) tooth surface areas (from Frayer, 1978, 1984; Brace, 1979) of the early and late Neandertals, Early and Late Upper Paleolithic Europeans, and recent Europeans and the average dates of these samples shows the gradual pattern of this tooth size reduction (Fig. 2). The early anatomically modern Europeans' tooth sizes were more similar to those of the late Neandertals than to those of the recent Europeans. Also, the late Neandertals with their projecting archaic faces had smaller tooth sizes than recent Australian Aborigines with non-projecting modern faces (Brace, 1995a,b,c). Therefore, the tooth size reduction alone did not result in the modern facial form.

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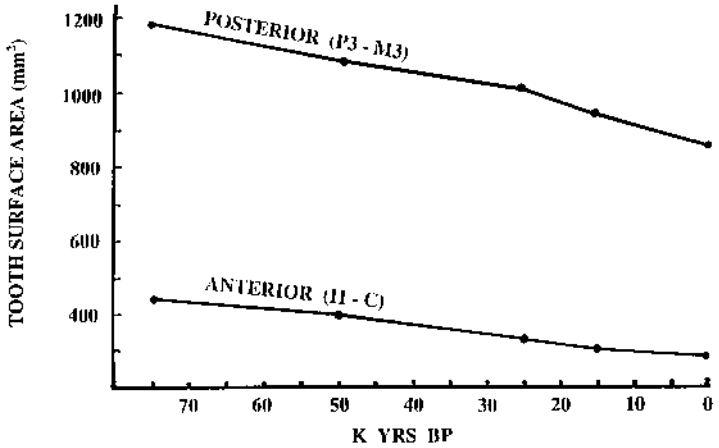


Fig. 2. Tooth surface area reduction over time

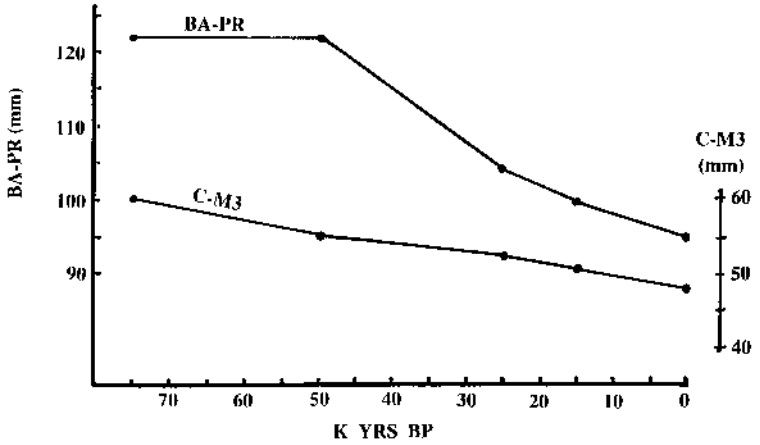


Fig. 3. Reduction of total facial projection (basion-prosthion length) and tooth row lengths (summed C-M3 mesio-distal lengths) over time

A plot showing reduction in the average summed canine-to-third molar lengths (representing the tooth row length) and basion-prosthion lengths (representing facial projection) (see Niskanen, 1990a for data sources) demonstrates that reduced tooth sizes resulting in shorter tooth rows did not cause reduction in facial projection (Fig. 3). Also, the lower facial/palatal lengths differ very little in the late late archaic and early modern humans (Lieberman, 1998). It appears that the reduction of total facial projection occurred independently from the tooth size/tooth row length reduction, and that the modern face emerged as a result of pulling back the tooth bearing parts of the face by over one centimeter (Niskanen, 1990a,b).

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A more important factor than tooth size reduction behind the emergence of the modern face may have been the facial size reduction through reduced use of the masticatory apparatus as a tool as reduced tooth wear of the anterior teeth indicates. Technological innovations – such as hafted tools – made the masticatory apparatus less frequently used as a tool. The result could have been a rapid facial size reduction and more modern-like facial configuration regardless of the tooth sizes (Brace et al., 1981; Skinner, 1981) because the facial size and shape are largely determined by the development of the masticatory muscles (Weijs and Hillen, 1986). This explanation conflicts with the observation that the late Neandertals did not have longer masseter muscle origin lengths and, therefore, masseter muscle sizes, than early anatomically modern Europeans (Trinkaus, 1986; Niskanen, unpubl.).

I do not think that the teeth-as-tools models explain the observed craniofacial transition because facial projection, tooth sizes, and masticatory muscle sizes changed independently from each other and with different rates. This mosaic evolution indicates major changes in selection directions and not just relaxation of selection pressures to maintain the level of robusticity (Niskanen, 1990a,b). This type of evolution is possible because the face is divided into different growth fields (Enlow, 1982) and because selection pressures and directions effecting different aspects of cranial anatomy are not necessarily the same (Brace, 1995b).

I think that the speech models explain this transition better. These models are based on reconstructions of vocal tract anatomy, which indicate that the archaic humans had limited abilities to produce speech sounds (Lieberman and Crelin, 1971). Selection for an anatomy more suitable for speech sound production could have resulted in modern craniofacial configuration and sudden emergence of cultural traits, such as art, long-distance exchange of raw material, regional stylistic variation of artifacts, and unquestionable evidence of rituals and religion (Krantz, 1980, 1981, 1994; see Klein, 1989, 1995 and Stringer and Gamble,1993 about this cultural revolution).

Cranial changes needed for improved speech sound production were reductions of the facial projection and lowering the cranial base (Krantz, 1980, 1981, 1994). According to Lieberman (1998), the facial projection reduced through shortening of the anterior-posterior length of the sphenoid bone from which the face grows anteriorly. This change in the early cranial base growth resulted in modern craniofacial configuration and made the vocal tract more suitable for speech sound production.

The sphenoid shortening and, therefore, the rapid evolution of anatomically modern humans from their archaic ancestors could have taken place through a mutational change of a regulatory gene or genes that regulate the growth and development of the anterior cranial base. Wilson (1975) has proposed that regulatory mutations have occurred during earlier stages of human evolution, making humans morphologically distinct from chimpanzees in relation to far lesser genetic distinctness. However, the new configuration cannot replace the earlier configuration unless it is more advantageous. In this case, the new configuration better able to produce speech sounds may have been favored by selection at the expense of the earlier configuration.

Gene flow could have spread genes producing anatomies better able to produce speech sounds between populations. However, populations that had completed this new adaptive transition probably had an advantage over those populations that had not yet started this transition and may have expanded geographically at their expense. For example, because

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this transition may have been completed in North Africa earlier than elsewhere, there may have been an expansion of African populations at the expense of Eurasian populations. However, it is unlikely that individuals having modern craniofacial anatomy were unable to produce fertile offspring with those having archaic anatomy. Dog breeds exhibit a tremendous amount of variation in their muzzle lengths. However, flat-faced bulldogs produce fertile offspring with long-muzzled breeds without any problems.

The speech models have been criticized due to uncertain reconstructions of soft tissues involved with the speech sound formation (Falk, 1975; Burr, 1976; Aiello and Dean, 1990; Brace, 1995c). In addition, the complex and integrated nature of the cranial anatomy make it unlikely that a single factor is responsible for the origin of the anatomically modern cranial form (Lieberman, 1998). However, in light of both the mosaic pattern of anatomical transition and the archeological evidence of improved communication abilities across this transition, I consider the speech model as a stronger single factor than the teeth-as-tool model behind the origin of the anatomically modern human craniofacial form.

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Evolution of Human Enamel Growth Analyzed by Confocal Microscopy

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INTRODUCTION

Tooth formation is the end result of a long developmental process, which results from interactions between oral epithelium and cranial neural crest-derived ectomesenchyme (Kollar, 1983; Ruch, 1984; Thesleff and Hurmerinta, 1981). The timing, rate and duration of these events vary along the tooth row and are attributed to the activity of numerous genes encoding growth factors and structural molecules. These activities are associated with the pattern of tooth development taking place in a staggered fashion along the dental arch, during a limited period of time (MacKenzie et al., 1992; 1991; Smith and Hall, 1990; Snead et al., 1988; Wagner, 1989; Weiss, 1993). However, little is known about how the timing and rate of dental development relate to final tooth size and form (Butler, 1956; Jernvall et al., 1994; Slavkin, 1990).

A hierarchical change in the timing and expression of regulatory genes can be postulated to account for the observed differences in homologous teeth on an evolutionary time scale. Over the course of human evolution, from ancient to modern times, there has been a decrease in molar tooth size. These size changes have been associated with a change in cusp pattern (Dahlberg, 1961; Smith, 1978, 1982, 1988; Wood, 1981; Wood and Abbott, 1983; Wood et al., 1983). The main change observed is a relative decrease in size of the distal portion of the crown (Dahlberg, 1945, 1961).

Enamel has two important properties, durability and structural complexity. Because of its durability teeth are preferentially preserved in the fossil record and its structural complexity reflects its development. To examine the relationship between tooth formation and changes in final tooth size we have analyzed the microstructural organization of mature molar enamel and particularly its arrangement at the occlusal surfaces of distinct regions. Confocal laser scanning microscopy (CLSM) was the method of choice to study mature mineralized teeth because it allows a non-destructive analysis.

MATERIALS AND METHODS

CLSM images were taken in modern and fossil teeth of hominids from Israel. On each CLSM image, three parameters of dental structure were studied at different enamel locations and these were 1) shape, 2) size, and 3) density of the enamel prisms.

Skeletal remains found in archaeological excavations in Israel that date from 6000-200 B.P. were used as representative of *Homo sapiens* samples and the remains of Qafzeh, dated to circa 90,000 B.P represented early *Homo*.

This study was carried out on 21 lower permanent molars, of which 18 were modern teeth and 3 were fossil teeth. Two modern teeth were excluded from this group because prisms were not visible in any cusp. None of the teeth had any observable wear facets, caries, cracks or other post-mortem damage. The developmental stage of each tooth was assessed both metrically and non-metrically (Smith et al., 1995). The mesiodistal (MD) and buccolingual (BL) crown widths as well as the mesiobuccal crown height were measured using a Digimatic needle-point sliding caliper (Mitutoyo) with a resolution of 0.01 mm and the product of the first two diameters was used as an index of crown size (Haydenblit, 1996). Measurements were taken according to the definitions described by Moorrees (1957).

CLSM images were taken through cusp tips of the protocone (mesio-buccal cusp) and hypoconulid (distal cusp) of the first permanent lower molars. Confocal optical sections (images perpendicular to the surface) were acquired from successively deeper sections in steps of 1µm. The above parameters were determined for the deep, intermediate, and superficial enamel of the cusp tips. The recording of the deep enamel region was limited until the image lacked sufficient contrast to record it, the superficial enamel level was examined at an area at the outer margin of the cusp, and the intermediate depth was defined as an area midway between the superficial and deep optical sections. To insure uniform magnification among the confocal images all enlargement factors (i.e. magnifying lenses in the microscope and zoom in the computer) were held constant. All the images were recorded using a Bio Rad MRC1024 confocal laser microscope using the 488nm line of a Kr/Ar laser, an Eclipse E800 Nikon microscope and a 60X/NA=1.4 Nikon Plan Apo objective (W.D. 0.21 mm). All the confocal images were obtained by a reflection mode. The principle of the confocal microscope is based on reflected light microscopy that optically scans a specimen by sending and receiving light through pinholes of variable apertures (Pawley, 1995). Thus, it is possible to focus on a chosen plane in a thick specimen while rejecting the light that comes from out-of-focus regions above and below that plane. The CLSM images were obtained directly from archaeological teeth. Each tooth was placed on a slide with plasticine and the tip of the cusp was placed directly perpendicular under the lens with immersion oil and a coverslip. A second drop of oil was placed between the coverslip and the objective lens.

Measurements of prism spacing followed the methods developed by Fosse (Fosse, 1968a-e). Briefly stated, up to 10 of each of four linear and one area measurements were collected randomly from each image. Three linear measurements (x, y, and d) were used to calculate the average ameloblastic cross-sectional secretory area (ASA), and the estimated prism density (EPD). Prism diameter (PD) and prism area (PA) data were collected to provide an estimate of prism size. To obtain an estimate of the prismatic matrix (PM), prism area was compared with the average ameloblastic area for the same region (PA/ASA x 100) (see Grine et al., 1987) for a detailed description of the formulae for these calculations). All measurements were analyzed with Scion image (a modified version from NIH image) and Lasersharp Software. In addition, depending on the

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alignment and packing of the ameloblasts, one of the three basic developmental prism pattern configurations (described by Boyde, 1965) was documented for each specific region.

Intra-observer error was assessed for both crown and enamel prism measurements. This was checked by repeated measures of 10 randomly selected teeth. The mean intra-observer measurement difference was 0.05 mm for crown measurements and 0.06 mm for enamel prism measurements. Statistical tests were performed using SPSS 8.0 for Windows 95.

RESULTS

For the recent archaeological samples the laser light was reflected from the outer surface of the tooth to about 100 to 180 µm (depending on each tooth) under the surface. Figure 1 illustrates prism shape for three different depths and shows the well-known prismatic structure of enamel. Distinction was made between prisms and interprismatic enamel. Pattern 3 prisms according to Boyde's (1964) classification were observed in all of the enamel surveyed. No significant differences were found among superficial, intermediate and deep reflections in ASA, EPD, PA and PA/ASA x 100 in either cusp (data not shown).

Since we are interested in looking at the changes between final tooth size and enamel formation we examined:

a) Prism size and spacing on the mesio-buccal and distal cusps of fully formed teeth.

b) Whether these prism parameters differ according to tooth size.

Student's t-test and non-parametric tests were used to examine the null hypothesis that there is no change in prism area or density between the mesio-buccal and distal cusps and that there is no correlation to tooth size.

Table 1 shows the mean values of prism parameters found in the mesio-buccal and distal cusps. Significant differences were found in ASA($P<0.01$), EPD($P<0.02$) and PA($P<0.02$) between both cusps and no significant difference was found in PA/ASA. Mean ASA and PA on the mesio-buccal cusp were larger than those of the distal cusp. Figure 2a shows the scatter plot between ASA against the crown area on both cusps. ASA on the distal cusp increases together with an increase in crown area. Linear regression analysis shows a significant if low correlation ($R^2=0.41$) between ASA and crown area in the distal cusp, but none for the mesio-buccal cusp where ASA does not increase. The value of EPD decreases as the crown area increases on both cusps (data not shown). This result was expected since ASA and EPD are intimately related. PA on both cusps increases as crown area increases (Fig. 2b). Linear regression analysis shows a significant if low correlation ($R^2=0.20$) for the distal cusp but there is no correlation between PA and crown area for the mesio-buccal cusp. To summarize, there is an increase in ASA and PA as the crown area increases, and both prism parameters, ASA and PA appear to be larger for the MB cusp than the D cusp.

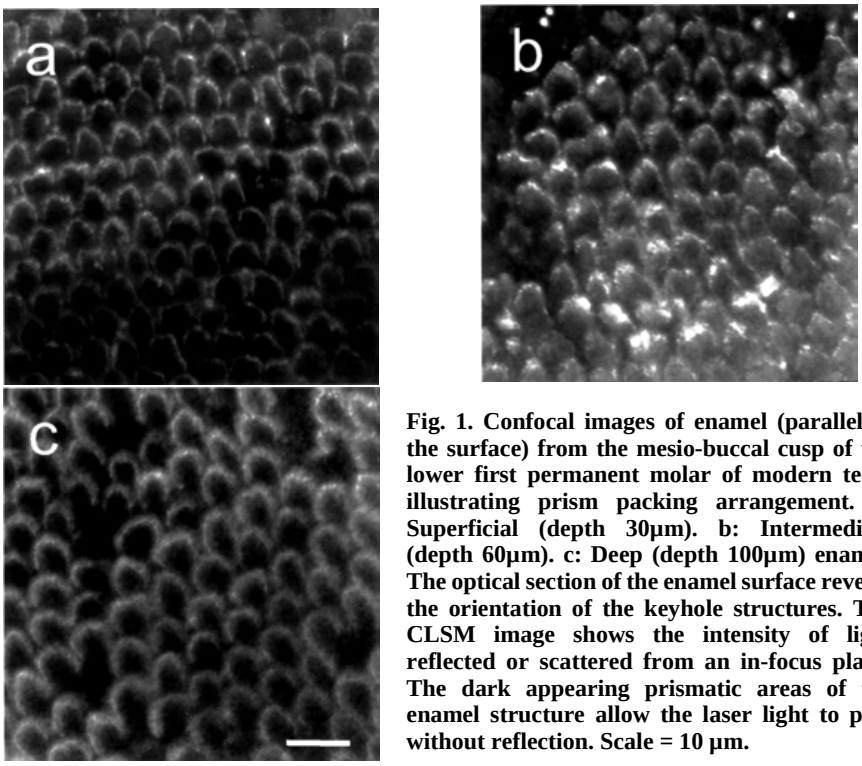


Fig. 1. Confocal images of enamel (parallel to the surface) from the mesio-buccal cusp of the lower first permanent molar of modern teeth illustrating prism packing arrangement. a: Superficial (depth 30µm). b: Intermediate (depth 60µm). c: Deep (depth 100µm) enamel. The optical section of the enamel surface reveals the orientation of the keyhole structures. The CLSM image shows the intensity of light reflected or scattered from an in-focus plane. The dark appearing prismatic areas of the enamel structure allow the laser light to pass without reflection. Scale = 10 µm.

Table 1. Comparison between ASA (ameloblast secretory area). EPD (estimated prism density), PA (prism area) and PA/ASA X 100[a].

	ASA (µm2)[b]			EPD(# per µm2)[b]			PA (µm2)[b]			PA/ASA X 100		
Cusp	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Mesio-buccal (n=18)	42.80	11.04	67.68-26.47	23805	4760	35284-14015	33.90	6.15	44.70-22.25	81.98	15.87	109.06-58.36
Distal (n=18)	37.00	6.58	46.41-20.72	26331	4993	39612-19031	30.99	5.39	46.88-21.47	82.43	11.44	102.75-60.28

[a]See text for explanation on prism parameters. [b]t-test, P<0.02. n=number of teeth. Means are based on the average of the superficial, intermediate and deep reflection images.

To further study if the prism parameters differ according to tooth size and/or cusp type, the relationship between tooth size and enamel prism spacing was examined. We have taken the value of 111 mm^2 (crown area, see Figure 2) as the cut-off point for dividing small and large teeth. There was a significant difference between small and large teeth on the distal cusp for ASA(P<0.03), EPD(P<0.01) and PA(P<0.01). On the mesio-buccal cusp PA(P<0.03) and PA/ASAx100 (P<0.03) (Figure 3). Our results suggest that large teeth have larger PA and ASA than small teeth.

To study enamel microstructure in fossil hominid teeth from Israel, confocal images were obtained from the mesio-buccal and distal cusps of teeth from Qafzeh. Figure 4 illustrates the appearance and quality of the confocal images obtained from the specimens of Qafzeh. From the outer surface of the enamel tooth to about 100 µm under the surface,

dental enamel was recorded by the CLSM as a horse-shoe microstructure. The interior of the prisms appears dark and the surrounding interprismatic margins are brighter due to the reflection of the laser light on the mineralized tissue as seen in Figure 1.

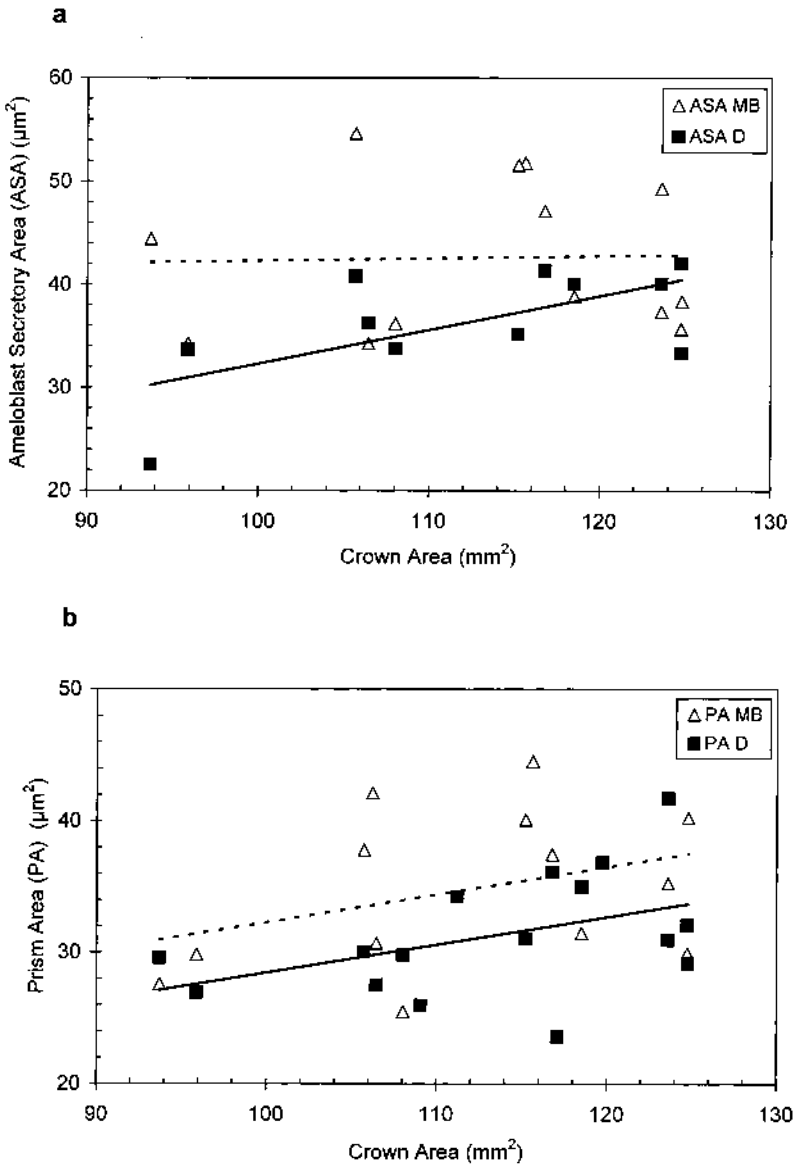


Fig. 2. Plot of correlations between prism parameters and crown area for the mesio-buccal (MB) and distal (D) cusps. a: Ameloblast secretory area (ASA) and crown area. b: Prism area (PA) and crown area. Note tooth size: small teeth include all teeth in which crown area is below 111 mm^2 and large teeth are above this value. Means are based on the average of superficial, intermediate and deep reflection images.

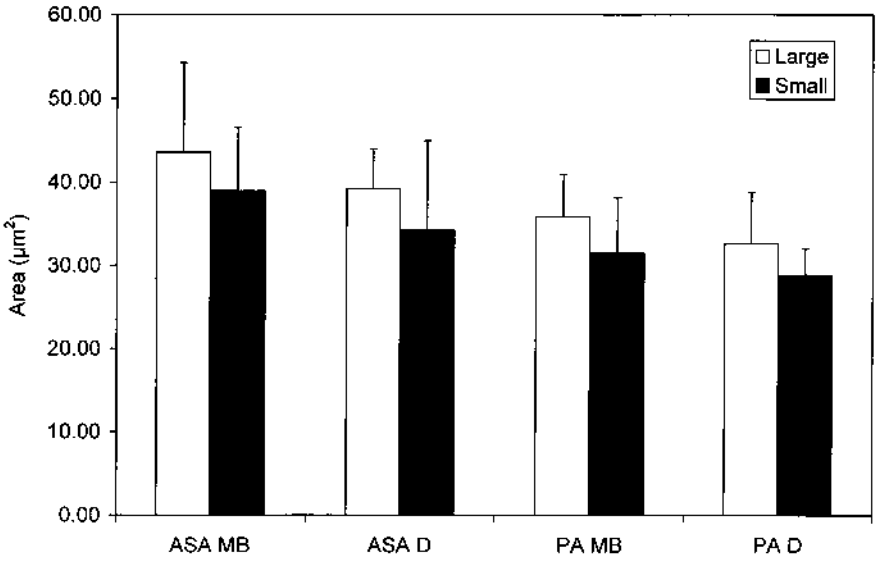


Fig. 3. Histogram showing the differences in ASA and PA of the mesio-buccal and distal cusps for large and small teeth. Means are based on the average of superficial, intermediate and deep reflection images. Small teeth, n=8, large teeth, n=10.

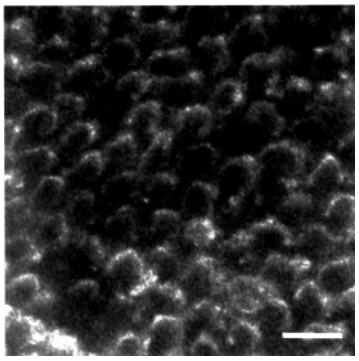


Fig. 4. CLSM image of enamel (superficial depth) from the distal cusp of a mandibular first permanent molar from Qafzeh (depth 30µm). Scale=10 µm.

DISCUSSION

Mature enamel has a complex three-dimensional structure (Hillson, 1986; Osborn, 1981). The enamel prism packing patterns reflect the past history of the position and movements of ameloblasts providing information about growth patterns in tooth development (Boyde, 1990) and on the relationships of fossil species to one another and to living forms (Boyde, 1989). Thus, the development of teeth is permanently recorded in their microstructure. This research, used as an exploratory study to analyze growth patterns of enamel, illustrates the potential of the non-invasive technique of confocal laser scanning microscopy as a means of examining the microstructure of recent and fossil human teeth.

The present study sample comprises 18 modern teeth. Enamel prisms were not visible in two teeth and this fact probably depends on the state of preservation of the teeth

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sampled. Furthermore, there is a big variation on the color of the crown of teeth sampled (from white to brown). Although no relation may exist between the external gross morphology and preservation of archaeological bone (Bell and Jones, 1991) there may be a correlation between external morphology, tooth microstructure and the ability of acquiring confocal images from teeth. Analysis on the relationship between the state of preservation of teeth and quality of images need to be further investigated.

Our working hypothesis is that the microstructure of enamel in modern teeth determines the macrostructure (size and form) of the mineralized mature teeth. We found that the mesio-buccal cusp contains prisms at a lower density and of larger size than the prisms on the distal cusp. Based on our results we propose that the decrease in tooth size may be the result of a reduction in ameloblast secretory area and prism area.

The novel CLSM technique for investigating the microstructure of teeth opens a wide field of three-dimensional, non-destructive analysis to study evolutionary growth and developmental processes in mineralized tissues of modern and fossil hominid teeth.

ACKNOWLEDGMENTS

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Dental Characters of the Pleistocene Hippopotamuses of Western Europe

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INTRODUCTION

Three hippopotamuses occurred in Western Europe during the Pleistocene: *Hippopotamus antiquus*, *H. tiberinus* and *H. amphibius*. The earliest occurrence of *Hippopotamus* in Western Europe is usually said to be represented by that of *H. antiquus* in the Lower Pleistocene layers of the Upper Valdarno (Central Italy) (Blandamura and Azzaroli, 1977; Faure, 1985; Mazza, 1991, 1995). Nevertheless, this first occurrence datum of the genus in Europe is doubtful. All the known hippopotamus remains from Upper Valdarno are part of ancient collections, of which we lack a detailed stratigraphic record. Hippopotamus bones were never recovered in recently discovered sites of the Upper Valdarno, such as Casa Frata (De Giuli and Masini, 1986), nor were they ever found *in situ* throughout the basin as isolated elements. The most ancient remains of *Hippopotamus* (*Hippopotamus antiquus)* found *in situ* are thus, at the moment, the ones found at Colle Curti (Colfiorito Basin, Central Italy) (Mazza, 1995) and Untermaßfeld (Eastern Germany) (Kahlke, 1984, 1985a, b, 1986, 1987, 1991, 1992), two sites more or less contemporaneous dated to the latest Early Pleistocene. Faure and Guérin (1979) and Faure (1983, 1985) stated that hippopotamuses were absent other parts of Western Europe during the Villafranchian, and Sutcliff (1985) stressed that in Great Britain hippopotamus is not known before the Cromerian. The present state of knowledge thus allows us to suppose that *Hippopotamus* probably was missing from the Villafranchian of all Western Europe and perhaps represents one of the newcomers which started to move into Europe at the very end of the Early Pleistocene, marking the so-called Villafranchian-Galerian turnover.

Hippopotamus antiquus is well represented during the first half of the Middle Pleistocene. At this time these pachyderms ranged from Spain as far east as Western Russia and Hungary and from Great Britain as far south as Italy and Greece.

At the very end of the Early Pleistocene another hippopotamus appeared in Western Europe, *H. tiberinus*, a species derived directly from *H. antiquus* or, if the late Early Pleistocene arrival of *H. antiquus* should be confirmed, a possible offspring from a stock, stemming from the *gorgops* stock of Africa, from which both *H. antiquus* and *H. tiberinus* had arisen (Mazza, 1991, 1995). *H. tiberinus* was smaller-sized than *H. antiquus*, and shows affinities with *H. gorgops* in its skull and with *H. antiquus* in its postcranial skeleton. Its known stratigraphic distribution ranges from the late Early Pleistocene to the early Late Pleistocene. Remains of this species are known, until now, only from Italy and Germany.

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The Eemian is marked by the abrupt and brief occurrence of *H. amphibius* which rapidly dispersed as far north as Great Britain and then as rapidly retreated as the ices of the last glaciation expanded. *H. amphibius* was represented by relatively large-sized specimens, larger in the average than those living today.

DESCRIPTION

Skull and Jaw Characteristics

The skulls of *Hippopotamus antiquus* and *H. tiberinus* are elongate and slender, compared to the shorter and more massive one of *H. amphibius*. The structure of the postorbital region and the arrangement of the nuchal crest are highly diagnostic. In *H. tiberinus* the postorbital region is very short, the occipital plane inclines forwardly and the nuchal crest is uplifted, the occipital condyles are very prominent, the parietal profile is steep, the sagittal crest is short and the temporal fenestrae are short as well and slope markedly downwards. In *H. antiquus* the postorbital region is more elongated, the occiput is more or less vertical, the occipital condyles are slightly prominent, the parietal profile slopes forwardly and the temporal fenestrae are more stretched backwards. In *H. amphibius* the postorbital region is elongated, the occiput inclines forwards and the occipital condyles are prominent, the nuchal crest projects backwards, the parietal profile slopes gently forwards or is almost horizontal and the temporal fenestrae are elongate. The orbits are faced slightly more anteriorly in *H. antiquus* and *H. tiberinus* than in *H. amphibius*. The mandible of *H. antiquus* has massive, but elongate horizontal rami, with flat or slightly concave basal profiles and the ascending rami are fairly low and show slightly protruding posterior profiles. Few fragmentary mandibles of *H. tiberinus* are known at the moment, but they recall the mandibles of *H. antiquus* in many respects, the only marked differences being the smaller size and more slender overall appearance, the narrower ascending rami and the coronoid processes slightly more inclined forwards. *H. amphibius* is characterized by having short and massive horizontal rami, with markedly convex basal profiles, and higher ascending rami, characterized by having the rear profile so convex as to protude considerably beyond the condyle. The coronoid processes are more elongated and the angular processes are more projected forwards than in *H. antiquus* and *H. tiberinus*. On the other hand, the rostral fan is comparatively broader in *H. amphibius* and in *H. tiberinus* than it is in *H. antiquus.*

Anterior Dentition

The anterior tusks of the European Pleistocene hippopotamuses are generally considered highly diagnostic for discriminating between the species. This commonly accepted opinion must be disproved. Most of the analyses of variance and of the principal component analyses performed on an overall sample of over 300 anterior tusks revealed no significant differences between the European Pleistocene species. The analyses of variance revealed a highly diagnostic difference only in the sample of the upper canines, *H. antiquus* having, compared to *H. tiberinus* and *H. amphibius*, the antero-posterior diameter of the upper canines which grows slower, as size increases, than the latero-medial diameter (Figs. 1 and 2). However the size ranges of the upper canines of the three

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species partly overlap. Lower canines are held to be very diagnostic. The size ranges of the lower tusks of the three named species amply overlap and the statistics performed on these teeth revealed low levels of significance. Another emphasized character, the arrangement and development of the enamel ridges and grooves on the two sides of the lower canines, was found to be frustratingly insignificant, all the possible morphotyes being shared, at variable frequencies, by all three the named species. The lower incisors are powerful in both *H. antiquus* and *H. amphibius*, while the second lower incisors tend to be comparatively weaker in *H. tiberinus*.

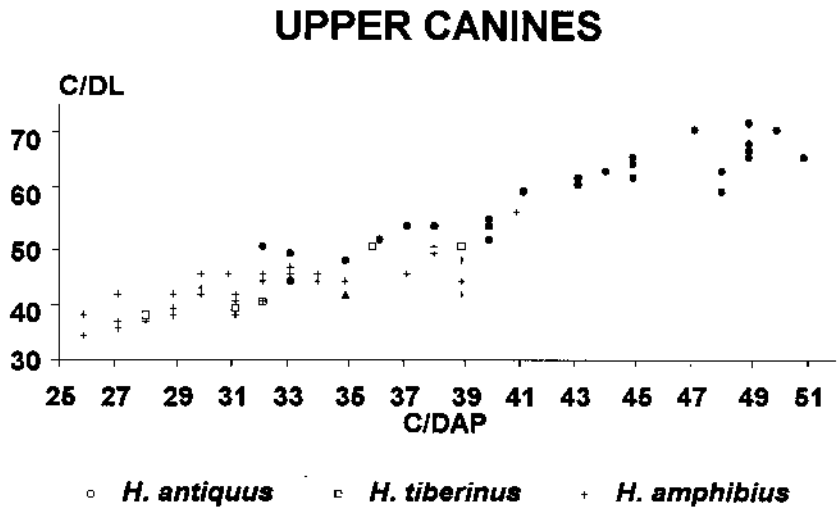


Fig. 1. Bivariate distribution of the antero-posterior diameter (C/DAP) vs. the lateral diameter (C/DL) of the upper canines.

Given the role played by tusks in confrontations between males, one may expect to find dimorphic differences in anterior teeth samples. However, Mazza (1995) observed that in none of the anterior tusk samples he had examined was there any particular clustering for size or morphologic characters. Startling was that in his lower canine sample the largest specimen of *H. amphibius* belonged to a female.

Cheek Teeth

The molar rows are more arcuated in *H. tiberinus* than in both *H. antiquus* and *H. amphibius*, and the palate is also narrower between the first molar alveoli. *H. antiquus* has proportionally longer premolar rows compared to *H. amphibius*. Its upper premolar row/upper molar row ratio ranges from 0.71 to 0.97 with a mean of 0.87. In *H. amphibius* the ratio ranges from 0.69 to 0.84 with a mean of 0.75. The ratio of the type skull of *H. tiberinus*, n. 321 from Maglianella, kept in the Museum of Natural History of Calci (Pisa), the only specimen of this species with complete cheek tooth rows, is 0.70. The importance of these ratios is however doubtful. A character which is usually held to be highly diagnostic is the presence and extension of the diastema separating the second and third premolars. This diastema is generally said to occur and to be well developed in *H.*

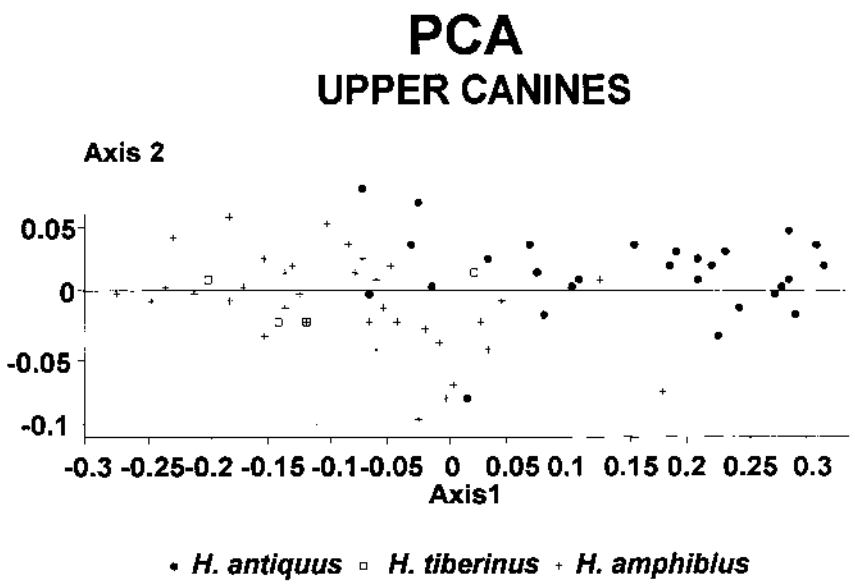


Fig. 2. Principal component analysis of the upper canines.

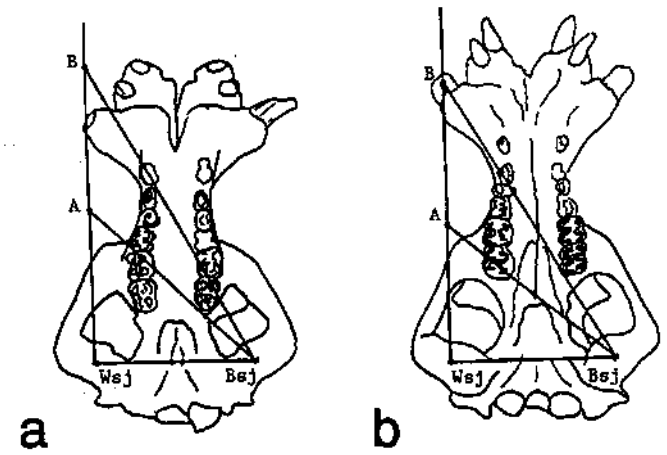


Fig. 3. Greaves' (1980) scheme applied to a) young and b) adult hippopotamus skulls (not to scale): AB = expected tooth row; Wsj = working side joint; Bsj = balancing side joint.

antiquus, while it lacks, or is slightly developed in *H. amphibius* and therefore it is considered an important means for discriminating between the two species (Bandamura and Azzaroli, 1977). Mazza (1995) has shown that diastemas tend to form as a hippopotamus ages. This is readily evident applying Greaves' (1980) schemes to the skulls of young, sub-adult and old adult specimens. Teeth at the highest efficiency fall within the triangle A-B-Bsj, A-B being the expected tooth row and Bsj the balancing side

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joint. In young and sub-adult specimens (Fig. 3, a), the premolar row is enclosed between the molar row, at its rear end, and the ideal line connecting the mesial extremity of the expected tooth row with the balancing side condyle at the fore end, this ideal line marking the limit beyond which the cheek teeth rapidly lose their efficiency. The premolar row is therefore confined between two rigidly established points. In these specimens the third upper molar, which is not yet emerged or is erupting, still lies outside the efficiency area A-B-Bsj. The third molars actually erupt quite late in these animals. It is not infrequent to find not yet completely erupted third molars associated to other already considerably worn cheek teeth. In young specimens a diastema is usually present between second and third premolars. As growth proceeds this diastema disappears but it may form again in aged individuals. Compared to young individuals, old hippopotamuses generally have completely worn-down second premolars, fairly worn third molars and a part of palate and of the horizontal rami of the mandibles extended behind the rear end of the third molars. In aged specimens the second premolars lie outside the efficiency area A-B-Bsj, while third molars fall within it (Fig. 3, b). It is therefore apparent that hippopotamus tooth rows shift progressively forwards as the animals age. This prolongs the efficiency of the dentition and consequently also the life span of the individuals. Researchers must be well aware of the deep ontogenetic modifications which occur in hippopotamuses. Caloi et al. (1980) provided a full complement of other age-dependent characters which can complete the picture.

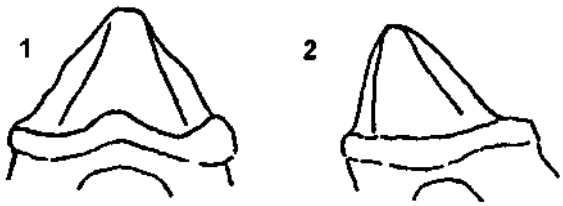


Fig. 4. Outlines of 1) upper and 2) lower premolars in latero-medial view. Not to scale.

Like in most mammals, the upper molars of hippopotamuses are more brachyodont than the lower ones. The first upper premolar may rarely be found, while the first lower premolar is constantly lacking. However, it is frequent to find only vestiges of the alveoli of these teeth. *H. antiquus*, *H. tiberinus* and *H. amphibius* have fairly similar upper premolars. The upper premolars of *H. antiquus* can be distinguished only for their very large size, which exceeds those of the upper premolars of both *H. tiberinus* and *H. amphibius*. *H. antiquus*, however, has a comparatively small fourth upper premolar.

On the other hand, these species are separated by marked dimensional and proportional differences in the lower premolars (Fig. 4). The premolar row/molar row ratio ranges from 0.67 to 0.90, with a mean of 0.77, in *H. antiquus*; from 0.59 to 0.79, with a mean of 0.67, in *H. amphibius*; it is about 0.7 in the mandible of the type skull of *H. tiberinus*, C. 601 from Maglianella (Central Italy) preserved in the Museum of Natural History of Calci (Pisa). *H. antiquus* has the largest-sized lower premolars. The second premolars of this species are proportionally more elongated than those of both *H. amphibius* and *H.*

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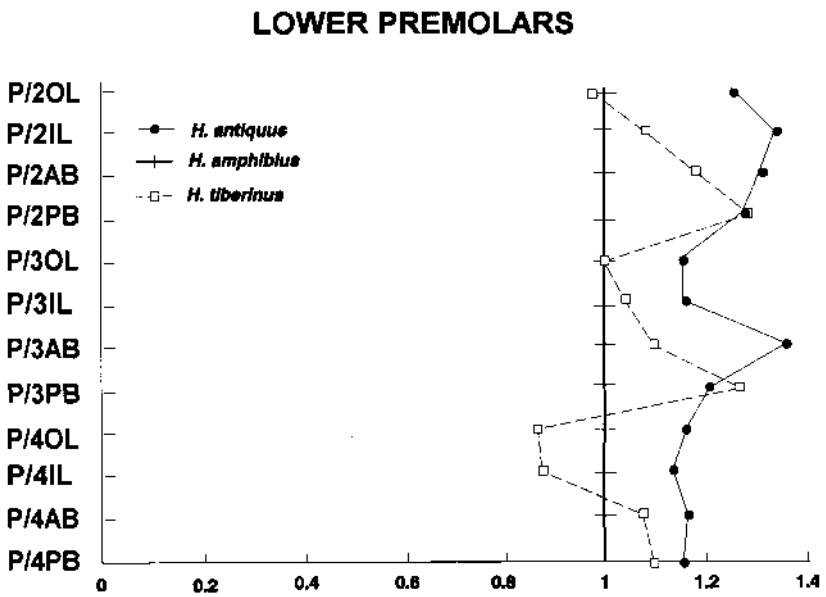


Fig. 5. Ratio diagram of the lower premolars. *Hippopotamus amphibius* used as reference. P/2: second lower premolar; P/3: third lower premolar; P/4: fourth lower premolar; OL: outer length; IL: inner length; AB: anterior breadth; PB: posterior breadth.

tiberinus, while the third premolars are wider than those *H. amphibius*. The fourth lower premolars of *H. antiquus* are large-sized replicas of the fourth premolars of both *H. amphibius* and *H. tiberinus*. The latter species has unusually wide lower premolars, compared to both *H. antiquus* and *H. amphibius*. Its fourth lower premolars, in particular, are shorter mesio-distally and broader latero-medially than those of the other two species. The proportions of the upper molars are similar in the three species, although *H. tiberinus* tends to have upper molars somewhat more slender than *H. amphibius*. *H. antiquus* and *H. tiberinus* have upper molars of comparable size; this means that *H. tiberinus* shows a peculiar combination of relatively small-sized premolars and large-sized molars, as opposed to both *H. antiquus* and *H. amphibius* which show a less marked dimensional difference between the premolar and the molar row. In the lower molar row, *H. tiberinus* has an unusually large first lower molar, larger than in both the other species, and a comparatively short third lower molar, while *H. antiquus* has a large third lower molar with a very robust posterior tubercle.

From the morphologic point of view, the cheek teeth of hippopotamuses show fairly repetitive patterns. Distinguishing isolated premolars may not be easy. Upper premolars have a more symmetrical triangular outline than lower ones (Fig. 5). This of course applies to all premolars but the upper fourth one, which appears roundish in occlusal view and bears a cruciform cusp which is the result of the fusion of two or even three distinct trefoil-shaped cusps. Fourth upper premolars are always rotated lingually. Other

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premolars which may be slightly rotated lingually are the third upper premolar, in *H. tiberinus*, and the fourth lower premolar, in *H. antiquus* and *H. amphibius.*

Isolated molars are also hard to distinguish from one another. Like in most mammals, the upper molars are broader than the lower ones which in turn are more slender and more rectangular in occlusal view. Molars may be distinguished on the basis of their absolute size, proportions and degree of wear. The first molars, for example, erupt much before the second and the third and they are therefore normally quite worn; second and third upper molars tend to be more trapezoidal than first upper molars in occlusal view; etc. On the other hand upper and lower molars share four trefoil cusp(id)s arranged more or less in same way, the lingual cusp(id)s converging occlusalwards towards the labial cusp(id)s, the anterior cusp(id)s straight but inclined slightly forwardly and the posterior cusp(id)s arcuated forwardly. The outlets of the valley between the mesial and the distal cusp(id)s may be either V- or U-shaped. In upper molars the labial outlets are often V-shaped and the lingual U-shaped in *H. amphibius* and both are U-shaped in *H. antiquus*. In lower molars U-shaped valleys are frequently observed in the first and second molars of *H. amphibius*, while in third molars V-shaped valleys tend to prevail. In the lower molars of *H. antiquus* this feature is highly variable. The cheek teeth of the presently known *H. tiberinus* specimens are in most cases too heavily worn to establish a dominant pattern. Another peculiar character of the European hippopotamuses is the so-called “comma-shaped” pattern (Mazza, 1995). This wear feature derives from a reduced development or from a complete absence of the posterior style of some cusp(id)s, so that the trefoil wear pattern results incomplete, giving the cusp(id) a comma conformation. The hypocone of third upper molars of *H. antiquus* and *H. tiberinus* is often comma-shaped, as also are the entoconid and, but rarely, the hypoconid of the third lower molars of fossil representatives of the three species; in present-day *H. amphibius* specimens comma-shaped cuspids are not very common.

Finally, a feature which is normally disregarded in the literature on hippopotamuses is the presence of cementum. This feature is not very common, but it does occur and deserves mentioning. The cementum is generally a thin veneer which covers the lower half of the cheek teeth crowns; a thick cementum cover is only occasionally observed. When present, the cementum is usually very thin on premolars. Only the third upper premolars and the third and fourth lower premolars of present-day *H. amphibius*, but more frequently of fossil representatives of this species, show variable (sometimes considerable) thicknesses of cementum. Commonly the molars of European hippopotamuses are free from cementum or bear a thin cementum cover. However, thick cementum may occasionally be found on the molars of *H. tiberinus* and *H. amphibius*, and especially in fossil representatives of the latter species. On the contrary, thick cementum is very rare on the cheek teeth of *H. antiquus*. At times, particularly in fossil representatives, cementum increases moving from the first to the third molars.

Anatomic-Functional Considerations

The cranial and jaw characters detailed in the previous sections shed light on the possible life habits of the European hippopotamuses. The skull may be assimilated to a lever with the fulcrum placed in correspondence with the occipital condyles (Fig. 6). In this lever system the power arm stretches progressively as the nuchal crest grows vertical; the effort

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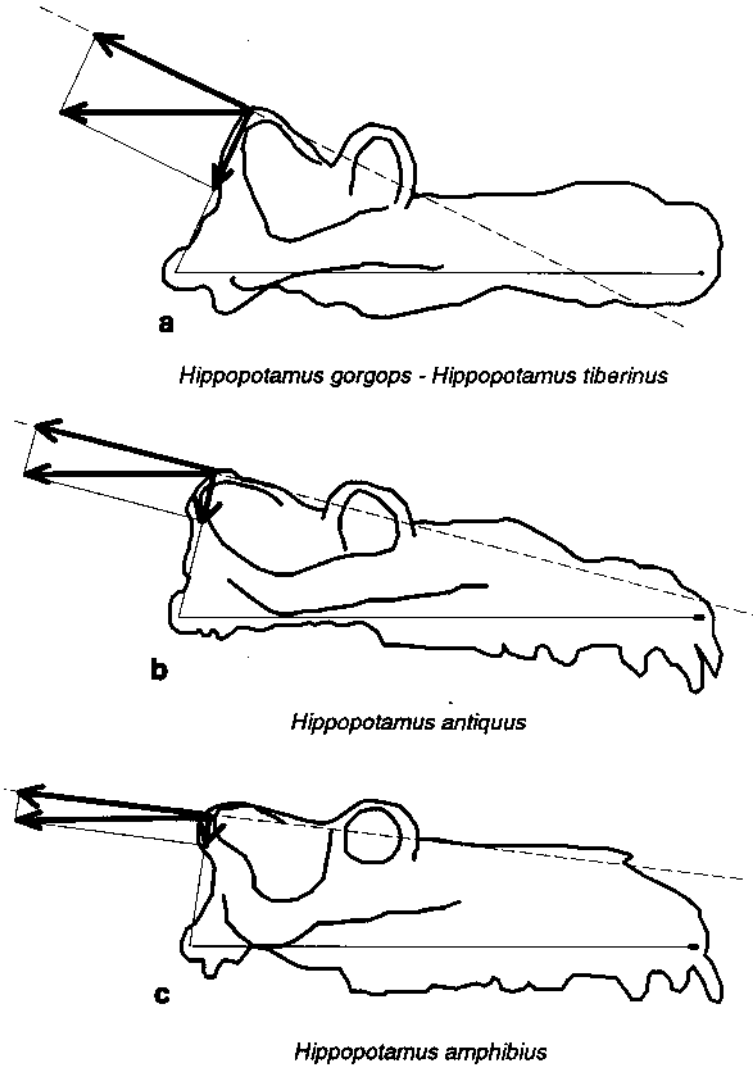


Fig. 6. Scheme depicting the forces applied to the nuchal crest-occipital condyles-muzzle lever system in the skulls of a) *Hippopotamus tiberinus - H. gorgops*; b) *H. antiquus*; c) *H. amphibius*. Not to scale.

of heaving the head correspondingly decreases. Comparing the skulls of the three species it is apparent that *H. tiberinus* had the shortest power arm, *H. amphibius* has the longest and *H. antiquus* had an intermediate condition. Therefore heaving the head was perhaps more effort for *H. tiberinus* than it was for *H. antiquus* and even more than it was for *H. amphibius*. This characteristic seems to be correlated with the relative dimension of the anterior tusks: hippopotamuses with more extended power arms in their cranial lever system can support larger-sized anterior tusks. *H. ampibius* is actually equipped with very robust tusks. The tusks are used by living hippopotamuses either as a deterrent in

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ritualized confrontations or as true weapons whenever these teeth are not enough persuasive. The aggressivity of living representatives is renowned. On the basis of the relative cranial and dental structures of the fossil representatives it can be supposed that intraspecific confrontations may have been an important means of hierarchic regulation also amongst the *H. antiquus* populations, while *H. tiberinus* was perhaps a less aggressive form or had a different ritualization of fights.

Finally, the distribution of the cementum on the teeth may provide information on the feeding habits of these animals. The common absence or slight amount of cementum on the molars of the European hippopotamuses tells us that these Pleistocene representatives perhaps fed mainly on plants with low silica content, as opposed to the living *H. amphibius*, which crops on abrasive grasses, as most probably also the fossil *H. amphibius* did. A thickening of the cementum is perhaps a first, rapid adaptive response to an increased content of abrasive items in the diet which may prelude to the development of a more hypsodont lateral dentition.

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Multidisciplinary Techniques for the Study of Tooth Fracture Behavior

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INTRODUCTION

Masticatory efficiency depends upon the ability of the dentition to apply concentrated bite forces to ingested foods while simultaneously resisting the dental stresses that may cause enamel fracture. Morphometric studies of the dentition indicate that tooth shape may match the physical properties of the most important dietary components such that dental features focus biting forces to the degree necessary for the fracture of specific foods (Lucas and Luke, 1984; Strait, 1993). For example, hard and brittle foods, such as hard-shelled nuts or invertebrates, may be more easily fractured with blunted tooth cusps and tough and elastic foods, such as vertebrate tissues and grasses, require sharp tooth edges (Lucas and Teaford, 1994). Over the lifetime of an individual, however, stress concentrations in the dental tissues may degrade tooth structure and occlusal shape, consequently altering the interaction between tooth and food as well as masticatory efficiency. Thus, the response of dental tissues to high stress concentrations emerges as an important component of masticatory function over an animal's lifetime. This chapter discusses how human tooth cusps respond to compressive loading, as well as how descriptive studies of tooth architecture can be combined with stress analysis techniques to establish how different dentitions resist breakage and process diverse foods throughout life.

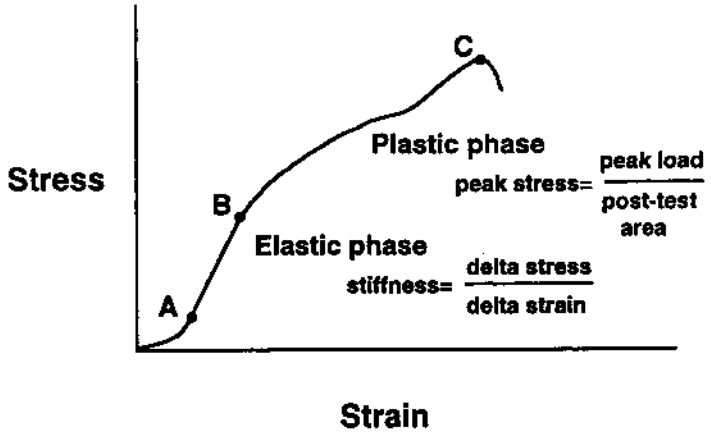


Fig. 1. Schematic stress-strain curve illustrating the elastic and plastic phases of material fracture, as well as the equations for stiffness and peak stress. Region A to B indicates the elastic phase and region B to C indicates the plastic phase. Point C corresponds with peak stress.

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MATERIALS AND METHODS

The protocone on 8 maxillary third molars was loaded compressively and the response of each cusp was recorded. Extracted teeth were stored in .9% saline prior to testing in order to prevent dehydration and the teeth were sprayed with saline solution during testing. A diamond saw was used to remove the roots at approximately 2mm below the dentino-enamel junction and produced an even base for loading the test specimen. In order to isolate the protocone, adjacent or interfering cusps were ground away with a dental drill. Each protocone was loaded compressively until failure at .5mm/min using an MTS non-hydraulic testing machine. Recorded loads and deformations were transformed into stress and strain values in order to calculate stiffness and peak fracture stress for each specimen (Fig. 1; Vincent 1992). For stiffness calculations an estimate of the loaded cusp surface area during the elastic phase was determined from a series of tests terminated at 100lbs. (444N). The area of the loaded cusp tip at 100lbs. was divided by the basal area of the tooth in order to produce a ratio of the loaded area relative to tooth size, and a mean of these ratios was determined. In subsequent uninterrupted tests the cusp tip area during the elastic phase was estimated by multiplying this mean ratio by the tooth's base. Because failure was characterized by vertical fracture rather than cusp tip crumbling, post-test tip area was used to determine fracture stresses at peak load.

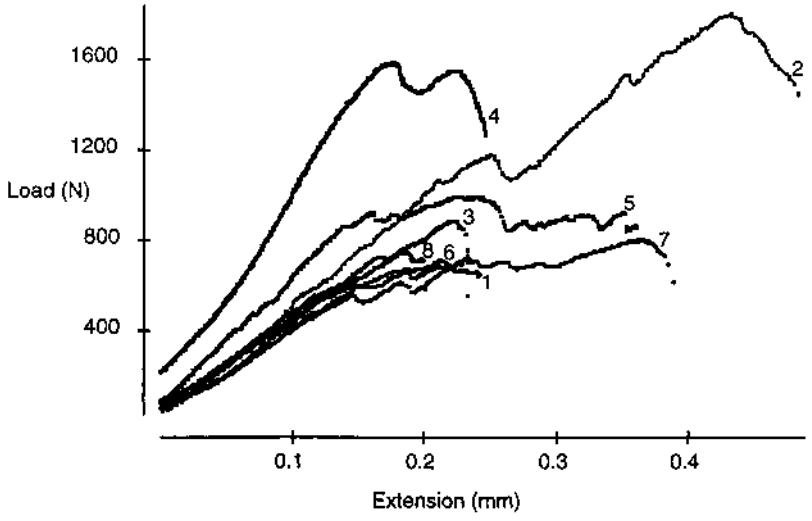


Fig. 2. Load versus extension data for compression tests of M^3 protocones.

Table 2. Stiffness values and estimated tip area loaded for the elastic phase of protocone compression testing.

Specimen	Tip area elastic phase* (mm^2)	Stiffness (N/mm^2)
1	0.25	22387.68
2	0.41	24669.84
3	0.33	27311.06
4	0.37	44025.53
5	0.32	45808.53
6	0.23	22535.02
7	0.25	18007.22
8	0.27	21820.38
Mean	0.31	28320.71
Standard Deviation	0.07	10583.85

*Estimated

Table 3. Peak stress values and loaded tip area at peak load.

Specimen	Post test tip area* (mm^2)	Peak stress (N/mm^2)
1	0.62	1106.09
2	3.23	559.89
3	0.82	1076.97
4	2.24	707.14
5	2.91	341.50
6	0.77	917.75
7	1.87	429.47
8	1.67	447.37
Mean	1.77	698.27
Standard Deviation	0.99	302.28

RESULTS

Overall, the cusps behaved with high stiffness and failed at high fracture stresses, however, substantial variation exists in the fracture behavior of the protocone under compressive loads. The load-extension data for the compressive tests indicate that each protocone initially responded to compressive loading with linear elasticity, during which the dental materials were not permanently deformed. The stiffness calculations for this phase of fracture indicate stiff behavior for each specimen; but, the high standard deviation for the calculated mean stiffness reflects high variation in the elastic behavior of the cusps (Table 1). Further loading of each cusp resulted in a transition from elastic behavior to plastic behavior. During the plastic phase the fracture recordings become irregular demonstrating the appearance of microfractures in the dental tissues. The dissimilarity of the curves in Figure 2 demonstrate the great variability in the plastic behavior of the cusps. The plastic phase ended with the total failure of the specimen following peak load, and although the stresses at peak load are generally high, they also exhibit substantial variation (Table 2.).

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DISCUSSION

The response of human tooth cusps to compressive loading is likely to be related to the condition of the dental tissues and the architecture of the cusp. A tooth may be described as a composite structure with enamel tissue covering the underlying dentine, and the characteristics of these hard tissues will determine the tooth's response to stress. For example, the presence of microfractures in the hard tissues could alter the fracture behavior of the cusp, potentially lowering the peak stresses experienced prior to failure (Kelly, 1995); thus, unobservable variations in the condition of the cusp may account for some of the variation in these data. Regardless of tissue flaws, enamel is more extensively mineralized than dentine and confers stiffness to the tooth crown (Waters, 1980). A narrow zone of dentine beneath the dentino-enamel junction (DEJ) appears even less mineralized than the remaining dentine and may have a high energy-absorbing capacity (Wang and Weiner, 1998). The relative proportions of enamel and dentine forming a cusp may therefore indicate the stiffness of the cusp under load. The great thickness of human enamel is well documented (Martin, 1985; Beynon and Wood, 1986), and may result in stiffer tooth crowns than in mammals with thinner enamel. Furthermore, variations in enamel thickness within the protocone of M^3 could also account for the high variation in stiffness observed between cusps. Describing the thickness of enamel relative to dentine may demonstrate the predicted relationship between enamel thickness and stiffness under compressive load within or between species.

The structural characteristics of enamel are also likely to affect the fracture behavior of cusps. The orientation of enamel prisms relative to applied stresses and the presence of Hunter-Schreger bands (HSB), or criss-crossing sets of prisms, have been observed to be a key feature of enamel fracture resistance in many mammals, including humans (Kawaii, 1955; Koenigswald et al., 1987; Rensberger and Pfretzschner, 1992). The prisms forming the enamel layer principally radiate from the dentino-enamel junction and can be distinguished from interprismatic material by major discontinuities in crystallite orientation (Koenigswald and Clemens, 1992). Prism boundaries are substantially weaker than prism cross-sections, and high tensile stresses perpendicular to the prism orientation are likely to generate cracks within prism boundaries (Rasmussen et al., 1976; Hassan et al., 1981; De Groot et al., 1990). Hunter-Schreger bands inhibit the propagation of cracks by expanding the radius of the crack tip and thereby reducing the stress concentration. HSB resist enamel fracture in the plane normal or oblique to the decussation plane, but offer no resistance to fracturing parallel to the decussation plane (Koenigswald, et al., 1987; Rensberger, 1993) The enamel prisms in human cusps follow a complicated course, with entwined prisms spiraling around a central prism, and this gnarled pattern may offer substantial resistance to wear and fracture (Moss-Salentijn and Hendricks-Klyvert, 1990). Variations in fracture stresses observed in human third molars could be associated with structural differences in the enamel. Scanning electron microscopy (SEM) of enamel structure provides the opportunity for examining the three-dimensional orientation of prisms and for predicting the relative fracture resistance of regions of the tooth crown, or of teeth in different species (Rensberger, 1995). SEM examination of fractured enamel surfaces may be a means of determining if structural variations in the enamel are associated with differences in fracture behavior.

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Materials testing of isolated enamel specimens can be used to test the predicted responses of different enamel structures to stress. For example, enamel specimens with a greater density of HSB may be predicted to fracture at higher stresses than enamel specimens with a lesser density of HSB. Similarly, enamels with different prism orientations may differ in their fracture behavior (Stanford et al., 1960). Materials testing has established the greater stiffness of enamel versus dentine; however, the values for elastic moduli obtained by different authors vary considerably and may be a result of structural variations in the tissue (Waters, 1980). A sample must consist of unflawed and structurally identical specimens, and the difficulties associated with specimen preparation have undoubtedly protracted research in this area. Using a standard microhardness tester, an indentation technique, the elastic modulus and fracture toughness of human enamel have been confirmed to vary according to microstructural orientation (Xu et al., 1998).

The shape of the molar crown, or in this study the specific contours of the protocone, are likely to affect the structure's behavior under stress, and strain gauge measurements may be an important means of defining the relationship between shape and stress distribution. Strain gauges are miniaturized deformation transducers that, when affixed to a surface, deform under an applied load in the same way as the structure to which they are attached. These deformations result in resistance changes in the gauge material that can be converted to strains (Swartz, 1991). Without strain gauges, a materials testing system records strains during compression of the cusp that describe the behavior of the whole lingual region of the crown; thus, it is difficult to attribute differences in strain to differences in gross morphology. On the other hand, strain gauge analyses can provide a more detailed picture of how small units of the enamel crown experience applied stresses. Because a strain gauge is applied to a discrete section of the crown, it provides information on structural deformation relative to only the enamel structure to which it is affixed. Within dentistry, strain gauge analysis has been used to assess how different clinical procedures affect the stress distribution and fracture potential of clinically altered teeth (Morin et al., 1988a; Linn and Messer, 1994; Saw and Messer, 1995; Walton et al., 1996). Future studies could measure strain in homologous regions of morphologically different teeth in order to demonstrate the effect of tooth shape on stress distribution.

Finally, the response of an intact tooth to stress forms a complex picture and finite element modeling provides the opportunity to isolate factors that affect stress distribution within tooth structure and to identify their individual and combined effects. In finite element modeling, a structure may be modeled with a simplified shape and stress regime, and the properties of different components of the model may be altered to assess their specific effects on stress distribution (Korioth and Verslius, 1997). The shape of the structure is modeled as a series of coordinates in space and these coordinates are connected into geometric elements; the elements are then assigned material properties. A stress regime is imposed upon specific elements and the deformation of elements throughout the model is recorded (Irons and Ahmad, 1980; Morin et al., 1988b). Thus, the shape of a tooth model and the material properties of the elements representing enamel and dentine can be varied to represent differences in architecture observed in mammalian teeth. Applied stresses result in enamel strains that can be attributed specifically to differences in tooth shape or in the material properties of the dental tissues. Alternatively, the tooth may be modeled realistically in order to confirm that regions of high tensile stress in the tooth enamel correspond with the observed distribution of fracture resistant

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enamel structure (Rensberger 1993, 1995). Furthermore, strain gauge measurements can be used to verify finite element models which predict a specific strain response under given stresses (Morin et al., 1988b; Amagarn et al., 1998). Replication of the applied tooth stresses in the laboratory, and strain gauge measurement of resulting enamel strains indicate the degree to which the tooth model replicates the behavior of the actual tooth under stress.

CONCLUSION

Human tooth cusps behave with high stiffness under compressive loads and sustain high stresses prior to failure. Architectural features of a tooth crown, such as characteristics of the hard tissues and cusp shape, are likely to determine fracture resistance of human cusps, as well as the observed variation in fracture behavior. Multidisciplinary techniques are proposed for the further investigation of how tooth crown architecture relates to its behavior under stress.

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Triconodont Mammals of the Aptian-Albian, Trinity Gr., Texas and Oklahoma

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INTRODUCTION

Triconodonts are of special interest in that the structure of their molars, each with three main, anteroposteriorly aligned cusps, is generally presumed to be primitive for mammals and near relatives of Mammalia, however the class is defined (Osborn, 1907; Jenkins and Crompton, 1979; Rowe, 1993; and references cited therein). The integrity of the Order Triconodonta, in which such dentally primitive taxa are conventionally placed, is problematic because of implied character contradictions among numerous anatomical systems, including the shoulder girdle (Jenkins and Crompton, 1979), tooth eruption and replacement (Jenkins and Schaff, 1988; Crompton and Luo, 1993), molar crown and occlusion patterns, basicranium, and jaw structure and ear ossicles (Allin and Hopson, 1991; Rougier et al., 1996). For this reason, we use the term "triconodont" in an informal, inclusive, nontaxonomic sense, and follow Jenkins and Crompton (1979; also Rougier et al., 1996) in their concept of the Triconodontidae, to which the species described here is referred.

Triconodonts, the most abundant and best known mammals of the Early Jurassic, are generally not very diverse, or with a few exceptions, well represented in Cretaceous faunas. *Astroconodon denisoni* Patterson (1951) from the Aptian/Albian, Antlers Fm., Trinity Group of north Texas, was described and illustrated, based upon two partial jaw rami, the holotype (FMNH PM 542) and paratype (UT TMM 1060-1). Patterson discussed it again in 1956, and in 1969 Slaughter described ten more specimens of the species (SMU 61759, 61982-4, 61988-9, 62011, 62014, and 62016-7). More recently Turnbull (1995) and Cifelli, Turnbull et al. (1997) illustrated some of these as well as other specimens from the Field Museum of Natural History, Southern Methodist University, and University of Oklahoma collections. There are now over 120 specimens of *A. denisoni* in those collections, including two recently recovered by Cifelli for the Oklahoma Museum of Natural History (OMNH 34478-9). All are very fragmentary, and all are from localities in the Paluxy Fm. or its lateral equivalent (Antlers Fm.) of Texas and Oklahoma (Fig. 1), considered to be of about the same age, see Winkler et al. (1989). Note too the greater extent of the outcrops in both states and that they also extend across southwest Arkansas, so hopefully more localities may be expected.

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CHEEK TOOTH FORMULA

Jaw fragments

We are grateful to Farish Jenkins who gave us the opportunity to compare our very fragmentary materials with the nearly complete MCZ specimens recently collected from the Cloverly Formation. This was a great help to us with tooth locus assignments.

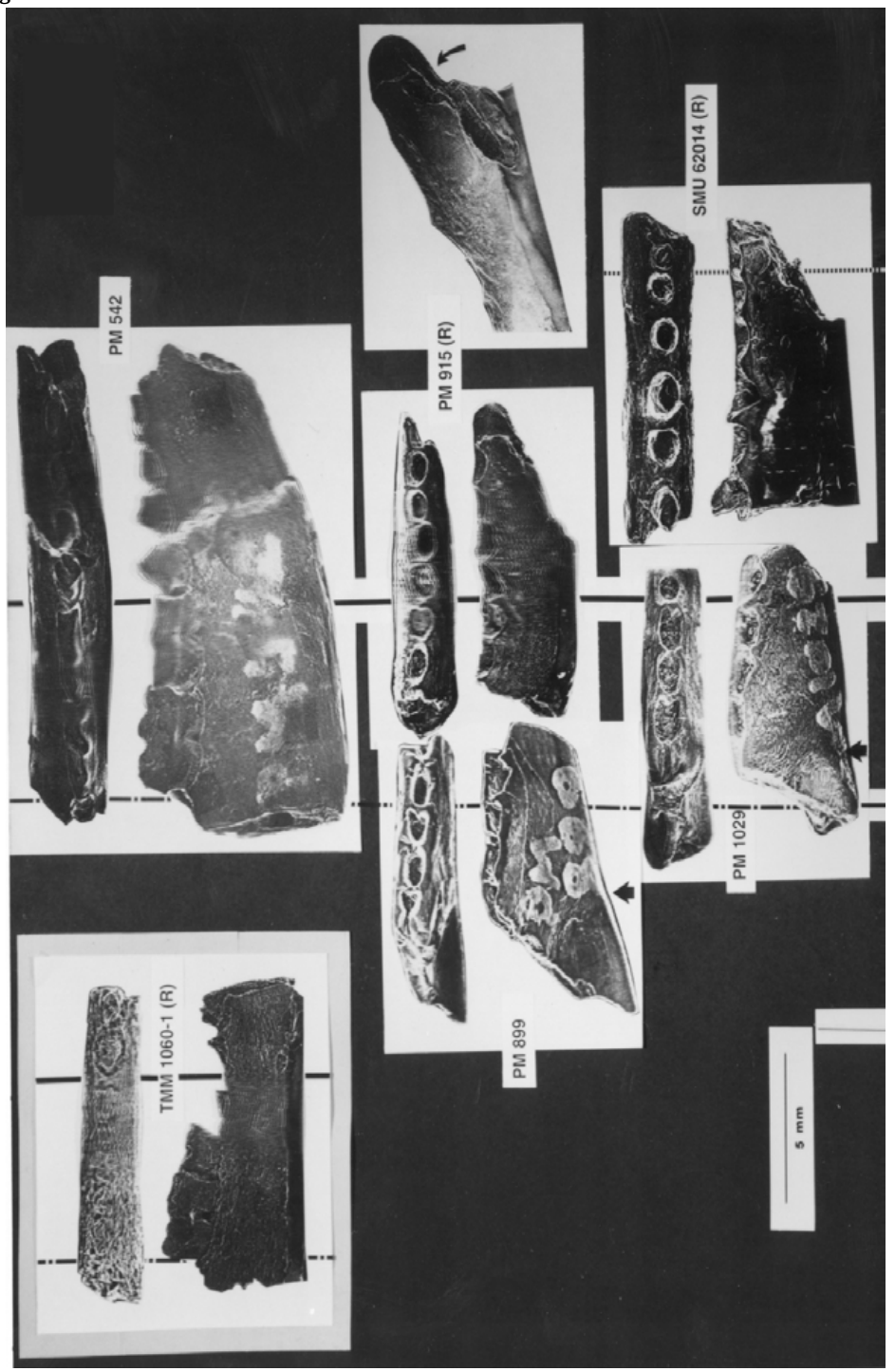


Fig. 1. Outcrop map of the Paluxy/Antlers Formations.

Based upon the few jaws with multiple alveoli and/or teeth, jaw depths at known tooth loci, and measurements of individual teeth, we are reasonably confident of the lower cheektooth formula of *A. denisoni* as being c 1, p 1-4, m 1-5. The following ramal fragments are critical for determining the lower dental cheek tooth formula, PM 542, TMM 1060-1 (both figured by Patterson, 1951, p. 43), PM 587, 588, 899, 915,1029, SMU 62014, 71744, and OMNH 34479. They constitute the core of our expanded hypodigm. All are shown at the same scale in Figure 2. Left rami are reversed (R) so as to appear as right. Vertical lines delineate tooth loci or other features, (mental foramen and the anterior edge of masseteric fossa in juvenile and adult conditions). The bold solid line marks the p4-m1 boundary which is distinguished by the more circular cross section of the posterior root of the last premolar (or its alveolus) followed by the typically grooved anterior root / alveolus, and crown features of the first molar. In 1951 Patterson noted that all *Astroconodon* molars have a distinctive tongue and groove arrangement whereby the ridge (tongue) of the posterior root and crown engages the groove of the following tooth. The dotted line between p1 and p2 calls attention to the mental foramen(a) which is (are) usually beneath p1(if single or twinned) and beneath p2 when a third is present (PM 587). Two vertical lines, dashed between m2-3, and thin between m4-5, mark those loci. An arrow marks the large lower canine alveolus. Assignments of specimens as shown are our best interpretations based in a few cases upon somewhat subjective criteria. Note the different locations of the front edge of the masseteric fossa,

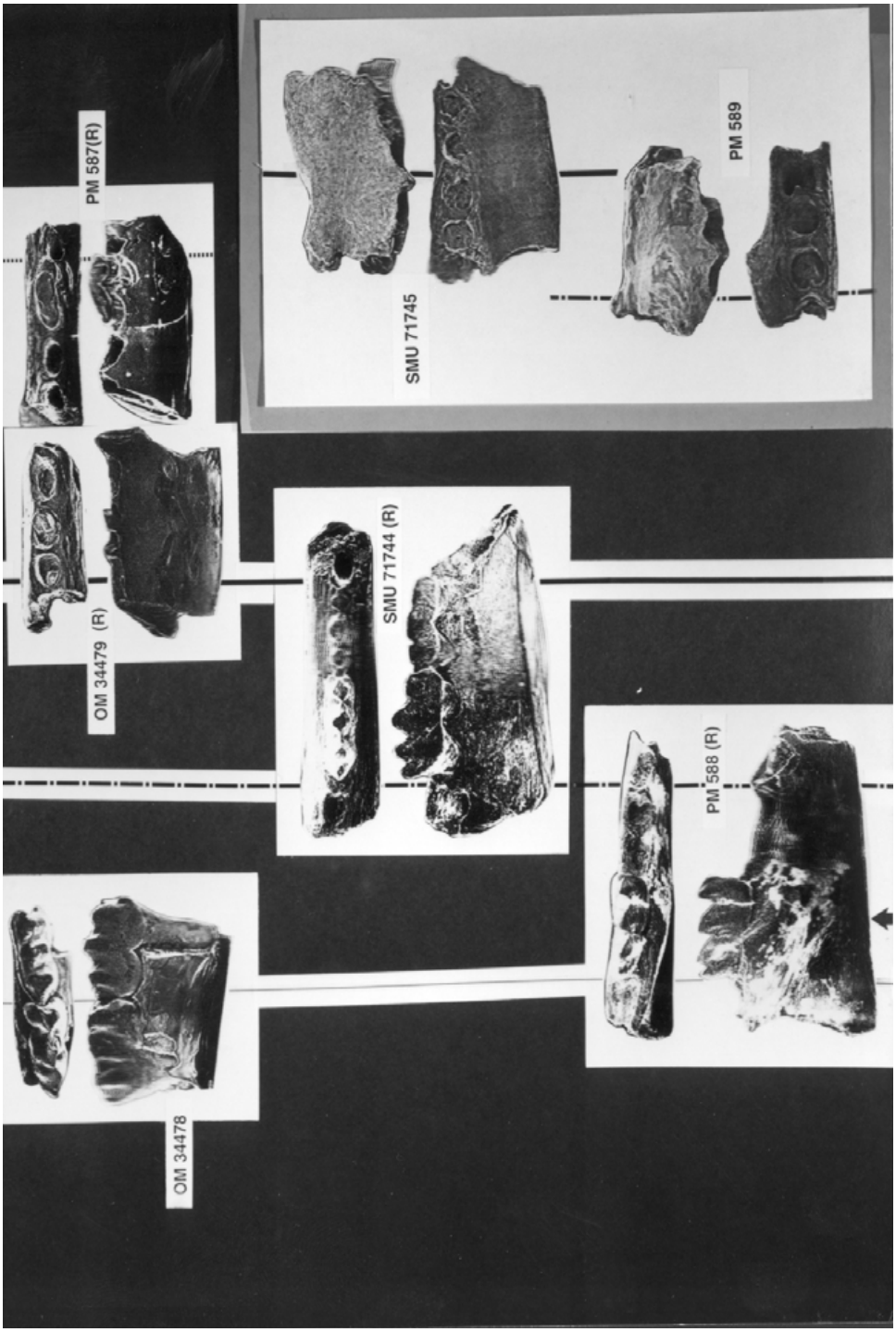
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Fig. 2.



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Fig. 2. Continued



with its bulging anterior margin (fat arrows). This is not a reliable landmark: It varies with age, and development of the jaw muscles. However, we feel that the combination of three features, great jaw depth, posterior location of the anterior edge of the fossa, and location of the m5 up on the base of the ascending ramus, all argue strongly for our assignment. Table 1 gives mean jaw depths beneath the midpoints of each tooth locus. These criteria led us to the lower cheektooth formula of *A. denisoni* (c 1, p 1-4, m 1-5). This molar formula is higher than in earlier Mesozoic forms, and is one tooth more per quadrant than Slaughter (1969) had hypothesized for *A. denisoni*. It is of interest to note that the holotype specimen is one of the largest of the lot, while the paratype, the sole other member of the original hypodigm, is among the smallest.

Table 1. Astroconodon densoni jaw and maxillary depths.

	Jaw Depth (mm)								
	p1	p2	p3	p4	m1	m2	m3	m4	m5
N, $\bar{x}$ (mean)	1, 3.4	3, 3.1	7, 3.33	8, 3.39	6, 3.73	6, 3.47	5, 4.16	3, 4.87	1, 5.4
Range	3.4	2.6-3.6	2.6-3.75	2.9-4.1	3.2-4.0	2.9-4.0	3.0-4.7	4.8-5.0	5.4
	Maxillary Depth								
	P1	P2	P3	P4	M1	M2	M3	M4	M5
Estimated Values	---	---	≈1.9	≈1.9	≈1.75 & 1.8	≈1.95	≈1.7	≈1.6	≈1.3

Table 2. Astroconodon denisoni cheektooth measures (mm).

	p1	p2	p3	p4	m1	m2	m3	m4	m5
N, $\bar{x}$ length	2,>1.8	1, 2.34	2, 2.65	2, 2.7	3, 2.67	3, 2.8	1, 3.0	---	---
Range	>1.8	2.34	2.55-2.75	2.5-2.9	2.6-2.75	2.6-2.9	3.0	---	---
Alv.[1], N, $\bar{x}$ length	1, 1.25	1, 2.2	2, 2.45	4, 2.59	2, 2.65	1. 2.7	2, 2.88	2,2.33	---
Range	1.25	2.2	2.25-2.65	2.5-2.85	2.4-2.9	2.7	2.7-3.05	2.25-2.4	---
N, $\bar{x}$ width	2, 0.53	1, 0.75	2, 0.95	3, 0.92	3, 0.87	4, 0.93	2, 1.08	---	---
Range	0.5-0.55	0.75	0.85-1.05	0.85-1.0	0.7-1.0	0.8-1.0	0.95-1.2	---	---
Alv.[1] N, $\bar{x}$ width	2, 0.73	3, 0.74	3, 0.83	5, 0.92	5, 0.81	4, 0.90	2, 0.80	3, 0.92	1, 0.7
Range	0.7-0.75	0.63-0.85	0.75-1.0	0.7-1.1	0.7-1.0	0.75-1.0	0.75-0.85	0.75-1.0	0.7
	P1	P2	P3P	P4	M1	M2	M3	M4	M5
N, $\bar{x}$ length	1, 2.21	---	2, 2.4	1, 2.46	2, 2.34	2, 2.9	1, 2.99	1, 2.9	1, 2.9
Range	2.21	---	2.39-2.41	2.46	2.23-2.44	2.7-3.0	2.99	2.9	2.9
N, $\bar{x}$ width	6, 0.56	3, 0.6	3, 0.77	5, 0.9	2, 0.87	2, 1.8	1, 1.19	1, 1.2	1, 1.26
Range	0.54-0.61	0.54-0.65	0.73-0.8	0.75-1.24	0.82-0.91	0.99-1.16	1.19	1.2	1.26

[1]Alv. = Alveolus

Using SEM photos (none reversed) we take a closer look at some of the more important specimens which led to the dental formula. With the holotype, PM 542, the partial right ramus seen at the top center of our Figure 2 (and in Patterson, 1951, figure 1, in which 1B, PM541 is now TMM 1060-1), there is little to add to his description beyond giving details of the wear facets of m1-2. Both p3 and p4 are broken, p3 with only its roots preserved, while p4 has its roots and those portions of the crown which are directly above the roots, thereby making a length measurement possible (Table 2). The m1 is unambiguously identified by its typical molariform crown outline and the fact that the preceding teeth lack that form, and are therefore the ultimate and penultimate premolars,

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interpreted as p4 and p3. The m1 is heavily worn; the wear surface extends well down to the base of the crown on the labial side at ~30^{o} angle to the vertical tooth axis, so that the cusps are deeply truncated. The m2 is also worn, but less so, and its cusps are less truncated. The wear angle is also a vertically shearing one at <30^{o}. A part of the anterior alveolus for the m3 is preserved just ahead of the posterior break plane of the jaw. The ramus is deep (Table 1) throughout its length.

The paratype, a left ramus fragment that Patterson (1951) illustrated and which is shown reversed in Figure 2 (top row left) has its p4 represented by roots and alveoli, the m1 by the anterior alveolus and posterior root and part of the crown, a very worn m2, and a bit of the anterior alveolus of m3. Slaughter (1969) was in error in calling this the type, a fact that must be taken into account when reading his discussion.

Figure 3 (left in row 2 of Fig. 2) shows PM 899, another right ramus fragment with 4 alveoli of three molars that we believe to be for molars 2-4. Our reasons for this assignment are: 1) the posterior alveoli of m2 and m3 are subequal, and m3 is very slightly the wider of the two, 2) the anterior alveolus of m4 is definitely smaller than that of its predecessor, 3) both of these conditions are reasonable for such an assignment, but it is possible, although less likely, that m1-3 are the teeth involved. However, if that were the case the m3 appears to be too small. The front of the masseteric fossa lies lateral to the last tooth of the three, about where it would be expected in an adult, in which case the m2-4 assignment (or even an m3-5 one) is best. On the other hand, jaw depth beneath the two anterior molars seems a bit shallow, which argues for the more anterior tooth position assignment.

The three photos of a right ramus, PM 915, seen to the center and right of that same row in Figure 2, are shown here as Figure 4. A ventrolateral view is included to show the rear of the canine alveolus (arrow). Tooth loci are readily identified because the line between p4 and m1 is such a landmark: the round posterior root of p 4 and the grooved anterior root of m1. It should also be noted that there is an *en echelon* alignment between the pairs of alveoli of p3 and p4, and that the roots of p1-2 must have been very short to judge by the rearward extent of the canine alveolus, where it and the posterior alveolus of p2 are confluent.

The left photo in the 3rd row of Figure 2 is of a juvenile or subadult. It is a right ramus fragment, PM 1029, in which the posterior alveolus of p4 is seen to be followed by alveoli of molars 1-2. Note that m2 is larger than m1, especially wider, and that at this young age the front edge of the masseteric fossa (arrow) lies well forward, laterally beneath m2.

The photo to the right in row 3, Figure 2, shows a left ramus fragment reversed (R), SMU62014. Here in Figure 5, it can be seen that the rear part of the upper surface of the canine alveolus is exposed, and that alveoli for p1-4 can be recognized. The anterior alveolus of p1 is partially broken away and only the anterior root and crown remnant of p4 are preserved. There does not seem to be the *en echelon* arrangement between p3-4, such as seen in PM 915, although the alignment axes of p1-3 are at slightly different angles with respect to one another. There is a mental foramen at the level of the posterior root of p2.

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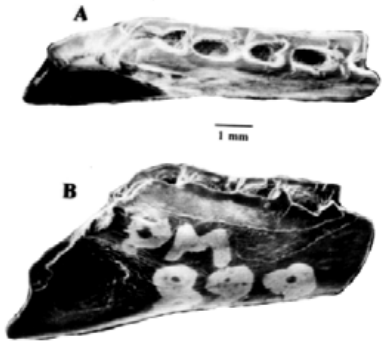


Fig. 3. PM 899, a right ramus fragment with alveoli of lower molars 2-4 shown in occlusal (A) and right lateral (B) views.

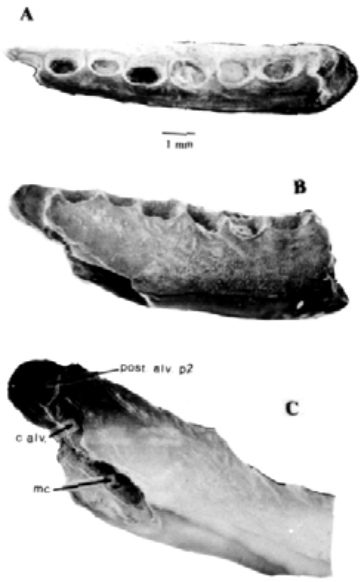


Fig. 4. PM 915, an edentulous left ramus fragment with alveoli of canine, p2-4, m1-2 shown in occlusal (A), left lateral (B) and left antero-lateral oblique (C) views. In C the rear alveolus of p2 (post. alv. p2) opens into the rear part of the canine alveolus (c. alv.), and the mandibular canal (mc) is exposed below.

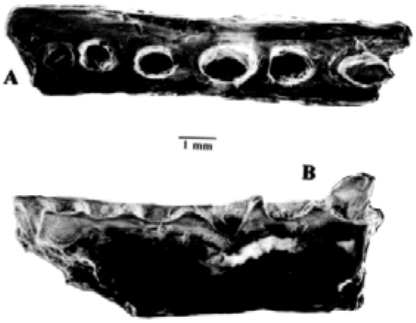


Fig. 5. SMU 62014, a left ramus fragment with part of the rear of the canine alveolus, and alveoli of p1-3, and the anterior root and part of the crown of p4, shown in occlusal (A) and left lateral (B) views.

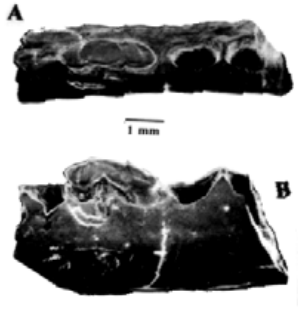


Fig. 6. PM 587, a left ramus fragment with posterior alveolus of p1, the very worn p2, and both alveoli of p3, shown in crown (A), and left lateral (B) views.

The 4th row of Figure 2 contains two Oklahoma specimens, OMNH 34478 and 34479 (R) and PM587, the first being a right maxillary fragment with two molars which will be discussed later. The other Oklahoma specimen, OMNH 34479, is edentulous, a left ramus fragment with the posterior alveolus of p3, both alveoli (and a bit of the anterior root) of p4, and the grooved front edge of the m1 alveolus. PM 587 a left ramus fragment shown reversed (4th row right in Fig. 2) is shown again in Figure 6. Present are the posterior alveolus of p1, the very worn p2, and both alveoli of p3. A bit of the canine alveolus can

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be seen in ventral aspect (not shown here). Small twinned mental foramina lie near the posterior part of the p1, and another is beneath and just behind the middle of the p2 locus. These features render the position assignment certain. Wear has almost obliterated the cusps in lateral view, but they are distinguishable in occlusal view.

In the 5th row on the left in Figure 2, SMU71744, a left ramus was shown reversed. Here in its normal aspect, Figure 7, it is enlarged to show details. The large round open posterior alveolus of p4, the most anterior locus represented, is followed by m1-2 and the open anterior alveolus of m3. The two teeth, while slightly less worn than those of the holotype, are very differently worn. On them wear is largely apical, with cusps a, b and c of both teeth worn to blunt, nearly horizontal wear facets. Some of these facets even incline slightly lingually. On the lateral face of m1, between cusps a and c, there is a near vertical facet (<30^{o} from vertical) that truncates the side of the crown to its base. No comparable deeply incised lateral (vertical) facet is on m2. Jaw depth, while considerable, is not quite as great as in the type (Table 1).

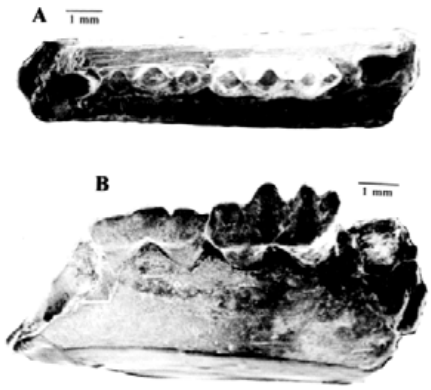


Fig. 7. SMU 71744, a left ramus fragment with the posterior alveolus of p4, m1-2 complete. The m1-2 is worn, its wear differing from that in the type by being mostly apical. It is shown in crown (A) and left lateral (B) views.

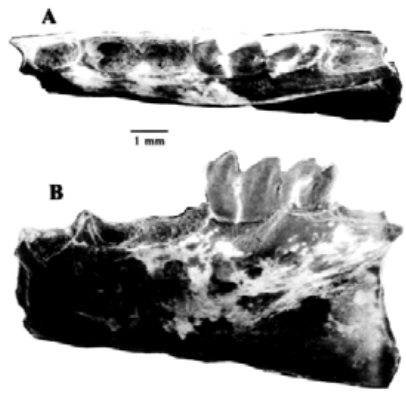


Fig. 8. PM 588, a left ramus fragment with the posterior alveolus of m2, both alveoli of m3, the fully erupted, unworn m4, and the anterior alveolus of m5. Note that the rear half of m4 and all of m5 are within the ascending ramus. The specimen is shown in crown (A) and left lateral (B) wiews.

Figure 2, row 6, shows PM 588 (R). Arguably it is the most posterior ramus fragment among all of the collections reported here. We interpret PM 588 to preserve the posterior alveolus of m2, both alveoli of m3, m4 in its fully erupted, pristine, unworn condition, and the anterior alveolus of m5 (seen well within the base of the ascending ramus which is largely broken away) Figure 8. The elongate shape of that anteriormost alveolus tells us we are dealing with a molar, not the p4. It is possible that the loci represented are those of m1-4, but two facts suggest otherwise: 1) the front edge of the masseteric fossa is situated at the level of the anterior part of the one in-place tooth, the m4, and 2) the considerable jaw depth at that locus (Table 1). Both weakly suggest our interpretation. (However, the jaw tapers rapidly, and is much shallower anterior to that point.) The

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presumed adult specimen of *Gobiconodon ostromi* illustrated by Jenkins and Schaff (1988) has this masseteric fossa feature located beneath m4-5, and jaw depth is greatest beneath these teeth.

Seven additional ramus fragments are present but are not discussed for they do not expand our understanding of the species although they do help to corroborate the assignments. They are PM 655; PM 914, PM 1003, and PM 1030 (the last three may belong to a smaller species); SMU 61987; SMU 62015 and SMU 62017.

Maxillary fragments

For the upper dentition, we have inadequate maxillary materials to conclusively demonstrate the formula. OMNH 34478 (left in 4th row Fig. 2) is our only dentigerous maxillary fragment. Its two teeth are either M4-5, or M3-4. First tentatively identified as M1-2, we now feel that that cannot be the case for the anteriormost tooth of the two appears to be too wide for it to be an M1 (Table 2), and it is very slightly wider than the following tooth which suggests the more posterior assignment. Figure 9 shows how this assessment is evident in comparison to other M1-5s. Also relatively low cusp heights lend support for the more posterior of the two possible assignments; the strongest support comes from posterior shallowing of the maxillary bone. Apical wear is apparent on the A, B and C cusps of the M4, and the beginnings of a more vertical shear seems to have just begun to develop on the lingual sides of these cusps, where the wear plane is inclined at ~45^{o} and is extensive both on and between the cusps. Cusps A and B of the M5 have suffered some breakage, but the B cusp has a "vertical" facet inclined at ~35^{o} to vertical. No wear shows on cusps C and D.

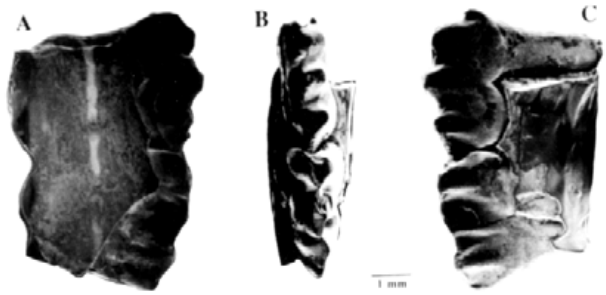


Fig. 9. OMNH 34478, a right maxillary fragment with M4-5 shown in right lateral (A), crown (B), and lingual (C) views. This is the only maxillary piece with teeth in the collection.

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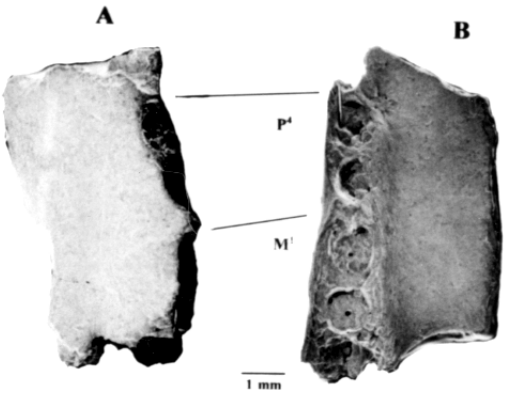


Fig. 10. SMU 71745, an edentulous right maxillary fragment with the posterior alveolus of P 3, alveoli and roots of P4-M1, and part of the anterior alveolus of M2, shown in right lateral (A), and occlusal (B) views.

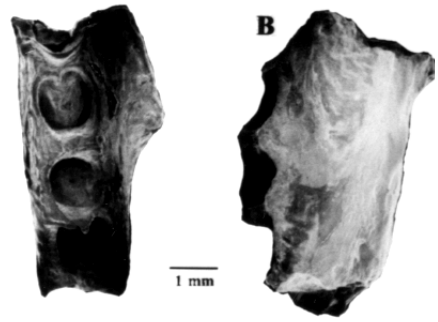


Fig. 11. PM 589, an edentulous maxillary fragment with part of the rear alveolus of M1, both of M2, and the anterior alveolus of M3. We are unsure of its proper identity; it could be a left (the favored interpretation shown here) in occlusal (A) and left lateral (B) views. The bony boss is, in this interpretation, the anterior buttress of the zygomatic arch. In the other interpretation it is a right maxillary with the bony boss being a small part of the palate.

There are two edentulous maxillary fragments, SMU 71745 and PM 589. They are shown in the outlined rectangle in the lower righthand corner of Figure 2. From these two maxillary fragments and the one specimen with teeth, and from tooth measurements and morphology of isolated teeth, we tentatively infer a cheektooth formula comparable to that of the lower dentition, i.e., C1, P1-4, M1-5.

Figure 10 shows SMU 71745, a right maxillary fragment, in right ventrolateral and palatal views. Part of the posterior alveolus of P3 is followed by alveoli and roots of P4-M1 and a bit of the anterior alveolus of M2. A considerable portion of the palate extends lingually from the alveolar edge, apparently to the midline. It is broken anteriorly, but appears to end naturally posteriorly, either at the anterior edge of a palatal foramen, or, less likely, at the posterior border of the palate.

Figure 11 presents one of two possible interpretations of specimen PM589. That shown is of a left maxillary (anterior at the top) in lateral and occlusal views. The three complete alveoli and two partial ones are: the posterior edge of the rear alveolus of M1, both alveoli of M2, and the anterior one of M3 plus the anterior wall of its rear alveolus. The bony boss labial to the rear of M1 and anterior root of M2 is the base of the anterior buttress of the zygomatic arch. Alternatively the specimen may be seen as a right maxillary fragment (same teeth) in which case the bony protuberance is a small part of the

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palate. In this last interpretation, the views are of the occlusal and medial aspects which show either the anterior edge of a palatal foramen or the rear of the palate to be situated somewhat farther back in this individual than in SMU 71745.

ISOLATED TEETH

Most specimens are isolated teeth, only a small selection of which are shown here. Unless otherwise indicated, each is included in the revised hypodigm. There is only one small indeterminate incisor tooth that possibly could have belonged to a triconodont, PM 643. It is not included in the hypodigm.

Only one canine has been recognized, SMU 71751. We are uncertain about its assignment: either a right lower canine, or left upper. It is elongate, more like the MCZ (Cloverly) lower canines which are longer than the upper Cs (Fig. 12A).



Fig. 12. Seven isolated teeth. (A) SMU 71751, the probable right lower canine. We think it is a lower, not an upper canine, because in the MCZ Cloverly specimens the lower canines are longer than uppers. (B) SMU 71750, a ? lower p1. (C) SMU 61983, an upper P1. (D) PM 653, a partial premolar, locus and side ? (E) PM 984, lower p2. (F) SMU 61987, a lower p3. (G) PM 882, an upper P3.

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Fig. 13. Six isolated teeth. (A) SMU 61984, an upper P4. (B) PM 1321, a molariform tooth, probably a dP4. (C) SMU 61985, a dP4. (D) PM 981, an m1 (or ? m2). (E) SMU 61986, an M1. (F) PM 883, an m2.

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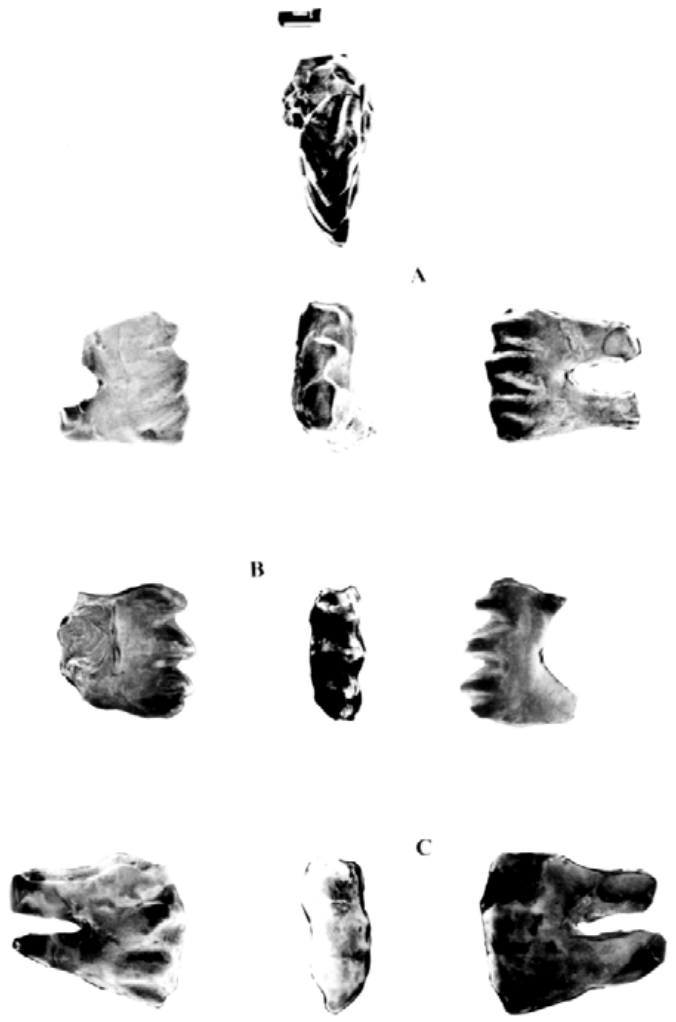


Fig. 14. Three isolated teeth. (A) SMU 62011, an M2 (or M3 or M4) shown in anterior (top), labial (left), occlusal (center), and lingual (right) views. (B) SMU 61759, an m3 (? or m4). (C) PM 885, M3 (or M4).

Premolars

One specimen may be a lower p1 (or p2 ?), SMU 71750 (Fig. 12B) and another that is very corroded may also be a p1 or p2, or even P1 or P2, SMU 62022.

Seven specimens are at least tentatively assigned as upper P1s, PM 595 (may be a smaller species and is not part of the hypodigm), PM1016, PM 1068, PM 1074 ?, PM 1138, PM 1139 and SMU 61983 (Fig. 12C).

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One small premolar (jaw and position undetermined), PM 653 (Fig. 12D), is not included in the hypodigm. In addition to the very worn tooth in the partial ramus, PM 587, there are three teeth that we score as lower p2**,** PM 984 (Fig. 12E), PM 1312 (may be a p3?), and SMU 62028. Ten teeth are scored as upper P2, only two without a query of some sort, PM 1137 and SMU 71738. The remainder are considered P2 or P3?, PM 1073, SMU 61731; (P3 or P2 ?), SMU 62010, SMU 62026, SMU 62030; (R P2 or L p2?), SMU 62038, SMU 71746, (corroded upper or lower 2nd or 3rd premolar ?) and SMU 72167.

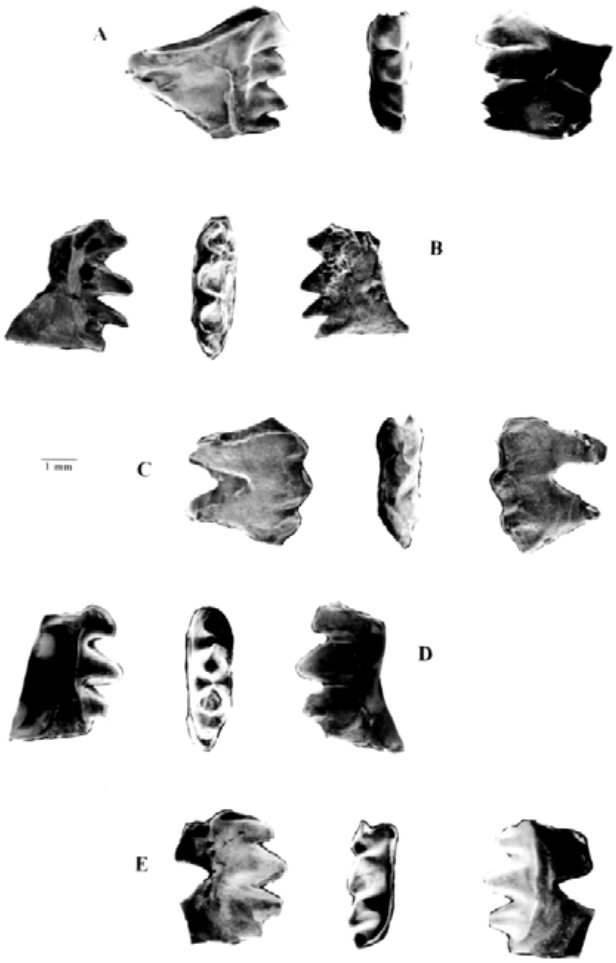


Fig. 15. Five isolated teeth. (A) PM 936, an m4, (B) SMU 62012, an M4 (or ? M5). (C) PM 585, an M5. (D and E) PM 934 and PM 928, both lower molars of uncertain locus. D has both apical and "vertical" shearing wear facets, while E shows early apical wear.

Lower p3s are, in addition to other teeth in ramus fragments, PM 542 (roots) and SMU 61987 (Fig. 12F), represented by five other specimens, SMU 71743, SMU 71760, PM 980, SMU 61989 and SMU 71741 (last three scored as? p3 or p4). Upper P3s are PM 652, PM 882 (Fig. 12G), PM 1329, and three scored as P3 or P4 ?, PM889, PM 927 and SMU 62027 (upper or lower ?).

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Both the holotype, PM 542, and paratype, TMM 1060-1, have p4 represented by roots. Three other p4s are PM 1044, PM 1141 and PM 1328. P4s are PM 888, PM 889 and SMU 61984 (Fig. 13A), which Slaughter (1969) called p3. Four other premolars (most fragmentary) are uncertain as to locus or even to the upper or lower series, PM 724, PM 881, PM 962, PM 995 and PM 1240.

Several very low-crowned molariform teeth may be deciduous, dp4s/dP4s, or less likely, they may be first molars. There are no ramal or maxillary specimens of the proper age stage to confirm the presence of any deciduous teeth within the Trinity sample. It seems highly likely from what is known in other triconodonts (*Triconodon mordax*, Simpson 1928 p. 79, fig. 24A: *Gobiconodon ostromi*, Jenkins & Schaff, 1988), and from the very presence of these isolated molariform teeth, that *A. denisoni* had at least one such tooth in each quadrant. Probable dp4 are PM 1067 and PM 1282, and dP4 are PM 656, PM 912, PM 939, PM 998 (? M1), PM 1321 (Fig. 13B), PM 1330, SMU 61985 (Fig. 13C) and SMU 62029.

Molars

With the lower first and second molars, we are on firm ground. Both holotype and paratype (PM 542, TMM 1060-1) and SMU 71744 (Fig. 7) have these teeth in place. Other m1s are: PM 935, PM 981 (?or m2) (Fig. 13D), PM 1077. The upper M1s are SMU 61986 (Fig. 13E) and SMU 62025 (both ? or M2), SMU 71417, and SMU 71748 (or M2?).

For lower m2s, in addition to those in jaws (Figs. 2, 7), there are three isolated teeth that are so assigned, PM 883 (Fig. 13F), PM 920, SMU 61988 (also called m2 by Slaughter, 1969). For M2s in addition to OM 34478 (Fig. 9) there are PM 1097 (? or M3), and SMU 62011 (? or M3 or M4) (Fig. 14A).

Three teeth are scored as m3s, SMU 61759 (? or m4) (Fig. 14B), SMU 62016, and SMU 71749; Only one M3 is recognized, PM 885 (? or M4) (Fig. 14C) unless the teeth in OM 34478 are M3-4, not M4-5 which we believe to be the case.

We suspect three teeth to be m4s, PM 936 (Fig. 15A), PM 1187 and SMU 61982. All are certainly posterior molars, and they have root fusion, so may be m5s. One with incipient root fusion we think is an m4, SMU 62045. M4s are few, OMNH 34478, PM 979, and SMU 62012 (Fig. 15B). Both single teeth could be M5, especially the latter with its root fusion. Other than the possible M/m5s just mentioned, we only scored one isolated tooth as M5, PM 585 (Fig. 15C).

Seventeen other teeth or partial teeth are recognized as lower molars, locus uncertain: PM 584, PM 593, PM 657 (low crown, unworn cusps), PM661, PM721, PM 890 (unworn), PM 898, PM 934 (interesting wear—apical and "vertical") (Fig. 15D), PM 926 (beginning apical wear) (Fig. 15E), PM 938, PM 996, PM1076 (small species ?, not in hypodigm), PM 1244, PM 1322 (unworn), SMU 71418 (possibly small species, not in hypodigm), SMU 71739, SMU 71740, and SMU 71742. Upper molars in this uncertain category are: PM 937, PM 964, PM 983, PM 1033 (short, wide with ~45^{o} apical wear facets merging with those of more "vertical" wear), PM1318 (? small species, not in hypodigm), PM 2329.

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We are left with two intriguing teeth, one that has but one root beneath an unbroken crown that resembles the posterior part of a lower molar, SMU 71747. It is not in the hypodigm. The other SMU 71750, is either a right lower premolar, ~p3, or as a left P3.

Tentative reconstructions of cheektooth dentitions

Figures 16, 17 and 18 are composites; outline drawings based upon most of the more certainly identified pieces. For the lower teeth and jaws (Fig. 16) we begin with the holotype (PM542), as it is the most extensive specimen of the lot. The other specimens directly included and discussed earlier are: the paratype TMM 1060-1, PM 587, PM 899, PM 915, PM 1029, SMU 61759, SMU 61984, SMU 61987, SMU 62014, SMU 71744, SMU 71750, SMU 71751, PM 587, OM 34479 and PM 588.

For the upper tooth series, because one of the edentulous pieces can be interpreted in two ways, we show each conception. In Figure 17, PM 589 is interpreted as preserving the bony boss of the anterior buttress of the zygomatic arch, while in Figure 18, that protuberance is interpreted as being a portion of the palate. The other edentulous element represented in the restorations is SMU 71745. It preserves a considerable part of one side of the palate ending either at its posterior edge, or at the anterior margin of a palatal foramen. The isolated teeth included in both restorations are: P1, SMU 61983; P3, PM 882; P4, SMU 61984; M1, SMU 61986; M2, SMU 62011; M3, PM 885; M4, SMU 62012; M5, PM 588; and dP4? PM 1321. In both Figures 17 and 18 we show OM 34478, the maxillary fragment with two teeth believed to be M 4-5.

Our ongoing, larger study involves the manner of occlusion and dental wear, which is less precise than that seen in most advanced tribosphenic mammals. It covers many more specimens than was possible for this report. Suffice it to say beginning wear on the cusps is apical, but this soon gives way to a more vertical wear which is usually found in comparable locations, determined by size, position and spacing of the cusps. But the wear facets are less consistent, and less well defined than in more advanced mammals.

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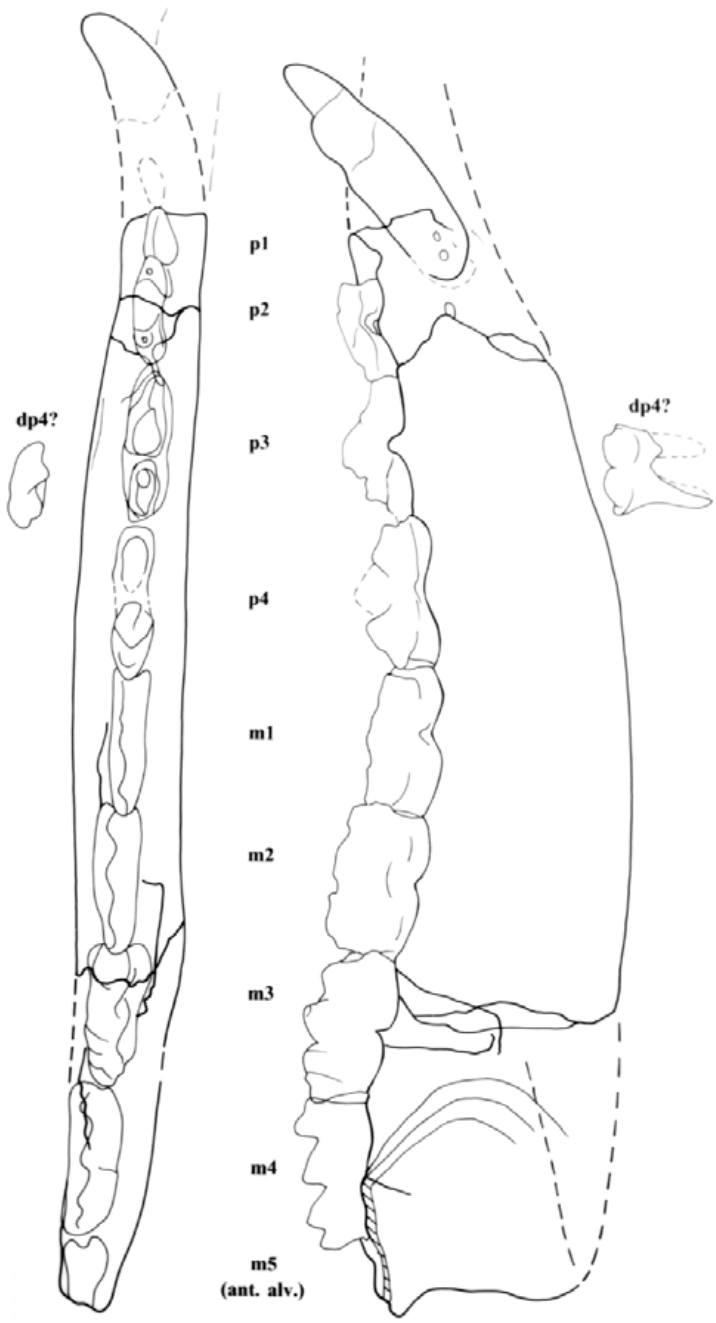


Fig. 16. Restoration of the lower jaw and dentition of *Astroconodon denisoni*. See text for listing of the specimens involved.

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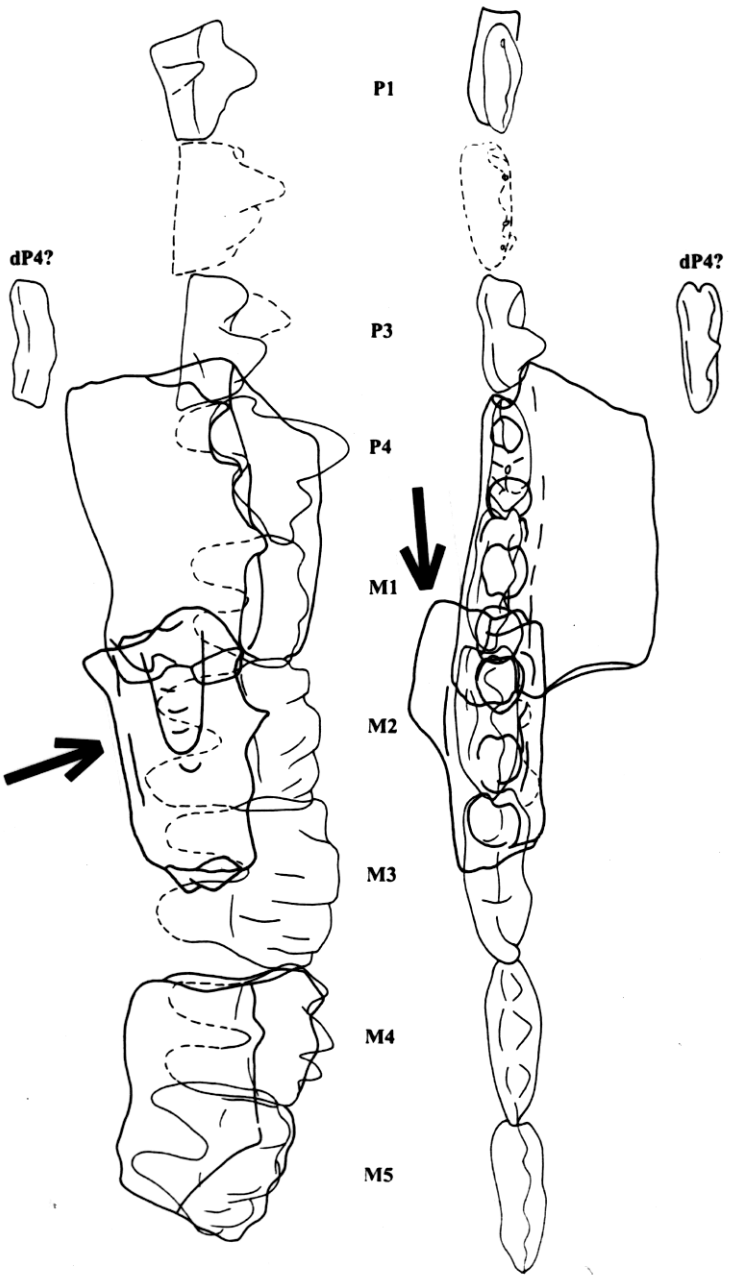


Fig. 17. Restoration of the upper dentition of *Astroconodon denisoni* in the favored interpretation of specimen PM 589 as preserving part of the anterior buttress for the zygomatic arch. See text for listing of the specimens involved.

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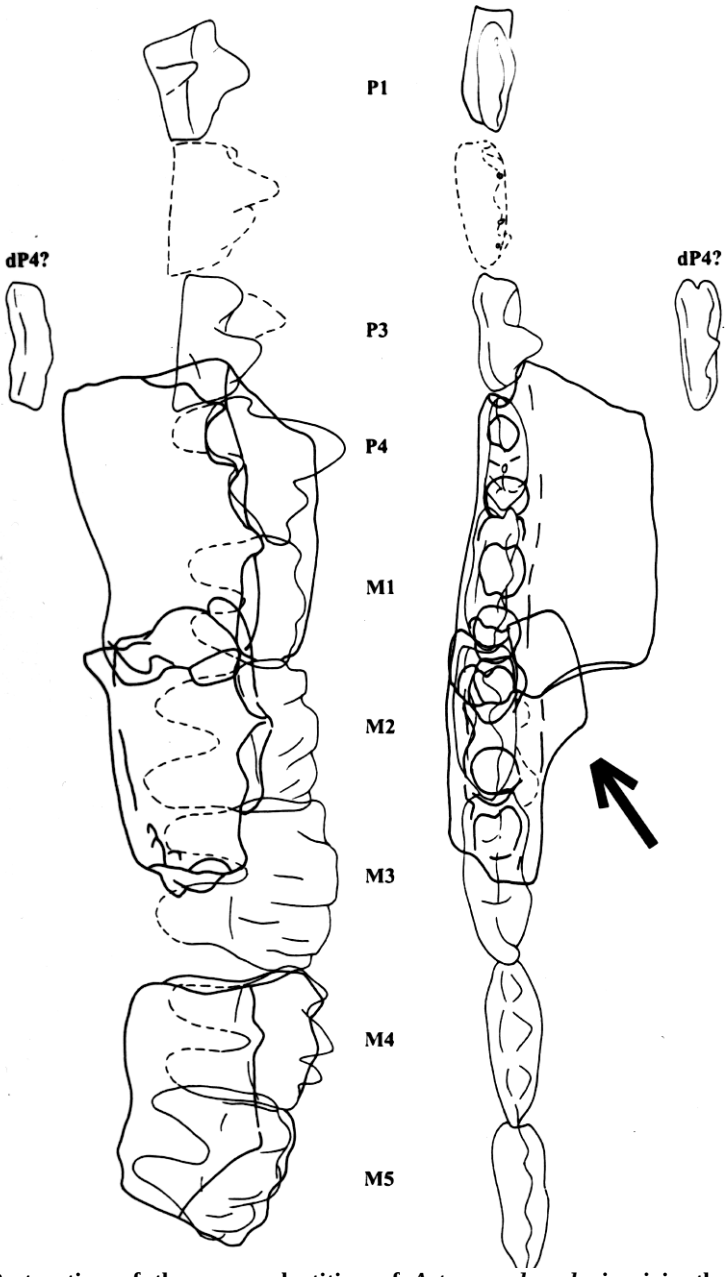


Fig. 18. Restoration of the upper dentition of *Astroconodon denisoni* in the alternative interpretation of specimen PM 589 as preserving part of the palate. See text for listing of the specimens involved.

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CONCLUSIONS

The messages from this interim report are: First, the *Astroconodon denisoni* materials, like the other mammal remains in the Trinity fauna (*Spalacotheroides bridwelli*, various therians, the multituberculates), are all fragmentary. No specimen contains more than two complete teeth, and most are isolated teeth or tooth fragments. Second, we have been able to arrive at the dental formula for *A. denisoni* using jaw and maxillary restorations. Third, these show that it had more molars (5) like Late Jurassic- earliest Cretaceous triconodonts, and more than the four Slaughter (1969) had hypothesized. By comparison to amphilestids, *Astroconodon* may be primitive in this regard. Fourth, that, while only touched on here, occlusal wear is fairly consistent, but less precise than that of higher mammals.

ACKNOWLEDGMENTS

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The Origin of Dancing and Grouping of Ameloblasts to Form Enamel Prisms and Hunter-Schreger Bands in Reptiles and Primitive Mammalia

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INTRODUCTION

Enamel prisms and Hunter-Schreger bands in mammalian teeth enamel including human enamel are considered to be the original form observed in the reptilian enamel. The purpose of the present study is to examine the origin and evolution of enamel prisms and Hunter-Schreger bands in reptiles and marsupials with a transmission electron microscope,TEM and a scanning electron microscope, SEM.

MATERIALS AND METHODS

Teeth of the fossil reptiles *Phytosaurus* sp., *Mosasaurus* sp, a recent reptile *Alligator mississippiensis*, recent mammalia *Monodelphis domestica*, and *Suncus murinus*, were used in this study.

1. SEM observation:

The teeth were polished in 3 dimensions and etched by 0.5 % HCl. The etched samples were gold-palladium coated by a IB-5 ion coater (Eiko Engineering Co., Japan), and observed using a S - 2700 (Hitachi) SEM at an acceleration voltage of 20 kV.

2. TEM observation:

The animals (*Alligator mississippiensis*, *Monodelphis domestica*, and *Suncus murinus*) were fixed by 2.5% glutalaldehyde (0.01 M cacodilate buffer, pH 7.4) with perfusion method. Jaws with teeth germs were dissected and decalcified by EDTA and post fixed with OsO4 (same buffer). After this, the samples were dehydrated by alcohol and embedded in Quetol 812. The ultra-thin sections were cut and stained with 1 % lead citrate. Ultra-thin sections were observed the JEOL JEM-1200EX transmission electron microscope (TEM).

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RESULTS AND DISCUSSION

The features of the crystal unit on the fossil reptiles were examined next. The crystal unit is bordered by a fissure, which is observed on etched enamel surface. Every unit has almost a universal crystal arrangement, one side of the unit showed a parallel arrangement and the other side was arranged perpendicular to this, between of these two were gradually changing crystals. These crystals abruptly changed their arrangement at the fissure. In *Phytosaurus* enamel, the units demonstrated a long pattern running almost parallel to the tooth axis, which may be formed by about 50 ameloblasts (Torii, 1998). *Mossasaulus* had around or oval type of unit which may be formed by about 15 ameloblasts. However, the size of units changed in every enamel layer (Fig.1). Pseudo enamel prisms have been reported in *Alligator mississipiensis*. (Sahni, 1987). However, these structures resembled the foregoing crystal units, because its size demonstrated that the structure is formed by several ameloblasts (Fig. 2a,b). This structure had a similar crystal arrangement to the crystal unit. These phenomena showed that the crystal unit formed by grouped ameloblasts is easily changed. One ameloblast also changed from one group to the other, so the size was changed in every enamel layer (Fig.3). The fissure showed the same structure as the prism sheath, which may contain a high percentage of enamel protein.

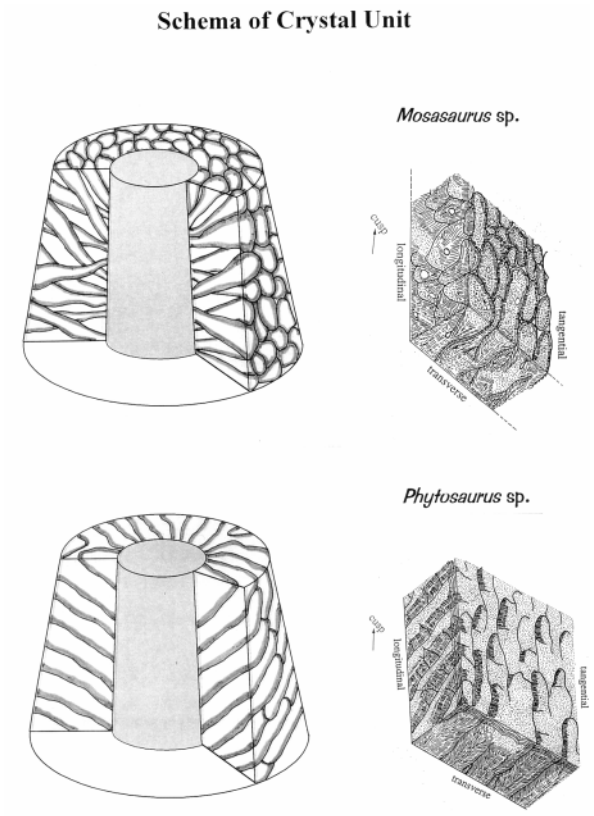


Fig. 1. Schema of crystal unit.

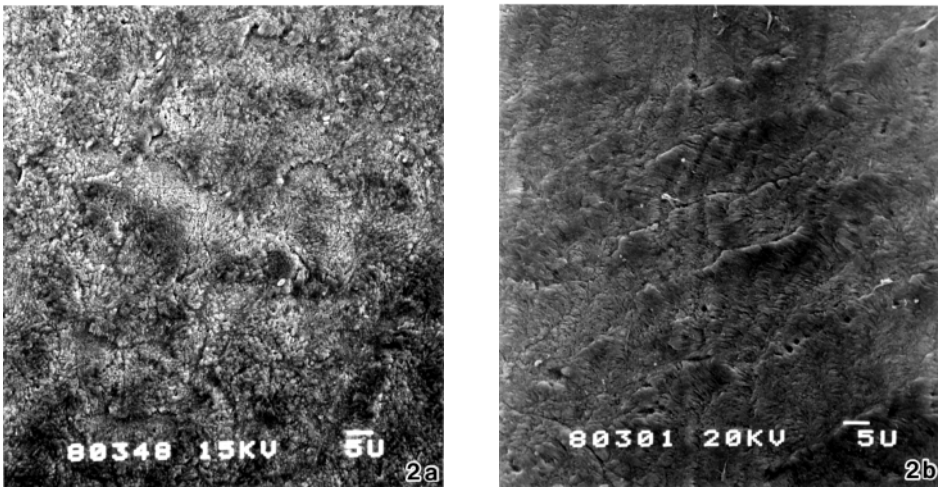


Fig. 2. a. *A. mississippiensis*. A tangential section. The crystal units could be recognized by the fissure, where the crystal orientation abruptly changed. Each unit had both a high density and a low density electron region. b. A longitudinal section. The fissure did not form a continuous line, showing that the size of crystal unit changed along with the development. Each unit had two arrangements of crystals, which were longitudinal and vertical at the unit axis. The arrangement of the crystals constructed incremental lines running obliquely to the furrows.

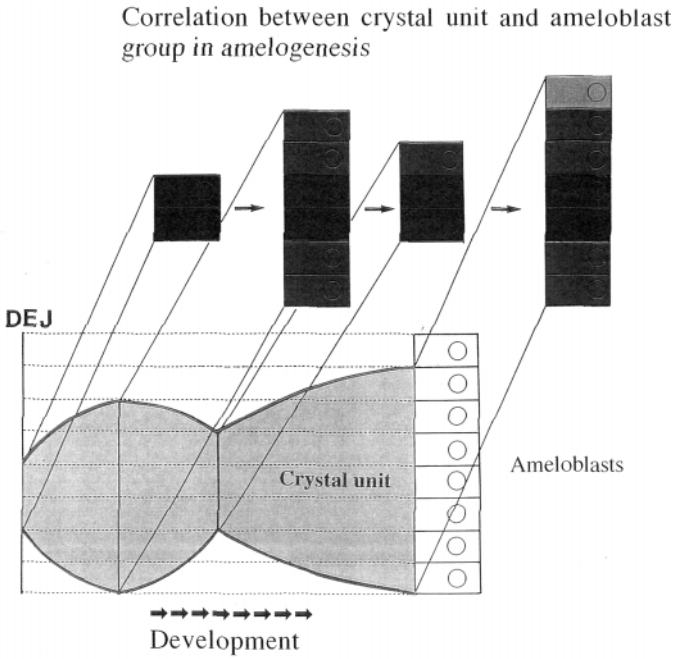


Fig. 3. Correlation between crystal unit and ameloblast group in amlegenesis. Crystal unit changed the form and the size in the enamel. A crystal unit formed by a group of ameloblasts, of which the members changed along with the development. Some of them left from a group and others came into a group.

On the initial developed enamel, an island from formed enamel is crystals deposited on the dentine surface at a short distance from the distal cytoplasmic membrane of ameloblast as is seen in mammalian enamel (Fig. 4). These islands grew and fused to each other as a crystal unit, of which the border showed abruptly changed enamel crystals such as the fissure (Fig. 5). Ameloblasts contained many rER, mitochondrias and secretory granules. The border of these ameloblasts did not fit the island border or fissure. The more developed enamel surface formed a scallop shape, so that the secretory end or distal end of ameloblast also formed many folding structures. The folding of the distal ameloblast surface caused the formation of a fissure, where the enamel crystals abruptly changed (Fig.6). However, the fissure did not fit the border of ameloblasts. In the next stage of amelogenesis, the developing enamel surface was smooth, but the fissure was observed with an abrupt change of the crystal arrangement (Fig.7). The border of the ameloblast did not closely adapt to the fissure. The fissure was observed clearly in the calcified enamel (Fig. 8). On the maturing enamel, the surface formed a slight curvature, and the fissure showed several ameloblasts forming the crystal unit (Fig. 9). The size of these ameloblasts was very similar. The projection of the Tomes' process of the ameloblasts and the process of odontoblasts penetrated into enamel to form the enamel tubules (Fig. 10) in the tubular enamel. This interaction between ameloblasts and odontoblast may control the movement of grouping and dancing of ameloblasts to form the more developed enamel structure of mammalia, such as enamel prisms and Hunter-Schreger bands.

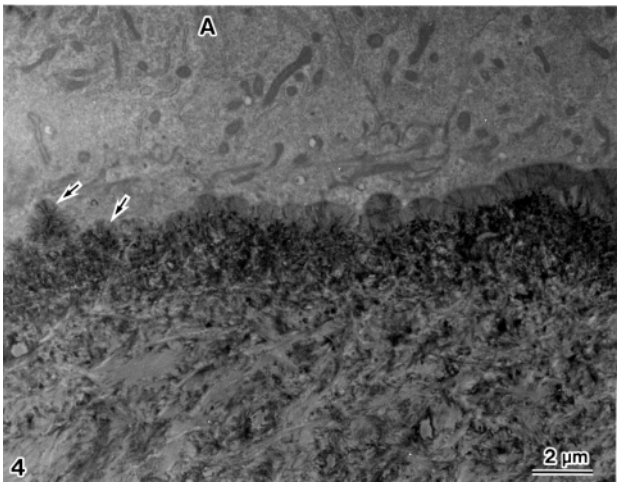


Fig. 4. The initial enamel crystals deposited on the dentine surface a short distance from the cytoplasmic membrane of ameloblasts (A). The enamel crystals formed an island(arrows). The right side is more developed enamel. The ameloblast had many mitochondria and secretory granules.

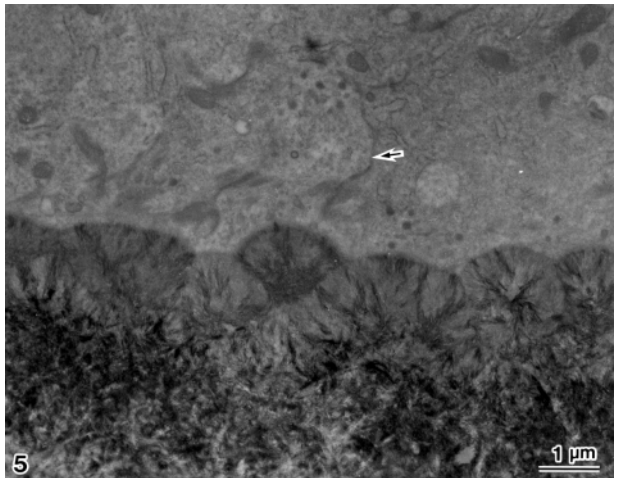


Fig. 5. The island-like crystal unit developed and fused together, as a result the developing enamel surface formed a scallop shape. The enamel crystal arrangement abruptly changed at the border of units develop to the fissure of crystal unit. The boundary of anameloblast that (arrow) didn't have a relationship to the border of unit. (*A. mississippiensis*)

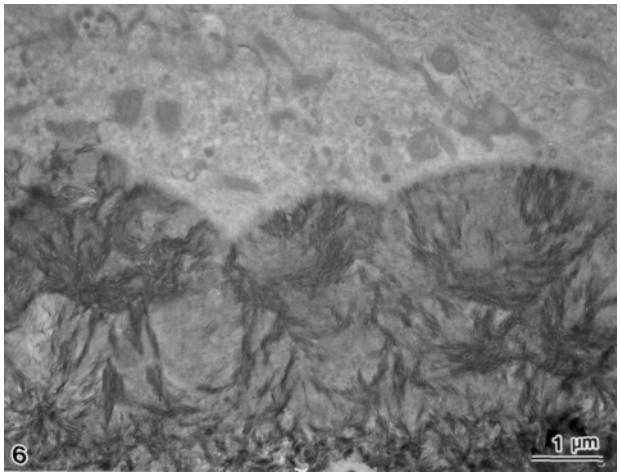


Fig. 6. *A. mississippiensis*. Crystal islands developed and fused with each other along with the development. The crystal arrangement was abruptly changed at the fissures, which corresponded with the fissure of crystal unit. These fissure were formed by the wrinkle projections of the distal cytoplasmic membrane of ameloblasts.

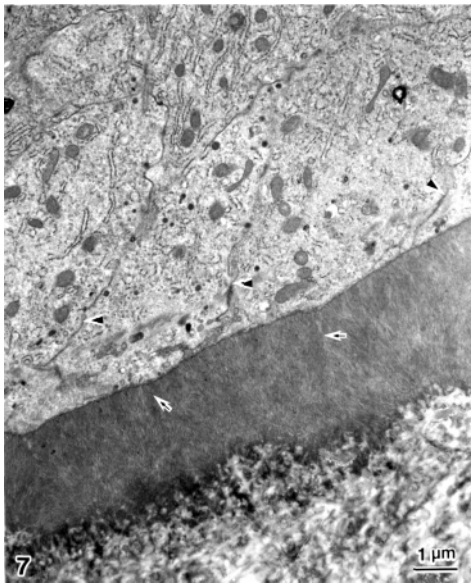


Fig. 7. The developing enamel surface formed more smoothly than the initial stage. The border of acrystal unit (arrows) was observed at the abrupt change of the crystal arrangement, which developed to the fissure of crystal unit. The boundary of the ameloblast (arrowheads) didn't adapt to the boundary of crystal unit. The ameloblast contained many RER, mitochondria and secondary granules in the cytoplasm. (*A. mississippiensis*)

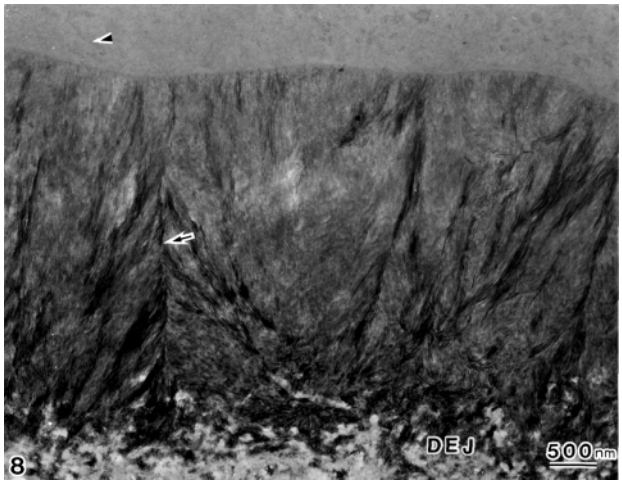


Fig. 8. *A. mississippiensis* The fissure (arrow) demonstrates the border of crystal arrangement. The fissure ran from DEJ to the developing enamel surface. However, the boundary of ameloblast (arrowhead) didn't closely adapt to this structure.

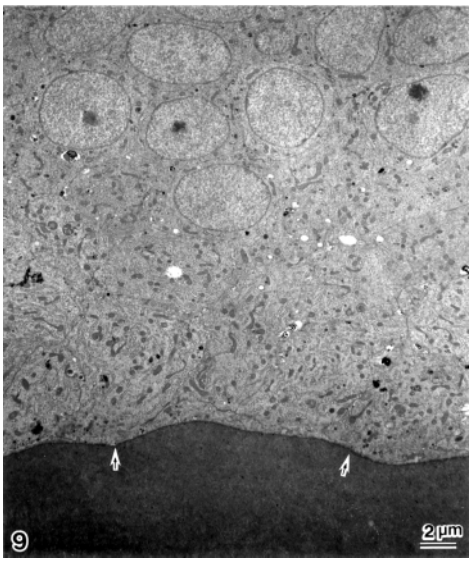


Fig. 9. Ameloblasts showed almost the same size. The boundary of the ameloblast didn't adapt to the fissure or border of crystal unit (arrows). The developing enamel surface showed slight scallops, which were formed by crystal units. A crystal unit was formed by a group of ameloblasts. (*A. mississippiensis*)

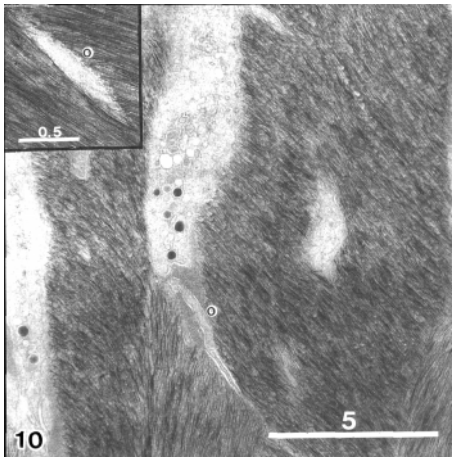


Fig. 10. The odontoblast process (o) penetrates into the enamel and contacts to the ameloblast. These processes contained microfilaments (in square) and formed enamel tubules (*Suncus*, Insectivora).

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CONCLUSION

1. Crystal units had a relatively uniform crystal orientation the same as the enamel prisms in mammalia. A crystal unit was formed by an ameloblastic group, of which the members changed along with the amelogenesis. This ameloblastic group developed into an ameloblastic aggregate to form the Hunter-Schreger bands.
2. In opossum enamel the enamel prism bending may show the original form of ameloblast dancing, which formed the Hunter-Schreger band, because this dancing arose under the influence of interaction between the ameloblast and the odontoblast, where the odontoblastic processes disappeared in enamel tubules.
3. The ameloblastic dancing and grouping may develop types of Hunter-Schreger bands. The island pattern was the most original grouping pattern showed by fossil reptiles. The sagittal pattern showed that the ameloblast grouping developed a sagittal direction was *Mosasaurus* sp. The horizontal pattern was the most generalized form in mammalia. This pattern was formed by the horizontal grouping of ameloblasts. This dancing and grouping might be the most effective way to form a complex tooth crystal pattern.

ACKNOWLEDGEMENTS

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The Third Upper Molar of the Würmian and Late Glacial Steppe Ass *Equus hydruntinus* from Italy

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INTRODUCTION

Paglicci Cave (Foggia, Apulia, Southern Italy), Polesini Cave (Rome, Latium, Central Italy) Settecannelle Cave (Ischia di Castro, Viterbo, Latium, Central Italy) are known to have provided a rich amount of stone implements and of bones. The Paleolithic deposits of Paglicci Cave range in time from 34,300 to 11,440 years B.P. (Palma di Cesnola and Bietti, 1983; Palma di Cesnola, 1988), while those of Polesini Cave are referable to the last part of the Late Glacial, from the end of Bölling (about 12,500 years B.P.) to Dryas III (about 10,500 years B.P.) (Radmilli, 1974; Bietti et al., 1983). Finally, the levels with Epigravettian culture facies of Settecannelle Cave range in time from 16,620 to 10,570 BP (Gnesutta, per. comm., 1997).

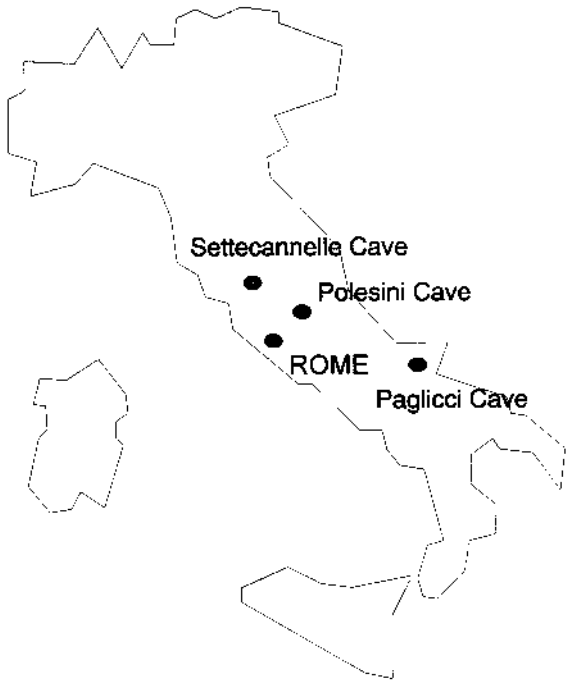


Fig. 1. Geography of the Paleolithic sites described in this paper.

MATERIALS AND METHODS

The *E. hydruntinus* remains are represented by teeth and postcranial elements. The latter are heavily fractured by intense human activity. Therefore, the study has been mostly focused especially on the morphometry of the third upper molar, because it showed the most evident variation (Rustioni, 1996a). The schemes proposed by Eisenmann et al. (1988) have been followed for the measurements and nomenclature. The specimens from levels with similar climatic and environmental conditions are grouped



Fig. 2. Right upper third molars: 1) specimen with isolated hypoglyph; 2) specimen with open post-fossette.

Table 1. Synthesis of the data reported in this paper.

M^3	N	Lo			lo			Lp			Ip			isolated hypograph		open post-fossette	
		max	mean	min	max	mean	min	max	mean	min	max	mean	min	N	%	N	%
Paglicci Cave	13	25.3	23.6	22.2	20.9	20.0	18.6	10.7	9.1	7.0	48.1	38.8	29.7	2	15.3	1	7.69
Polesini Cave	31	25.5	23.0	21.0	22.5	20.1	17.2	11.8	9.4	8.0	49.0	41.1	32.9	4	12.9	14	45.1
Settecannelle Cave	15	24.8	23.4	20.0	22.6	19.5	17.8	11.4	10.1	8.7	52.5	43.4	35.2	3	20.0	2	13.3

N=Number of specimens; Lo=Length; lo=Breadth; Lp=Protocone length; Ip=Protocone Index (Lp X 100/Lo)

DISCUSSION

In Paglicci Cave *E. caballus* is always dominant with respect to *E. hydruntinus*, with the exception of level 18b (Laugerie interstadium) characterized by similar percentages and in the levels from 6c to 2 where *E. hydruntinus* is the best represented equid. The Steppe ass dominates over horse at Polesini Cave and mostly at Settecannelle Cave. This confirms the more temperate-Mediterranean character of *E. hydruntinus* as compared to *E. caballus*. In fact, during the last part of the Late Pleistocene the Adriatic side was affected by the cold climatic condition of the Balkan area (Masseti et al., 1995).

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The dimensional analysis carried out to distinguish the specimens from different climatic-environmental moments, did not show appreciable differences in the skeleton. However, the limb bones (metapodial bones, astragali and phalanges) from Paglicci Cave are larger than those from Polesini Cave. The post-cranial elements from Settecannelle Cave are not well represented.

The premolars seem to be the most variable dimensionally. From a morphological viewpoint attention was mostly focused on M3. The sample from Paglicci is fairly poor (13 specimens), but in a single M3 from level 2 the post-fossette is open posteriorly.

The relatively rich sample from Polesini (31 specimens) has shown a similar percentage of teeth with isolated hypoglyph coming from the levels 11-9 (13.3%), referable to the Dryas II, and from levels 8-2 (12.5%), referable to the Alleröd.

A higher number of specimens with open post-fossette (43.75 %) was found in the levels referable to the Alleröd, while those from the Dryas II levels reach only 20%. Some specimens from both levels show the post-fossette imperfectly closed.

E. hydruntinus is relatively well represented in the levels 16-12 (from 16,620 to 15,700 years BP) of Settecannelle Cave. However, only fifteen upper third molars are recovered. In particular the percentage of specimens with isolated hypoglyph is 20%.

In some present-day representatives of the genus *Equus*, the isolated hypoglyph reaches the maximun percentage in *E. africanus* (64%), which never shows the post-fossette open posteriorly. This character is more frequent in *E. hemionus* than in other *Equus* species (Eisenmann, 1986). *E. hydruntinus* from Polesini shows some teeth with post- fossette open posteriorly comparable with those of the present day *E. hemionus*.

Equus hydruntinus: systematics and dispersal events

The systematics of *E. hydruntinus* is uncertain. However, the European Steppe ass is more similar to stenonid and zebroid horses than to the fossil and extant asses and hemiones (Forsten, 1986). In some fossiliferous sites, the European wild ass is the only representative of *Equus* present. In others, it is usually associated with a "caballoid" horse, but never with other asses.

The wild ass appears in Europe during the last part of the Middle Pleistocene, probably derived from a *Equus stenonis*-like stock (Forsten, 1986). The Steppe ass became frequent in the Late Pleistocene, especially during the Würm glaciation and disappeared in the Holocene without any apparent descendents. *E. hydruntinus* finds are frequent in Europe and in the Mediterranean area. The find from Kazakhstan (Hisarova, 1963) is the easternmost representative of the species. The finds from some North African Late Pleistocene archaeological sites, from Northern American Rancholabrean localities and from some Late Pleistocene deposits of China referred to the steppe ass, are doubtful (the North African remains are similar to those of the true ass; cfr. Churcher, 1972). For these reasons, I believe that *E. hydruntinus* is a result of a local European evolution.

E. hydruntinus appears in Italy during the last part of the Riss glaciation, as testified by the scanty remains (fragmentary metacarpal bone and isolated upper and lower cheek teeth) from the Paleolithic site of Campo Verde (Latium, Central Italy) (Mazza et al., 1992). This taxon is well represented in the prehistoric sites of the Late Pleistocene mainly in the Adriatic side of the Italian Peninsula. In Northeastern Italy *E. hydruntinus* is not well represented and is documented for the first time in the levels corrisponding

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approximately to the Dryas II pollen zone (Riparo Tagliente) (Masseti et al., 1995). The geographic distribution of *E. hydruntinus* seems to have been drastically reduced during the Holocene. The European wild ass apparently survived in Basilicata (finds from Rendina) and Sicily at least up to the Neolithic (Bökönyi, 1974; Wilkens, 1989; Rustioni, 1996b).

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The Features of Enameloid Formation During Odontogenesis in Teleosts

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INTRODUCTION

The definition of "enameloid" given by Poole (1967) and Ørvig (1967) is all-inclusive, and is suitable for describing well-mineralized tissues covering a tooth surface which do not correspond to the true enamel, in lower vertebrates. Therefore, it is quite possible that "enameloid" contains several hard tissues whose origins differ. It is assumed, for instance, that the developmental process of elasmobranch enameloid is distinct from that of teleost enameloid in many respects, while they resemble each other after the maturation stage (Sasagawa, 1989, Sasagawa and Akai, 1992). However, we have very little data about the enameloid distributed in a huge number of lower vertebrates. The distinction and comparison of and the relationships among the enameloid of lower vertebrates are the subjects of ongoing studies in our laboratories.

Regarding biomineralization, enameloid is an analogue of the mammalian enamel, because the degree of mineralization and the structure of the enameloid are quite similar to that of true enamel in mammals, despite the differences in their developmental processes.

Studies of the enameloid formation in teleosts may provide important evidence concerning 1) biomineralization, 2) tooth morphogenesis and 3) the phylogeny of the dental hard tissues in vertebrates. In the present article we elucidate the prominent fine structural and the histochemical features during cap enameloid formation in teleosts, and compare them with that in elasombranchs.

THE PROCESS OF CAP ENAMELOID FORMATION

The first stage in teleost enameloid development is the enameloid matrix formation in which the organic matrix is formed between the odontoblasts and the dental epithelial cells. The second stage is the enameloid mineralization, in which predentine appears beneath the enameloid , and mineralization begins at the boundary between the enameloid and the predentine and then spreads to the apex of enameloid. The third stage, beginning after the arrival of the mineralization at the surface of the enameloid, is the enameloid maturation. At the stage of enameloid maturation, the degree of mineralization in the enameloid is markedly increased, and most of the organic matrix in the enameloid has degenerated and disappeared. The mineralization in dentine and the formation of collar

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enameloid also occur in the third stage. At the later stage of enameloid maturation in *Oreochromis* and *Tilapia*, iron is deposited in the outermost layer of the highly mineralized cap enameloid (Sasagawa, 1992).

The tooth germs at the stage of enameloid maturation are usually found, but those at the stage of enameloid mineralization are rarely seen. The frequency of the emergence of the tooth germs at the first stage is intermediate between these. The frequency of the appearance of tooth germs may reflect the length of each stage.

ORGANIC MATRIX IN THE CAP ENAMELOID

Abundant collagen fibrils found in the enameloid matrix at the early stages is a marked feature of teleost enameloid (Fig. 1). The origin of these collagen fibrils is not clear. Prostak and his colleagues (Prostak and Skobe,1984,1985,1986; Prostak et al.,1993) postulated that the bulk of the collagen fibrils in teleost enameloid was of ectodermal origin, because procollagen granules were found in the inner dental epithelial (IDE) cells of a cichlid fish after the injection of colchicin. The procollagen granules stained by phosphotungstic acid (PTA) were also visible in the IDE cells of tilapia, in which there were well-developed organelles concerning secretory function (Sasagawa, 1995). It is certain that the IDE cells at the stage of enameloid matrix formation in teleosts are able to produce collagen fibrils. Conversely, the odontoblasts contained well-developed rough endoplasmic reticulum (rER) and Golgi apparatus, and also several procollagen granules in the Golgi area at the same time (Fig. 2). There were many odontoblast processes in the enameloid matrix, and many collagen fibrils were situated around the processes, suggesting their close relationship (Sasagawa, 1995). The ectodermal-origin collagen theory is problematic in that no evidence has been obtained showing the migration of large numbers of collagen fibrils to the enameloid through the basal lamina possessing defined lamina dense (Fig. 3). Most of the collagen fibrils in the enameloid are from the odontoblasts, as several researchers had speculated (Kerr, 1960; Shellis and Miles, 1974, 1976; Yamashita and Ichijo, 1983), whereas the IDE cells synthesize collagen fibrils, and a number of the ectodermal collagen fibrils migrate to the enameloid.

Herold and co-workers (1980, 1989) and Slavkin and his colleagues (1983) reported the existence of proteins in enameloid that are of ectodermal origin i.e., amelogenins and enamelins, by means of immunohistochemistry. However, neither amelogenins nor enamelins was detected by the immunogold electron microscopy using different antibodies (Ishiyama et al., 1993). It is necessary to determine the molecular biological characteristics of the enameloid proteins to solve this problem.

Figs 1-6:

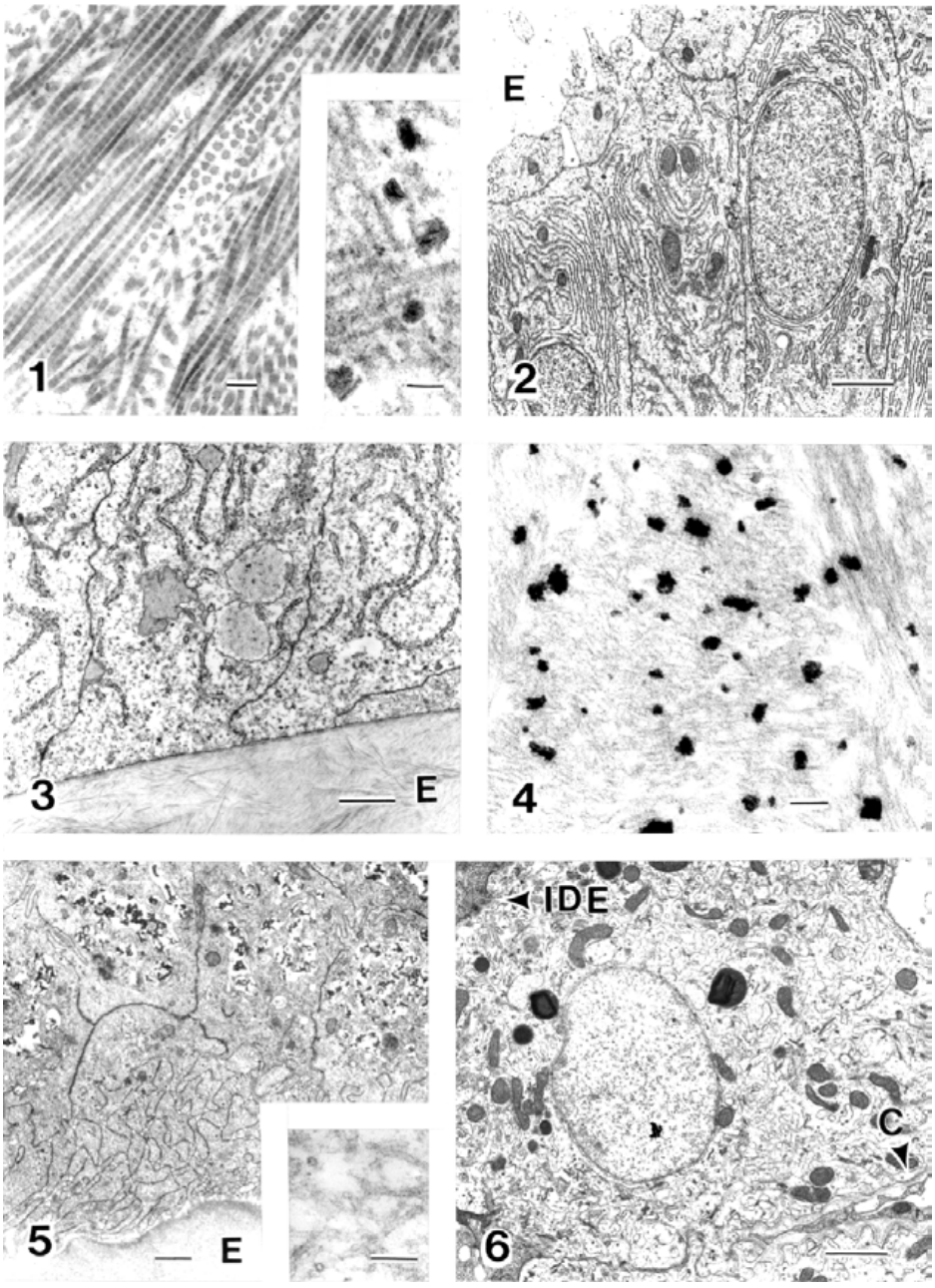


Fig. 1. Transmission electron micrograph shows the collagen fibrils in the enameloid at the stage of enameloid matrix formation. *Oreochromis niloticus*. The section was not demineralized, and was stained with uranyl acetate and lead citrate (U-Pb). Bar=200nm. Inset shows the matrix vesicles (MVs) containing fine crystals among the collagen fibrils. *Tilapia buttikoferi*. Not demineralized, U-Pb. Bar=100nm.

Fig. 2. Odontoblasts at the stage of enameloid matrix formation. Abundant rER and well-developed Golgi apparatus are present around the nuclei. Many projections extend into the enameloid matrix (E). *Oreochromis*. Demineralized in a 2.5% solution of EDTA-2Na, U-Pb. Bar=2μm.

Fig. 3. Distal cytoplasm of IDE cells and the outermost layer of the enameloid (E) at the late stage of enameloid matrix formation. The distal end is straight, and several organelles are scattered in the distal cytoplasm. *Oreochromis.* Demineralized, U-Pb. Bar=1μm.

Fig. 4. Non-specific acid phosphatase activity at the points that usually correspond to the MVs in the enameloid matrix at the middle stage of enameloid maturation. There are no marked collagen fibrils, but fine filamentous substances are seen in the enameloid. *Tilapia.* Demineralized, U-Pb. Bar=100nm.

Fig. 5. Distal cytoplasm of the IDE cells at the middle stage of enameloid maturation. Ruffled border is characteristic of this stage. The electron-dense substances are visible in the vesicles near the ruffled border. E:enameloid. *Oreochromis.* Demineralized, U-Pb. Bar=1 μm. Inset shows the tubular structures in the enameloid matrix. *Oreochromis*. Demineralized, U-Pb. Bar=100nm.

Fig. 6. The ODE cells at the stage of enameloid maturation. Labyrinthine canalicular sER occupy most of the cytoplasm. C:capillary, IDE:IDE cells. *Oreochromis*. Demineralized, U-Pb. Bar=2μm.

MATRIX VESICLES APPEAR IN THE ENAMELOID

Matrix vesicles (MVs) probably play important roles during cap enameloid formation in teleosts. At the stage of enameloid matrix formation, there are large numbers of MVs in the enameloid, and many of them possess slender crystals (Fig. 1, inset). It is certain that the MVs are the site of the initial mineralization in the cap enameloid (Sasagawa, 1988, 1995), as in the predentine (Yamada and Ozawa, 1978).

At the stage of enameloid mineralization and the early stage of enameloid maturation, a number of MVs that contain no crystals but show an electron-dense substance are still found in the enameloid matrix that has begun to degenerate (Sasagawa, 1995), and acid phosphatase (ACPase) activity was detected at the MVs during these stages (Sasagawa, 1998a), implying that some of the MVs enclose lysosomal enzyme and are involved in the degeneration of the enameloid matrix (Fig. 4). Although this proteolytic activity was detected in the enameloid from the enameloid mineralization stage to the enameloid maturation stage (Shimoda, 1989), the exact mechanisms of the degeneration that occurs in the enameloid matrix including abundant collagen fibrils remain to be identified.

DEGENERATION OF THE ORGANIC MATRIX IN THE ENAMELOID

A ruffled border appears at the distal ends of the IDE cells, which contain many mitochondria and lysosomal bodies, but less rER, at the stage of enameloid maturation (Fig. 5). The lysosomal bodies found in the IDE cells during the enameloid mineralization stage and the enameloid maturation stage show definite ACPase activity (Sasagawa, 1997a, 1998a). Many projections from lateral cell membranes and well-developed interdigitations between the IDE cells are visible from the central to the

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proximal region of these cells. At the early and middle stages of enameloid maturation, marked alkaline phosphatase (ALPase) activity is localized at the lateral and proximal cell membrane of the IDE cells (Inage et al., 1984; Sasagawa, 1998a). In the outer dental epithelial (ODE) cells, the cytoplasm is filled with well-developed labyrinthine canalicular smooth endoplasmic reticulum (sER) (Fig. 6). Capillaries, together with ODE cells, penetrate the layer of IDE cells in some places (Garant, 1970; Sasagawa, 1997a). As a result, the outline of the layer of dental epithelial cells is convoluted. These ultrastructural and histochemical findings indicate that the dental epithelial cells are engaged in the degeneration and removal of the enameloid matrix.

Fine tubular structures of 16nm in thickness are often visible in the outer layer of the enameloid near the IDE cells possessing a ruffled border after demineralization (Fig. 5, inset). Ultrastructural findings suggest that the tubular structures are remnants of the organic matrix in enameloid.

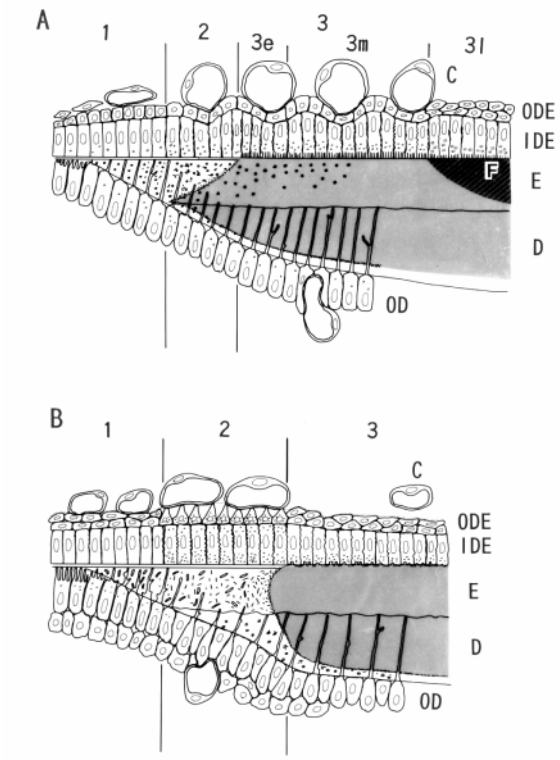


Fig. 7. Diagrams showing the odontogenesis in elasmobranchs and teleosts. A: *Tilapia*, teleost, B: *Heterodontus*, elasmobranch, 1: stage of enameloid matrix formation, 2: stage of enameloid mineralization, 3: stage of enameloid maturation, 3e: early stage of 3, 3m: middle stage of 3, 3l: late stage of 3. C: capillary, D: dentine, E: enameloid, F: iron accumulation, IDE: inner dental epithelium, OD: odontoblasts, ODE: outer dental epithelium, shadow means mineralized area.

COMPARISON OF THE ENAMELOID FORMATION BETWEEN ELASMOBRANCHS AND TELEOSTS

Ultrastructural and histochemical studies of the histogenesis of the enameloid in elasmobranchs have been performed by several researchers, and the details of the differences in enameloid formation between elasmobranchs and teleosts have been revealed (Sasagawa, 1989, 1993; 1998b; Sasagawa and Akai, 1992). Figure 7 is a schematic presentation of the enameloid formation in elasmobranchs and teleosts. When we compare teleost enameloid, actually cap enameloid, with elasmobranch enameloid in developmental aspects, the major points that differ between the two are as follows:

1. In teleosts, abundant collagen fibrils occupy the enameloid matrix in early stages. Many MVs are present among the collagen fibrils. In elasmobranchs, however, the organic matrix in enameloid mainly consists of large numbers of tubular vesicles (TVs) that are thought to be derived from odontoblasts. While two kinds of fibrous elements, i.e., collagen fibrils and electron-dense fibrils with stripes at intervals of 17nm are present in elasmobranch enameloid, the quantity of both is not great.
2. Although both the TVs in elasmobranchs and the MVs in teleosts are the sites of initial mineralization, the process of mineralization that occurs in the enameloid is different between the two fishes (Sasagawa, 1998b).
3. In teleosts, well-developed organelles concerning secretive function, i.e., rER, Golgi apparatus and secretory granules, are found in the IDE cells at the enameloid matrix formation stage. In elasmobranchs, in contrast, such organelles tend to develop at the stages of enameloid mineralization and maturation rather than at the stage of matrix formation.
4. Marked ruffled borders appear at the distal ends of the IDE cells at the stage of enameloid maturation in teleosts. The IDE cells also contain many lysosomal bodies and mitochondria in the distal cytoplasm. In elasmobranchs, conversely, there is no ruffled border, but many vesicles are present in the distal cytoplasm of the IDE cells at the stages of enameloid mineralization and maturation, when the organic matrix in the enameloid degenerates and disappears. It is probable that the IDE cells are involved in the removal and degeneration of enameloid matrix in the later stages of both teleosts and elasmobranchs, but the function of the IDE cells is distinct between the two fishes.
5. The ODE cells that include well-developed labyrinthine canalicular spaces and the penetration of capillaries into the dental epithelial cell layers, which are characteristic at the stages of enameloid mineralization and maturation in teleosts, are not found in elasmobranchs. In teleosts, the cytoplasm of the ODE cells showed neither marked ALPase activity nor ACPase activity (Sasagawa, 1998a), while the ODE cells at the stage of enameloid mineralization in *Heterodontus*, an elasmobranch, contained many granules which showed intense ACPase activity (Sasagawa, 1997b).
6. The ALPase activity is clearly localized at the proximal and lateral cell membrane of the IDE cells from the later stage of enameloid mineralization to the middle stage of enameloid maturation in teleosts, suggesting that the IDE cells are engaged in the removal of materials (Sasagawa, 1998a). The ALPase activity is also found in elasmobranchs at the same sites, but the stage is different. In elasmobranchs, the activity

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appears from the late stage of enameloid matrix formation to the end of the enameloid mineralization stage (Sasagawa, 1997b). Hence, the period when the ALPase activity is expressed in the IDE cells of teleosts is later than that in elasmobranchs.

7. In teleosts, the accumulation of iron in the outermost layer of the cap enameloid is often observed (Sasagawa, 1992). However, no such phenomenon has been observed in an elasmobranch, to our knowledge. This disparity may be one of the differences in the mechanism of later enameloid formation between teleosts and elasmobranchs.

Regarding the histogenesis of dental hard tissue, elasmobranch enameloid and teleost enameloid possess many differing features, as mentioned above, and they are distinct from mammalian enamel. We postulate that the enameloids found in living pisces are not an ancestral hard tissue of mammalian enamel, but rather an analogue.

ACKNOWLEDGEMENT

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Decussation Pattern of Hunter-Schreger Bands and Arrangement of Prisms in Mammalian Tooth Enamel

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INTRODUCTION

The microstructure of the enamel, such as enamel prism and Hunter-Schreger bands (HSB), has been known to differ between taxa. Kawai (1955) reported that the enamel structure of the cheek teeth differ between orders of mammals. HSB consist of adjacent zones of enamel prisms which are oriented in contrasting directions. HSB have been divided into five types according to our studies: horizontal HSB, which is the most common arrangement, vertical HSB, island-like HSB, uniserial HSB and irregular HSB (Kozawa, et al., 1991). In this study, details of HSB have been examined and two different patterns of the relationship between a band and the included prisms are described. They indicate the existence of different arrangements of prisms in the bands. The appearance of HSB may imply the arrangement and motion of ameloblasts during enamel development, so the patterns of ameloblast groups and their dancing determine types of HSB. The types of HSB may have evolutionary and taxonomic implications, because they are related to tooth morphology. In recent years the biomechanic and functional morphological implications of HSB have become apparent as well. The relationship between horizontal, vertical and island-like HSB types is discussed here in respect to ontogeny and phylogeny.

MATERIALS AND METHODS

The following mammalian teeth were prepared for this study: *Deinotherium*, for island-like HSB, *Rhinoceros* and *Pyrotherium* for vertical HSB, and *Canis* (dog), *Sus* (pig), *Giraffa*, *Tapir*, *Desmostylus*, and *Desmostylella*. (*Desmostylella* is of uncertain taxonomic affinity. In this study a small and multicusped molar resembling that of *Desmostylus* was assigned to *Desmostylella*.) for the horizontal HSB. The teeth were embedded in polyethylene resin and sectioned tangentially, vertically and horizontally. An oblique tangential section from the dentinoenamel junction (DEJ) was prepared to understand the transition of arrangement of HSB. After polishing and slight etching with 1/20N HCl the specimens were sputter-coated with gold. These specimens were analyzed by reflective light microscope. The enamel microstructure was examined by scanning electron microscope (SEM).

RESULTS

In tangential sections, the island-like HSB of the *Deinotherium* molar were characterized by the appearance of small patches under a reflective light microscope. This type of HSB did not have a belted appearance. In SEM observation of tangential section, this feature could be recognized as an assemblage of bundles of prisms (Fig. 1). Prism bundles were oriented in different directions and seemed vortices in parts. In longitudinal section, branches of prism bundles appeared irregularly between bundles which had similar optical feature.

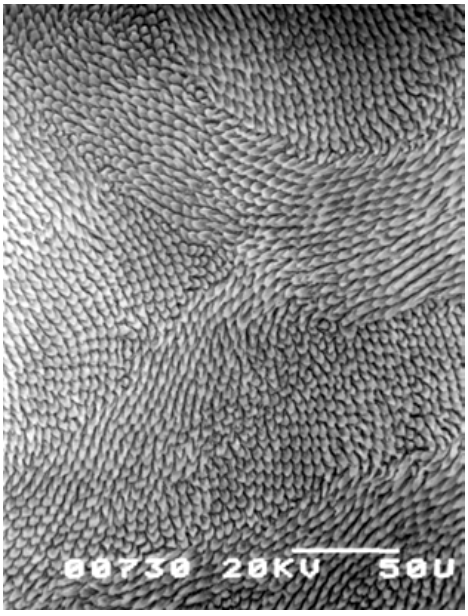


Fig. 1. SEM micrograph of *Deinotherium*, tangential section of molar. This feature can be recognized as assemblage of bundles of prisms, but island-like type of HSB does not show a belted appearance. The boundaries of bundles have various angled and shaped borders and seem to form vortices in parts.



Fig. 2. Light micrograph of *Pyrotherium*, tangential section of molar. Undulating vertical HSB can be seen in tangential enamel surface. The boundary between bands is not clear cut and prism orientation is slightly different between bands. *Phyrotherium* shows a transition from island-like type to vertical type of HSB according to pattern of bands of HSB.

Looking at the molar of *Pyrotherium*, undulating vertical HSB could be seen in tangential enamel surface (Fig. 2). In transverse sections, the HSB could be observed in the inner three fourths of enamel. The bands bifurcating near the DEJ merged into a band in the middle of enamel, but the width of the band changed on its way to the surface. The boundary between bands was not clear cut and prism orientation was slightly different between bands. As a result, the appearance of this vertical HSB seemed to include the island pattern decussation. In both tangential and transverse sections of *Rhinoceros*, vertical HSB could be perceived clearly in the inner three fourths of enamel. Two or three lines of prisms, which appeared as cross cut surfaces, composed the boundary between

bands. Each band consisted of quite distinct prisms rows. The arrangement of the prisms belonging to each band was in the opposite direction. The appearance of prism bundles had a complicated arrangement near the DEJ.

Dog (*Canis*) and Pig (*Sus*) both possessed horizontal HSB. The boundary between bands of HSB was distinguished by the different direction of adjacent bands under a SEM observation. Pig enamel was more than twice the thickness of dog enamel and was a little clearer than that of dog (Fig. 3). In *Tapir* and *Giraffa* enamel, both bands of HSB ran parallel in longitudinal section. Prisms composing the band gradually changed their inclinations on their way. The boundary between bands was not so clear by means of shifting to adjacent bands under SEM observation. Appearance of prism bundles showed island-like arrangement near the DEJ. In desmostylians, the transferring pattern of bands of HSB was perceived clearly meaning that the prisms changed directions they moved into another band. In this taxon, appearance of HSB was very similar and both bands of HSB ran parallelly in the course of an S curve in longitudinal section. Orientation of prisms composing a band of HSB inclined distinctly to the direction of the band in *Desmostylus* exclusively (Fig. 4). The boundary between bands was obscure because the prisms shift to adjacent bands. Each of the bands was usually the same width and was composed by about eight prisms in width. *Desmostylella* had a very similar appearance of HSB in this study.

In the zone from the DEJ until the commencement of banded feature of HSB, prisms started with varying inclinations at the DEJ and bent in different directions in both vertical and horizontal HSB. Prisms assemblage formed irregular borders which were produced by different inclination from adjacent prism groups The prisms seemed to form vortices at group borders. The appearance of this zone resembled island-like pattern decussation (Figs. 5 and 6). The breadth of the zone depended on the portion of the DEJ.

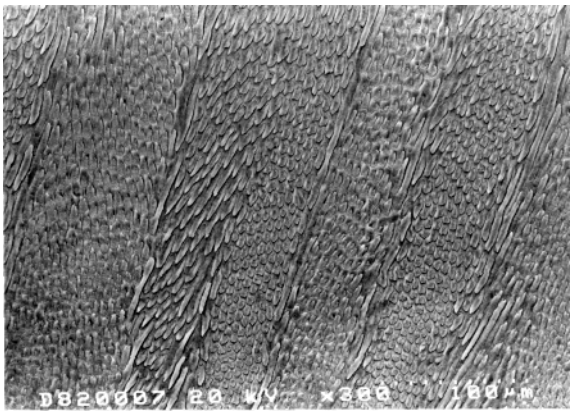


Fig. 3. SEM micrograph of *Sus* (pig), longitudinal section of molar. Boundary between bands of horizontal HSB is clear by means of different direction from adjacent bands. This feature shows a separate pattern of bands, which has a common inclination between a band and prisms belonging to the band. Moreover, appearance of enamel prism groups merging and dividing can be observed in the bands. These bands show various inclinations, widths and directions of bifurcation. Dog(*Canis*) has similar feature of horizontal HSB.

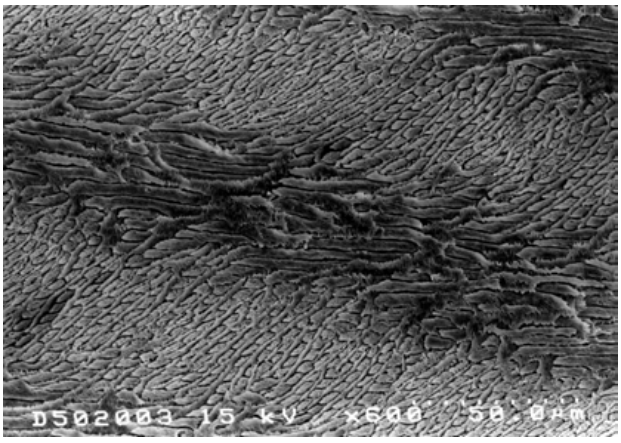
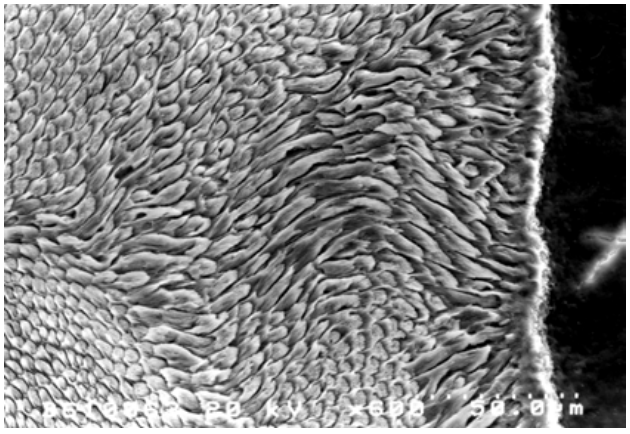
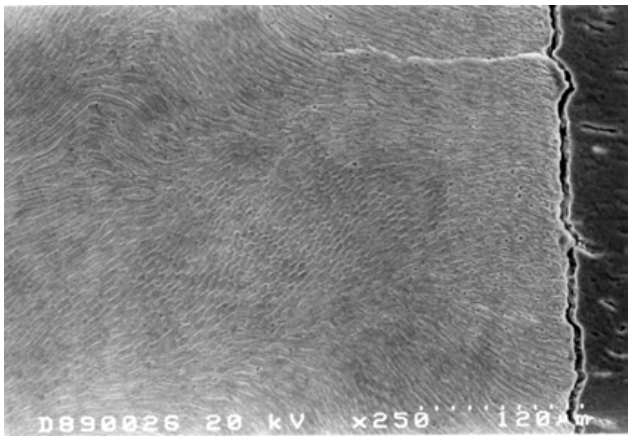


Fig. 4. SEM micrograph of *Desmostylus*, longitudinal section of molar. Transferring pattern of bands of HSB is perceived clearly. Orientation of prisms composed a band of HSB inclines distinctly in the direction of the band. The boundary between bands is obscure because the prisms shift to adjacent bands. Each of the bands is usually the same width and is composed by about eight prisms in width.





Figs. 5. and 6. SEM micrograph of *Rhinoceros*, transverse section of molar (Fig. 5) and of *Tapir*, longitudinal section of molar (Fig. 6). In the zone from the DEJ until the commencement of the banded feature of HSB, prisms start with varying inclinations at the DEJ and bend in different directions in both vertical and horizontal HSB. Prisms form assemblage with irregular borders, these are produced by different inclination from adjacent prism groups The prisms seem to be feature of vortices at group borders in place. The appearance of this zone resembles to island-like HSB. The breadth of the zone depends on the portion of the

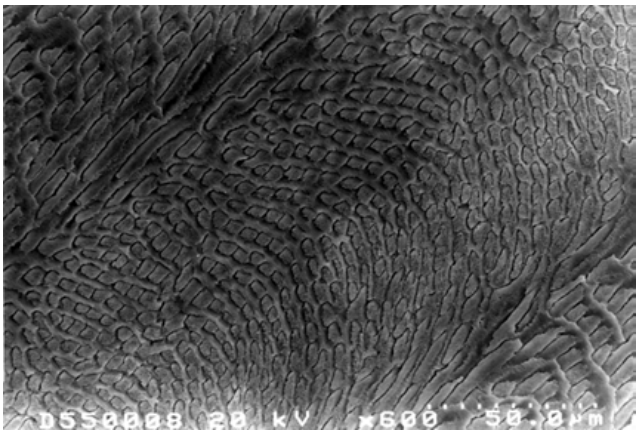


Fig. 7. SEM micrograph of *Desmostylella,* longitudinal section of molar. *Desmostylella* has very similar appearance to HSB to *Desmostylus*. Bands of HSB bifurcate at a certain frequency on their way by means of dividing a prism group. At the boundary between bands, change of prism orientation occurs gradually.

After the onset of the characteristic banded feature, bands of HSB bifurcated at a certain frequency on their way by means of dividing a prism group in both patterns of bands (Figs. 3 and 7). At the boundary between bands, a change in prism orientation occurred gradually. Some of the branches of a band disappeared on their way.

DISCUSSION

Appearance of island-like HSB has been described in *Barytherium* and *Deinotherium* (Kozawa, et al., 1991) and this type of HSB does not have a belted appearance. In the relationship between prisms and bands of horizontal HSB, there are two different ways that prisms change from a band to another or prisms continue to be a band during enamel development. These differences are indicated by the distinction between orientations of a band and prisms included the band. The separate pattern has a common inclination between a band and prisms belonging to the band. In the transferring pattern the prisms run through bands at a certain angle. Thus, the bands of HSB are formed not by prism groups which are arranged at earliest stage of development but by similar optical feature of prisms passing through the band. HSB which have two kinds of bands or zones traditionally, so called diazone and parazone, have different origins derived from either the separate pattern of bands or the transferring pattern of bands.

In this study, the separate pattern includes HSB of a dog (*Canis*) and a pig (*Sus*) while the transferring pattern embraces HSB of *Desmostylus*, and *Desmostylella*, and *Paleoparadoxia* (Suzuki, et al., 1995), which belong to desmostylians (Ijiri, 1939a,b; Kozawa, 1974; Kozawa, 1984).

Moreover rhinoceros, which was described in detail by Boyde and Fortelius (1986), has the separate pattern of bands of vertical HSB. On the other hand, prisms of *Pyrotherium* enamel which have vertical HSB run a complicated course between the bands. It seems that moving of prisms resembles the pattern observed in island-like HSB than the transferring type of bands.

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In the separate pattern, bifurcated and intermittent bands of HSB indicate the prism groups are produced alternatively. Besides, bifurcated and intermittent bands of HSB are observed in the transferring pattern. For this reason, it is confirmed that the existence of prism groups is maintained in the transferring pattern of bands. The degree of assemblage of prism groups and the moving of prisms forms the characteristic feature of HSB types.

In both vertical HSB and horizontal HSB, decussation of island-like pattern could be seen in the zone close to the DEJ. After this thin layer, the characteristic pattern of these HSB is formed. Appearance of enamel prism groups merging and dividing can be observed in the bands on their way. These bands show various inclinations, widths and directions of bifurcation.

In *Pyrotherium* having vertical HSB in the molar enamel, the width of band is not uniform and the boundary between bands is not so clear. In tangential section, an island-like pattern can be seen in places. It may be noticed that both HSB type and band pattern of *Pyrotherium* enamel has a transitional phase from island-like HSB to vertical HSB.

Island-like HSB are yet to be seen in living mammals and vertical HSB can be seen in only a few members (Fortelius, 1984). Vertical HSB of rhinocerotoides has been discussed from a biomechanical view (Rensberger and von Koenigswald, 1980; Rensberger, 1995) and both descriptive and experimental studies on the ontogeny of vertical HSB are needed.

It may be concluded that both vertical and horizontal HSB are derived from simple island-like HSB formed by simple ameloblastic group dancing, in terms of ontogeny and phylogeny. In enamel having horizontal HSB the manner of forming bands of HSB is varied. As a result, horizontal HSB have been adapted to differentiation of cusps and diversity of their morphology in the great majority of mammalian dentitions.

These enamel structure have been explained by the ameloblastic mobility. It is reported that each zone or band of HSB is formed by a bundle of prisms which have a common orientation. The arrangement of the ameloblasts, the spatial relationship of Tomes' processes to the enamel prisms, the form and size of the cells and the way in which the ameloblasts dance determine the number, shape, size and direction of the prisms within the developing enamel layer. The prisms initially arise at the DEJ and then differentiate into small groups which may be the origin of HSB. Each group of ameloblasts arranges to a certain extent, merges into new groups, and separates into another groups in the ameloblast layer. Little is known about the ameloblastic activities that produce such groups.

Ameloblastic dancing has improved transferring pattern of bands in horizontal HSB, which may be advantageous to composing complex feature of mammalian molars.

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Dental Characters of the Turolian Fossil Artiodactyls from Scontrone (Abruzzo, Central Italy)

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INTRODUCTION

A rich Vallesian (Lower Tortonian) vertebrate community was found in 1990 at Scontrone (National Park of Abruzzo, central Italy). Remains of reptiles (crocodiles and chelonians) and continental mammals were recovered in calcarenite layers. The macromammals from Scontrone resemble closely those found in the late 70's in fissure fillings of the Gargano penisula (Rustioni et al., 1992). Unlike those from Scontrone, the Gargano assemblages include also micromammals, such as *Prolagus*, *Microtia* and other rodents.

The Scontrone mammalian community includes a lutrine carnivore and some artiodactyls, among which *Hoplitomeryx* (Leinders 1983) and a larger-sized *Amphimoschus*-like cervid (Rustioni et al., 1992).

Unfortunately, scanty cranial specimens and few upper dentitions have been encountered until now, most of which belong to young individuals. Quite more abundant and varied are the mandibles and lower dentitions of adult or even aged individuals.

MATERIALS AND METHODS

The specimens studied here are the following: juvenile left maxillary (SCT 18); fragmental right maxillary (SCT 59); fragmental right maxillary (SCT 125); fragmental mandible (SCT 16); fragmental left mandible (SCT 20); fragmental right mandible (SCT 29); fragmental right mandible (SCT 50); fragmental right mandible (SCT 51); fragmental left mandible (SCT 58); fragmental left mandible (SCT 60); fragmental left mandible (SCT 67); fragmental right mandible (SCT 71); fragmental left mandible (SCT 72); fragmental right mandible (SCT 73); fragmental right mandible (SCT 76); fragmental right mandible (SCT 77); fragmental left mandible (SCT 78); fragmental right mandible (SCT 79); fragmental right mandible (SCT 80); fragmental left mandible (SCT 81); fragmental left mandible (SCT 82); fragmental right mandible (SCT 83); fragmental left maxillary (SCT 86); fragmental right mandible (SCT 88); fragmental left mandible (SCT 89); fragmental right mandible (SCT 99); fragmental left mandible (SCT 100); fragmental left mandible (SCT 102); fragmental right mandible (SCT 103); fragmental right mandible (SCT 112); fragmental left mandible (SCT 155); fragmental left mandible (SCT 177); fragmental right mandible (SCT 195). Several isolated cheek teeth were also found, but they have been overlooked in the present study. (See Figs. 1 and 2. and Tables 1 and 2.)

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Hypsodonty is usually calculated by height of crown x 100/length, although a numerical boundary between brachyodont and hypsodont teeth has not been established. Heintz (1970) also introduced another index, breadth x 100/length, which can be considered a numerical representation of our definition of brachyodonty/hypsodonty. The brachyodonty and hypsodonty of the Scontrone cheek teeth could not be quantitatively assessed because of their heavy wear. Therefore, Mazza and Rustioni (1996) considered brachyodont the teeth with labio-lingual breadth at the collar considerably exceeding the labio-lingual breadth at the occlusal plane and hypsodont those with the two breadths comparable. This derives from the fact that in brachyodont teeth the labial and lingual walls converge occlusalwards, while in hypsodont teeth they are approximately parallel. In this view, upper cheek teeth are normally slightly more brachyodont than lower cheek teeth. Lower cheek teeth therefore provide a more reliable picture of hypsodonty/ brachyodonty than do upper cheek teeth. The cheek teeth from Scontrone are cementum-free. Cementum seems to be correlated with feeding on grasses or on plants with a relatively high silica content, rather than with a particular dental structure. It seems to be indipentent from brachyodonty or hypsodonty, although it is more frequently observed in hypsodont teeth. Some herbivores have brachyodont teeth, in the sense given above, and in some cases their teeth are covered with a variable amount of cementum, like in the small-sized gomphotherid from Gebel Zelten (Libia) and in several hippopotamuses.

Solounias and co-workers (1994; 1995) observed that tooth microwear is not a reliable tool for assessing the dietary habits of ruminants, since it reflects the last diet of an animal before death and not necessarily its usual diet. They (Solounias et al., 1995) considered the morphology of the masticatory apparatus far more reliable in discriminating between browsers and grazers. For this reason they recommend concentration on the structure of skulls and mandibles. Mandibles are quite well represented at Scontrone, while skulls are very fragmentary. The only taxon which could be studied in detail is *Hoplitomeryx*, because a complete skull of this genus is known from Gargano.

Dimensional and morphological similarities in the dentition represented the criterion adopted to attribute some maxillaries and mandibles to the same taxon.

RESULTS

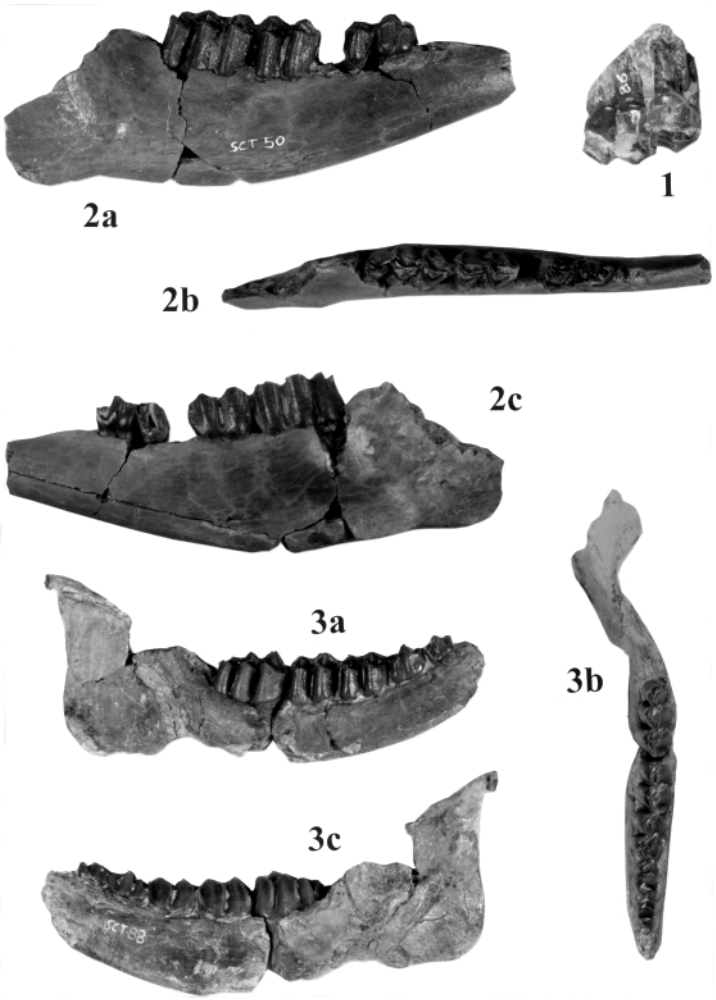


Fig. 1.

1: cf. *Amphimoschus*,	**left maxillary,**	**lingual view.**	**SCT 86 x 1.5**
2a: *Hoplitomeryx* sp.	**right hemimandible,**	**lateral view.**	**SCT 50 x 1.4**
2b:	**occlusal view**		
2c:	**lingual view**		
3a: Taxon A	**right hemimandible**	**lateral view**	**SCT 88 x 1.5**
3b.	**occlusal view**		
3c.	**lingual view**		

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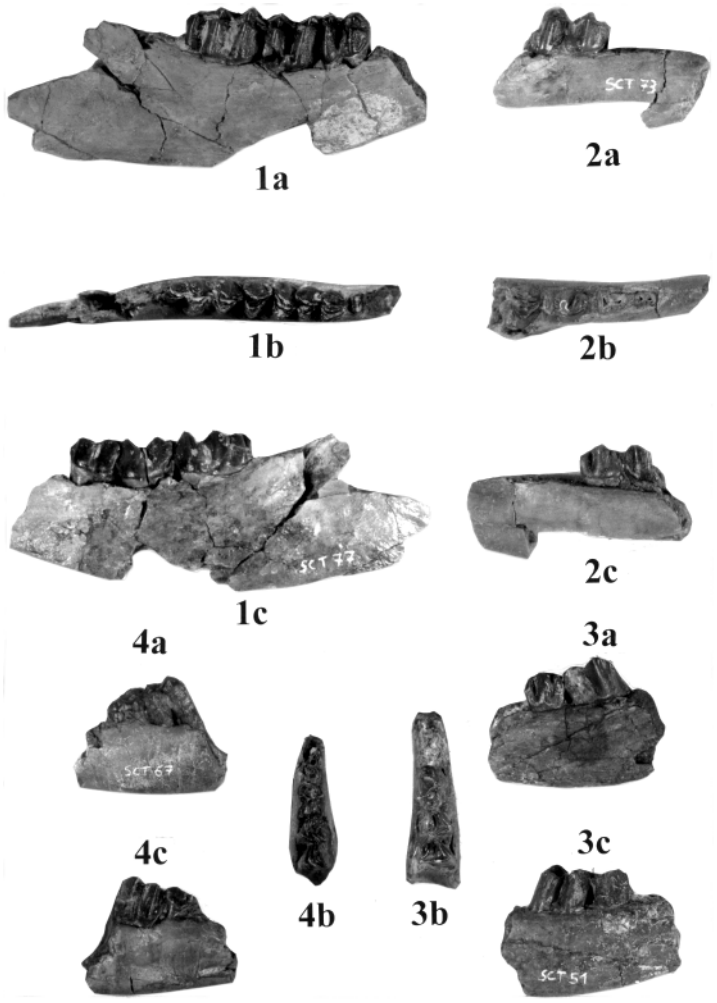


Fig.2.

1a. Taxon A	**right hemimandible**	**lateral view**	**SCT 77 x 1.2**
1b.	**occlusal view**		
1c.	**lingual view**		
2a. Taxon A	**right hemimandible**	**lateral view**	**SCT 73 x 1.3**
2b.	**occlusal view**		
2c.	**lingual view**		
3a. Taxon B	**right hemimandible**	**lateral view**	**SCT 51 x 1.6**
3b.	**occlusal view**		
3c.	**lingual view**		
4a. Taxon B	**left hemimandible**	**lateral view**	**SCT 67 x 1.7**
4b.	**occlusal view**		
4c.	**lingual view**		

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cf. Amphimoschus

Maxillary SCT 18

A fragmentary left maxillary of a large-sized juvenile artiodactyl, approximately as large as a present-day fallow deer, still preserves the emerging third upper premolar, the last deciduous upper premolar, which is being replaced by the emerging fourth upper premolar, and the first two molars. The teeth are brachyodont. The deciduous fourth upper premolar is molariform and has a very robust paracone labial rib, no metacone rib, a well developed parastyle and mesostyle, and a weak metastyle. The protocone is more developed than the hypocone and its rear ala is bent forwards. The rear alae of the paracone and of the protocone fuse with wear with the anterior ala of the hypocone. A small entostyle is present, while no cingula occur.

The labial enamel wall of the definitive third upper premolar has robust styles and paracone ribs. The tooth has a weak protocone fused to a well developed hypocone. The metacone is also weak. Two marked spurs are present on the lateral enamel walls of the lingual cusps, one on the anterior ala of the protocone, the other on the posterior ala of the hypocone. The tooth lacks cingula and entostyle. The labial enamel wall is smoother than the lingual one.

The two molars look very much alike, the second upper molar being practically an enlarged replica of the first molar. Both bear a robust parastyle, an even stronger mesostyle and a considerably developed paracone rib. On the other hand the metacone rib is lacking. The lingual crescents have enlarged rear alae. Cingula and entostyles are not present. A very narrow fissure, which extends down to the enamel collar, separates the lingual crescents. The labial enamel wall is smoother than the lingual one.

Maxillary SCT 86

The small maxillary fragment bears the fourth premolar and the first molar. The teeth are heavily worn and very brachyodont. The premolar is unmolarized. The first molar has a very reduced entostyle. Both teeth have robust and prominent paracone ribs, while the metacone ribs are slightly developed and do not form any prominence on the labial enamel walls. The styles are also very robust and jutting. No cingula occur. Also the cement is absent, the teeth having an overall smooth enamel.

Mandible SCT 20

This fragmental left mandible was explicitly called "*Amphimoschus*-like" by Rustioni et al. (1992) and Mazza and Rustioni (1996) for its resemblace to *Amphimoschus artenensis* (Mayet, 1908), although the teeth borne by the specimen from Scontrone have smoother enamel. SCT 20 is a large-sized representative, approximately the size of the maxillary SCT 18. Few other isolated third lower molars are attributed to this taxon. The horizontal ramus has a fairly marked pachyostosis. The specimen still preserves its complete molar row. The brachyodont molars all bear ectostylids, progressively smaller from the first to the third molar. The lingual crescents are directed obliquely backwards and asymmetrical. Weak cingula are present at the bases of the protoconids. The lingual enamel walls are smooth, the buccal are rough. The metaconid ribs are more robust than entoconid ribs. The third molar has a distinctly bi-cusped third lobe.

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Hoplitomeryx sp.

Maxillaries SCT 59 and SCT 125

Rustioni et al. (1992) and Mazza and Rustioni (1996) have reported the occurrence of *Hoplitomeryx* among the artiodactyls from Scontrone. Unfortunately, the author of this genus, Leinders (1983), failed to provide description of the teeth of these peculiar animals; he only reported that *Hoplitomeryx* lacks the second lower premolar. SCT 59 and SCT 125, the fragments of two right maxillaries, have been attributed to *Hoplitomeryx* because of the resemblance of their cheek teeth with those borne by the mandibles ascribed to this genus, mandibles which are described below. SCT 59 and SCT 125 still bear the second and third molars. The teeth are relatively more hypsodont than those of other arctiodactyls from Scontrone. The paracone ribs are prominent, while the metacone ribs are slightly marked. The lingual crescents have a peculiar trapezoidal outline in occlusal view. The styles are well developed, but not as robust as those of the upper teeth attributed to taxon A. The second molars bear a very small entostyle, while the third molars lack such structure. Cingula and cement are absent.The lingual enamels are rough, while the labial ones are smooth.

Mandibles SCT 16, SCT 29, SCT 50, SCT 58, SCT 71, SCT 80, SCT 81, SCT 89, SCT 102 and SCT 195

Based on Leinders' (1983) indications, the lower cheek teeth formula of *Hoplitomeryx* seems to be 2.3. Ten mandibles (SCT 16, SCT 29, SCT 50, SCT 58, SCT 71, SCT 80, SCT 81, SCT 89, SCT 102 and SCT 195) are tentatively referred to *Hoplitomeryx* because of their resemblance to that illustrated by Leinders (1983).

These mandibles are sinuous in both dorsal and ventral view. The horizontal ramus is slender in latero-medial view and it tapers considerably towards the symphyseal area. The alveolar corpus is latero-medially massive. These specimens have shallow masseterine fossae and deep pterygoid fossae. The ventral rim of the masseterine fossa and the mandibular scissure are well marked. The rear gonion extends beyond the condyle; the latter is short and directed obliquely outwards and backwards, and slightly inwards and downwards. A high mental foramen is elongated forwards by a deep groove.

Adopting the criterion introduced above, the teeth borne by these mandibles appear fairly hypsodont. In all the teeth the labial enamel is considerably rougher than the lingual one.

The non-molarized fourth premolar bears a small paraconid and a slightly split metaconid. The metaconid is connected to the protoconid and the entoconid to the hypoconid by lophids directed obliquely backwards. A weak entostylid occurs. A shallow vertical groove is present on the buccal wall at the transition from the trigonid to the talonid. No cingula can be observed.

The "pli-palaeomeryx" is not present on the molars. The lingual crescents and the triangular labial ones are obliquely directed backwards and inwards. The mesostylids are more robust than the parastylids and metastylids. The molars have fairly prominent metaconid and entoconid ribs. A small ectostylid occurs between the protoconid and the hypoconid of all the molars. No cingula are present. The third molar has a characteristically bi-cusped third lobe, with the labial lobe larger than the lingual one.

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The mandibles described in this section, as we have seen, have slender, depressed horizontal rami and very convex basal profiles, all features suggestive of a browser ruminant. A complete and unpublished skull of *Hoplitomeryx*, found in the Gargano, and the reconstruction illustrated by Leinders (1983), consistently shows depressed maxillary fossae and an acute angle between the ideal line connecting the rear to the anterior end of the lateral dentition and the ideal line running from the rear end of the lateral dentition to the fore border of the orbit, which, according to Solounias et al. (1995) are other typical features of browser ruminants (Fig. 3).

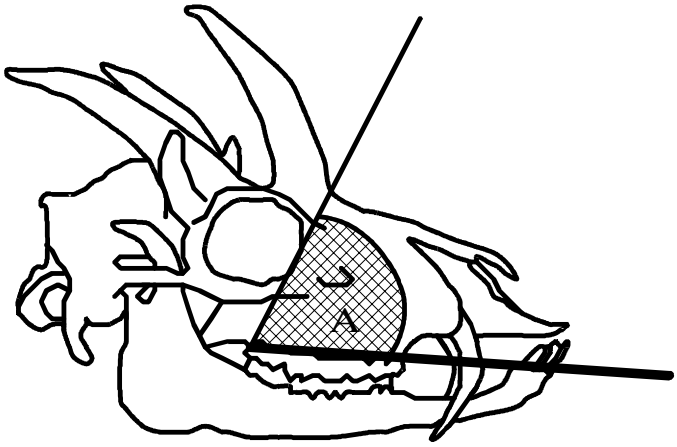


Fig. 3. Skull of *Hoplitomeryx* - the acute angle A, between the ideal line from the rear to the anterior end of the lateral dentition and the ideal line from the rear end of the lateral dentition to the fore border of the orbit is a typical feature of browser ruminants, according to Solounias et al., (1995).

Taxon A (unnamed)

Maxillaries SCT 70

This maxillary fragment still preserves its molar row, although the first molar is incomplete.The teeth are very brachyodont and their ribs do not form any kind of prominence on the labial enamel walls, which thus appear smooth. Also the sharp styles do not form any relief on the labial walls. The lingual crescents are U-shaped. There is a tiny entostyle issuing from a slightly developed cingulum at the base of the hypocone of the second molar. The enamel is smooth all over and there is no cement.

Mandibles SCT 60, SCT 73, SCT 76, SCT 77, SCT 88, SCT 103, SCT 155 and SCT 177

These mandibles also have sinuous horizontal rami, but the horizontal ramus is more depressed and more slender than in *Hoplitomeryx,* in particular latero-medially and the ventral border of the masseterine fossa and the mandibular scissure are far less evident. In ventral view, the horizontal ramus has a sharp ventral border.

The cheek teeth formula is 3.3. The teeth are more brachyodont than in *Hoplitomeryx*. The premolar row is decideadly reduced especially for the small dimensions of the second and third premolar. The fourth premolar is unmolarized, with a robust parastylid and an extremely weak paraconid; the two structures fuse together with wear. The metaconid is

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connected to protoconid and the entoconid to the hypoconid by lophids almost perpendicular to the sagittal axis of the tooth. The enamel surfaces are smooth. The transition from the trigonid to the talonid is marked by a very deep vertical groove on the labial enamel wall. The fourth premolar bears no cingula. The lingual crescents of the molars are directed obliquely backwards like in *Hoplitomeryx.* Ectostylids are present on all the molars. The difference in height of the lingual and buccal cuspids is lesser than in the molars of *Hoplitomeryx.* Very robust ribs and stylids occur on the lingual walls of the molars. They are wear-resistant structures, which give a trough-like wear outline of the molars in medial view. A weak cingulum occasionally occurs at the base of the protoconid, especially on the first molar. The buccal enamel walls are rough, while the lingual ones are smooth. The "pli-palaeomeryx" is never present.

Taxon B (unnamed)

Mandibles SCT 51, SCT 67 and SCT 79

These fragmental mandibles have very hypsodont cheek teeth. They bear an unmolarized fourth premolar with paraconid and parastylid fused together. The lophids connecting the metaconid with the protoconid and the entoconid with the hypoconid are obliquely directed backwards. A robust entostylid occurs, while there are no cingula. The trigonid is separated from the talonid by a very deep vertical groove and the outer enamel wall is rough. The first two molars are very similar to one another. The distinctly triangular buccal crescents of these molars are directed obliquely backwards and a distinct ectostylid occurs between the protoconid and hypoconid. The difference in height of the entoconid and hypoconid is lesser than in *Hoplitomeryx*. The entoconid has a prominent lingual rib. The metastylid is sharp.

Taxon C (unnamed)

Mandibles SCT 72 and SCT 83

Both fragments still bear their second and third molars, although both second molars are incomplete and the third molar of SCT 83 is badly preserved. A peculiar feature of these mandibles is that their horizontal rami show a marked pachyostosis. The teeth are brachyodont without ectostylids. The ribs and the styles of the teeth are slightly prominent on the lingual enamel walls. The rear alae of the protoconids are fused to the anterior alae of the entoconids. The labial crescents are obliquely arranged backwards and have a V-shaped outline in occlusal view. The posterior lobe is bicusped, with the labial lobe exceeding in size the lingual lobe.No cingula nor cement occur. The enamel is rough.

Table 1. Measurements of upper cheek teeth (mm.)

Maxillary	SCT 18	SCT 59	SCT 70	SCT 86	SCT 125
4th deciduous tooth Length	12.0				
4th deciduous tooth Breadth	12.5				
3rd premolar Length	11.6				
3rd premolar Breadth	12.0				
4th premolar Length				10.8	
4th premolar Breadth				14.3	
1st molar Length	16.1			14.9	
1st molar Breadth				16.0	
2nd molar Length	18.3	13.9	10.3		13.0
2nd molar Breadth		12.7	12.9		12.5
3rd molar Length		15.8	11.9		
3rd molar Breadth		13.9	10.8		

Table 2. Measurements of lower cheek teeth (mm.)

Mandibles	SCT 16	SCT 29	SCT 50	SCT 58	SCT 71	SCT 80	SCT 81	SCT 89	SCT 60	SCT 73	SCT 76	SCT 77
LP/2-P/3												
LP/3-P/4									19.2			
LP/3-M/3		57.5							59.1?			
LP/4-M/3			60									
LM tot		44.0	40.8				41.9		38.9		36.9	36.5
P/3L		6.4							6.6			
P/3l		4.0										
P/4L	8.3	8.1	8.9						8	7.8		
P/4l		6.0	5.7						4.6	5.5		
M/1L	11.5	10.8	10.2				11.7	12.2	10.4		10.6	10.3
M/1l		7.2					9.7	7.1	6.8			7
M/2L	12.9	13.2	12.5	14			12.3	14.1	11.7		11.3	11.8
M/2l		7.5	7.7	7.9			10.1	7.3	7.6			8
M/3L	18.3	19.1	18.1		19.6	20.1	18.7		16.3		14.3	16.1
M/3l		7.5	7.8		8	8.6	9.6		7.8			8

LP/3-M/3 toothrow length from P/3 to M/3; **LP/3-P/4** toothrow length from P/3 to P/4;
LP/3-M/3 toothrow length from P/3 to M/3; **LP/4-M/3** toothrow length from P/4 to M/3;
LM tot molar row length: M/1 - M/3;
P/3L third premolar length; **P/3l** third premolar breadth;
P/4L fourth premolar length; **P/4l** fourth premolar breadth;
M/1L first molar length; **M/1l**first molar breadth;
M/2L second molar length; **M/2l** second molar breadth;
M/3L third molar length; **M/3l** third molar breadth.

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CONCLUSIONS

The observations outlined above attest to the occurrence of at least five different taxons, one relatively large-sized representative, about the size of a present day fallow deer, and four smaller ones, ranging from a size comparable with that of a present-day hydropote approximately to that of a roe deer. The cheek teeth of most of these animals are brachyodont and lack cingula and cementum. The dentitions of these representatives recall those of present-day fallow or roe deer, therefore of browser and browser/grazer rather than true grazer artiodactyls, which is also the life habit suggested by the skull and mandible architecture of *Hoplitomeryx*.

The artiodactyls from Scontrone are suggestive of discontinuous forest cover, perhaps rich in bushes, dominated by tender vegetation.

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Mature Enamel Morphology and Forming Enamel Dynamics: Crystal Orientation, Crystal Continuity, Prism Diameter, Retzius Lines, and Prism Cross-Striations

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INTRODUCTION

The structure of mature dental enamel tells a story, the story of its development. This is because evidences of its growth process are permanently recorded in its mature structure (Risnes, 1998). The structure of enamel primarily affords information on various aspects of the ameloblasts; on their shape, size, packing pattern, direction of movement, speed of movement/rate of secretion, life span, and rate of recruitment. It is advisable that one always, when studying the structure of mature enamel, seeks to relate it to development, ontogenetic or phylogenetic, or to function. On the other hand, studies of enamel development need to be interpreted within the frame set by the structure of mature enamel. It is this reciprocal appreciation of mature enamel morphology, forming enamel dynamics, and enamel function that forwards progress in the collective knowledge and understanding of dental enamel.

The present study presents some new evidences on mature enamel structure related to amelogenesis.

MATERIALS AND METHODS

Rat mandibular incisors for transmission electron microscopy were obtained by dissection after anaesthesia and perfusion fixation of the animals according to Karnovsky (1965). The teeth were demineralized according to Warshawsky and Moore (1967), rinsed in the buffer, post-fixed in 2% OsO_4 for 30 minutes, dehydrated in a graded series of ethanol, and embedded in Epon®. The apical part of the incisor containing the zone of enamel secretion was sectioned into about 2 mm thick transverse sections, which were reembedded in Epon® and oriented such that the sections would be cut parallel with the prisms, i.e. obliquely transversely. Ultrathin sections were cut in a Leica ultracut UCT ultramicrotome, stained with uranyl acetate and lead citrate, and observed in a Philips 400T transmission electron microscope operated at 60 kV.

The erupted part of rat incisors and human extracted teeth were sectioned and ground along predetermined planes according to the method of Risnes (1981, 1984). The specimens were brushed lightly under running tap water, etched with 0.1 or 1% nitric acid for 15-30 seconds, air-dried overnight, sputter-coated with 30 nm gold-palladium and observed in a Philips 515 SEM (scanning electron microscope) operated at 15-20 kV.

RESULTS

Crystal orientation and crystal continuity

Developing rat incisors cut obliquely transversely and parallel with the long axis of the prisms (Fig. 1) demonstrated that the prism crystals were not necessarily oriented perpendicular to the secretory aspect of the Tomes' process. The prism crystals could deviate as much as 60-70^{o} from a perpendicular orientation relative to the secretory surface of the Tomes' process.

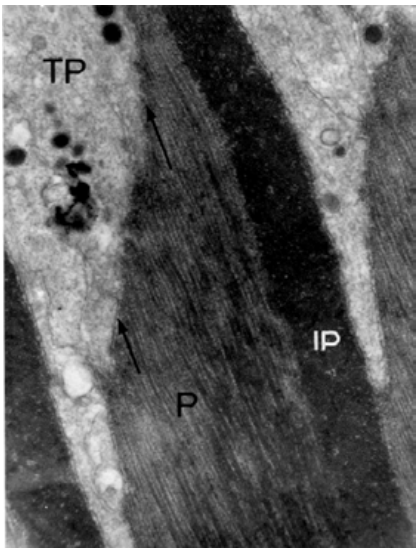
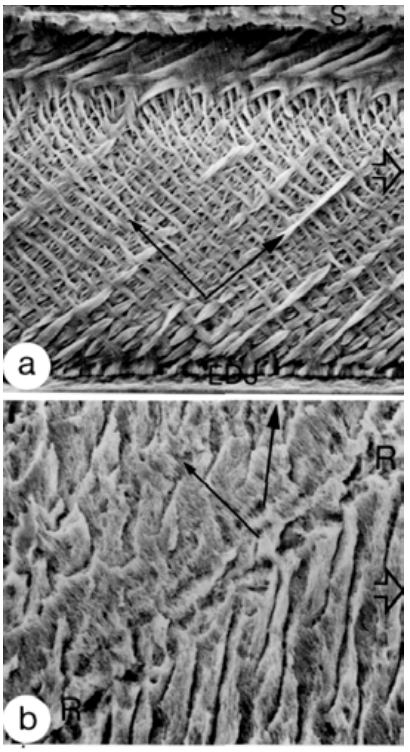


Fig. 1. Transmission electron micrograph of rat incisor demonstrating that the crystals of prisms (P) may be oriented obliquely (arrows) to the secretory aspect of the Tomes' process (TP). IP = interprism. X 14000.



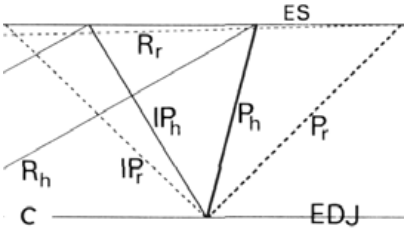


Fig. 2. a. SEM micrograph of longitudinally sectioned rat mandibular incisor showing orientation of prisms/prism crystals (large arrow) and interprism crystals (small arrow). Incisal direction toward right (open arrow). EDJ = enamel-dentin junction. X 380. b. SEM micrograph of mature human permanent enamel showing orientation of prisms/prism crystals (large arrow) and interprism crystals (small arrow), the latter perpendicular to a Retzius line (R-R). Incisal direction toward right (open arrow). X 635. c. Schematic representation of orientation of prism crystals in rat (P_r) and man (P_h), interprism crystals in rat (IP_r) and man (IP_h), and Retzius lines in rat (R_r) and man (R_h) in longitudinally sectioned enamel. EDJ = enamel-dentin junction, ES = enamel surface.

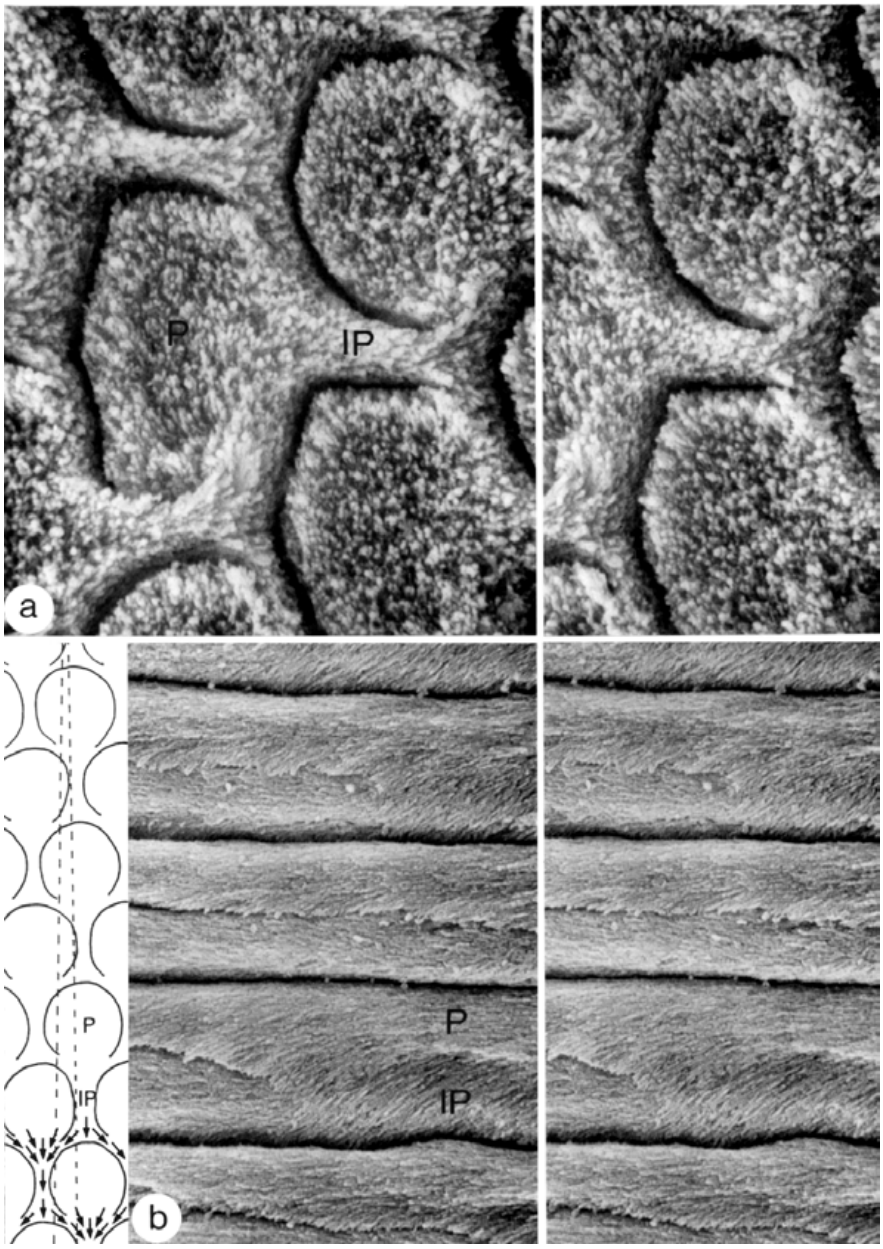


Fig. 3. SEM stereo-pair micrographs of mature human deciduous enamel. a. Transversely sectioned prisms. Crystals deviate cervically from prism (P) to interprism (IP). X 6500. b. Longitudinally sectioned prisms. Crystals deviate cervically from prism (P) to interprism (IP). X 2500. Inset: stippled lines indicate plane of section along left and right border of the micrograph, arrows indicate streaming of interprism crystals deduced from SEM stereo-pair micrographs.

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Both in rat (Fig. 2a) and in man (Fig. 2b) the orientation of crystals in prisms and interprism was distinctly different; the interprism crystals deviated in a cervical direction relative to the prism crystals, more in rat than in man, about 90^{o} and 45^{o} respectively. In man the interprism crystals tended to be oriented perpendicular to the Retzius lines (Fig. 2b).

The rate of growth in length of crystals during enamel formation equals the distance grown divided by the time spent. The geometry of enamel structure indicates that prism crystals form more rapidly than interprism crystals in man, but not in the rat (Fig. 2c). In both species, however, interprism crystals reach the enamel surface at a more cervical position than the prism crystals that they were positioned adjacent to at the EDJ.

In acid-etched specimens of mature deciduous human enamel crystal orientation could be judged by stereo-pair SEM micrographs both in specimens sectioned transversely (Fig. 3a) and in specimens sectioned longitudinally (Fig. 3b) relative to the long axis of the prisms. It was evident that crystals deviated from the prism into the interprism intervening between the two cervically situated prisms. As the deviating interprism crystals approached the convex border of the next prism, the peripheral crystals deviated to each side of this prism, passing between it and the immediately adjacent incisally situated prisms. However, the central crystals of the interprism tail abutted on the next prism without changing direction and apparently ended at the prism sheath. The pattern of cervical streaming of crystals from prism to interprism and within the interprism as judged from the stereo-pair SEM micrographs is indicated schematically in Figure 3b.

Prism diameter

In a supernumerary human tooth, a mesiodens, a conspicuous hypoplasia was present incisally; the enamel outside a distinct Retzius line had not been formed at the incisal tip (Fig. 4a). A distinct enamel shoulder bordered the hypoplastic defect cervically. The prisms of this shoulder exhibited a curved path, bending incisally from the distinct Retzius line (Fig. 4b). The distance between the centers of prisms visible on the surface of the enamel shoulder was about 8 μm (Fig. 4c). A sector of about 190 μm at the distinct Retzius line contained the same prisms as a sector of about 330 μm at the surface of the shoulder (Fig. 4b). This increase in linear dimension corresponds to an increase in the distance between prism centers from 4.5 to 8 μm, identical to the increase observed in the present study. The angulation of the prisms relative to the enamel surface was about the same as that relative to the distinct Retzius line. On approaching the shoulder surface, prism borders became indistinct in the longitudinal plane. Close to the bottom of the hypoplastic defect a pattern of incremental lines with a periodicity of about 2 μm could be observed (Fig. 4c).

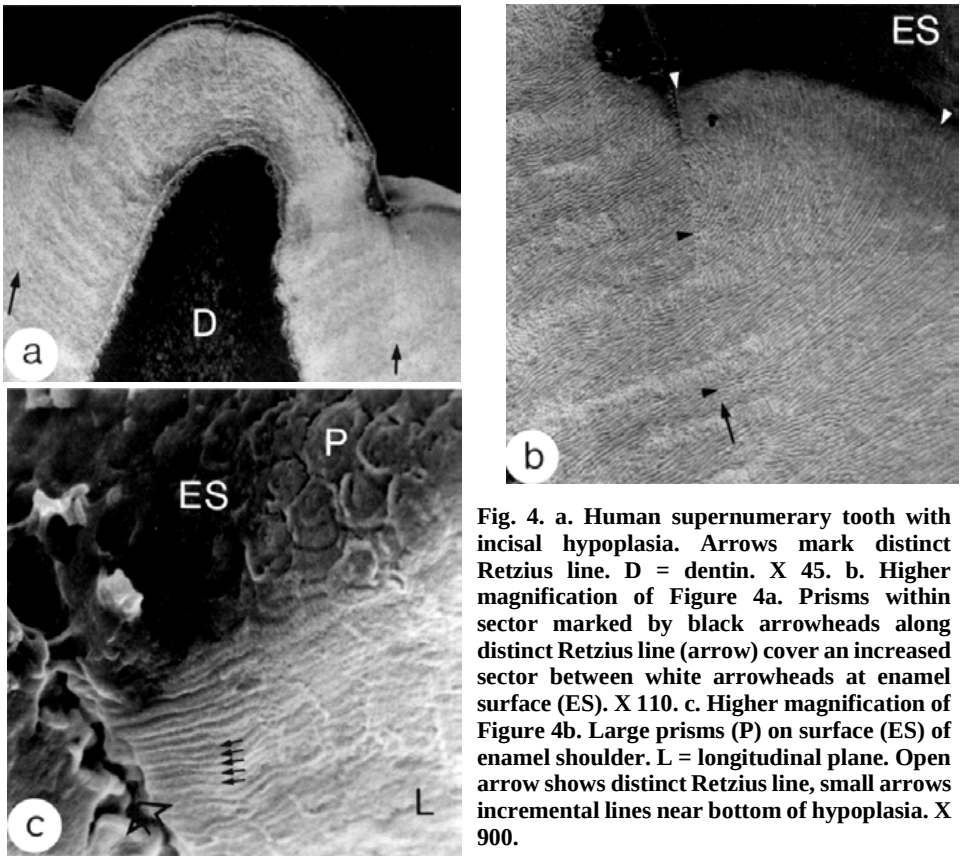


Fig. 4. a. Human supernumerary tooth with incisal hypoplasia. Arrows mark distinct Retzius line. D = dentin. X 45. b. Higher magnification of Figure 4a. Prisms within sector marked by black arrowheads along distinct Retzius line (arrow) cover an increased sector between white arrowheads at enamel surface (ES). X 110. c. Higher magnification of Figure 4b. Large prisms (P) on surface (ES) of enamel shoulder. L = longitudinal plane. Open arrow shows distinct Retzius line, small arrows incremental lines near bottom of hypoplasia. X 900.

Retzius lines

Some of the regularly spaced Retzius lines in the outer enamel of a human permanent tooth (Fig. 5a) were characterized by a temporary loss of distinct prism profiles. This was evident both in a transverse tooth plane cutting the prisms longitudinally (Fig. 5b) and in a tangential tooth plane cutting the prisms transversely (Fig. 5c).

A thicker, non-periodic Retzius line in the inner enamel was also characterized by an obscuring of prism identity with absence of prism sheaths (Fig. 5d).

Prism cross-striations

Prism cross-striations were readily visible in an acid-etched specimens of human permanent enamel (Fig. 6). Typically, the cross-striations curved cervically from prism to interprism, maintaining a more or less perpendicular relationship to the crystals.

Ameloblasts with Tomes' processes could be schematically inserted into the SEM micrograph in a staircase-like pattern, using the cross-striations and prism sheaths as guiding lines (Fig. 6). There was a correspondence in width between the three to four last prism segments produced by three neighbouring imaginary ameloblasts.

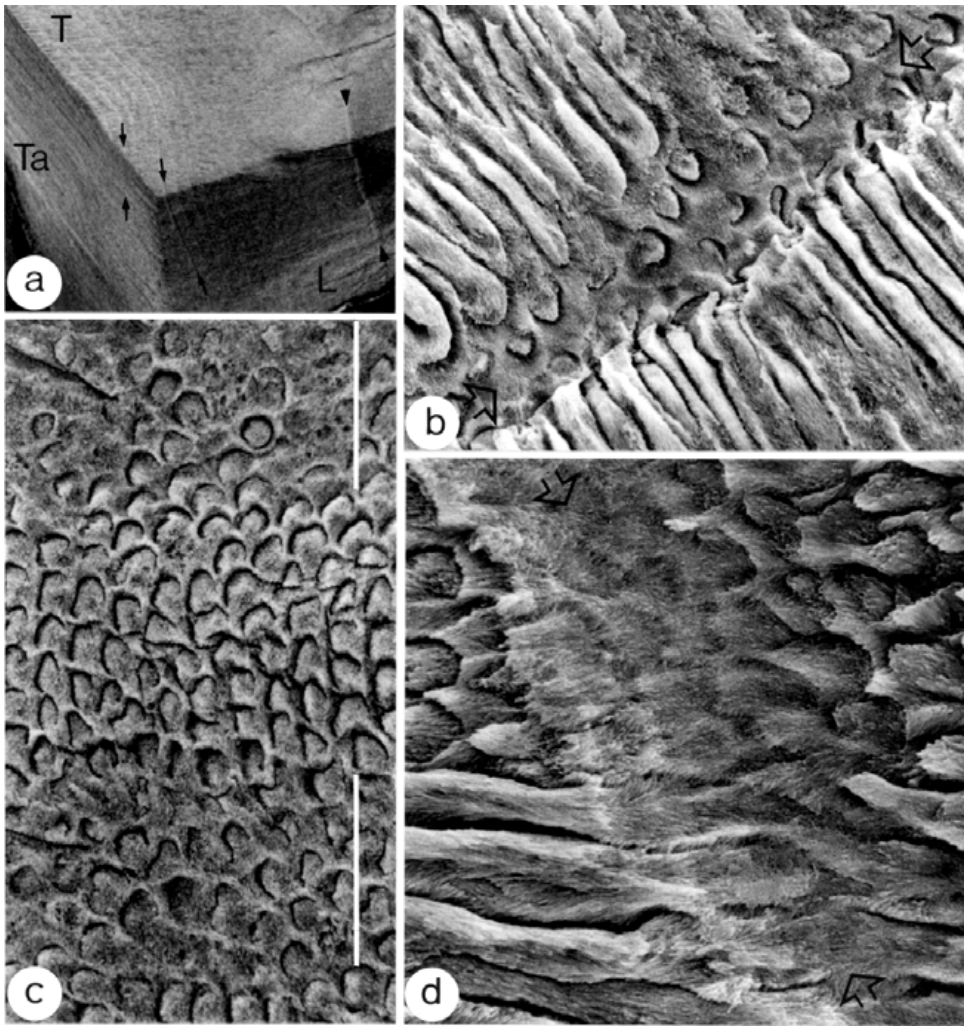


Fig. 5. SEM micrographs of mature human permanent enamel demonstrating structure of Retzius lines. a. Human premolar sectioned along longitudinal (L), transverse (T), and tangential (Ta) planes. Thin, regularly spaced Retzius lines in outer enamel can be followed from one plane to the next (small arrows). Thick Retzius line (arrowheads) in inner enamel can be followed from longitudinal to transverse plane. X 45. b. Appearance of regularly spaced Retzius line (open arrows) in outer enamel of the transverse plane in Figure 5a. Prism profiles are temporarily lost. Direction toward enamel surface is toward left and upwards. X 890. c. Appearance of two regularly spaced Retzius lines (bars) in outer enamel of the tangential plane in Figure 5a. Prism profiles are temporarily lost or indistinct. X 890. d. Thick Retzius line (arrows) in inner enamel of the longitudinal plane in Figure 5a. Obscuring of prism identity. Direction toward surface is toward left. X 1780.

Prism cross-striations occur at intervals along the long axis of the prisms. The geometry of the prism cross-striations indicate that cross-striations will also be visible in obliquely sectioned and nearly cross-cut prisms (Fig. 7).

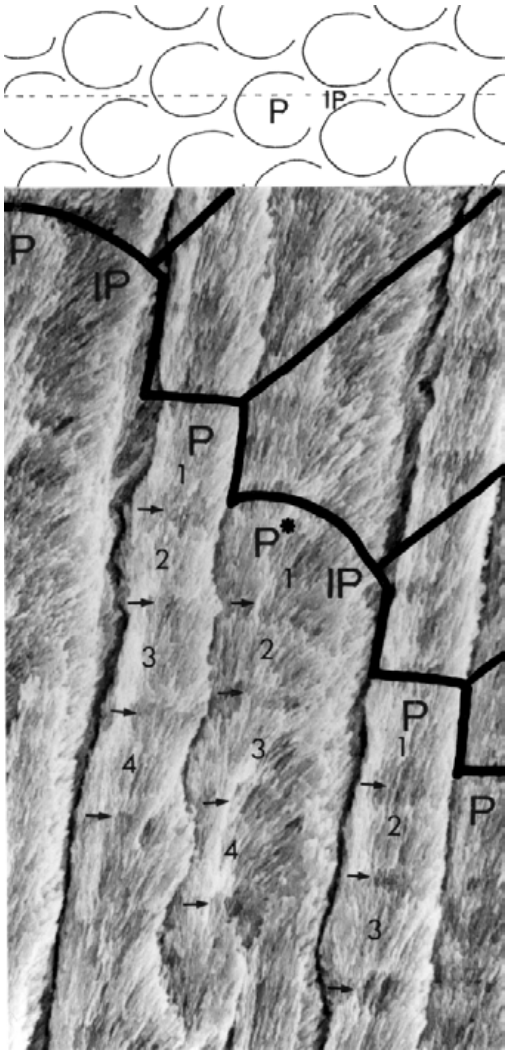


Fig. 6. SEM micrograph of prisms in longitudinal section of human permanent tooth. Plane of section indicated in inset. Ameloblasts are schematically inserted using prism sheaths and prism cross-striations as guiding lines. Cross-striations curve cervically from prism (P) to interprism (IP) whereby crystals maintain a near perpendicular orientation relative to the secretory aspect of the imaginary ameloblasts. Cross-striations (arrows) indicate previous segments (1-4) produced by the imaginary ameloblasts. There is a correspondence in width between corresponding segments (1=3=4>2). X 2150.

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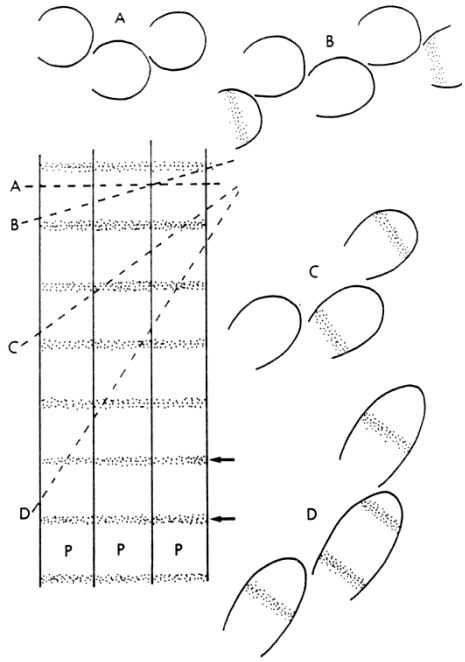


Fig. 7. Schematic representation of three prisms (p) with cross-striation (arrows). A, B, C, and D indicate appearance of prism profiles and cross-striations in sections cut along the corresponding planes.

DISCUSSION

Crystal orientation and crystal continuity

Crystal orientation is fundamental to enamel structure, being responsible for the distinction between prisms and interprism. The factors governing crystal orientation during enamel secretion is not fully understood. It has been contended that crystals tend to be oriented perpendicular to the secretory aspect of the ameloblasts (Boyde, 1965). When ameloblasts are inserted schematically into an SEM micrograph of enamel, letting the cross-striations and prism sheaths represent former ameloblast-enamel interfaces (Fig. 6), the notion of crystals being oriented perpendicular to the secretory aspects of the ameloblasts tends to hold good in human enamel. However, since during rat incisor amelogenesis prism crystals may be oriented distinctly obliquely to the secretory aspect of the Tomes' process as shown in the present study (Fig. 1), there must be additional or other factors influencing crystal orientation. Emphasis has lately been put on the spatial configuration of the enamel matrix at the molecular level (Fincham et al., 1994).

It has been suggested that enamel crystals are very long and that they may extend through the whole thickness of enamel from the EDJ to the enamel surface (Fearnhead, 1971; Leblond and Warshawsky, 1979; Warshawsky and Nanci, 1982; Daculsi et al., 1984). The present study indicates, in accordance with the view of others (Osborn, 1973;

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Boyde, 1976, 1989), that some crystals end at the prism sheaths. Mature dental enamel is relatively uniformly mineralized throughout its thickness. As some crystals end within the enamel, full mineralization can be obtained either by additional growth in thickness and width of the remaining crystals or by initiation of new crystals throughout amelogenesis. If new crystals are not initiated throughout enamel formation, there will be a decrease in the number of crystals per area and an increase in the diameter of individual crystals toward the enamel surface in mature enamel. This has been found not to be the case (Abrigo, 1972, cited after Boyde, 1989); Daculsi and Kerebel, 1978; Daculsi et al., 1984). Consequently, new crystals must be initiated during enamel formation. Since crystals are constantly depleted from the prism domains as they deviate into the interprism domain, new crystal initiation must primarily occur within the prism domains.

Provided that some of the crystals within a prism remain within the prism throughout the enamel thickness, geometrical considerations indicate that a continuous prism crystal and a continuous interprism crystal which were neighbours at the EDJ, will become widely separated at the enamel surface; the interprism crystal ends up in a more cervical position than the prism crystal. While the prism crystal has been related to one and the same ameloblast throughout amelogenesis, the interprism crystal has been related to a large number of ameloblasts, at least the number of ameloblasts fitting into the linear distance between the prism crystal and the interprism crystal at the enamel surface. In human enamel with a thickness of 1.5 mm and an incisal inclination of prisms of 15^{o} one continuous interprism crystal would be related to about 250 ameloblasts. In rat mandibular incisor enamel with a thickness of 120 μm, an incisal inclination of prisms of 45^{o}, and a cervical inclination of interprism crystals of 45^{o}, the number of ameloblasts related to one continuous interprism crystal would be about 60. In human enamel interprism crystals, being oriented perpendicular to the incremental growth lines (Retzius lines), would elongate at a slower speed than prism crystals since they have grown a shorter distance in the same time. Both prism crystals and interprism crystals elongate a longer distance relative to the same Retzius line than indicated by the two-dimensional geometrical scheme, the prism crystals since the prisms undulate in the transverse plane in accordance with the Hunter-Schreger bands and the interprism crystals since they have to pursue an undulating path between the prisms (Fig. 3b). In the rat mandibular incisor the elongation rate of prism and interprism crystals are almost the same.

Prism diameter

Conflicting evidence exists concerning the diameter of prisms at the enamel surface compared with that at the EDJ, both an increased diameter (Skobe and Stern, 1980) and a constant diameter (Radlanski et al., 1986) has been found. Theoretically, if the number of ameloblasts is constant during amelogenesis, the tangential diameter of ameloblasts and prisms would have to increase from the EDJ to the enamel surface, since the latter represents a larger surface than the former. The present study has shown that when ameloblasts are forced to cover a larger area, as the shoulder bordering a hypoplastic defect, their diameter may increase resulting in prisms with increased diameter, and that this may occur without a change in prism angulation.

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Retzius lines

Previous evidence has suggested that staircase-type Retzius lines are characterized by a temporary overgrowth of interprism at the expense of prisms, probably involving a constriction of the Tomes' processes (Risnes, 1990). The indistinctness of prism identity observed here both in periodic Retzius lines in the outer enamel and in a non-periodic, thick Retzius lines in the inner enamel strengthens the notion that an affacement of prism identity is an important aspect of some Retzius lines and possibly reflects a lability in the expression of the Tomes' processes. This in accordance with the tendency for Tomes' processes to regress toward the end of enamel formation resulting in aprismatic superficial enamel.

Prism cross-striations

Ample evidence suggests that prism cross-striations exist, although indications of the opposite have been presented (Weber and Glick, 1975; Warshawsky et al., 1984). However, their nature is not clear. Their relationship to another prism periodicity, the prism varicosities/undulations, is also obscure (Risnes, 1998). Circumstantial evidence from the present study links the cross-striations to a rhythmic deposition of enamel along the prisms. Existing evidence indicates that this rhythm is diurnal (Schour and Poncher, 1937; Risnes, 1986; Bromage, 1991).

It has been argued against the cross-striations as a true prism periodicity that nearly cross-cut prisms may also exhibit cross-striations. However, geometrical considerations in the present study predict the appearance of cross striations also in obliquely and nearly cross-cut prism profiles.

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Aspects of Tooth Crown Development in Common Chimpanzees (*Pan troglodytes)* with a Note on The Possible Role of Sexual Dimorphism in Canine Growth

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Introduction

Some of the earliest studies to document details of dental development in our closest living relative, the chimpanzee, date back to the late nineteenth century and research into this aspect of hominoid life history has continued unabated to the present day (e.g., Keith, 1899; Zuckerman, 1928; Krogman, 1930; Schultz, 1935; Bennejeant, 1940; Nissen and Riesen, 1945, 1964; Oka and Kraus, 1969; Tarrant and Swindler, 1972; Moxham and Berkovitz, 1974; Dean and Wood, 1981; Kraemer et al., 1982; Anemone et al., 1991, 1996; Beynon et al., 1991; Conroy and Mahoney, 1991; Seibert and Swindler, 1991; Simpson et al., 1992; Kuykendall et al., 1992; Chandrasekera et al., 1993; Smith, 1994; Smith et al., 1994; Conroy and Kuykendall, 1995, Smith and Tomkins, 1995; Winkler, 1995; Kuykendall, 1996; Kuykendall and Conroy, 1996; Reid et al., 1998b). As a result, more is known about chimpanzee dental development than for any other greater or lesser ape. In one sense, however, this is simply a reflection of how little we know about dental development in the gorilla, the orangutan and especially the gibbon (see Smith et al., 1994; Dirks, 1998).

A detailed understanding of the timing and pattern of dental development in extant hominoids is important as this feature is used widely for constructing paleobiological inferences from our earliest fossil ancestors (e.g., Conroy and Vannier, 1991a, b; Conroy and Kuykendall, 1995). Despite its usefulness, there are still several problems associated with using this feature in comparative studies of hominoid life histories. Due to the high levels of interpopulation variation present in modern humans, we are unable to judge which modern human population (if any) best represents a comparative reference for our species, or how levels of variation in other extant hominoid species, which are still largely undocumented, affect our comparisons. This all begs the question of whether or not a "total developmental pattern" for any species can be identified as species demonstrate mosaic patterns, both in their morphology and in their growth and developmental processes. Thus, it is unlikely that any single detail of such complex developmental patterns would faithfully characterize a given species for purposes of comparison with

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isolated fossil specimens and that the previous use of a comparative dichotomy between ‘human’ versus ’ape’ patterns of dental development in describing those of fossil hominids is inadequate.

Despite these caveats, a great deal can still be learned not only about general life history profiles but about the fundamental mechanisms of growth and development in extant apes and early hominids from comparative studies of dental chronology. Dental tissues are ideal structures for investigating both the rate and pattern of development. Because teeth preserve a faithful record of their ontogeny, they also allow us to relate changes in each of these to the timing of other events during an animals growth. Our present knowledge about the embryonic development and morphogenesis of different tooth types, as well as about the control of dental patterns at the molecular level, is coming close to identifying which molecular pathways have changed during vertebrate evolution (Kontges and Lumsden, 1996). As a result, it is only in the formation of dental tissues that we are presently close to uncovering the proximate mechanisms of growth and development, the modification of which forms the basis of evolutionary change (Macho and Wood, 1995). It is from this perspective that we approach the analysis of dental development in our closest living relative, the common chimpanzee (*Pan troglodytes*) using well-established histological techniques.

To date, most studies on chimpanzees concentrate either on the emergence times of teeth, on the sequence of mineralization stages of the teeth as revealed from radiographs or from dissections, or less successfully, on both at the same time. Recently, and against an established background of information about the periodicity of incremental markings in human and primate tooth tissues, there has been steady progress in understanding from histological studies how human, fossil hominid and other primate teeth grow (e.g., Dean and Wood, 1981; Bromage and Dean, 1985; Beynon and Wood, 1986; 1987; Beynon and Dean, 1988; Beynon et al., 1991a, b; Dean and Beynon, 1991; Dean et al., 1993a, b; Reid et al., 1998a, b). We have also begun to understand more about human and hominoid tooth root growth (Dean et al., 1992; Dean et al., 1993a, b; Reid et al., 1998a, b) and about rates of dentine formation in primates (Dean, 1993; Dean and Scandrett, 1995). As a direct result of these histological studies of tooth growth, one can now confidently predict that more could be learned about the different mechanisms of enamel and dentine growth in primates using histological approaches. Histological studies of developing dentitions are much more limited with respect to the numbers of individuals they can include and the degree of variation they can portray than are large, longitudinal, radiographic studies. On the other hand, they are able to resolve some issues such as defining the end of enamel formation and the initiation of mineralization with greater precision. (In past studies, authors described the various stages of tooth growth in several different ways. In this study, the term "onset of mineralization" is equivalent to "initiation of crown formation" whereas the term "crown formation" refers to "enamel cap formation".)

Although many studies document aspects of chimpanzee dental development, confusion still exists about both the times of initial mineralization and time it takes to form each anterior tooth crown in chimpanzees. As far back as the 1920s, it was recognized that the duration of the stages of tooth development are the same in chimpanzee as in humans (Zuckerman, 1928). (Not only do nearly all of the subsequent studies support this view, but at the same time recognize the similarities in circumnatal

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development among all extant hominoids (Oka and Kraus, 1969; Kraus and Jordan, 1965; Tarrant and Swindler, 1972; Moxham and Berkovitz, 1974; Seibert and Swindler, 1991; Swindler, 1985). While more recent studies focusing on tooth emergence (Conroy and Mahoney, 1991) and tooth formation (Dean and Wood, 1981; Beynon et al., 1991) demonstrate a long time lag between first permanent molars and permanent central incisors in chimpanzees, others do not (e.g., Anemone et al., 1991; Kuykendall, 1996). Thus, one aim of this study is to document the times of enamel formation in chimpanzee anterior teeth using data derived from a histological analysis. A second aim is to document the times for the onset of initial mineralization and duration of crown formation of all permanent teeth in chimpanzees more precisely as well as construct a schedule for the pattern and timing of dental development in this one hominoid species. In particular, we focus on: (i) the chronology of the developing dentition; (ii) the rate of tooth root formation; (iii) the degree of sequential molar overlap; and (iv) the possible role of sexual dimorphism in canine development. One final overall aim is to try to document aspects of dental development in chimpanzees with sufficient confidence and in new and novel ways such that we can eventually make reliable comparisons between chimpanzees and other great apes. At present, it is not possible to say with any confidence how gorillas, chimpanzees or orangutans differ in any aspect of their dental development, yet it is expected that these taxa should differ from one another even in some small ways. Only when a clear picture of growth and development (not only for dental but other postcranial variables as well) emerges on chimpanzees and other great apes can paleoanthropologists comment on similarities and/or differences in the patterns of growth and development in early hominids more precisely.

Materials and methods

One adult, two juvenile and one infant chimpanzee specimen were used in this study. For a description of the developmental status of each of the four specimens, see Reid et al. (1998b). In total, 14 maxillary and 28 mandibular teeth, including an additional 3 isolated molar teeth from chimpanzee specimens of unknown origin, were radiographed, dissected from the jaws where necessary, photographed and measured. The teeth were then embedded in polyester resin and 150-180 μm thick longitudinal sections were taken from the midline axial plane in anterior teeth and from the buccolingual planes of both the mesial and distal cusps in posterior teeth. The sections were then lapped to a final thickness of 100 μm, polished and prepared for polarized, incident and transmitted light microscopy according to the techniques outlines in an earlier study (see Reid et al., 1998a, b). A total of 56 sections representing all maxillary and mandibular permanent teeth were then analyzed to detail aspects of the chronology of dental development in this one hominoid species.

Determining the periodicity, or the number of short-period incremental lines (i.e., cross striations) between adjacent long-period lines (striae of Retzius) for each specimen is necessary for estimating the time taken to form each tooth crown. Two of the chimp individuals had a periodicity of 7 while the others had a periodicity of 8. Measurements of the total number of striae of Retzius, daily secretion rates (i.e., cross striation repeat intervals) and the periodicity were also recorded for each tooth section. Crown formation

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times were determined by summing the time taken to form the cuspal (i.e., appositional) and imbricational (i.e., lateral) regions of each tooth (Table 1). Cuspal and imbricational enamel formation times (in years) were determined according to the following formulae (see Reid et al., 1998 for further explanations):

1. Cuspal enamel formation times (after Risnes, 1986):

$$\frac{\textit{cuspal enamel thickness } X \text{ } 1.15}{\textit{mean daily rate of secretion}} \times \frac{1}{365}$$

where 1.15 is the value used to correct for prism length as a result of decussation, and the mean daily rate of secretion is determined by averaging the recorded measurements of cross striation spacing in the inner, middle and outer regions of the cusp tip from X250 photomontages.

2. Imbricational enamel formation times:

$$\frac{\textit{number of striae}}{365} \times \textit{periodicity}$$

In order to establish the precise dental chronology for each specimen, tooth sections were examined microscopically seeking accentuated striae in the enamel. Accentuated striae corresponding to hypoplastic episodes are present in both the mandibular and maxillary teeth of all animals (Table 2). These striae are used to register teeth of differing developmental stages at the same time period (see Beynon et al., 1991). A neonatal line is present in each of the first molars and is used to calculate the amount of crown formed prenatally from prism lengths (i.e., the distance from the EDJ to the neonatal line) and daily secretion rates. The timing of accentuated striae in the first molar crowns were calibrated in years from the neonatal line. Teeth of differing developmental stages within each individual chimpanzee specimen are registered and cross-matched with one another using accentuated striae corresponding to accentuated lines associated with stress, hypoplasia or metabolic disturbances (see for example Dean, 1987, 1989; Macho et al., 1996; Hillson and Bond, 1997). This information was used to construct a bar chart of the timings of crown initiation and completion and the overall sequence of tooth development for maxillary and mandibular teeth in *P. troglodytes* (Figs. 1a-d).

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Table 1. Cuspal, imbricational and total crown formation times in years (Mean±SD) derived from values of all the sections available for each tooth type (not just the 4 individuals).

Tooth	Cuspal Enamel	Imbricational Enamel	Total Crown Formation Time
		Mandible	
I_1	0.51±0.09	4.14±0.31	4.66±0.23
I_2	0.52±0.04	4.78±0.38	5.33±0.37
C	0.55	6.55	7.10
P_3	0.73±0.10	4.29±0.37	5.01±0.45
P_4	0.70±0.10	3.20±0.08	3.90±0.14
M_1	0.65±0.14	2.20±0.38	2.85±0.26
M_2	0.92±0.16	2.80±0.35	3.69±0.28
M_3	0.90±1.13	3.14±0.48	4.04±0.45
		Maxilla	
I^1	0.47	5.17	5.64
I^2	0.55	3.95	4.50
C	0.46	6.88	7.14
P^3	0.49	3.81	4.30
P^4	0.50	3.11	3.61
M^1	0.53±0.08	2.20±0.23	2.73±0.31
M^2	0.60	2.96	3.56
M^3	0.70	2.78	3.48

Table 2. Ages at crown initiation and crown completion (CC) or age at death in years for all teeth from each of the four P. troglodytes individuals used in this study.

Specimen	Crown Initiation	Accentuated Striae[a]	Total No. of Striae[b]	Crown Formation	Age at CC/Death
Animal #1					
I_1	0.15	2.95	232	4.45	4.60
1_2	0.20	2.95	261	5.00	5.20
C	0.40	2.95	284	5.45+	5.85+
P_3	1.10	2.95	240	4.60	5.70
P_4	1.40	2.95	216	4.15	5.55
M_1	-0.05	2.95	125	2.40	2.35
M_2	1.80	2.95	190	3.65	5.45
M_3	3.60	---	177	3.40	7.00
Animal #2					
I_1	0.46	2.05	244	5.35	5.81
1_2	0.66	2.05	235	5.15	5.81
C	0.57	2.05	290	6.36 inc	6.93
P_3	1.40	2.05	185	4.05	5.45
P_4	1.75	2.05	178	3.90	5.65
M_1	-0.15	2.05	139	3.05	2.905
M_2	1.67	2.05	130	2.85	4.52
M_3	3.62	---	151	3.31 inc	6.93
I^1	0.26	2.05	183	4.00	4.26
I^2	1.25	2.05	203	4.45	5.70
C	0.42	2.05	297	6.51 inc	6.93
P^3	1.21	2.05	201	4.40	5.61
P^4	1.67	2.05	162	3.55	5.22

Table 2. Continued.

M^1	-0.15	2.05	105	2.30	2.15
M^2	1.40	2.05	146	3.20	4.60
M^3	3.84	---	141	3.09 inc	---
Animal #3					
I_1	0.30	---	226	4.95	5.25
1_2	0.70	---	224	4.91 inc	5.61 inc
C	0.45	---	236	5.16 inc	5.61 inc
P_3	1.20	---	202	4.41 inc	5.61 inc
P_4	1.95	---	164	3.60	5.55
M_1	-0.15	---	121	2.65	2.50
M_2	1.95	---	167	3.66 inc	5.61 inc
Animal #4					
I_1	0.15	0.45	213	4.09 inc	4.24
1_2	0.19	0.45	211	4.05 inc	4.24
C	0.38	0.45	202	3.86 inc	4.24
P_3	1.11	---	163	3.13 inc	4.24
P_4	1.40	---	148	2.84 inc	4.24
I^1	0.21	0.45	209	4.01 inc	4.24
I^2	0.86	---	176	3.38 inc	4.24
C	0.38	0.45	201	3.86 inc	4.24
P^3	1.27	---	155	2.97 inc	4.24
P^4	1.19	---	159	3.05 inc	4.24
M^1	-0.15	0.45	112	2.30	4.24

[a] The number in this column indicates the time (in yrs) from the initiation of crown mineralization to an accentuated striae most likely associated with developmental stress periods and/or hypoplasias. These accentuated lines were also used to cross-match and register teeth within individuals in order to gauge the pattern and chronology of dental development (see text).

[b] The total number of striae multiplied by the periodicity of each individual yields the total amount of time taken to form the imbricational (i.e., lateral) region of the tooth. When added to the age at crown initiation, this provides the age at crown completion or death.

Abbreviations: "inc": tooth formation is incomplete; "+": indicates attrition so that estimates of crown formation/age at death are slightly greater than the values reported here.

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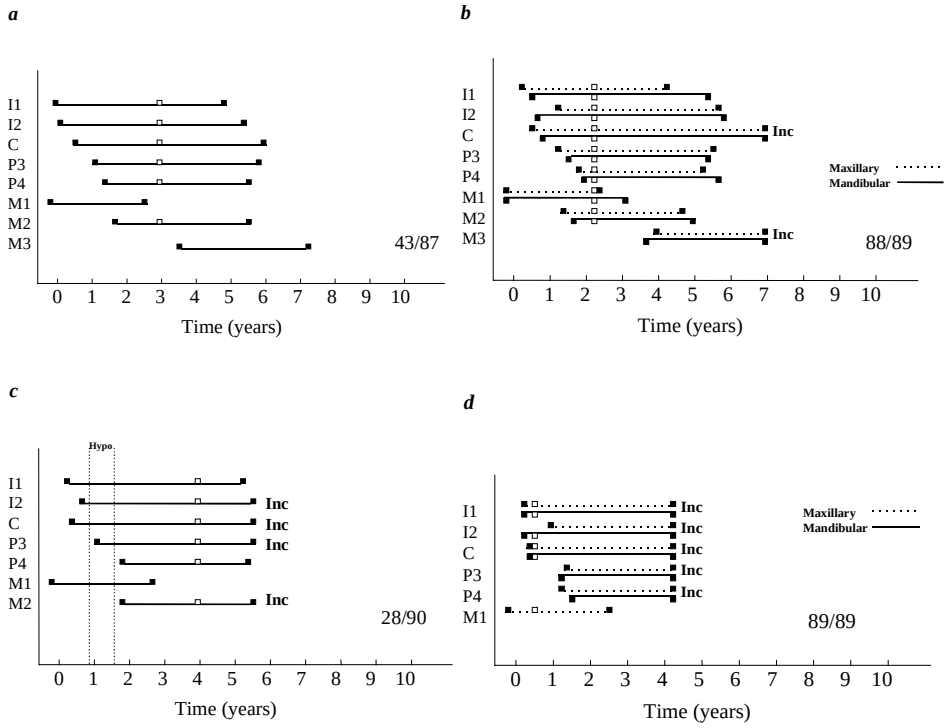


Fig. 1. a-d. Bar charts of dental development for each *Pan troglodytes* specimen. Closed squares indicate the onset of mineralization and crown completion, respectively, for each tooth. Open squares represent the appearance of accentuated striae (see Table 2) while the dotted lines for specimen 28/90 (Animal no. 3) represent the duration of a hypoplastic episode ("Hypo") occurring from 0.95 to 1.65 years of age. Animal reference numbers 1, 2, 3, and 4 correspond to specimen numbers 43/87, 88/89, 28/90, and 89/89, respectively.

RESULTS

Dental chronology

Winkler (1995) has drawn attention to the fact that radiographs may fail to reveal the early stages of tooth formation in great apes and that these stages are better demonstrated by direct dissection of the specimens. Additionally, Beynon et al. (1991) have pointed out that dissections of great ape tooth germs reveal some stages of formation in some tooth types that follow a different sequence to those known for modern humans. For example, the root bi- or trifurcation of great ape upper premolar teeth began to form before the end of enamel formation (Beynon et al., 1991). All this, together with the problems of defining stages of tooth formation sufficiently accurately on radiographs (Liversidge, 1995; Beynon et al., 1998) has now generated a more variable picture for the timing of mineralization stages in chimpanzees than for the timing of tooth emergence. This is

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surprising since there is fundamental recognition that data for tooth mineralization stages are better than tooth emergence data for estimating both skeletal maturity and chronological age (Gleiser and Hunt, 1955).

Three previous radiographic studies have estimated crown formation times of chimpanzees. However, the first was a cross-sectional radiographic analysis of a museum sample of immature *Pan*, *Pongo* and *Gorilla* jaws, and may not be appropriate as a standard for *Pan* only (Dean and Wood, 1981). The other studies on tooth growth and crown formation times in *Pan* (Anemone et al., 1991, 1996; Kuykendall, 1996) used longitudinal radiographic methods and present data comparable with those presented here. Crown formation times of all teeth, however, were consistently lower than those in the present study; these careful radiographic studies appear to underestimate crown formation times of *Pan* when compared with the present histological study. The underestimations are considerable for most tooth types: I_1 2.5 yrs., I_2 3.1 yrs., C 1.6 yrs., P_3 1.9 yrs., P_4 0.1 yrs., M_1 0.8 yrs., M_2 1.1 yrs. and M_3 0.3 yrs (Table 1). In the present study, initial crown mineralization of both I_1 and I_2 is estimated between birth and six months, I_2 taking a longer time to complete its crown than I_1. In the radiographic study, initial mineralization of both incisors was estimated between six and nine months and took 2.4 years to complete their crowns. When compared with the present study, the radiographic studies show a delay in initial mineralization and an early completion of crowns of all teeth. This finding is consistent with the observations made by Beynon et al. (1991) on *Gorilla* and *Pongo.*

Crown heights are much greater in incisors and canines than in premolars and molars. The greater difference in the crown formation times between the radiographic and present study in anterior teeth probably relates directly to their longer crowns. Thinning of cervical enamel of anterior teeth, when compared with the molar teeth with their short crowns and relatively bulbous cervical enamel, undoubtedly results in a different radiographic appearance of the cervical enamel. The thin cervical enamel is likely to be "burned out" or missed in radiographic images therefore reducing radiographically determined crown formation times (Beynon et al., 1998).

Aside from this potential problem associated with tooth geometry, assessments of the stage of crown completion via standard radiographic techniques, or histological methods, can vary depending on which region of the tooth is subjected to observation. When enamel formation ends at the same point in time along both the buccal and lingual cervices, estimates of crown formation times should be equal regardless of which cusp is examined. However, if there is a disparity between the initial times of cusp mineralization and/or a cervical margin which continues to form for longer on the buccal or lingual aspect, then estimates of total enamel formation time will differ when made on different aspects of the same tooth. This is especially problematic in analyses of molariform teeth as it seems to be often assumed that crown completion occurs simultaneously across the entire tooth crown. All of this is made worse in radiographic studies where only the mesial or distal cervical margins can be imaged in molar teeth; both of these tooth regions complete enamel formation earlier than either the buccal or lingual aspects.

Rate of Root Formation

Estimates of the times and rates of root formation are not possible in the sample of chimpanzee teeth studied here. Data are available on root lengths (see Reid et al., 1998b; Table 2) and these provide a useful, albeit preliminary, estimate of how much tooth root is formed between the time of crown completion and the age at gingival emergence. Rates of root formation differ depending on which part of the root is examined in apes (Dean and Wood, 1981). Root length cannot then be used as a direct measure of rate of growth. A comparison between the ages at the end of crown formation (from our histological analysis) and at gingival emergence (from Kuykendall et al., 1992) does provide a broad measure of the time the first part of the root took to form (Table 3; Fig. 2). For most *Pan* teeth, about a year elapses between crown completion and gingival emergence. Previous radiographic studies (Anemone et al., 1991, 1996; Kuykendall et al., 1992, Kuykendall and Conroy, 1996) document as much as three years for this period of root formation. It is the earlier completion of crown formation reported in these radiographic studies that underlies the longer estimates for the period of root formation in the time up to gingival emergence. The implication from this study is that chimpanzee teeth have a greatly reduced time for root growth before emergence occurs. This is in direct agreement with the findings of Dean and Wood (1981) based on observations of the incremental lines in the roots of great ape teeth. Future investigations of tooth root growth will focus specifically on how rates of formation regulate the time at which teeth are able to emerge into the mouth. It is clear that the major differences that exist between modern humans and *Pan* lie in the first part of the root formation rather than in the total period of crown formation.

Table 3. A comparison of root formation times (RFT) at the time of eruption[a] taken from the literature and those derived from histological data on crown initiation, crown completion and gingival emergence ages in P. troglodytes. All data in years.

Tooth	Crown Initiation	Crown Formation	Age @ Cr. Completion	Age @ Eruption	RFT (pres. study)	RFT (literature)
I1	0.27	4.66	4.93	5.55	0.62↔≈3.0	
I2	0.50	5.33	5.83	5.89	0.06↔≈3.0	
C	0.45	7.10	7.55	7.96	0.41↔≈2.5	
P3	1.20	5.01	6.21	7.40	1.19↔>3.0	
P4	1.63	3.90	5.53	6.76	1.23↔>3.0	
M1	-0.12	2.85	2.73	3.15	0.42↔≈1.5	
M2	1.81	3.69	5.60	6.63	1.03↔≈2.5	
M3	3.61	4.04	7.64	≈10.5	≈3.0↔>3.0	

[a]Root formation times are calculated as the age at eruption minus the age at crown completion. Median gingival emergence ages from the literature are complied from: Krogman (1930); Schultz (1940); Clements and Zuckerman (1953); Nissen and Riesen (1945, 1964); Anemone et al. (1991), Kraemer et al. (1982); Conroy and Mahoney (1991); Kuykendall et al. (1992).

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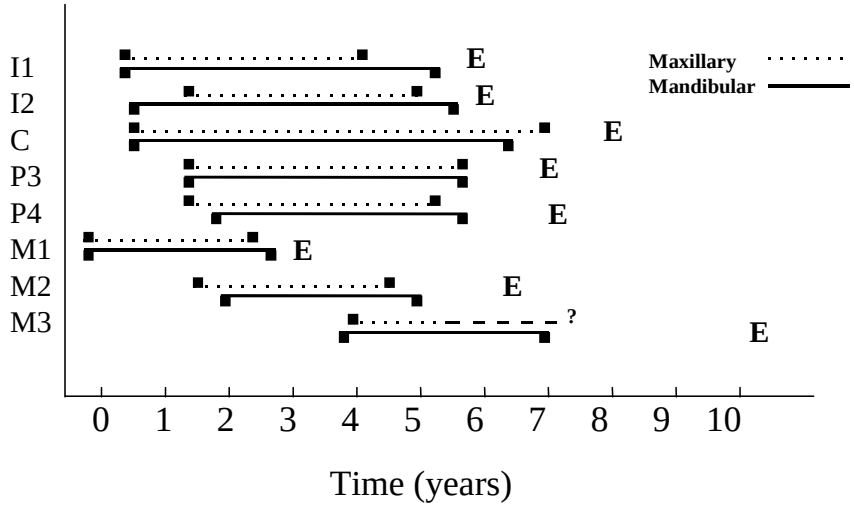


Fig. 2. Bar chart of dental development in *Pan troglodytes* with emergence times for the maxillary and mandibular teeth. Symbols for the onset of mineralization and crown completion are as in Fig. 1, however, are based on mean values for all of the specimens included in this study. Median gingival emergence from Table 3. The dashed line and question mark for M^3 indicates that no histological data are available for the time at crown completion for this tooth. E = emergence through the gingiva.

Sequential Molar Overlap

Previous studies of tooth crown development in primates have commented on the degree of sequential overlap in the formation of molar crowns. Dean and Wood (1981) did not record overlap between either M_1 and M_2 or M_2 and M_3 in a mixed sample of great apes. Subsequently, Swindler (1985) published data for *Macaca nemestrina* where there was also no overlap between successive molars. This has now been confirmed for certain other catarrhine species including *Papio cynocephalus* and *P. hamadryas anubis* (Reid and Dirks, 1997). Studies on *Theropithecus* (Swindler and Beynon, 1993) and *Pan* (Anemone et al., 1991, 1996; Kuykendall et al., 1992, Kuykendall and Conroy, 1996), however, have all noted considerable sequential molar overlap between M_1/M_2 and M_2/M_3 formation and this is confirmed in the present study for *Pan*. Some data for *Pongo* (Winkler, 1995) and *H. sapiens* (Fanning and Moorrees, 1969; Reid et al., 1998a) do tend to suggest more variation in the degree of sequential molar overlap than is often appreciated. Future research may tie each of these observations in with the degree of facial prognathism, with molar tooth size, and with the space available within the jaws at molar crown initiation. Overlap in molar crown formation also has the potential to increase the time between crown completion and gingival emergence by initiating enamel formation earlier. As such, it may be involved in determining how fast the first part of root formation is required to grow in order to achieve gingival emergence "on time" in the context of the general dental development schedule.

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Canine Development and Sexual Dimorphism

Sexual dimorphism, in the very widest sense, has clear links with various aspects of life history such as sexual selection, habitat and social structure (e.g., Darwin, 1871; Leutenegger and Kelley, 1977; Clutton-Brock and Harvey, 1977; Leutenegger, 1978, 1982; Jungers, 1985; Kay et al., 1988). The adaptive reasons for canine dimorphism, in particular, is complex as many behavioural and ecological factors, such as the varied social and mating systems present in primates, female-female competition for food resources, predation pressure, etc., might all be expected to influence the degree of expression for different reasons (see Plavcan and van Schaik, 1994). Regardless of the social or behavioural basis of canine dimorphism, what is clear is that the important morphological variable to be considered is canine projection above the occlusal plane. As most histological studies of tooth growth focus on crown height, studies on the rate and time of attainment of tooth height in canine-dimorphic primates can contribute much as they directly reflect this behaviorally important character.

Very little is known about the histology of developing canines in male and female great apes compared to what is known about the degree of sexual dimorphism present for many metric aspects of these teeth such as crown height, crown base area, etc. From an ontogenetic point of view, it is unclear whether these metric differences, such as crown height, between male and female chimp canines come about due to alterations in the total period of crown formation, the rate at which the crown forms, or some combination thereof. Such questions can be addressed by using information from incremental lines as these allow us to ascertain accurate data on the age of initiation, the total period of crown formation and the rate at which crown formation proceeds.

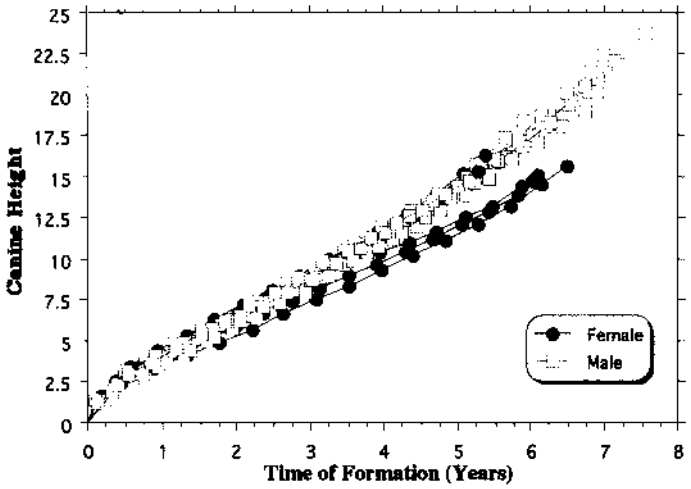


Fig. 3. Bivariate plot of the ontogeny of sexual dimorphism in canine crown formation for a total of 12 chimpanzee individuals (male=7; female=5).

In general, male and female chimps initiate mineralization of mandibular canines at roughly the same age (approximately 0.45 years for males and 0.47-0.52 years for females; Kuykendall, 1996; see also this study Table 2). Mean crown formation times for

mandibular canines have been reported to be, on average, higher in males (6.83 and 5.96 years for males and females, respectively; Ranges for male and female canine crown formation times (in years) are 5.92-7.75 for males and 5.11-7.20 for females though this difference is not statistically significant (Kuykendall, 1996). As data from these studies suggest that few or no differences exist in the total *period* of crown formation, it is reasonable to assume that perhaps female and male canine teeth grow at different *rates*. In order to test the hypothesis that differences in rate and/or time of development are present within chimpanzees a total of 12 reliably sexed chimpanzee canines were analysed to examine the ontogeny of sexual dimorphism in this one hominoid species. The accumulation of crown height, measured as the linear distance along the EDJ in intervals of 20 striae of Retzius, appears in Figure 3. When multiplied by periodicity, it was possible to relate the ontogeny of crow height accumulation for a given period of time in each specimen (chimpanzees have a periodicity range of 6-9, which is quite similar to modern humans, 7-9). Male and female chimps exhibit similar growth trajectories early on in the ontogeny of canine crown formation (Fig. 3), though cuspal formation times do not differ between sexes. At roughly 3 years of age, however, some females begin to show slower rates of formation and overall tend to be spread over a greater range of high and low rates of growth throughout. Total canine crown formation times also differ between sexes with a range for males of 5.91-7.58 years while that for females is 5.28-6.49 years; male and female crown formation times are significantly different, $p<0.05$). These observations are consistent with the idea that the growth curves in male chimps may reflect greater selection pressure, though more testing is needed on other general of extant hominoids.

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Absence of Sexual Dimorphism in Enamel Thickness of Human Deciduous Molars

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INTRODUCTION

Tooth crown dimensions can be viewed as developing from two main sources, (1) size of the dentin component, established at the end of the bell stage, and (2) thickness of the enamel, an ectodermal tissue whose deposition begins late in the bell stage. These temporal and structural distinctions (dentin is an ectomesenchymal derivative) suggest that dentin and enamel thicknesses need not necessarily covary. So too, while males characteristically have larger teeth than females (e.g., Garn et al., 1967; Harris and Bailit, 1987), it does not follow that the dimorphism is contributed proportionately by the enamel and the dentin. The works of Stroud et al. (1994, 1998) and Harris and Hicks (1998) are perhaps the only studies to definitively test for a sex difference in enamel thickness, and they all found no difference in enamel, even though mesiodistal crown dimensions were quite discernibly dimorphic.

Several radiographic studies, notably those by Alvesalo and coworkers (e.g., Alvesalo and Tammisalo, 1981; Alvesalo, 1985; Alvesalo et al., 1985, 1987) have shown from examination of persons with aneuploidies of the X or Y chromosome that sex-linked genes modulate tooth size, but the nature of the effect is different for the X and the Y. The X chromosome possesses enamel-promoting (amelogenin) genes on its always-active region and addition of X's (e.g., the sequence 45,XO to 46,XX to 47,XXX) results in increasing marginal enamel thickness, but with negligible changes in dentin dimensions. The Y chromosome, in contrast, has an approximately systemic effect on up-regulating growth of both enamel and dentin – as well as other body parameters such as stature (e.g., the sequence 46,XY to 47,XYY, and so on). On the other hand, not so much is known about the nature of tooth size in karyotypically normal males and females. Is the well-documented sex difference in crown size due to enamel thickness (as speculated by Moss and Moss-Salentijn, 1977, Moss, 1978, and Alvesalo et al., 1987), a difference in dentin, or some combination of both?

Purpose of the present study was to measure the mesiodistal crown components of deciduous molars in order to test for sexual dimorphism in enamel and dentin thicknesses. Data were collected in vivo from standardized radiographs. The sample also was partitioned into American blacks and whites to see whether the larger teeth of blacks is due to differences in enamel, dentin, or both.

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MATERIALS AND METHODS

Standardized bitewing radiographs were taken on pediatric dental patients as part of routine screenings. The sample (n = 270 children) was distributed proportionately between blacks and whites (127 and 143) and between males and females (129 and 141). The bitewing film captures the buccolingual images of all four deciduous molar crowns on one side. Kodak Ultra-Speed film was used, with settings of 70 kVp, 8 mA, and 0.3 sec. Films were examined to assure they met two selection criteria (Mass et al., 1996): One, radiographs with overlapping shadows of adjacent teeth were eliminated. Two, only radiographs with maximal overlap of buccal and lingual cusps of the same tooth were analyzed. In addition, teeth with carious lesions or restorations obscuring relevant landmarks were not measured. The left or right film was selected at random for use, or, if there was a difference, the side with more sound teeth or better film contrast was used. The source-to-film distance averaged 7.0" with a source-to-subject distance of 6.5", so magnification was about 7.7%. Dimensions were not corrected for magnification in this report.

Assignment as to American black or white was made by the child's parent. Admixture estimates of whites with American blacks are on the order of 20 to 30% for Northern and Western samples (e.g., Glass and Li, 1953; Glass, 1955; Roberts, 1955; Roberts and Hiorns, 1962) but are generally below 10% for Southeastern samples (e.g., Pollitzer, 1958; Workman et al., 1963; Pollitzer et al., 1964, 1970; Blumberg and Hesser, 1971). Children in the present study were born in western Tennessee, northern Mississippi, and eastern Arkansas. Children of mixed-race marriages and children of other ethnic groups were eliminated from consideration.

Each radiograph was mounted in a plastic, reusable 35 mm slide mount. Opaque removable photographic cropping tape was used to hold the film in position and mask unfilled areas of the slide mount. The "slide" was placed in a Polaroid SprintScan 35® film scanner interfaced to a Macintosh computer. The SprintScan is driven by software in PhotoShop® (Adobe Systems), and the image was captured as a gray-scale image in PhotoShop. Scanning resolution was 2,700 dots per inch.

Contrast and brightness were optimized for each image, and screen magnification was increased to about 8X (though the screen image does not affect the image stored in RAM). Mesiodistal crown dimensions were obtained with a read-out precision of 0.1 mm. Three variables were measured on each molar (Fig. 1): maximum mesiodistal crown diameter assessed perpendicular to the tooth's long axis, mesial enamel thickness, and distal enamel thickness (Alvesalo and Tammisalo, 1981; Alvesalo et al., 1987). Enamel thickness was measured at the same height established for maximum MD crown length. Mesiodistal width of the dentin (including the pulp chamber) was obtained by subtracting the two enamel widths from overall crown diameter.

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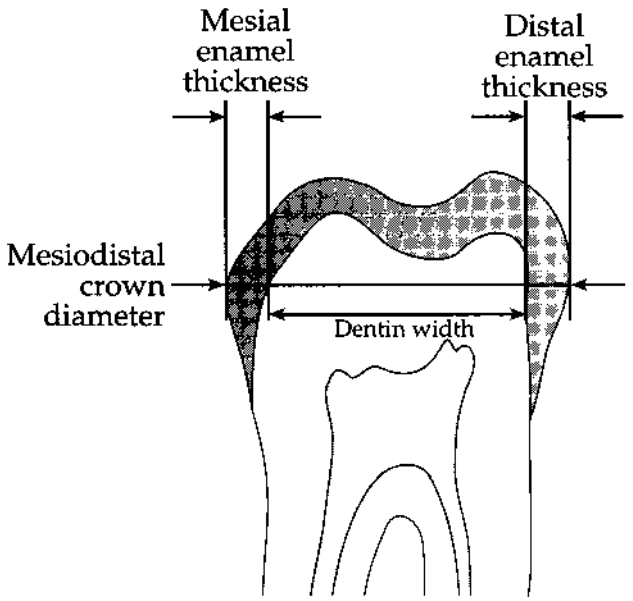


Fig. 1. Schematic of a mandibular molar in lateral view showing the three mesiodistal variables: mesial enamel width, distal enamel width, and crown diameter. A fourth variable "dentin width" was obtained by subtracting the two enamel widths from crown diameter.

Statistical analysis consisted of two-way factorial analysis of variance, testing for race (black, white) and sex differences, and principal components analysis to assess the statistical interrelationships among the variables.

Intraobserver reliability was quantified from double determinations. With one repetition, reliability in terms of a components of variance analysis of variance is the intraclass correlation (Winer, 1971). In this test, $r_i = 0.974$, so about 3% of the variance was due to repeatability error.

RESULTS

Crown and Dentin Diameters

Both the mesiodistal crown diameters and mesiodistal lengths of the dentin components of the molar crowns were highly significantly different between blacks and whites and between males and females within each ethnic group (Table 1). The F-ratios were larger between races than between sexes, and they were appreciably larger for the dentin components of crown size than for overall mesiodistal crown diameters of the teeth. The black-white difference averaged 3.3% for crown diameters and 5.3% for dentin thicknesses. Indeed, each of the matched crown-dentin comparisons had a higher percentage difference for dentin ($P = 0.008$ by a paired sign test).

Table 1. Statistics for deciduous molar component widths.

		Blacks			Whites			F-Ratios		
Tooth†	Sex	n	$\bar{x}$	sd	n	$\bar{x}$	sd	Race	Sex	Interaction
					Mesiodistal Crown Diameter					
Um1	M	59	8.04	0.46	70	7.72	0.52	33.8**	16.9**	0.4
	F	68	7.82	0.56	73	7.42	0.50			
Um2	M	57	9.78	0.61	69	9.59	0.47	10.0**	9.6**	0.2
	F	65	9,59	0.59	72	9.34	0.56			
Lm1	M	58	8.84	0.44	70	8.59	0.49	13.0**	24.0**	0.6
	F	68	8.51	0.54	73	8.33	0.48			
Lm2	M	59	11.20	0.58	70	10.77	0.44	32.8**	11.8**	0.2
	F	66	10.93	0.66	73	10.56	0.60			
					Dentin Thickness					
Um1	M	59	6.91	0.49	70	6.49	0.50	55.9**	13.7**	0.5
	F	68	6.72	0.51	73	6.23	0.50			
Um2	M	55	8.15	0.50	69	7.85	0.52	23.3**	10.1**	0.0
	F	65	7.96	0.52	72	7.63	0.52			
Lm1	M	54	7.79	0.44	69	7.37	0.48	40.1**	22.5**	0.7
	F	67	7.46	0.49	73	7.15	0.44			
Lm2	M	59	9.47	0.53	70	8.94	0.43	51.9**	12.4**	1.6
	F	66	9.17	0.54	73	8.80	0.54			
					Enamel Thickness: Mesial					
Um1	M	59	0.54	0.11	70	0.59	0.13	20.2**	3.3	0.7
	F	68	0.50	0.10	73	0.58	0.10			
Um2	M	56	0.74	0.15	69	0.81	0.17	16.3**	0.4	0.0
	F	65	0.72	0.15	72	0.80	0.14			
Lm1	M	58	0.49	0.08	70	0.58	0.13	35.9**	0.2	0.9
	F	68	0.50	0.11	73	0.56	0.11			
Lm2	M	59	0.76	0.14	70	0.84	0.14	12.6**	0.9	2.0
	F	66	0.77	0.14	73	0.80	0.12			
					Enamel Thickness: Distal					
Um1	M	59	0.59	0.09	70	0.63	0.14	5.4*	0.2	0.5
	F	68	0.59	0.11	73	0.62	0.10			
Um2	M	55	0.89	0.18	69	0.93	0.17	0.5	0.0	1.1
	F	65	0.92	0.16	72	0.91	0.18			
Lm1	M	54	0.59	0.10	69	0.63	0.13	10.2**	2.1	0.1
	F	67	0.57	0.12	73	0.62	0.11			
Lm2	M	59	0.97	0.17	70	0.99	0.15	0.1	0.1	2.0
	F	66	0.99	0.16	73	0.96	0.16			

†Abbreviations are: Maxillary (U), mandibular (L), molar (m), male (M), and female (F). *$0.01 > P > 0.01$. **$P < 0.01$

Sexual dimorphism averaged 3.0%. There was no discernible difference in the magnitude of sexual dimorphism between races or between the total crown and dentinal components, but the deciduous first molar was significantly more dimorphic than the second ($P = 0.02$ by a paired sign test).

Table 2. Statistics comparing mesial and distal marginal enamel thicknesses.

		Blacks			Whites			F-Ratios		
Tooth	Margin	N	$\bar{x}$	sd	N	$\bar{x}$	sd	Race	Margin	Interaction
Um1	Mesial	127	0.52	0.10	143	0.58	0.12	13.7**	128.0**	9.2**
	Distal	127	0.59	0.10	143	0.62	0.12			
Um2	Mesial	120	0.73	0.15	141	0.81	0.15	5.7**	438.8**	20.8
	Distal	120	0.91	0.17	141	0.92	0.17			
Lm1	Mesial	121	0.49	0.10	142	0.57	0.12	24.0**	172.9**	9.6**
	Distal	121	0.58	0.11	142	0.63	0.12			
Lm2	Mesial	125	0.76	0.14	143	0.82	0.13	2.2	685.7**	21.6**
	Distal	125	0.98	0.16	143	0.98	0.15			

*0.05 > P ≥ 0.01. **P < 0.01

Table 3. Correlation matrix for the eight enamel variables and four dentin variables.†

	Um1_MEn											
Um1_DEn	0.725*	Um1_DEn										
Um1_Dent	-0.239*	-0.170*	Um1_Dent									
Um2_MEn	0.623	0.561*	-0.145	Um2_MEn								
Um2_DEn	0.571	0.572*	-0.031	0.760*	Um2_DEn							
Um2_Dent	-0.150	-0.170*	0.555*	-0.193*	-0.154*	Um2_Dent						
Lm1_MEn	0.587	0.542*	-0.236*	0.521*	0.498*	-0.161*	Lm1-MEn					
Lm1_DEn	0.536	0.535*	0.068	0.553*	0.506*	-0.024	0.743*	Lm1_DEn				
Lm1_Dent	-0.094	0.061	0.640*	0.052	0.009	0.533*	-0.296*	-0.048	Lm1_Dent			
Lm2_MEn	0.498*	0.529	0.046	0.580*	0.515*	-0.033	0.529*	0.562*	0.023	Lm2_MEn		
Lm2_DEn	0.393*	0.418*	0.061	0.500*	0.492*	0.101	0.325*	0.451*	0.145*	0.671*	Lm2-DEn	
Lm2_Dent	-0.180*	0.130	0.557	-0.080	0.024	0.671	-0.222*	-0.096	0.685*	-0.093	0.037	Lm2_Dent

†Abbreviations are: Maxillary (U), mandibular (L), distal (D), mesial (M), Enamel (En), dentin (Dent). Sample size (i.e., cases with complete data) was 252.

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Marginal Enamel Thickness

None of the eight F-ratios testing for a sex difference in enamel thickness was statistically significant (Table 1), even though maximum crown diameters were clearly dimorphic. On the other hand, enamel was significantly thicker in whites than blacks, especially for the mesial marginal dimensions.

Mesial and distal thicknesses were compared with repeated-measures analysis of variance (Table 2), and, in each of the four molars, enamel was significantly thicker on the distal margin. On average, the distal marginal enamel was 25% thicker than the mesial enamel. There also was a consistent race difference, but it was the whites (with smaller mesiodistal crown diameters) who had the thicker enamel. The race difference (whites > blacks) was obvious for the maxillary and mandibular first molars, but it was far more subtle on the second molars. The reader will note that the race-by-margin interaction terms also were statistically significant. This is because the degree of black-white difference was much greater for the mesial marginal thickness (11.6% thicker in whites) than the distal margin (3.5% thicker in whites) so there is a lack of additivity between race and margin. Tests within margin and within race were calculated, and they showed that the main effects shown here were not biased to any important extent, so the two-way models are reported to illustrate the interaction.

Principal Components

Since the patterns of variation between sexes and races are different for enamel and dentin, the question arises whether these two tissue types are related metrically. The correlation matrix (Table 3) possesses a wide range of values, from -0.30 to 0.76, and most correlations (45/66) were significantly different from zero ($\alpha = 0.05$). All 21 of the nonsignificant correlations were between an enamel variable and a dentin variable.

Principal components analysis (Gorsuch, 1983) was calculated using the eight marginal enamel thicknesses along with the four mesiodistal dentin diameters. Communalities – the portions of the variance accounted for by the components – were all on the order of 0.6, but they were slightly higher for the four dentin dimensions (Table 4).

Two principal components were extracted with eigenvalues above one (Guttman, 1954; Kaiser, 1960), which agrees with the position of the inflection point on a scree plot of descending eigenvalues (Cattell, 1966). Scanning the component scores (Table 4), which are the correlations of the variables with the component, discloses a clear dichotomy: Enamel thicknesses are heavily weighted on PC 1 and the four dentin diameters are heavily weighted on PC 2. None of the dimensions had a variable complexity score above 1.2. This analysis shows that all eight measures of enamel thickness are positively intercorrelated to moderate degrees – as are the four dentin measures – but there is little statistical dependency between the enamel and dentin structures.

Table 4. Communalities and rotated principal component loadings.

Variable	Communality†	PC One	PC Two
	Maxillary First Molar		
Mesial Enamel	0.647	0.784	-0.180
Distal Enamel	0.624	0.779	-0.131
Dentin	0.664	-0.083	0.811
	Maxillary Second Molar		
Mesial Enamel	0.683	0.822	-0.085
Distal Enamel	0.635	0.797	-0.003
Dentin	0.654	-0.072	0.805
	Mandibular First Molar		
Mesial Enamel	0.622	0.741	-0.270
Distal Enamel	0.616	0.784	-0.038
Dentin	0.740	0.010	0.860
	Mandibular Second Molar		
Mesial Enamel	0.626	0.791	0.036
Distal Enamel	0.530	0.697	0.211
Dentin	0.735	-0.063	0.855
Eigenvalue		4.968	2.810
Percentage Variation		41.4	20.4

†Communalities are final solutions, not starting estimates

Factor scores were analyzed with two-way analysis of variance (Table 5), and there was a highly significant difference in enamel thickness (PC 1) between races, with white children exhibiting the greater thicknesses. There was, again, no suggestion of a sex difference in the enamel variables. Dentin thickness (PC 2), on the other hand, was highly significantly different both between blacks and whites and between sexes. In overview, then, these multivariable results ratify the same contrasts derived from the univariate analyses.

Table 5. Statistics comparing factors scores.

Principal		Blacks			Whites			F-Ratios		
Component	Sex	N	$\bar{x}$	sd	N	$\bar{x}$	sd	Race	Sex	Interaction
One	M	50	-0.99	4.26	68	1.63	5.49	13.6^{**}	0.6	0.5
	F	62	-1.09	4.62	72	0.75	4.25			
Two	M	50	2.32	2.52	68	-0.66	2.66	76.4^{**}	16.0^{*}	0.0
	F	50	2.32	2.52	68	-0.66	2.66			

*0.05 > P ≥ 0.01. **P < 0.01

DISCUSSION

Attainment of the mature size and surface morphology of the dentin, which occurs at the end of the bell stage of tooth development, and subsequent amelogenesis are largely independent events. Enamel (derived from ectoderm) is initially deposited on cusp tips

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late in the bell stage, but size of the dentin (derived from ectomesenchyme) is established well before enamel forms on all cusps and before its coalescence on the occlusal table (Kraus and Jordan, 1956; Oöe, 1981). The temporal and structural differences between dentin and enamel may parallel the statistical independence of mesiodistal dentin diameters and marginal enamel thicknesses found in the present study.

Sexual Dimorphism

One might suppose that males would have thicker enamel than females since their crown diameters are significantly larger (e.g., Thomsen, 1955; Moorrees et al., 1957; Hanihara, 1976; Moyers et al., 1976). If enamel scaled isometrically with dentin within a population, then both of these tissues would be sexually dimorphic. In fact, though, there is no evidence that enamel is systematically thicker in either sex. Marginal enamel (mesial + distal) constitutes about 16% of the mesiodistal crown diameter in both boys and girls. The implication is that, whatever control mechanism governs the greater growth of the dental papilla that becomes the dentin and pulp chamber of a molar, it is different from that controlling the rate and duration of amelogenesis. Stroud and coworkers (1994, 1998) came to the same conclusion, though they examined permanent teeth. Macho (1995) reviewed the human and nonhuman primate literature, and he too reported no sex difference aside from some statistically nonsignificant tendencies. While there do not appear to be published data for the deciduous dentition, Alvesalo and coworkers (1981, 1985) measured dentin and enamel thicknesses on maxillary canines and incisors and showed, as here, that mesiodistal crown diameters are significantly dimorphic between sexes, but there is no discernible sex difference in enamel thickness.

This finding discloses that the crown size difference exploited for forensic purposes of sex identification of infants and children occurs during formation of the dental papilla, not during subsequent amelogenesis. Multivariable analyses (Black, 1978; DeVito and Saunders, 1990) suggests that sex can be correctly determined about two-thirds to three-fourths of the time from deciduous crown diameters. This is a lower percentage than obtained from the permanent dentition (e.g., Ditch and Rose, 1972; Garn et al., 1977), though variability in the degree of sexual dimorphism between populations (as well as between primary and permanent dentitions) may confound this comparison.

Since males and females have equivalent marginal enamel thicknesses but males have larger crown diameters, it might follow that males have *proportionately* thinner enamel. In fact, there is no statistically significant difference in relative size between males and females, and this is attributed to the low correlations between enamel and dentin thicknesses (Table 3). Having a large dentin component (as in males) does not predispose for thicker than average marginal enamel.

Moss and Moss-Salentijn (1977; Moss, 1978) offered a carefully reasoned conjecture as to why males have larger crown diameters than females. They suggested that studies of tooth mineralization show that males take somewhat longer to undergo crown formation (e.g., Moorrees et al., 1963), so there is more time for enamel deposition. Moss and Moss-Salentijn specifically addressed the sex difference in crown size of the permanent canines, but the implication is that the sexual dimorphism in crown dimensions is mainly attributable to thicker enamel in males. Moss' suggestion does not account for other sexually dimorphic facets of tooth size, such as greater pulp widths and longer root

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lengths in males than females (Garn et al., 1978, 1979; Shields et al., 1990; Woods et al., 1990). As evidence has accumulated, we now see that the sex difference is established at an earlier stage of tooth development than that of enamel formation and, at least in humans, enamel thickness is statistically equivalent in the two sexes. The size and shape of the mature tooth crown is defined by the junction between the inner enamel epithelium (from which ameloblasts arise) and the dental papilla (Oöe, 1981; Avery, 1994). One noteworthy consequence of this is that genetic and environmental stresses that affect crown size and/or increase bilateral asymmetry necessarily have their impact prior to the end of the bell stage. These stressors can be chromosomal (e.g., Barden, 1980a,b; Alvesalo and Kari, 1977; Townsend et al., 1984), genetic (e.g., Schalk-van der Weide, 1992; Hertzberg et al., 1994), or environmental (e.g., Sciulli et al., 1979; Garn et al., 1979; Heikkinen et al., 1994), but only those teeth that have not yet completed the bell stage are susceptible to growth disturbances, generally down-regulation. There are, of course, some intrinsic diseases that affect enamel thickness, such as aneuploidy (Alvesalo and Tammisalo, 1981; Varrela et al., 1988) and some Mendelian traits (e.g., Wright et al., 1994; Mass et al., 1996), but examples appear to be uncommon, and it remains to be seen whether enamel thickness is as responsive to stressors as dentin.

Extensive work by Alvesalo and his colleagues shows that genes on both the X and Y sex chromosomes modulate tooth size. Data indicate that genes on the X chromosome promote enamel formation but do not noticeably affect dimensions of the dentin. The Y chromosome, on the other hand, promotes both dentin and enamel formation, probably by enhancing mitotic rates (e.g., Alvesalo et al., 1987; Alvesalo and Laine, 1992).

Black-White Differences

It has been established that American blacks have significantly larger tooth crown diameters than American whites. This is apparent for the permanent teeth (Henderson, 1975; Richardson and Malhotra, 1975; Hanihara, 1976; Macko et al., 1979), and it also holds for the primary teeth based on more limited information (Hanihara, 1976; Moyers et al., 1976). There is no substantial explanation for this racial difference. It is attractive to cite the dental reduction that has occurred in European groups over the past 50,000 years (e.g., Brace 1963, 1967; Brace and Mahler, 1971; Brace et al., 1991; Frayer, 1977, 1978), but the microevolutionary record is sparse for Subsaharan African peoples. This, at present, precludes assessments of when the microevolutionary divergence in tooth size between blacks and whites occurred. Moreover, the genetic changes driving the phenotypic changes are wholly speculative.

The present study shows that molar crown size contributes to black-white differences in two ways: Greater growth of the formative tooth in blacks during the pre-mineralization stages produces a larger dental papilla that goes on to form the dentin and pulp chamber. It would seem that the racial difference is one of proliferative rates since blacks spend less time in all phases of tooth mineralization than whites (Harris and McKee, 1990) yet their crowns and roots are larger. Greater size of the dentin in blacks is partially offset by thicker enamel in whites. Whites have considerably thicker enamel on the mesial margin ($\overline{x}$ = 11% greater), but it also is thicker on the distal surface ($\overline{x}$ = 4%). It

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would be of interest whether the cross striations (or lines of Retzius) differ in the deciduous teeth of blacks and whites since there is evidence here that the rate and/or duration of enamel formation are different.

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Electron Microscopic Observations of Tooth Development in the Opossum (*Didelphis virginiana*) with Specific Regard to Enamel Developing Processes, von Korff's Fibers and Collagen

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INTRODUCTION

Teeth are complex mineralized structures whose development can give clues to both the life history of a living organism and to the evolution of these specialized structures. All vertebrate teeth have four main structures: the enamel, the dentin, the pulp and the cement. There are three main cell types responsible for the mineralization of teeth: the ameloblasts, odontoblasts and cementoblasts. Odontoblasts arise from pulp or dental papilla cells. In embryonic or non-erupted juvenile teeth ameloblasts cover the external tooth surface and are responsible for secreting the underlying enamel. Inside the developing enamel is dentine, which is formed by odontoblasts residing in the pulp at the core of the tooth. The opossum is an ideal animal to study original tooth development because of its primitive position in mammalia. Of interest to this study are three structural features of opossum teeth.

First is the development of enamel tubules. These tubules are microscopic spaces in enamel formed by cellular extensions or processes. In human teeth these tubules become mineralized as the cellular processes recede. However, in marsupials the tubules and processes remain intact throughout the life of the tooth. The origin of enamel tubules is controversial. Boyde and Lester (1967) reported that the cellular processes arise from ameloblasts, while Fosse, Risnes and Holmbakken (1973) wrote they that originate from odontoblasts; still others think that the processes arise from both cell types.

Second is the existence of von Korff's fibers. The details of von Korff's fibers have never been reported in marsupials. These fibers are bundles of collagenous fibers that pass from the dentin matrix into the dental pulp between the odontoblasts. The existence of these fibers is controversial because they were often believed to be artifacts (Ten Cate et al., 1970).

The third feature is the distribution of different types of collagen in opossum teeth. In order to determine the distribution of collagen in opossum teeth, immunocytochemistry using chicken anti-collagen type I and III was performed on ultrathin sections of neonate opossum teeth.

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MATERIALS AND METHODS

A. Structure

Tooth germs were obtained from opossum neonates (*Didelphis virginiana*) of 14, 21, 28, and 35 days old. Animals were killed by perfusion fixation or decapitation. The jaws containing tooth germs were dissected out and immersed in fixative. The fixative used for perfusion and immersion was 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). All specimens were postfixed in 1% osmium tetroxide solution buffered with 0.1 M sodium cacodylate (pH 7.4). After postfixation they were dehydrated in ethanol series and embedded in Epon-812. Ultrathin sections were cut using a diamond knife, stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM) (HITACHI H-8000).

B. Immunocytochemistry

Animals were killed as described above and were fixed in 2% paraformaldehyde with 0.5% glutaraldehyde or 4% paraformaldehyde with 0.01% glutaraldehyde in Sorensen's buffer (pH 7.4). After ethanol dehydration specimens were embedded in LR-White. Sections were treated with 5% EDTA (pH 7.4) for 3 min and then washed 2 times with distilled water. They were then etched with 2.5% sodium metaperiodete for 3 min and washed with distilled water 2 times. The sections were treated 3 minutes in 1% BSA and incubated overnight at 4 with either 1:50 dilution of rabbit anti-chicken collagen type I and II antiserum or with rabbit preimmune serum. The following day, the sections were washed 3 times in Sorensen's buffer and placed in 1% BSA for 3 min. They were then incubated in 1:20 dilution of goat anti-rabbit antiserum conjugated with 15nm gold beads for one hour. Sections were then washed in Sorensen's buffer and twice in distilled water. They were then stained briefly with 2% uranyl acetate and washed 2 times with distilled water.

RESULTS

A. Developmental

Three weeks after birth, ameloblasts were arranged in a well defined cell layer. The cells had a clear plasma membrane and were slightly elongated with a tapered end in contact with the enamel. The tapered end is known as a Tome's process. The cells had oval shaped nuclei and many mitochondria were located in the tapered end. The rER appeared swollen and run along the cells' long axis abundantly and at the base of the cell. At the same time, odontoblasts were also in a layer. The cells and nuclei had a similar shape as ameloblasts. The rER were well developed and bag-like. They were evenly distributed through out the cytoplasm except the odontoblast process.

At four weeks, the ameloblasts were still arranged in a layer, however the plasma membrane was not smooth, and cells appeared shrunken, possibly because of hypertonic conditions of the fixative. The nuclei had moved towards the base of the cells. The odontoblasts were still in a layer, however their plasma membrane that was in contact

with predentin shows small invaginations and vesicles. At this stage the rER was well developed into tubular shapes, and multi-layered. Collagens were seen throughout the dentin, which was partially crystallized. Also in dentin were odontoblast processes, which were surrounded by hydroxyapatite crystals. Collagens were seen in the predentin as bundles in regular patterns. Furthermore, enamel was made of crystals, shorter than those in dentin that form a ribbon like pattern.

Odontoblast tubules extended two thirds of the way across the enamel layer from the pulp, while the ameloblast processes extended two thirds of the way across the enamel from the tooth exterior. (Fig. 1)

The ameloblasts' projections in the tubule were characterized by the presence of large particles and vesicles in their cytoplasm. However, the odontoblasts' processes had fine fibers and small, electrondense materials. (Fig. 2)

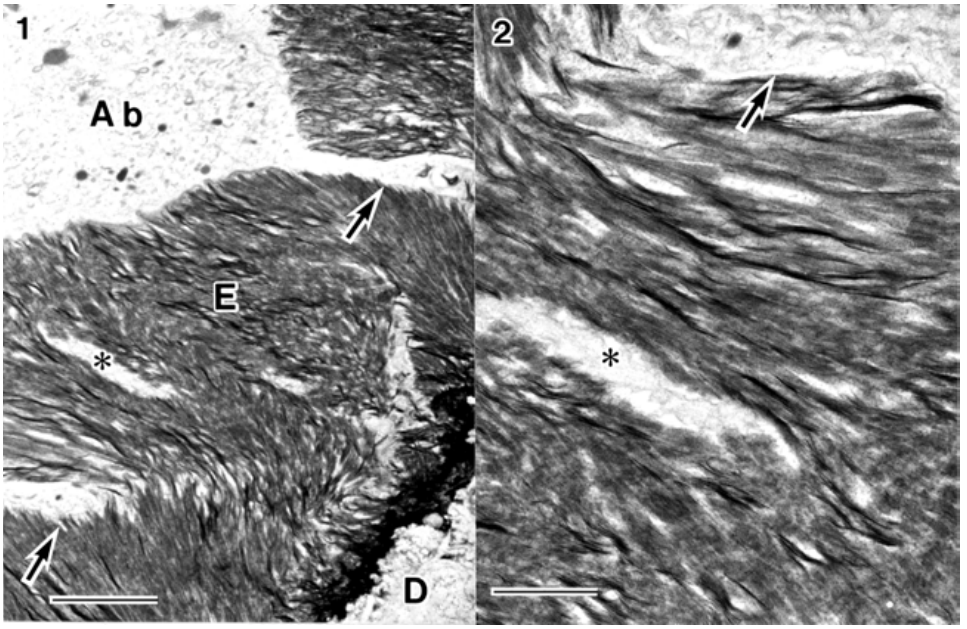


Fig. 1. Odontoblast tubules (*) extend across the enamel layer from the DEJ, while ameloblast processes (arrows) extend two thirds of the way across the enamel from the tooth exterior. Ab: ameloblast. E: enamel. D: dentin. Bar = 1.3m.
Fig. 2. High magnification of Fig.1. Odontoblast tubules (*) contains fine fibrils, but ameloblasts processes (arrow) has vesicles and granules. Bar = 500nm.

B. von Korff's fibers

This study shows that opossum (*Didelphis virginiana*) possesses von Korff's fibers in its predentin. The von Korff's fibers were made from bundles of collagen fibers and were found in the intercellular spaces between odontoblasts processes the predentin and the dentine. They were also present in those intercellular spaces near the basal lamina, and also in preodontoblasts. Compared with other collagens, which were arranged randomly, von Korff's fibers seemed to be arranged parallel to the long axis of odontoblasts. (Fig. 3)

The von Korff's fibers were 50-70nm thick while other collagen fibers are 20-40nm thick. (Fig. 4) The von Korff's fibers were clearly less abundant between opossum odontoblasts than between the odontoblasts of other mammals and human teeth germs.

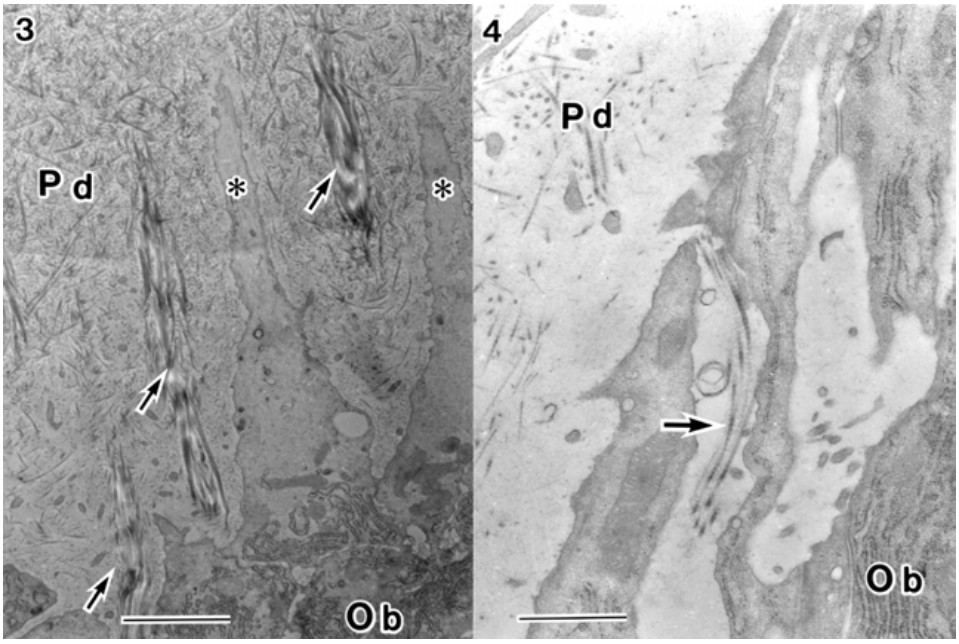


Fig. 3. Von Korff's fibers (arrows) from bundles of collagen in predentin. * : odontoblast tubules. Bar = 2.5m.
Fig. 4. Fine Von Korff's fibers (arrows) are found between odontoblast. Ob: odontoblast. Pd: predentin. Bar = 1.0m.

C. Collagen

Immunocytochemistry using anti-collagen anti-bodies indicates that type I collagen has a specific distribution in opossum teeth germs. (Fig. 5) As in most other animals, type I collagen is found in dentin and predentin. In addition to this many vesicles in odontoblastic process show the collagen. A rebelling reaction of collagen type III in enamel is suspected. While type III collagen was found one third layer of the predentin in adjacent to the odontoblast (Fig. 6), and around the odontoblast processes in the dental tubule, as well as the dental papilla. (Figs. 7 and 8)

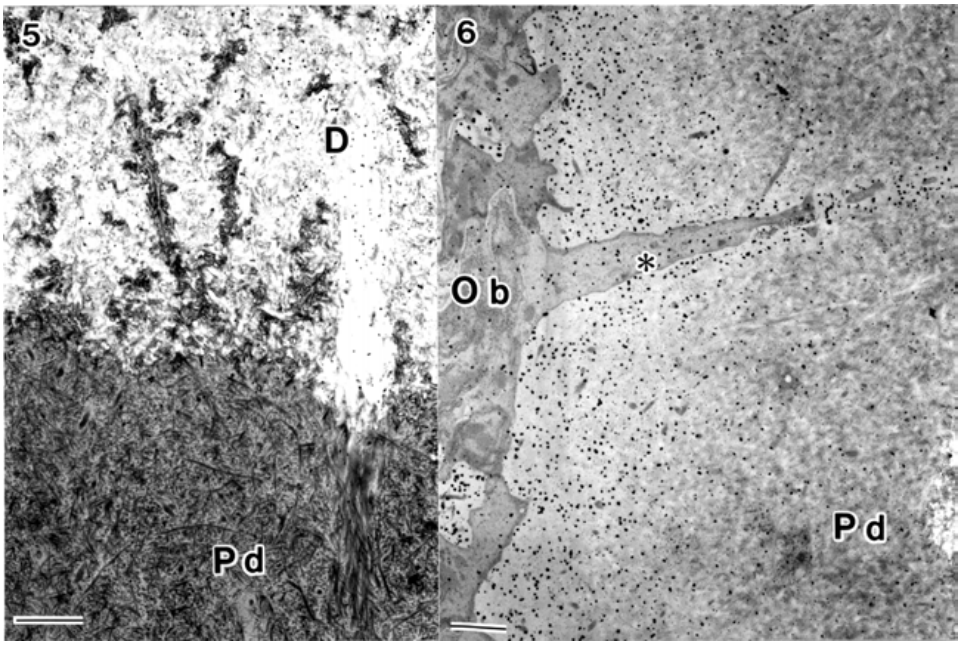


Fig. 5. Section was stained for type I collagen. Positive labeling reaction can be observed in predentin and dentin. Pd: predentin. D: dentin. Bar = 2m.

Fig. 6. Section was stained for type III collagen. Positive labeling reaction can be observed in the one third area immediately adjacent to the odontoblast, around the odontoblast processes, and between the odontoblasts. Ob: odontoblast. Pd: predentin. * :odontoblast tubules. Bar = 2m.

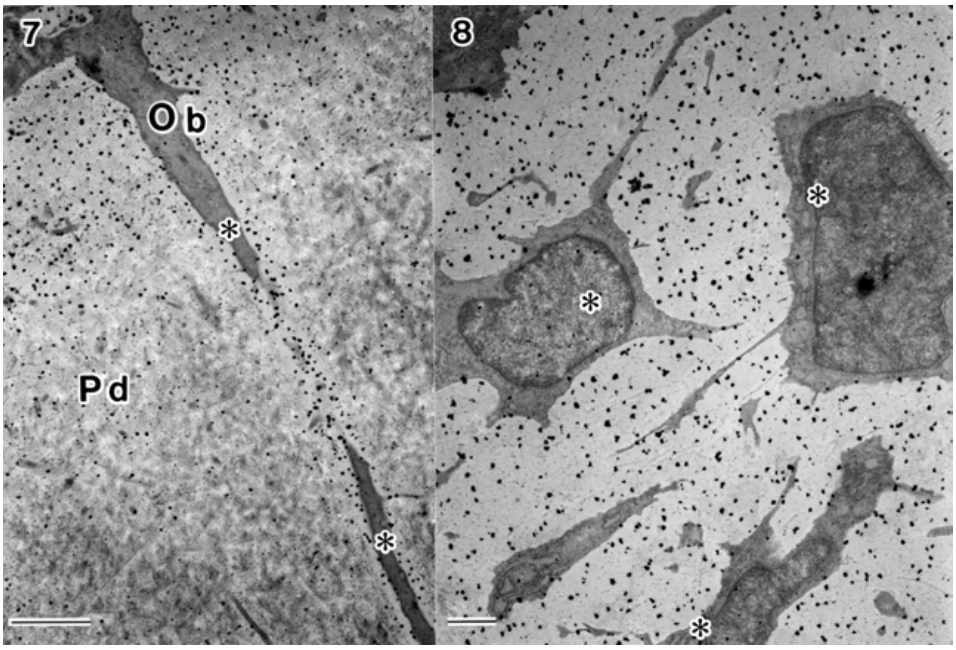


Fig. 7. Section was stained for type III collagen. Positive labeling reaction can be observed around the odontoblast processes. Ob: odontoblast. Pd: predentin. * : odontoblast tubules. Bar = 2m.

Fig. 8. Section was stained for type III collagen. Positive labeling reaction can be observed between dental papilla cells. * : dental papilla cell. Bar = 1.0m.

DISCUSSION

A. Development

Based on structural evaluation Kozawa and Tateishi (1983) assume that enamel tubules, in the opossum, are formed by both ameloblasts and odontoblasts coming into contact with each other. Other research indicates that humans do not have enamel tubules, but porcines are known to have enamel tubules. Kozawa, and Tateishi (1983) and Iwasa (1997) found tubules that run from odontoblasts to the enamel surface by examining opossum teeth (*Monodelphis domestica*) in the SEM. However, the cellular origin of these tubules could not be determined. This research shows that odontoblasts extend very close to the surface of the enamel. Also ameloblastic processes are shown to be close to odontoblastic processes. However, it is not known whether or not these tubules join to form single, long tubules, that span the enamel layer. Further analysis using serial sections is required to determine if ameloblasts and odontoblasts truly fuse together.

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B. von Korff's fibers

von Korff fibers are fan shaped collagenous fibers that come from dental papilla cells, pass between odontoblasts, and go into the predentin. This type of structure was found in this study. There are two possibilities regarding the structure of von Korff's fibers; one is that these fibers exist in large invaginations of odontoblasts. The other is that von Korff's fibers pass through the area where the tight junction existed. Serial sections may help to determine if any of these conditions are correct. Where do von Korff's fibers synthesize, originate and where do they terminate? Are von Korff's fiber localized in a particular region of the tooth or are they found everywhere? Finally, what type of collagen are von Korff's fibers in opossum? Immunolocalization was not performed on von Korff's fibers. However, Ohsaki and Nagata (1994) recently reported that von Korff's fibers in the rat were collagen type III

C. Collagen

Further research will determine if type I collagen exists as part of the enamel crystal matrix or if the localization detected in this research is artificial. Tung (1985) reported that type III collagen was a component of either dentin or predentin. What role, if any, type III collagen has in dentinogenesis is unclear.

ACKNOWLEDGEMENTS

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Long-Period and Short-Period Incremental Lines in Dentin of Alligatorid Teeth

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INTRODUCTION

The crocodilia are the only non-mammalian vertebrates in which the teeth are attached to the jaws by a true periodontal ligament (Poole 1961; Soule 1967; Westergaard and Ferguson,1990). It is well established that crocodile teeth possess a number of important anatomical and histological structures which correspond to structures in mammals, thus representing the most primitive pattern of thecodont attachment (Poole 1961; Soule 1960; Westergaard and Ferguson, 1990). The mode of development and the histological structures of alligatorid teeth parallels with that of mammals in many respects.

The various types of incremental lines in mammalian dentin have been debated for many years ago (Schour and Hoffman, 1939a,b; Schour 1953; Dean and Scandrett, 1996). Not much attention was paid to the morphology of dentin growth layers in the histological literature, but it was discussed a great deal by zoologists (Klevezal, 1996). The short-period incremental lines or von Ebner's lines are considered to be daily markings, and are indicative of circadian rhythms (Dean and Scandrett, 1996; Miani and Miani, 1971; Yilmaz et al., 1977). Klevezal (1996) showed that the growth layers of the first order in mammals were the annual layers, that of the second order were formed monthly, and that of the third order were daily layers or circadian ones. Ohtsuka and Shinoda (1995) found that the circadian increments and the ultradian increments coexisted in rat incisor dentin. However, there has been little report on the short-period incremental lines and long-period incremental lines of dentin in the Alligatorid teeth. This present study was designed to examine the periodicity of the short-period and long-period incremental lines using both the tetracycline and the calcein injection methods.

MATERIALS AND METHODS

Alligatorids (*Caiman latirostris* and *Caiman crocodilus*) were bred in water tanks at 28-30 °C. Of their teeth used in this study, 5 *Caiman latirostris* were from 1 month old to 1.8 years of age, weighing from 70 g to 2.4 kg, and the craniocaudal length was from 26 to 86 cm while 7 *Caiman crocodilus* were from 2 months old to 1.5 years old, weighing from 60 to 365 g with a craniocaudal length of 25 to 47 cm. Tetracycline and calcein were administered to the animals as follows: 1) The tetracycline (TC) was orally administered to *Caiman latirostris* at twice monthly intervals according to the lunar variation for one year. 2) The TC and calcein (CAL) were orally administered to *Caiman crocodilus* at twice monthly intervals according to the lunar variation for one year. TC was

administered on the new moon and CAL was administered on the full moon. 3) The TC and CAL were orally administered to *Caiman crocodilus* at weekly intervals for one year. CAL was orally administered to some *Caiman crocodilus* at 3 day intervals for 2 weeks.

Each exfoliated tooth was fixed in 10% formalin. After fixation, the teeth were ground parallel to the longitudinal axis of the tooth. The ground sections (15-50μm) were observed with the polarized light microscopy, fluorescent microscopy, microradiography, desktop X-ray analytical microscopy (XGT-2000, Horiba, Japan) and confocal laser scanning microscopy. Chronological analysis was made by counting the number of incremental lines and measuring the widths of adjacent incremental lines using a confocal laser scanning microscopy (Leica TCS4D, Leica, Germany). Some decalcification specimens were stained with hematoxylin and eosin staining, and Powers and urea silver impregnation.

RESULTS

Long-period incremental lines and short-period incremental lines were observed in the dentin of *Caiman crocodilus* with hematoxylin and eosin staining. Long-period incremental lines were observed clearly in dentin with urea silver impregnation. Short-period incremental lines were observed in dentin with Powers silver impregnation. The width of short-period incremental lines in dentin was 16-21μm. In the ground section, short-period incremental lines were also observed in dentin by the polarized light microscopy. Dark incremental lines in dentin (arrows) were observed in the transmitted image of the ground section of dentin in *Caiman crocodilus* (Fig. 1a). TC labeling lines (yellow) and CAL labeling lines (red) were observed in the superimposed image (Fig. 1b). TC was orally administered on the new moon. TC labeling lines corresponded to the dark incremental lines of the transmitted image. The rate of dentin formation per day was 9.8μm. As compared with the dark incremental lines that observed at the full moon of about 14 day intervals, the incremental lines that were observed at the new moon of about 28 day intervals were darker, wider and had lower calcification using the confocal laser scanning microscopy and microradiography. But the difference in the mineral content was small using the desktop X-ray analytical microscopy.

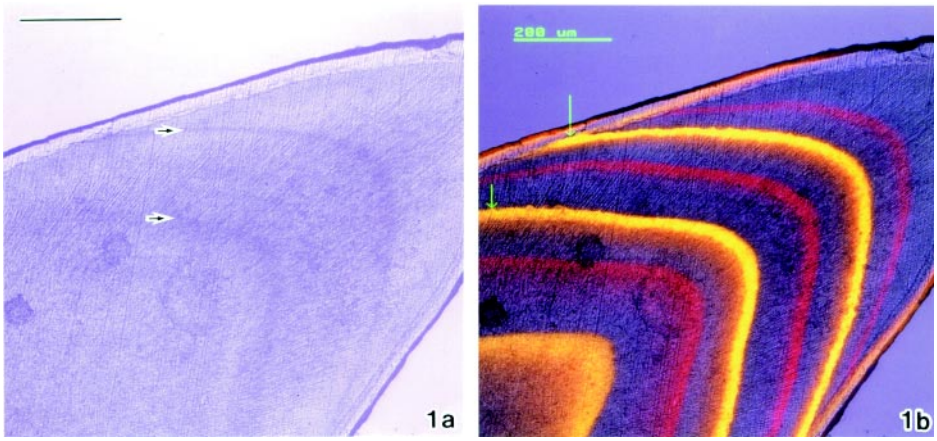


Fig. 1. Confocal images of ground section of dentin in *Caiman crocodilus.* Dark incremental lines in dentin (arrows) were observed in the transmitted image (a). TC labeling lines (yellow) and CAL labeling lines (red) were seen in the superimposed image(b). TC was orally administered on the new moon. TC labeling lines corresponded to the dark incremental lines of transmitted image. Bar: 200 m.

TC labeling lines in dentin were observed in the superimposed image (Fig. 2 a) of the ground section in *Caiman latirostris.* Between the TC labeling line (new moon) and the TC labeling line (full moon), 6 narrower incremental lines (arrows) were shown in the superimposed image (Fig 2b). The narrower incremental lines were spaced at 2 days apart. The rate of dentin formation per day was 11-14μm. The TC labeling lines in the superimposed image (Fig 3a, arrows) corresponded to the dark incremental lines of the transmitted image in *Caiman crocodilus* (Fig 3b, arrows). The rate of dentin formation per day was 8.5-11μm. Between the CAL labeling line (glow: full moon) and the TC labeling line (green: new moon), 6 narrower incremental lines (arrowheads) were shown in the superimposed images (Fig 3a). Between the TC labeling line (green: new moon) and the CAL labeling line (glow: full moon), 6 narrower incremental lines (arrowheads) were shown in the transmitted images (Fig 3b).

The dark layers were observed between the TC labeling lines in the fluorescent image of the ground section of dentin in *Caiman latirostris* (Fig 4). Between the TC labeling lines, 6 narrower incremental lines (arrows) were shown in the inner layer and 5 narrower incremental lines (arrows) were shown in the outer layer. The rate of dentin formation per day was 9-10μm. The rate of dentin formation varied between 6μm -12.5μm per day. The average rate per day in these Alligatoroid teeth was 9.48±1.77μm.

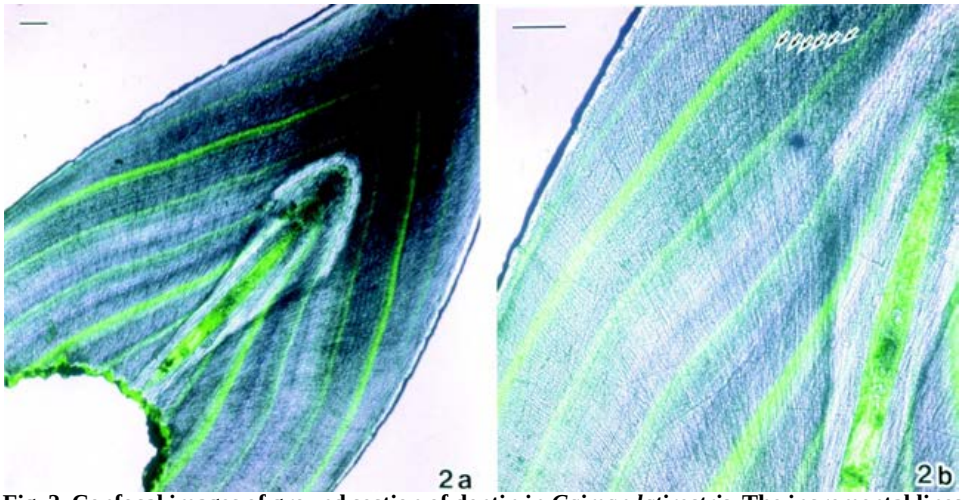


Fig. 2. Confocal images of ground section of dentin in *Caiman latirostris.* The incremental lines and TC labeling lines were observed in the superimposed image (a). Between TC labeling line (new moon) and TC labeling line, 6 narrower incremental lines (arrow) showed in the superimposed image (b). The narrower incremental lines were spaced 2 days apart. Bar: 100 μm.

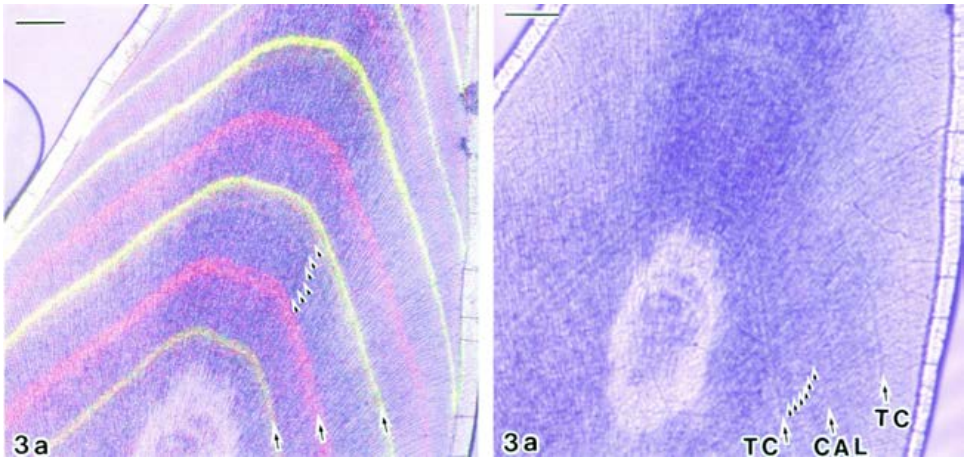


Fig. 3. Confocal images of ground section of dentin in *Caiman crocodilus.* TC labeling lines in the superimposed image (a, arrows) corresponded to the dark incremental lines of transmitted image (b, arrows). Between CAL labeling line (glow: full moon) and TC labeling line (green: new moon), 6 narrower incremental lines (arrowheads) showed in the superimposed images (a). Between TC labeling line (green: new moon) and CAL labeling line (glow: full moon), 6 narrower incremental lines (arrowheads) showed in the transmitted images (b). Bar: 100 μm.

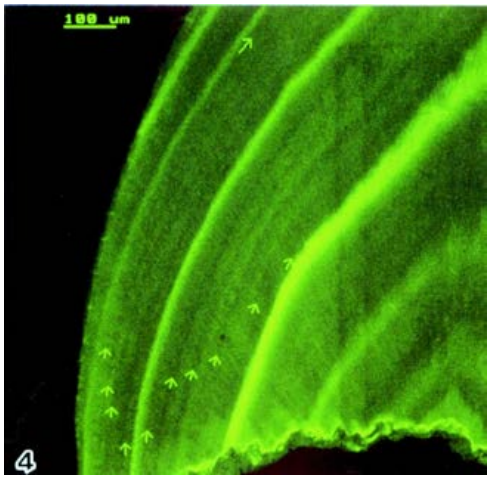


Fig. 4. Confocal images of ground section of dentin in *Caiman latirostris.* Dark layers were observed between TC labeling lines in the fluorescent image. Between TC labeling lines, 6 narrower incremental lines (arrows) showed in the inner layer and 5 narrower incremental lines (arrows) showed in the outer layer.

DISCUSSION

The present study reveled that the periodicities of incremental lines in dentin of Alligatorid teeth are about 2 days, about 14 days, and about 28 days apart (Fig. 5). The optical differences between the principal bands of a growth layer is determined by differences in the content and distribution of the mineral component of the dentin (Klevezal, 1996). This permeability is related to the size and distribution of calcospherites. The more transparent layers consisted of bigger calcospherites and the less transparent layers consisted of smaller calcospherites (Klevezal, 1996). By the polarized light microscopy observation, differences in optical density are also probably determined by the orientation and distribution of the organic matrix.

Schour (1953) reported that the pairs of dark and light increments were of uniform width (approximately 16µm), occurred in dentin of all species studied, and gave to the dentin its characteristically regular incremental calcification pattern. The growth rate of the organic stroma of the tooth was 2-16µm per day, depending on the type of tooth (Schour and Hoffman, 1939b). Thus, the calcification rhythm described by them was either of a daily nature or over a span of several days (Klevezal and Kleinenbrg, 1967). The width of these layers in different teeth and in different species varies from 2 up to 30µm (Klevezal, 1996). In the Alligatorid teeth dentin, the average rate per day was 9.48±1.77µm. The formation of the daily layers was determined not only by the daily changes in the calcification rate but also by daily changes in the growth rate of dentin.

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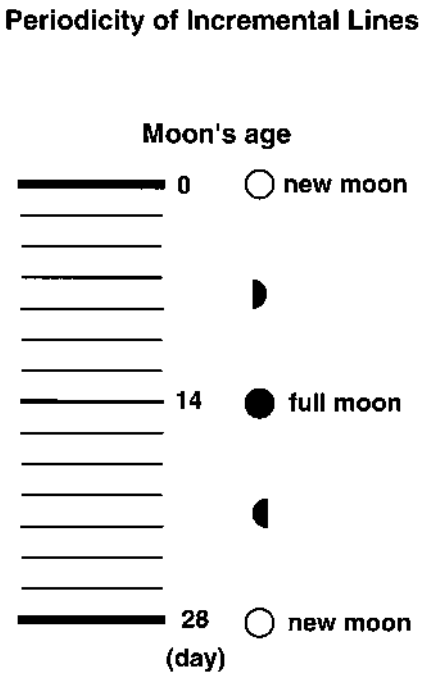


Fig. 5. Periodicity of incremental lines.

Rosenberg and Simmons (1980) showed the spectra of structural and compositional rhythms with a range of periodicities which extended from a matter of hours (ultradian) to days (infradian) and circadian (approximately 24 hrs). The low-frequency structural rhythms could represent infradian periods ranging between 2 and 30+ days (Rosenberg and Simmons, 1980). There were 12-13 growth layers per one annual growth layer of dentin. Their layers were formed with monthly periodicity and termed lunar layers (Klevezal (1996). In *Lynx lynx*, mean period of the layer formation was 14-15 days (Klevezal (1996). For the first time, the present study showed that the lunar incremental lines (about 28 days period) and the half lunar incremental lines (about 14 days period) existed in the dentin of Alligatorid (*Caiman latirostris* and *Caimon crocodilus*) teeth. The 28 day (lunar) period incremental lines were more hypocalcified than the 14 day period (half lunar) incremental lines. The lunar incremental lines were formed at the new moon. The factors that determined these periodicities should be studied in the future. In mammals, daily incremental lines or circadian incremental lines are formed in dentin (Klevezal, 1996; Ohtsuka and Shinoda, 1995). However, circadian incremental lines were not observed in Alligatorid teeth on this present study. This may be because of the difference in species and in the rate of dentinogenesis. Further investigation is necessary. Klevezal and Kleinenberg (1967) concluded that the formation of the growth layers in dentin is determined by an inhibition of the growth of an individual, as whole, and by an inhibition of growth of these tissues. These intervals may be related to the fundamental biorhythm of the animals and the periodicity of odontoblast activity. We think that the

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circadian and infradian incremental lines may be independent of each other and are driven by different oscillatory mechanisms. More data will be needed for the implication, so that the findings will be become clearer.

ACKNOWLEDGEMENTS

We would like to thank Mr. T. Yamamoto, head of the breeding section of the Atagawa Tropical and Alligator Garden, for guidance of crocodile and the staffs of the breeding section of the Atagawa Tropical and Alligator Garden, for their considerable assistance. A portion of this work was supported by the Nihon University School of Dentistry at Matsudo Research Grant (Suzuki Grant) for 1998 (98-0002) and the Frontier Science from the Ministry of Education, Science, Sports and Culture of Japan.

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Developmental Study of Monophyodont Teeth in Northern Fur Seals (*Callorhinus ursinus*) Using 3-D methods

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INTRODUCTION

In most mammals, monophyodont teeth are considered as part of the primary (deciduous) dentition (Colyer, 1936; Ooe, 1962, 1979), however, the first and fifth teeth in northern fur seals have been classified as part of the secondary (permanent) dentition on the microscopic anatomy (King, 1983; Kubota et al., 1962; Kubota and Matsumoto, 1963; Kubota and Togawa, 1970). The study aims to elucidate the position of the monophyodont teeth in northern fur seals from the developmental aspect by using a computed three-dimensional reconstruction method.

MATERIALS AND METHODS

Two northern fur seal embryos (body length 13.5cm, 24.0 cm) were fixed with 10 %v/v formalin, and decalcified with 10 % formic acid. The heads of the animals were dissected and embedded in paraffin wax. Serial horizontal sections (4µm) were made and stained with haemotoxylin and eosin. The tooth germ and dental lamina in each section were identified histologically using light microscopy. The upper right tooth germs and dental laminae of specimens were reconstructed by a three-dimensional computer-aided method with light microscopy. The contours of both the inner and outer tooth enamel epithelia of the tooth germ were traced on transparent paper and they were reconstructed in 3-D images with OZ95 (Rise Corporation, Japan).

RESULTS AND DISCUSSION

We observed serial horizontal sections of the upper right canine and first postcanine tooth. The first generation (deciduous) tooth germ of the first post canine developed from the same dental lamina of the first generation (deciduous) tooth germ of canine. When the first post canine tooth germ developed, the dental lamina of the first generation (deciduous) tooth germ of canine separated from that of the first post canine tooth. Each dental lamina of every kind of tooth developed separately, so that the developing margin of the dental lamina showed a wavy shape. The secondary tooth germ could not be recognized on the first post canine dental lamina. The second generation (permanent) tooth germ of the canine developed in the canine series of the dental lamina (Figs 1 and

2). Both the primary tooth series and the permanent series developed from the same dental lamina, which developed from the previous dental lamina continuing to the oral epithelium.

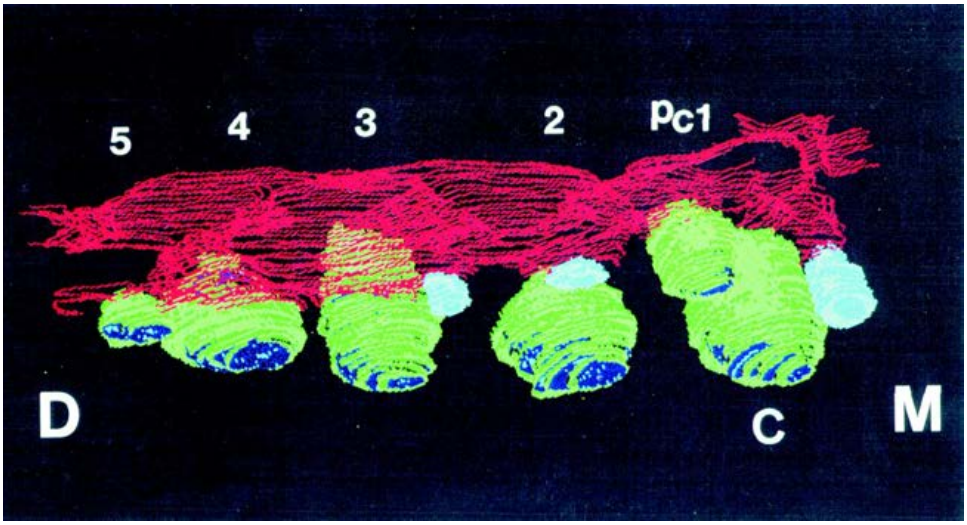


Fig. 1. Reconstruction of the upper right dental lamina (arrows) and post canine teeth germ (pc), lingual aspect. M: mesial, D: distal, Green: the primary (deciduous) teeth, Blue: the secondary (permanent) teeth, Red: dental lamina.

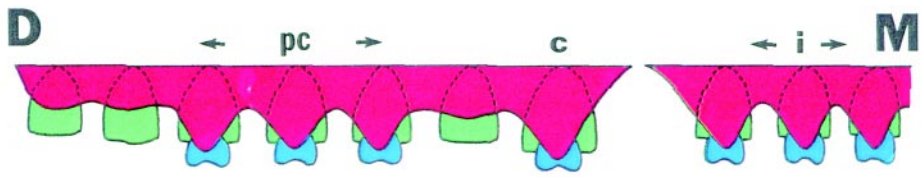


Fig. 2. A diagram of Fig.1. Each kind of teeth series such as the Primary (deciduous) and secondary (permanent) teeth, developed from same dental lamina (arrows), which developed from the previous dental lamina continued to the oral epithelium. M: mesial, D: distal, Green: the primary (deciduous) teeth, Blue: the secondary (permanent) teeth, Red: dental lamina

Reconstruction of the upper right shows the first and secondary generation tooth germ of the canine (Fig. 3). In the canine series, the first generation tooth germ developed on the elongated dental lamina, which developed deeply into the jaw and formed the secondary generation tooth germs on the margin (Fig. 4). Horizontal sections of the upper right canine series are shown in figure 5. The developing third generation tooth germ can be observed on the distal side of the secondary generation tooth germ of canine.

The secondary tooth germ could not be found on the first post canine tooth germ (Figs 6 and 7). The fifth post canine tooth germ directly developed on the dental lamina, which developed from the first generation tooth germ of the fourth post canine tooth. However, the secondary tooth germ could not be observed on these tooth germs (Fig. 8). It is concluded the first and fifth post canine tooth belong to the first (deciduous) tooth series not the secondary (permanent) or successional tooth, as in the case of molars in most other mammals.

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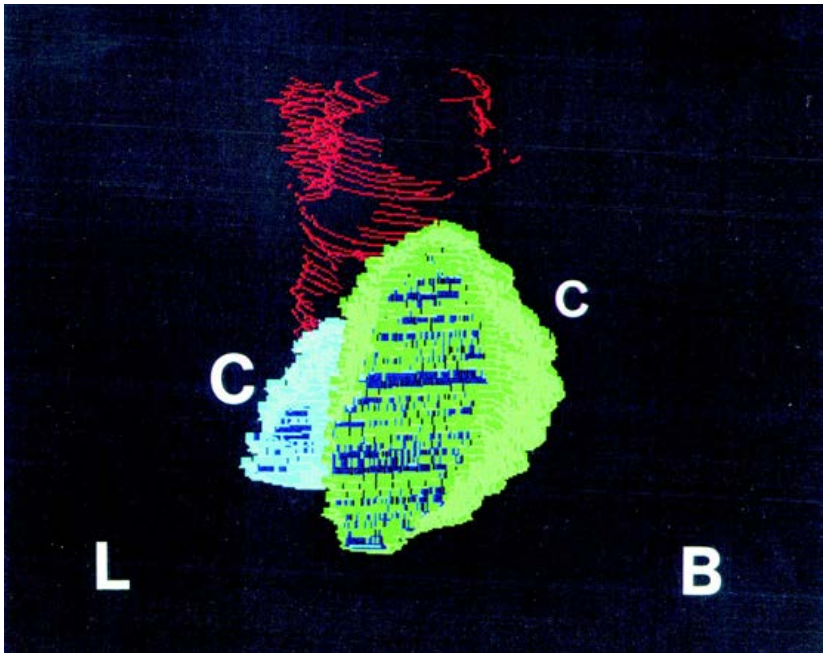


Fig. 3. Reconstruction of the upper right, the first and secondary generation tooth germ of canine. Mesial aspect. B: buccal, L: lingual, Green: the primary (deciduous) teeth, Blue: the secondary (permanent) teeth, Red: dental lamina.

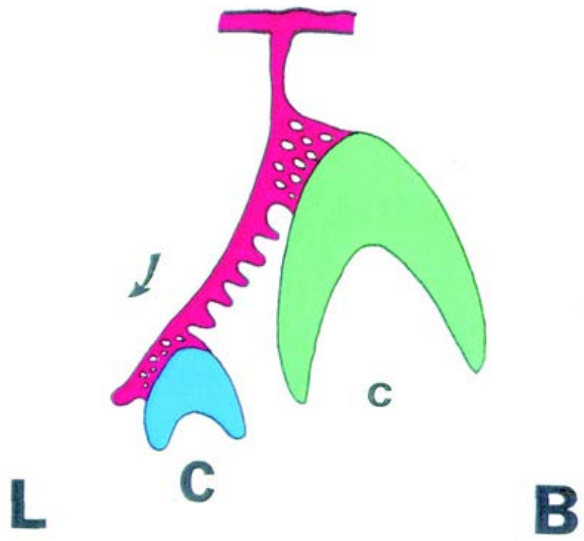


Fig. 4. A diagram of fig. 3. On the canine series, first generation tooth germ (c) developed on the elongated dental lamina (arrows), which developed deeply and formed the secondary generation tooth germ (C) on the margin. B: buccal, L: lingual Green: the primary (deciduous) teeth, Blue: the secondary (permanent) teeth, Red: dental lamina.



Fig. 5. Horizontal sections of the upper right canine series. The developing third generation tooth germ (arrows) could be observed on the distal side of the secondary generation tooth germ of canine (C). B: buccal, L: lingual.

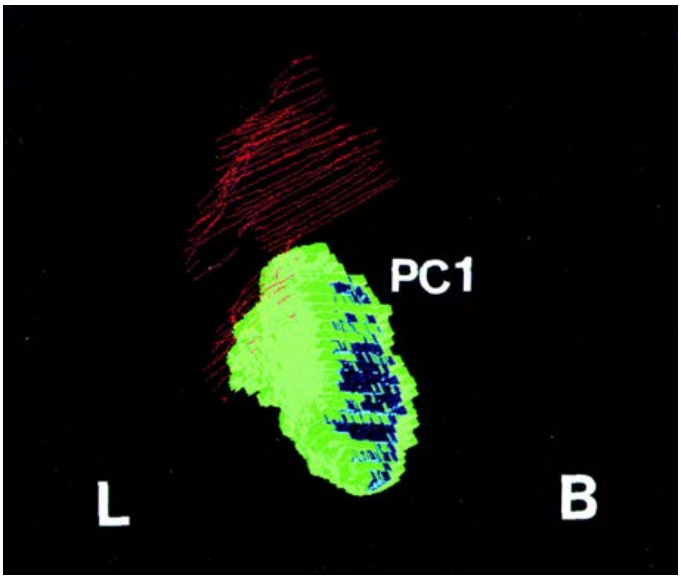


Fig. 6. Reconstruction of the upper right, the first post canine tooth germ (pc1). Mesial aspect. B: buccal, L: lingual, l: dental lamina Green: the primary (deciduous) teeth, Blue: the secondary (permanent) teeth Red: dental lamina.

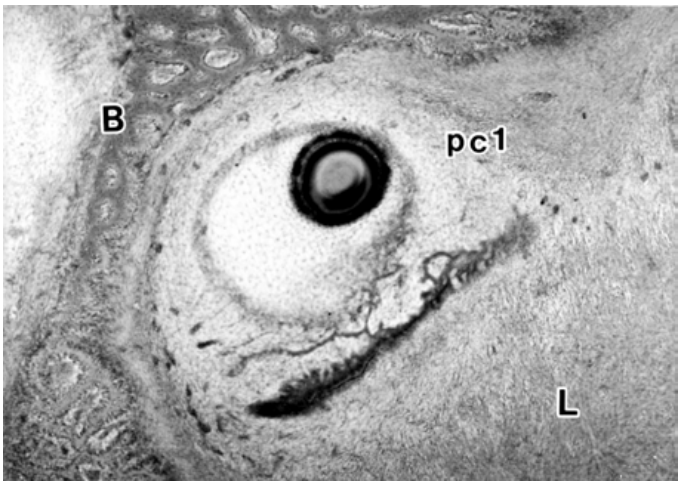


Fig. 7. Horizontal sections of the upper right, the first post canine tooth germ (pc1). The secondary tooth germ could not be found on these tooth germs. B: buccal, L: lingual.

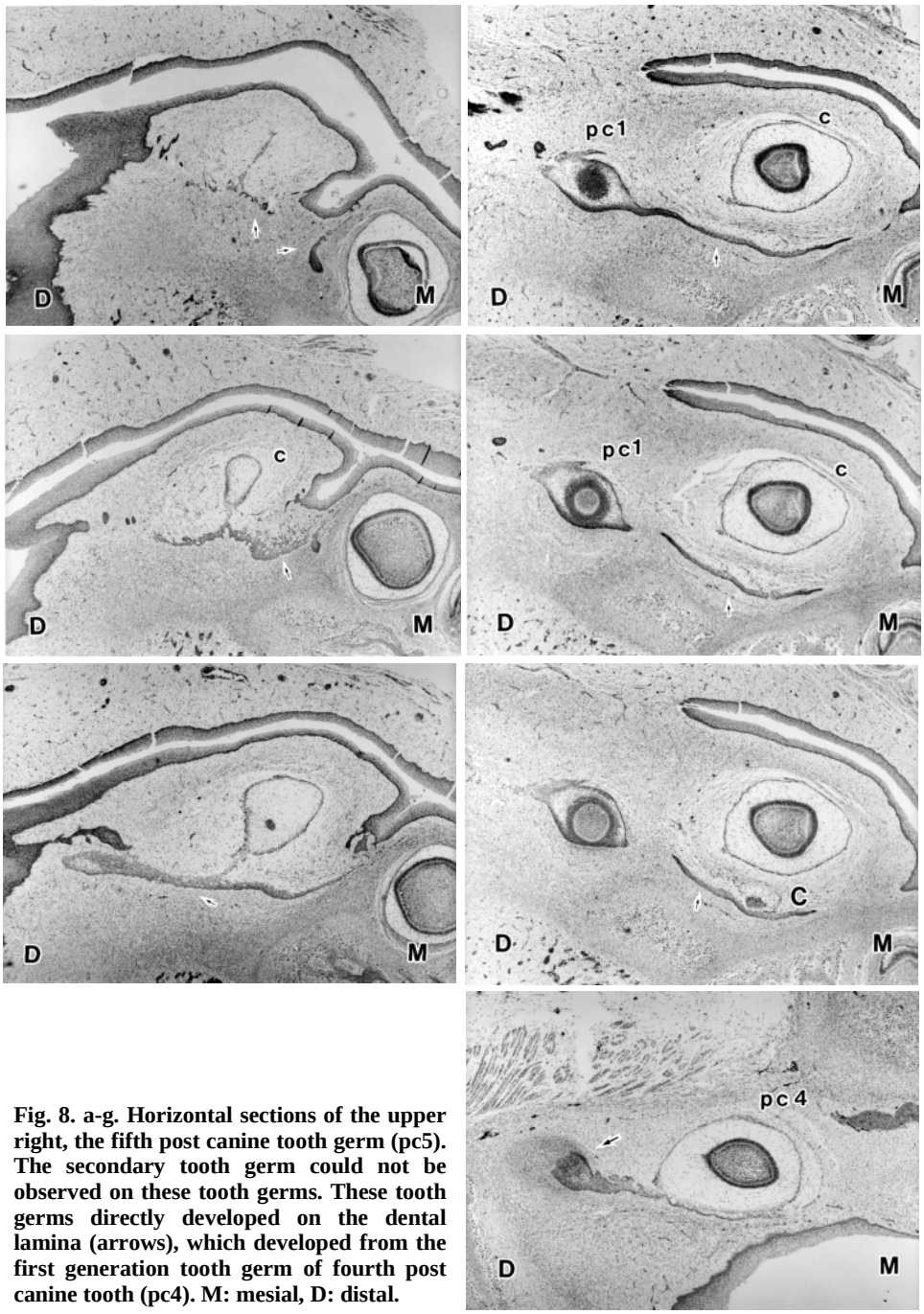


Fig. 8. a-g. Horizontal sections of the upper right, the fifth post canine tooth germ (pc5). The secondary tooth germ could not be observed on these tooth germs. These tooth germs directly developed on the dental lamina (arrows), which developed from the first generation tooth germ of fourth post canine tooth (pc4). M: mesial, D: distal.

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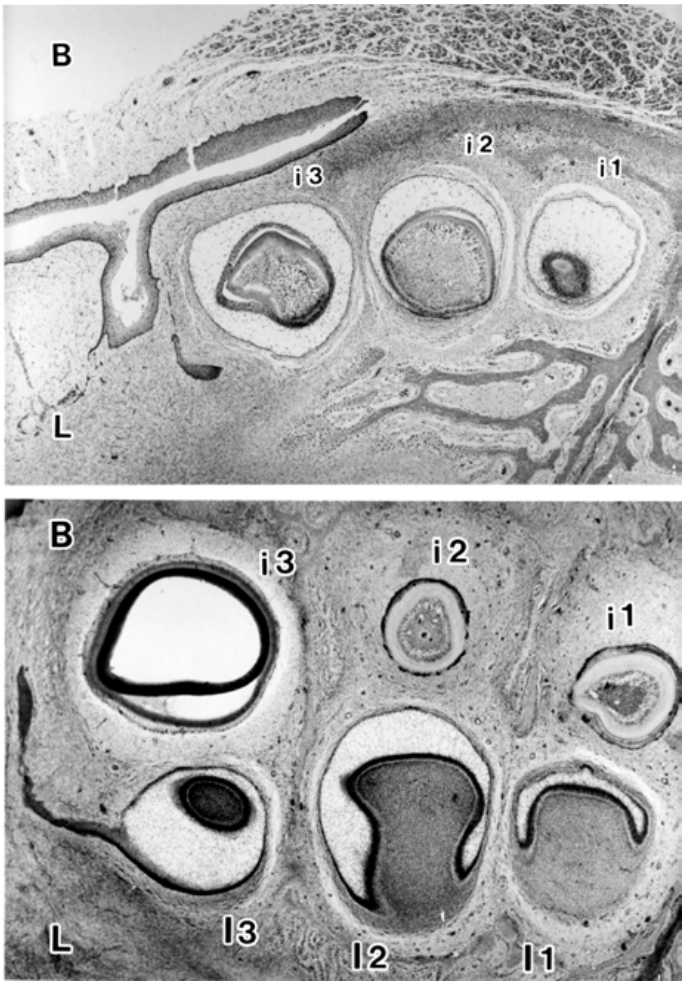


Fig. 9. a, b. Horizontal sections of the upper right incisor teeth group. The first generation tooth germ of incisor tooth formed one cusp, but the secondary tooth germ developed two cusps. B: buccal, L: lingual, a: body length 13.5cm, b: body length 24.0 cm.

The first generation tooth germ of the incisor tooth formed one cusp, but the secondary tooth germ developed two cusps (Fig. 9). It is suggested that the first generation tooth form degenerates earlier than the secondary tooth, because the tooth is deleted in the embryonic period being devoid of function. This may be the only case in mammals.

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Annulations in Root-Cementum of Human Teeth – Fact or Artifact?

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INTRODUCTION

When ground sections of roots of human premolars are examined with a light microscope, sometimes fine alternating light and dark lines can be seen in the cementum. The number of these lines or annulations is believed to be age-dependent and is used in palaeontology and forensic medicine to determine the age of the tooth and the age-at-death of an individual respectively (Naylor et al., 1985; Charles et al., 1989; Xiaohu et al., 1992; Kvaal and Solheim, 1995). Regardless of whether there is a connection between age and number of the lines in cementum it is of some interest to understand what could be the cause of this light-microscopical phenomenon. Therefore, we looked for ultrastructural features on the electron microscopical level or differences in mineralization that could explain the appearence of annulations in cementum in the light microscope.

MATERIALS AND METHODS

We examined 5 mineralized transversal sections from the middle third of roots of different human premolars (freshly extracted or dried material) by conventional light microscopy (LM), confocal laser scanning microscopy (CLSM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) combined with energy dispersive x-ray-analysis (EDX). To make the results obtained by light- and electron microscopy comparable, we examined the same ground section in water, embedded it in plastic and cut semi-thin and ultra-thin sections from this ground section. This allowed us to assign TEM-micrographs to a distinct area of the ground section. We repeated this procedure with different ground sections. In the same way we compared the same specimen area in a conventional light microscope and in a confocal laser scanning microscope.

Sawing and grinding of ground sections. Preparation followed mainly the method described by Naylor et al. (1985). We cut sections (thickness 100 – 150 µm) with a diamond blade in a Buehler low speed saw, ground them to a thicknes of about 100 µm and polished them with different grinding papers. To remove adherent abrasives we rinsed the ground and polished sections in water, sometimes in an ultrasonic bath.

Fixation, dehydration and plastic embedding: Ground sections were fixed in 2.5% glutaraldehyde at 5°C overnight and in some cases subsequently in 1% OsO_4 for 1 hour. After fixation sections were dehydrated in 30%, 50%, 70% and absolute ethanol and infiltrated with Spurr`s resin (ERL). The resin was polymerized at 60° for 12 hours.

Semi-thin and ultra-thin sectioning: Semi-thin sections were cut with Diatome histo-diamond knives with a Leica RM 2165 and ultrathin sections with Diatome diamond knives on a Reichert Ultracut.

Staining of ground-, semi-thin and ultrathin sections: Ground- and semi-thin sections were examined unstained or after staining with toluidine blue or methylene blue/ azur II. Ultrathin sections were examined in TEM without any staining or after staining with 2% uranyl-acetate sometimes followed by 0.5% tannic acid.

Microscopy and energy dispersive X-ray analysis: For conventional LM a Zeiss Stereomicroscope SV 11 and a Zeiss Photomicroscope, both equipped with a JVC video camera and coupled to an image processing device (ImageC, Imtronic) were used. Micrographs were taken on Kodak Professional 100.

CLSM was carried out with a Leica CLSM-Diaplan on unstained ground sections. TEM investigations were done in a Zeiss EM 902. For SEM and EDX of ground sections a Zeiss Gemini with a Link Isis EDX device was used.

RESULTS

Ground sections and semi-thin sections in LM: The visibility of annulations varied greatly in different ground sections of the same tooth or in different teeth. They were hardly ever visible over the whole cementum in all ground sections and staining had no influence on this. Semi-thin sections from a ground section (thickness 0.5-2 µm) showed a kind of granular structure. The lines, visible in the ground sections had almost completely disappeared (Fig. 1a,b). This result was independent of whether semi-thin sections are stained or not.

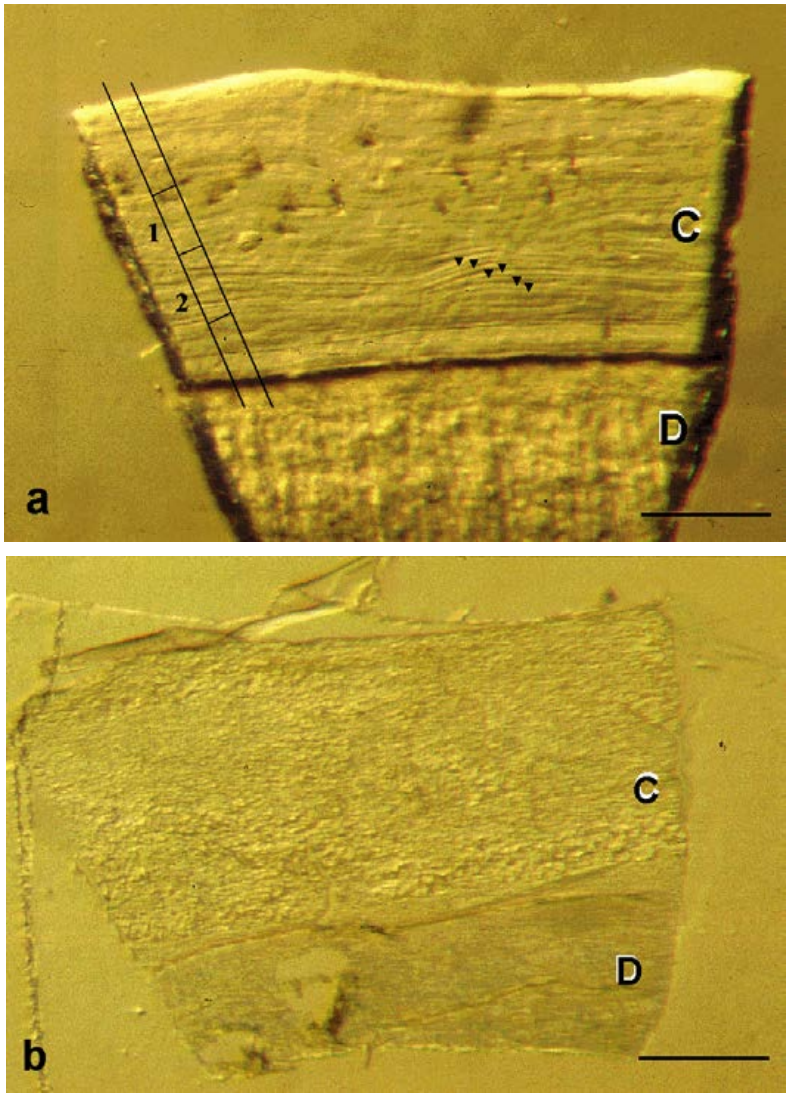


Fig. 1. (a) Mineralized ground section (thickness ≈100 µm) from the middle third of the root of a human premolar after infiltration with ERL but before polymerization. The areas 1 and 2 indicate the region from where the TEM micrographs were taken. (b) Unstained semi-thin section (section thickness ≈1µm) of the ground section after polymerization. Instead of annulations, visible in ground section (some are marked by arrows in 1a), a granular structure is the predominant impression in the semi-thin section. Zeiss Stereomicroscope, bright field mode. C=cementum, D=dentine. Bars = 100 µm.

Ground sections in LM and CLSM: CLSM-images differed from the images taken by conventional bright-field LM. (Fig. 2a,b). In addition to the lines which had been visible in LM, several fine lines were only visible in CLSM. These lines could be followed up in optical sections over a range of 30 µm in depth of the ground section.

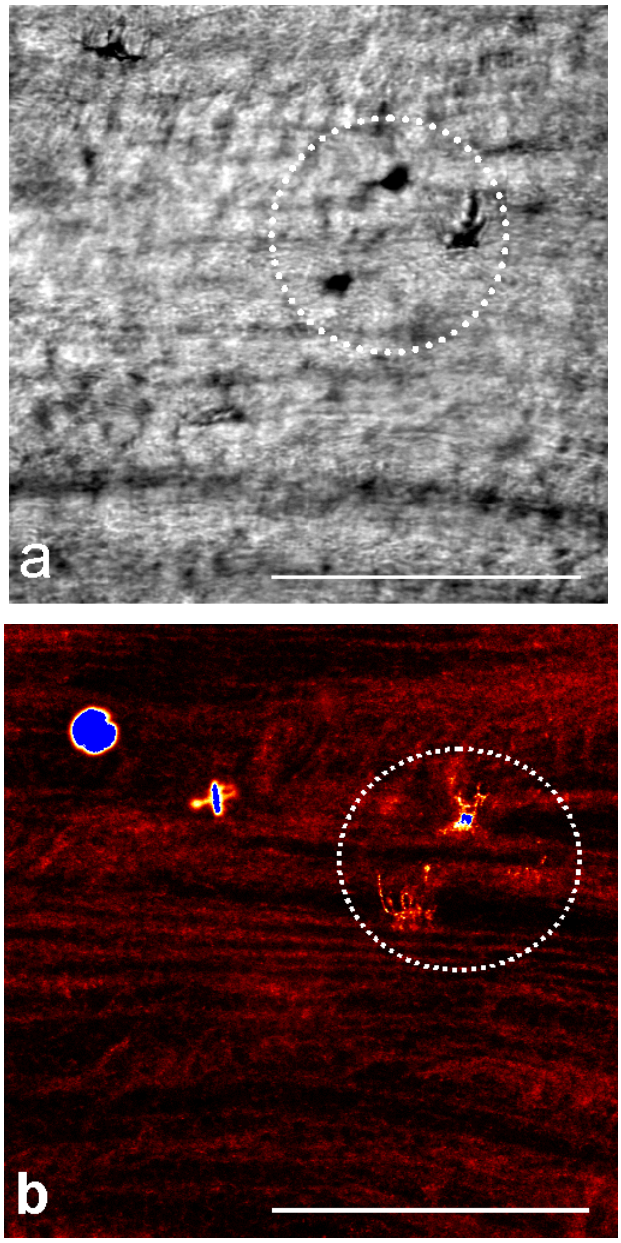


Fig. 2. Conventional LM (bright field mode) (a) and optical section taken with a CLSM (b) of the same specimen area (a triplet of cementocytes is marked). Lines that are visible in CLSM micrographs are missing in bright-field LM. Bars = 100 µm.

LM of a ground section and TEM of an ultrathin section from this ground section: From the ground section in Figure 1a, a series of TEM-micrographs, beginning at the surface of the root and ending in the dentine was recorded and compared with the LM image of the ground section. Two areas in the ground section have been selected. One area where only very weak lines are visible and another area where strong lines can be seen (Fig. 1a). TEM micrographs of these areas (Fig. 3) looked quite similar. In both areas there were dense, plaque-like structures, which seem to be cross-sectioned fibre bundles. The distribution of these fiber bundles was largely the same in the two areas. There was no obvious connection between these fibre bundles and the light microscopical visible annulations. Only in one area in TEM micrograph was there a short "line-up" of fibres, but this was amazingly in the area where no distinct annulations were visible in LM of the ground section

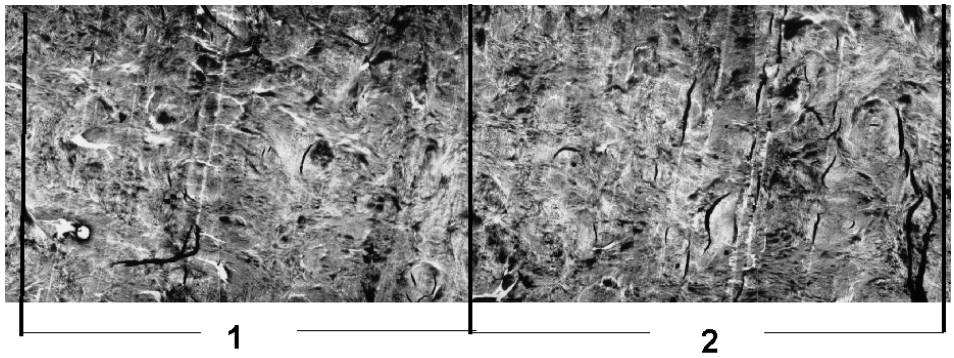


Fig. 3. TEM of an ultrathin section (section thickness ≈ 80 nm) from the ground section shown in fig. 1a. Part of a series of 11 TEM-micrographs covering the area marked in Fig. 1a as area 1 and 2. The area is about 90 µm long.

Ground sections in SEM (EDX-analysis): EDX-linescans recording the P Ká- and Ca Ká-signal intensity over cementum of ground sections always showed only a small variation in the calcium- and phosphorus-signal intensities. Because signal intensities are directly influenced by surface structure and by changes of the electron beam current, the small peaks are not believed to be significant for different amounts of these elements in cementum.

DISCUSSION

With different methods we got different, partially contradictory results. The fact that the annulations are not reproducibly visible over the whole root-cementum area and not in any ground section of every examined tooth did not make things easier. The most amazing result was the disappearance of the annulations after semi-thin sectioning. Instead of lines there was only a granular structure. On the other hand, this granular structure of the cementum seen in these semi-thin sections fits well with the results from TEM, where dense areas, probably cross-sectioned fibres, reflect this granular structure. The lines visible in ground sections re-appeared in CLSM-pictures although the optical

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section thickness was approximately the same as the section thickness of the semi-thin sections so we would have expected a quite similar appearance. A granular structure was never seen in CLSM pictures. Finally TEM-micrographs from a defined area in the ground sections showed structures of different density, which in principle could be the cause for the light microscopical phenomenon, but their distribution in the cementum seems to be rather random.

CONCLUSION

There is no simple explanation for the light microscopical phenomenon ”cemental annulations”.The question put in the title remains unanswered. The annulations might represent real structural features of cementum or a kind of superstructure like Hunter-Schreger Bands. Shroeder (1986) presumes, that fibres, which abruptly change their course in cementum are the cause of lines of different optical density in root cementum. At least it still cannot completely be excluded that annulations are mere "optical artifacts" in unusually thick sections.

ACKNOWLEDGEMENT

We thank Mrs. B. Scheidereiter and Mrs. Anne Kähler for their photographic work.

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The Measurement of Tooth Morphology: Development and Validation Of a New Image Analysis System

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INTRODUCTION

New techniques of measuring tooth morphology are required to enhance investigation of developmental anomalies of the dentition and for diagnosis and aetiological determination. Tooth measurement has applications in clinical orthodontics, in anthropology and in developmental biology. Current measurement techniques include manual measurement of erupted teeth on study models (Moorrees et al., 1957; Hunter and Priest, 1960; Bolton, 1962; Lavelle, 1970). This provides data from two-dimensional linear measurements. Other techniques are the Optocom (Van der Linden et al., 1972; Moyers et al., 1976), reflex metrography (Takada et al., 1983; Richmond, 1987), reflex microscopy (Speculand et al., 1988; Lowey, 1993), image analysis (Brook et al., 1983) and laser scanning (Soma et al., 1992; Kuroda et al., 1996).

Whichever technique is used, the reliability of each measurement system is affected by many factors. Sources of measurement error include the type of device or technique used, skill of the operator, impression and casting procedures if study models are used, or quality of other materials and the condition of the tooth as well as the gingivae (Lundstrom, 1954; Hunter and Priest, 1960; Lavelle, 1970; Potter et al., 1981; Kieser et al., 1985; Cohen et al., 1995; Yuen et al., 1997). By controlling these factors and improving the measurement technique, greater accuracy is possible.

In order to provide a wider range of data for tooth dimensions than can be obtained by manual methods and to enhance comparisons between individuals, groups and various methods, a computerised Image Analysis technique was developed and described (Brook et al., 1983). The technique involved a television-based system in which a dental study cast was placed on a macrostand. Linear, periphery and area measurements of tooth crowns were obtained from video images of occlusal and buccal surfaces. In another study, Brook et al. (1986) measured the mesio-distal dimensions of the teeth of 50 male students using this image analysis system. A comparison of this technique with the manual method revealed that, in general the image analysis technique produced slightly larger mean estimates than did manual measurements. In general that image analysis system produced more variability than did the manual method.

Some of these measurement techniques involve operator subjectivity or technical problems, for example calliper placement on crowded teeth. Automation of measurement to a degree can also present limitations; for example Brook et al. (1986) acknowledged the difficulty of coping with imbricated teeth and the curvature of the arch in the anterior segments.

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The aim of this study is to present a new image analysis system with increased accuracy and ease of use for tooth measurement recently developed in the Department of Child Dental Health, School of Clinical Dentistry, University of Sheffield. This paper investigates its validity against the gold standard of manual measurement and examines the reproducibility gained by three operators. This work forms part of a large departmental project investigating normal and abnormal development of the human dentition.

MATERIALS AND METHODS

The new system utilises a frame-mounted 32-bit digital camera. Using separate acquisition and analysis software the system captures and analyses images from individual teeth, clinical photographs, radiographs and study models. For tooth measurement using study models, the specimen is placed on a calibrated variable stage under standard illumination. This can be rotated in three planes allowing imaging from different orientations. The system therefore permits buccal, occlusal and lingual images of the teeth that can be captured individually for greater accuracy. Each image takes approximately 5 minutes to produce. The camera chip has a resolution of 1.5 mega pixels and the images are displayed in an array of 1012 x 1524 pixels for analysis (on a 17 inch 32-bit true colour monitor). The camera has a minimum frame interval of 0.25 seconds.

The permanent teeth of 5 sets of upper and lower study models, selected from the Charles Clifford Dental Hospital records of adult white Caucasian patients, were used for measurement. In all cases dental models were made from Erkoflex (EVA) impression material and poured in Kaffir D stone (British Gypsum). The laboratory work was performed by one trained operator and followed manufacturing instructions.

The clinical crowns of central and lateral incisors, canines, first and second premolars and first and second molar teeth for both maxillary and mandibular arches were measured. The third molars as well as any teeth that were partially erupted, had carious lesions or restorations, were crowded or exhibited any evidence of tooth wear or model damage were excluded from measurement. For both image analysis and manual measurement, each set of study casts of a single subject were systematically investigated.

Imaging Procedure

The study model was placed on a platform. This was adjustable in three planes and the study model was held securely in place by a spring and two positioning screws. Each individual tooth was imaged separately, from both the buccal and occlusal aspects, using a 32-bit digital camera (Kodak/Nikon DCS 410). This was mounted horizontally above the study model on an adjustable rod. The study cast was illuminated with four multi-directional spot lights surrounding the cast. A length of steel rule was placed adjacent to the particular tooth surface being imaged. The camera was connected to a PC (Pentium II-266 MMX, Viglen Ltd, UK) and Adobe Photoshop (V 4.0, Adobe Systems Ltd, Europe) was used to acquire the images from the camera.

The buccal images of teeth on each cast were captured first, followed by the occlusal images. For each image the lens (90mm Elicar macro) of the camera was focused parallel to the particular tooth surface of interest and either parallel to the long axis of the clinical

crown for buccal images or at right angles to the long axis of the clinical crown for occlusal views. Following acquisition each image was then given an appropriate file name and saved.

Analysis procedure

The images were imported into Image Pro Plus (V 3.01, Media Cybernetics, USA) which was used to make the measurements. Each image was firstly calibrated using the steel rule and the following (linear and area) measurements were then made.

Buccal view:

1. The perimeter (P). This was the maximum tooth surface periphery from this particular view and was traced. The measurement was then determined from this trace.
2. The mesio-distal (MD) dimension. This was taken as the maximum distance between the mesial and distal proximal surfaces of tooth crown, lying on the trace determined in 1). In circumstances where the tooth was not in an optimal position (e.g. slight crowding or rotation was present) or adjacent teeth were missing, the measurements were taken from the anatomical positions where the tooth contact should occur.
3. The occluso-gingival (OG) dimension. This was the distance between the occlusal surface and the gingival level of the crown, perpendicular to MD at its midpoint.
4. The area (A). This was the surface area bounded by the perimeter trace (i.e. P) and was determined from the trace made in 1).

Occlusal view:

As for the buccal measurements:

1. The perimeter, using the same techniques and definitions as for the occlusal view.
2. The mesio-distal dimension was determined from the occlusal aspect, following the same criteria as for the buccal view.
3. The bucco-lingual (BL) dimension. This was the greatest distance between the buccal and lingual surfaces of the crown and was also perpendicular to and bisected the line defining the MD dimension.
4. The area, using the same techniques and definitions for area in the buccal view.

The Classical Manual Technique

Electronic digital callipers (Mitutoyo Manufacturing Co. Ltd., Japan) were used for the manual tooth measurements. The callipers were capable of measuring to 0.01mm. The blades were modified with sharpened flat metal beaks fitted to the tips. Linear measurements only were performed, according to the criteria of Moorrees et al. (1957). The following measurements were made.

Buccal aspect:

The mesio-distal dimension was determined from the buccal view with the manual technique. Similar to the image analysis method, this was defined as the maximum distance between the mesial and distal proximal surfaces of tooth crown. The calliper beaks were held at right angles to the tooth surface and parallel to the long axis of the clinical crown. Again, in cases where there was a tooth missing or the tooth was not in its optimal position, the measurements were taken from the anatomical points where the normal contact would occur.

Occlusal aspect:

1. The mesio-distal dimension was taken, using the same definition and criteria mentioned above.
2. The bucco-lingual dimension was obtained as the greatest distance between the buccal and lingual surfaces of the crown, judged as being perpendicular to and crossing the midpoint of the mesio-distal dimension.

For both manual and image analysis techniques, the measurement value of each crown variable in millimetres (mm) was obtained from double determination. For any difference between each pair of linear values (i.e. MD, BL or OG) equal to or greater than 0.3 mm, the measurements were repeated. If the discrepancy reappeared, the data was considered separately for a possible explanation. Perimeter differences of greater than or equal to 2mm were also treated in the same way, with their associated area values being used for statistical analysis.

Analysis of Data

The objective of this study was to determine the intra-operator repeatability of tooth measurement, with the aim of testing the validity of the new image analysis system in comparison to a manual technique. The data was derived by three operators (1 to 3). The limits of agreement were calculated for each of the operator's differences between measurements (Bland and Altman, 1986). These are displayed as a proportion of the mean measurement made. The Coefficient of Reliability (Houston, 1983) of the difference between first and second occasion measurements was also determined for intra-operator reproducibility.

RESULTS

The results of the manual and image analysis measurements for the three operators are shown in Tables 1 and 2. The tables display the maximum "percentage measurement error", or extent of disagreement, recorded within the operators for each measurement in each view using both techniques and Houston`s Coefficient.

The minimum coefficient value recorded for measurements using either techniques was 0.97.

Table 1. Intra-operator summary of manual measurement.

	BUCCAL ASPECT	OCCLUSAL ASPECT	
% measurement error	MD	MD	BL
Operator 1	6.89%	5.76%	6.46%
Operator 2	3.89%	3.50%	4.13%
Operator 3	3.40%	3.25%	3.79%
Coefficient of reliability	≥0.9829	≥0.9919	≥0.9902

Table 2. Intra-operator summary of image analysis measurement.

BUCCAL VIEW				
% measurement error	MD	BL	AREA	PERIMETER
Operator 1	3.54%	4.57%	**1.9%**	**6.88%**
Operator 2	2.00%	3.00%	3.57%	1.04%
Operator 3	2.10%	2.30%	**12.42%**	**1.59%**
Coefficient of reliability	≥0.9978	≥0.9967	≥0.9788	≥0.9770
OCCLUSAL VIEW				
% measurement error	MD	BL	AREA	PERIMETER
Operator 1	3.28%	3.21%	2.22%	2.03%
Operator 2	2.22%	3.51%	**15.17%**	**1.25%**
Operator 3	2.79%	2.93%	4.88%	2.24%
Coefficient of reliability	≥0.9973	≥0.9961	≥0.9868	≥0.9987

*See discussion for explanation of bold figures.

The manual measurements show percentage errors ranging from 3.25 to 6.89%. In general, the percentage measurement errors for the image analysis technique are smaller overall and for each operator, in comparison to the manual technique (ranging from 1.9% to 4.88%). There are however three percentage errors exceeding 6.88% for the image analysis technique, concerning perimeter and area measurements which are considered below.

DISCUSSION

The results show that this new image analysis system permits accurate measurement of tooth dimension with comparable or better reproducibility than the gold standard of manual measurement. This digital camera produced detailed images with high resolution. The measurements possible using such a system give a wider range of data than can be achieved using manual techniques. The measurements can also be made simultaneously. This will permit greater data comparisons that may reveal more significant trends between tested groups. Further data handling may involve combining data from both views of each tooth.

It is possible to image more than one tooth at a time, ensuring that the relevant surfaces are not obscured by crowding or tilting of teeth for example. The time required to image one tooth surface with this system however is rapid, allowing sufficient images to be gained for comparative purposes and provides greater image accuracy for measurement.

The intra-operator reproducibility assessment reveal coefficients of reliability exceeding 0.9 for all linear measurements of both buccal and occlusal views for all three operators. This demonstrates the high degree of accuracy of the system and ease of use. This is also reflected in the small values for “percentage measurement error”, or maximum extent of disagreement. Although lower, the coefficient values for area and perimeter measurements and the values for extent of disagreement were still of an acceptable level (see later). These measurements were determined from operator tracing of the tooth surface and do demonstrate a high level of accuracy.

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For the manual measurements, a relatively high degree of accuracy was also revealed. The greater differences shown buccally for the MD dimension in comparison to MD occlusally are likely to be due to differences in calliper beak placement and model orientation by the operators. Access of the calliper beaks was sometimes difficult, due to individual tooth positioning and their relation to the adjacent teeth and this can affect measurement. This may result in larger mean estimates of tooth dimension in comparison to the IA measurements, although our study was concerned with reproducibility of measurement. The occlusal view was reported by the operators to be the easier view to measure and this may explain the lower percentage errors for the MD dimension in comparison to the buccal view.

Using the image analysis method tooth orientation was constant such that the view of each tooth measured by all operators was the same. This is obviously not as controlled during manual measurement. The BL dimension does show greater differences manually indicating greater accuracy of measurement using image analysis. Slight loss of model material can occur during manual measuring; this may be reflected in the larger percentage differences for the manual technique. Any obvious loss however resulted in the model being discarded.

For the image analysis additional measurements to the manual technique made in this study include area and perimeter determination of the tooth surfaces. In general, the differences between area measurements were greater than perimeter measurements. This was to be expected as the area value is a function of the perimeter traced and expressed in square units.

In the occlusal view (Table 2) the results for perimeter show the percentage measurement error, or maximum degree of disagreement, to be in a range of 1.04-6.88% within operators. The highest value in this range actually gave a low percentage error for its corresponding area measurement (1.9%; bold figures). Considering both buccal and occlusal views, the largest percentage measurement error for area (12.42 and 15.17%) gave corresponding low values for perimeter discrepancies (1.59 and 1.25% respectively). The mismatch in these results is a consequence of the methodology of the analysis programme calculating the area from the perimeter trace. These two measurements have a quadratic relationship as shown in Figure 1. Due to this relationship greater differences in perimeter values for small teeth (P2-P1) will result in proportionally smaller differences in area values (A2-A1), hence the first finding described here. Smaller differences in perimeter values for larger teeth (P4-P3) will result in larger corresponding area differences (A4-A3), explaining the two larger area errors in the results.

Within this particular study we were not concerned with the reproducibility of the area measurements as such, as they are determined from the trace each operator makes. However, due to the discrepancies highlighted here it is important that we view our area data at this stage with caution. One or more “average-size” differences recorded between first and second occasion perimeter measurements of small teeth by operator 1 will have led to the large perimeter error seen. Likewise, a small difference, or differences, recorded between perimeter measurements for larger teeth will have resulted in the corresponding elevated area differences. Because we have combined all tooth types in determining our measurement error, it is not possible to identify the exact cause of these results. In future

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studies increasing the number of teeth measured will allow separation of results for individual tooth types, so permitting identification of a perimeter threshold that leads to such large area differences.

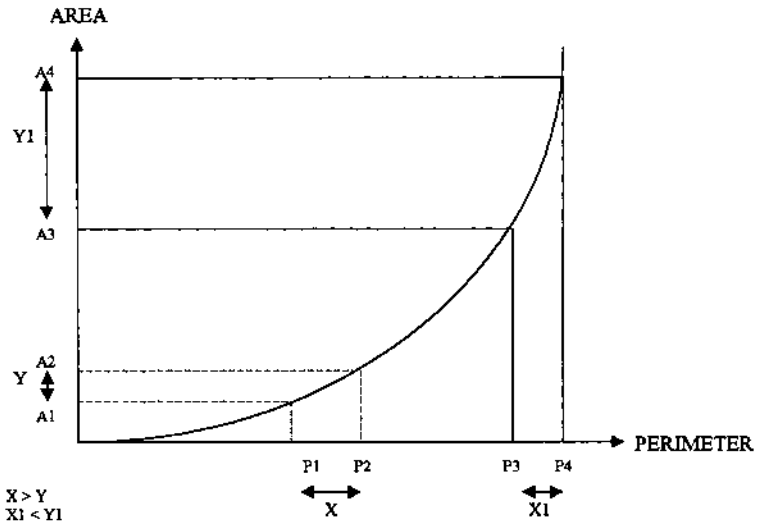


Fig. 1. Graph to show relationship between perimeter and area. Perimeter difference P1 to P2 (size X) is greater than corresponding area difference A1 to A2 (size Y) for small teeth. Perimeter difference P3 to P4 (size X1) is smaller than corresponding area difference A3 to A4 (size Y1) for larger teeth.

The new system produced higher levels of reproducibility in comparison to Brook et al. (1986) and individual tooth imaging can cope with imbricated, rotated teeth and arch curvature, by imaging individual teeth rather than a complete arch (Lowey, 1993). The images were acquired by one operator for this study. Further validation of our new system will involve assessment of the imaging procedure prior to measurement. In this study validation of simple measurements has been shown. Inter-operator reproducibility is also to be determined and further development of this system will permit investigation of more extensive tooth measurements.

CONCLUSION

The new system permits accurate and reproducible measurement of the teeth of dental study casts. Our findings validate the new technique against that of classical manual measurement. A major advantage of this image analysis system is that it provides far more information concerning tooth dimensions, thus increasing discrimination in comparisons between groups in aetiological studies.

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A Computerized Approach to Reconstruction of Growth Patterns in Hominid Molar Teeth

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INTRODUCTION

Recent attempts to reconstruct life histories of fossil hominids have focused on the dentition as a reliable source of information on developmental rates. This as it is assumed that dental maturation is highly synchronized with general body development (Dean et al., 1986; Smith, 1991). Most of the research carried out on dental development in fossil hominids has focused on studies of the later stages of crown and root development, estimated from enamel apposition rates (Beynon, 1992; Beynon and Dean, 1987; Beynon and Wood 1987; Ramirrez-Rozzi, 1993) or crown and root development estimated from radiographs or single C-t scans (Conroy and Vannier, 1987; Faerman et al., 1994; Mann et al., 1990; Skinner and Sperber, 1982). However it is also possible to study earlier stages of the ontogeny of these fossil teeth, since the dentine-enamel junction (DEJ) provides a permanent record of the balance achieved between cell growth and differentiation at an early stage of development. Moreover, this can be compared to the final shape of the crown seen at the outer enamel surface (OES), so providing information on two successive phases of growth within a single tooth.

The potential value of the DEJ for studying ontogeny and phylogeny has long been realized (Butler, 1968; Korenhof, 1960; Kraus, 1952, Kraus and Jordan, 1965), but its application to fossils has been limited until now by the difficulty of visualizing the DEJ without destroying either dentine or enamel. The development of three dimensional imaging systems has removed this obstacle, and Smith et al., (1997) used serial C-t scans to develop a three dimensional model for comparison of selected locations at the DEJ and OES of modern and fossil teeth.

In this paper we describe a new computerized three dimensional model derived from serial C-t scans, that can be viewed from any angle or superimposed on any other tooth for direct comparison. The model is extremely flexible and was designed to obtain information on global size and shape change in teeth. It has been applied here to analysis of growth in the first permanent (M1) and second deciduous (DM2) molars. We propose that the shape and size changes observed reflect growth trajectories within the teeth and can be used to reconstruct the ontogeny of tooth development in both living and fossil hominids. The rationale for using C-t scans and the accuracy of the method has been discussed in detail in Smith et al., (1997). The great advantage of the method is that it is non-invasive, can be applied to both recent and fossil teeth and provides excellent

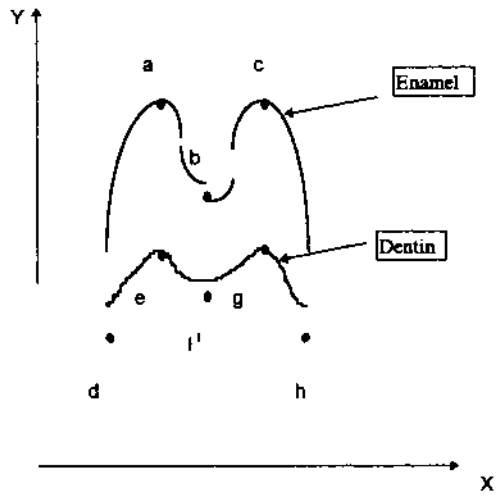
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delineation of both the DEJ and OES. The main limiting factor is the accuracy of identification of data points, but this can be estimated by repeated scanning and measurement of selected teeth.

MATERIALS AND METHODS

Mandibles of 14 infants recovered from archaeological excavations in Israel and dating to between 6000 B. P. and 200 A.D. were used in this study. Selection was based the presence of an unworn DM2 and unerupted M1 with at least ¾ of the crown complete. Serial C-t scans were then taken along the mesio-distal axis of the lower DM2 and M1. The C-t scans were made using an Elscint 2400 model, with scans 1.2 mm thick, but taken at 0.5 mm intervals to give intercalated scans. Using a work station and optimizing window settings for each reading (i.e., separate readings for the enamel-air and enamel-dentine boundaries), X and Y co-ordinates were registered for 8 sites on each scan as shown in Figure 1. The third coordinate, z, was calculated from the distance between successive slices. All scans were re-measured on separate occasions. Where differences exceeded 3%, teeth were re-scanned and if easily duplicable measurements could not be obtained they were excluded from analysis. When all scans of any one tooth had been examined in this way, the co-ordinates were entered into a data base and used to create a three dimensional image using the program described below.

Fig. 1. The <u>C-t scan:</u>
The CT scan results in a series of 2-dimensional images (slices) of a tooth profile. Such an image would typically look like that shown below



Although each point across the lines can be measured, there are several key-points in each section (points 'a' to 'h' in figure 1) that are sufficient to supply all the information needed.
Normally, the CT scan resolution (distance between sections) is 0.5 mm, and a typical tooth scan will result in about 20 slices.

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General description:

The input to the program consists of two file-types:

1. A tooth topology-file, which contains information on the structure of the teeth and the method used to measure them. Hence different tooth types can be handled by the program.
2. A tooth data-file (or files), which contains a set of 3-dimensional tooth-coordinates obtained by performing measurements on the images created by the C-t scan. The result of such input is a 3-dimensional description of the enamel and the dentine surfaces of the tooth, and the program can work with one or more teeth.

The program analyzes the structure of the tooth, and performs the following computations:

1. Computes a reference plane which is an "average plane" for all the coordinates measured at the base of the tooth. The coordinate system is then transformed in such a way that further measurements will be related to that plane.
2. Detects the cusps: here the program applies an algorithm to find cusps on the enamel and dentine surfaces. Consequently a set of cusp-vectors are defined as the vectors connecting matching enamel and dentine cusps. When working with two teeth, a "center of gravity" is calculated for each one and the two teeth are aligned together so that their centers-of-gravity are united; their reference planes are parallel and the sum of distances between every two matching cusps (one of each tooth) is minimal (using mean-square analysis).

Calculations and output:

The program calculates:

1. All distances and angles in the 3-dimensional figure of the tooth, and in particular distances between cusps, angles between cusp-vectors and angles between cusp-vectors and the reference-plane (or the normal vector to that plane).
2. Average thickness of enamel, enamel and dentine areas, etc.

When working with two teeth, comparisons between them can also be performed.

For example, each of the following calculations can be made:

- Ratios between average magnitudes of each tooth (e.g. - comparing average vector lengths).
- Angles between matching vectors in the two teeth.

Computation of deformation vectors, which are vectors between two matching cusps of the two teeth.

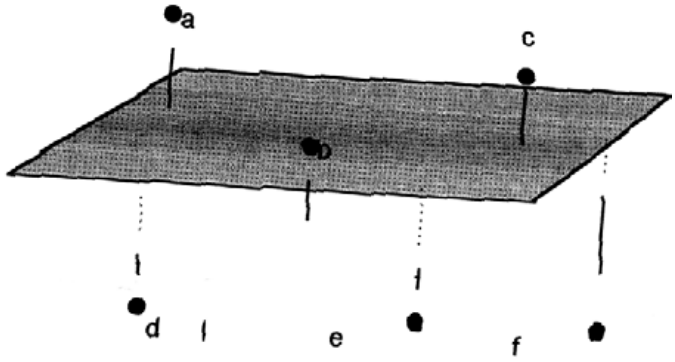
Visualization:

The image of the tooth is visualized by using interactive 3-dimensional graphics. This means that a 3-dimensional image appears on the screen, that the user can rotate, zoom or move, select and remove independently the enamel surface, dentine surface, reference-plane, cusp-vectors, and deformation vectors (in the case of two teeth). When working with two teeth, the user can manipulate each one of them separately or together.

Implementation:

The program can be used to measure a single tooth, to compare two teeth or to compare a tooth with a pre-defined tooth pattern. The C-t scan results in a series of 2-dimensional images (slices) of a tooth profile shown in Figure 1. In order to find the best matching plane for all these points, a least-square analysis is used to find the plane that minimizes the sum of distances to all points (Fig. 2).

Fig. 2. Computing the reference plane:
The contact points between the enamel and the dentine surfaces provide a set of points that denote the base surface of the tooth. However, these points are not exactly on the same plane, but rather distributed above and below any plane we would try to draw.
The situation is demonstrated here.



In order to find the best matching plane for all these points, a least-square analysis is used to find the plane that minimizes the sum of distances to all points.
Cusp detection:
Refers to point 'a' in figure 1. For each CT scan section, this point is the "left maximum of the enamel surface". By taking all the a's from all the sections, we get a set of such points, and we can locate enamel cusps by finding maximum points within this set.

Cusp detection:

Refers to point 'a' in Figure 1. For each C-t scan section, this point is the "left maximum of the enamel surface". By taking all the a's from all the sections, we get a set of such points, and we can locate enamel cusps by finding maximum points within this set. In this way, however, only points that were specifically measured can be located (as mentioned above, the resolution of the C-t is normally 0.5 mm). Generally, the real cusp might be in between two of the measured points. The situation is demonstrated in Figure 3: To gain a more accurate location of the cusp, an interpolation curve through the points around the cusp was computed (like the curve in Figure 3), and the cusp was taken to be the maximum of that curve. Assuming that the natural contour of the tooth is quite smooth, the error for cusp location drops from 0.5 mm to about 0.2 mm. The same technique is used for points 'c', 'd', and 'h' in Figure 1 to locate the other cusps.

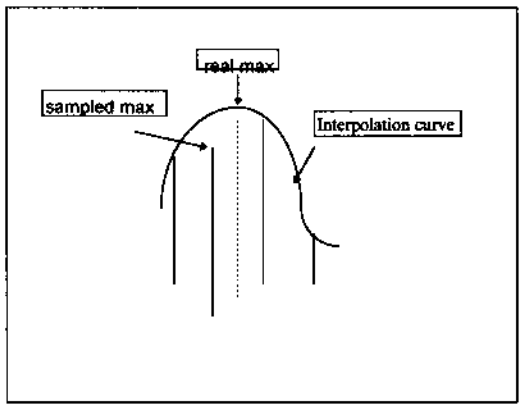


Fig. 3. the maximum falls between sampled points.
To gain a more accurate location of the cusp, an interpolation curve through the points around the cusp was computed (like the curve in fig. 3), and the cusp was taken to be the maximum of that curve.
Under the assumption that the natural contour of the tooth is quite smooth, the error for cusp location drops from 0.5 mm to about 0.2 mm. The same technique is used for points.

Computation of center of gravity:

There are a number of different options: the global center-of-gravity (the average point of all measured points) or cusps center-of-gravity (the average point of selected cusps).

Analyses:

From the large number of possible computations, the following variables were selected in order to estimate size and shape change between the DEJ and OES of the DM2 and M1: intercusp distances; surface area and shape of the pentagon joining cusp tips at the DEJ and OES and % area occupied by the DB-DI-DL triangle at each surface (Figure 4).

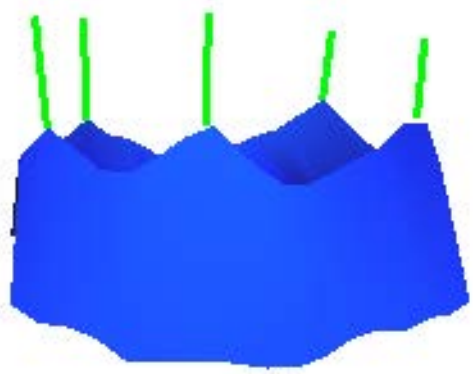


Fig. 4. Reconstruction of M1, with enamel removed, showing DEJ and vectors joining cusp tips at DEJ and OES.

Specifications:

Enamel / Dentine pentagon area:

The area of the enamel / dentine pentagon's projection onto the reference plane.

Enamel / Dentine DB-DI-DL triangle area:

The area of the enamel / dentine projection onto the reference plane.

Enamel / Dentine pentagon angles:

Angles between neighbor edges of enamel / dentine pentagon.

Enamel / Dentine pentagon angles:

Angles between neighbor edges of enamel / dentine pentagon.

Cusp tips:

MB-Mesio-buccal; DB- Disto-buccal; DI-distal; DL-Disto-lingual; ML-mesio-lingual: "e" is used as prefix to denote cusp tip at the OES and "d" is used to denote cusp tip at the DEJ.

RESULTS

One mandible was excluded from the study because of poor definition, and in a second the DM2 was damaged, so that data are presented for 12 DM2 and 13 M1. The computerized image of a typical M1 is shown in Figure 4. Here the enamel has been removed, leaving the dentine shell and vectors joining enamel and dentine cusp tips. The area of the pentagon defined as the projection between cusp distances on the reference plane, was significantly larger at the OES than that at the DEJ in both teeth (Table 1; p=0.001 in the DM2 and p=0.000 in the M1), and the OES and DEJ of the M1 were significantly larger than that of the DM2. In the DM2 the area occupied by the DB-DI-DL triangle at the OES deceased slightly relative to the area it occupied at the DEJ in 10/ 12 cases, but the differences were not statistically significant. In the M1, all 13 cases showed a decrease in the area occupied by the DB-DI-DL triangle at the OES and the differences were highly significant (P=0.001). This is reflected in shape differences in the two teeth shown in the pentagon joining cusp tips in Figure 5. In the DM2 there was a small but significant decrease in the DB angle at the OES (P=0.05). In the M1 the changes were more pronounced as the tooth assumed a more rectangular form. This is emphasized in the marked increase in the DB angle and reduction in DL angle, as the DI cusp approximates the mesio-buccal side of the tooth. Table 2 gives the mean distance of all cusp tips from the tip of the eMB cusp. Notably, the eMB-eDI and eMB-eDL distances are similar in the DM2, but very different in the M1. In this tooth the eMB-eDI distance is significantly less than the eMB-eDL distance.

Table 1. Pentagon area and shape change.

	M1 (n=13)		DM2 (n=12)	
Total Area				
	Enamel	Dentine	Enamel	Dentine
$\overline{X}$	32.1	27.1	23.3	20.5
SD	3.98	2.40	3.20	3.00
P	0.001		0.05	
	%DB-DI-DC			
$\overline{X}$	25.50	28.90	29.00	30.00
SD	6.43	7.20	5.70	5.00
P	0.001		0.05	

In M1, the percentage area of the triangle DB-DI-DL was always smaller at the OES and the differences were statistically significant. In DM2 the percentage area of the triangle DB-DI-DL was smaller in 10/12 cases, but the differences were not statistically significant.

Table 2. Distance of all cusps from tip of MB cusp on outer enamel surface

		On dentine		On enamel	
DISTANCE		Dm2	M1	Dm2	M1
MB-DB	Mean	3.57	4.25	3.79	4.55
	Variance	0.14	0.19	0.15	0.18
MB-DI	Mean	6.37	6.71	6.36	6.87
	Variance	0.41	0.3	0.24	4.55
MB-ML	Mean	3.86	5.08	3.80	4.98
	Variance	0.16	0.33	0.14	0.22
MB-DL	Mean	6.65	7.59	5.89	7.47
	Variance	0.24	0.12	0.21	0.13
MB-MB	Mean	1.1	1.64		
	Variance	0.05	0.02		

DISCUSSION

Kraus and Jordan (1965) showed that the DM2 and M1 resembled one another in the early stages of morphogenesis, but differed in the relative ordering of morphogenesis and mineralization. The DM2 was characterized by early and rapid expansion of the talonid, and mineralization in the MB cusp of the DM2 began at an early stage of development, before all cusp tips were clearly defined (15 I/2 weeks *in utero*). Final definition of intercusp relationships at the DEJ defined as coalescence of centers of mineralization of individual cusps was completed by the 32nd week *in utero.* In the M1 mineralization commenced at a later stage of morphogenesis, after initial definition of all cusps. Kraus and Jordan (1965) reported that in one specimen studied by them, the first signs of

mineralization were seen at 28 weeks *in utero*, but that the average age for the initialization of mineralization in the M1 was 32 weeks in *utero*. There is relatively little information available on the later stages of morphogenesis and timing and sequence of mineralization at the interphase defining the DEJ in the M1, and most of that available is based on radiographic studies, summarized by Liversidge (1995). Moorrees et al., (1963) reported that cuspal coalescence in the lower M1 occurred at around 7 months, which would suggest that the process took twice as long as in the DM2. Thus the spatial and temporal patterning and rate of cell division and maturation appear to differ in the two teeth from an early phase of morphogenesis.

The results obtained here are in agreement with previous studies on decalcified teeth (Korenhof 1960) and cross sectional studies of tooth germs from archaeological samples (Smith et al., 1995) for the extent and pattern of differences observed between the DM2 and M1 at the DEJ. Butler (1968), reported that human fetal deciduous molars showed a marked increase in intercusp distances during the last stages of mineralization preceding coalescence. He theorized that this was due to the mineralized cusp tips tilting apart with continued growth at the cuspal base and Osborn (1993) presented a computerized simulation of cell division that showed how localized differences in cell division may affect the height and inclination of cusp tips. Experimental studies have now demonstrated that the spatial and temporal ordering of cell division and function within the tooth germ is regulated by a large number of genes (Slavkin and Diekwisch, 1996; Snead et al., 1988), so that small differences in their ordering and duration of activity can markedly modify tooth form.

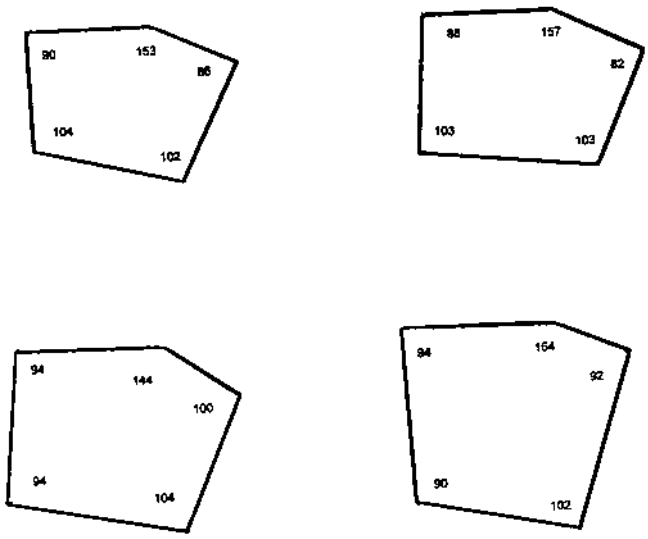


Fig. 5. Mean values for area and shape of pentagons joining cusps at the OES and DEJ, upper left-DM2 DEJ, Upper right DM2 OES. Lower left -M1 DEJ; Lower right-M1 OES.

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In the three dimensional models of human teeth examined here, the angles between vectors joining individual cusp tips at the DEJ and OES, are assumed to define their direction of growth and produce size and shape differences between them. In this study they were defined by changes in intercusp distances and shape of the pentagon joining cusp tips at the DEJ and OES respectively. As shown in Tables 1, 2 and Figure 5, they demonstrate that there are significant differences in growth trajectories of the modern DM2 and M1. The relative reduction of the area defined by the DB-DI-DL triangle on the OES of the M1, reflects the reduced growth of the distal moiety of the tooth. The differences between the two teeth are not simply a function of size, but reflect specific differences in growth and maturation within the tooth germ.

We propose that the observed changes in cuspal divergence as measured by shape and size change at the DEJ and OES, provide a means of reconstructing the presumed pattern of growth. The continuous rapid growth of the talonid in the DM2 prior to coalescence with other cusps, is reflected in the marked divergence of cusps shown in our study and demonstrated by the extent and localization of change in size and shape at the OES. The change observed in the location of the distal cusps in the M1 is the result of differences in mitotic rates at the base of the developing DI and DL cusps, with the latter growing at the expense of the former.

CONCLUSIONS

The model developed for this study constitutes an innovative technique for studying growth trajectories in modern and fossil teeth. The specific results presented here support the hypothesis that evolutionary reduction in molar tooth size, characterized by talonid reduction is associated with reduction of mitosis in the later stages of morphogenesis, preferentially affecting the distal portion of the tooth.

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Dimensional Measurements of Enamel Lesions by Computerised Image Analysis and Processing

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INTRODUCTION

White areas of the enamel surface can be developmental (*developmental white opacities)* or result from post-eruptive demineralisation (*demineralised white lesions*). A large number of causes of developmental enamel defects both environmental and genetic have been described (Small and Murray, 1978). The total prevalence of enamel defects, which include both opacities and hypoplasia, in a population of British schoolchildren was 68%; 10.5% of individuals had 10 or more teeth effected (Brook et al., 1997).

White areas due to enamel demineralisation occur during and after orthodontic treatment as an adverse effect (Artun and Brobakken, 1986). The phenomenon has become a significant clinical problem in orthodontics since the introduction of directly bonded orthodontic brackets (Zachrisson, 1977). The frequency is reported to vary from 4.9% (Gorelick et al., 1982) to 84% (Mizrihi, 1983). Mitchell (1992) in a longitudinal study found it occurred in 18.5% of patients, the average percentage of teeth affected was 1.6%. Willmot (1997) reported that in a University Clinic in Sheffield, 8.9% of labial tooth surfaces after fixed appliances therapy showed some post-orthodontic demineralised white lesions. Most of the lesions were small and this study highlighted the fact that some of the white areas counted by the researchers may have been developmental defects present before orthodontic treatment commenced; these could only be differentiated by reference to comparable pre-treatment photographs.

Most in-vivo studies of post-orthodontic demineralised white lesions have relied on visual inspection and have been based on subjective clinical assessment (Mitchell, 1992; Ogaard, 1989; Van der Linden and Dermaut, 1998). Therefore in studies of post-orthodontic demineralisation it is important to distinguish between developmental white opacities and demineralised white lesions thought to be associated with fixed orthodontic appliances. Computerised image analysis has been used to measure the size, shape, position and degree of whiteness of these post orthodontic demineralisation lesions using both analogue and digital images (Willmot, 1996). In these studies the mean area of the labial white lesion was of the order of 2 square millimetres but there was considerable individual variation. It has also been shown (Willmot, 1997) that it was possible to distinguish with statistical confidence the whiteness seen after post-orthodontic demineralisation from normal enamel and define its site on the tooth. More recently, Willmot and Benson (1998) have reported that using computerised image analysis of conventional clinical photographs with normal white light in vitro demineralisation lesions created on the enamel of extracted teeth can be reproducibly discriminated.

The aim of this study is to develop methods of differentiating between demineralised white enamel lesions associated with fixed orthodontic appliances from pre-existing developmental white opacities using computerised image analysis and processing.

MATERIALS AND METHODS

Image acquisition was from clinical photographs of pre and post-orthodontic patients. The clinical photographs were taken with a Yashica Dental Eye II camera (Kyocera Yashica [UK] Ltd., 4 Bennet Road, Reading RG2 0QX) using standardised settings of distance, illumination and aperture. The images were captured by a frame grabber using a Fotovix converter (Fotovix IIIS, Film video processor, Model TF-156WE, Tamron Co. Ltd., Japan) and SM Camera III software (SM Camera II software, Fast Multimedia. A.G., Imagination Software). The image was converted into Tagged Image File Format (TIFF) for subsequent image processing.

The images were processed with Optimetric (Bioscan Ltd) and Image Pro Plus (Media Cybernetics) software. Figure 1(a) shows a labial developmental enamel opacity acquired as a TIFF file and unprocessed. Figure 1(b) shows the same image processed with linear contrast reduction and three passes of a Laplace edge filter enabling a discrimination of the opacity edges to be seen.



Fig. 1. (a). Unprocessed TIFF Image of Enamel Opacity on labial Surface of /1.

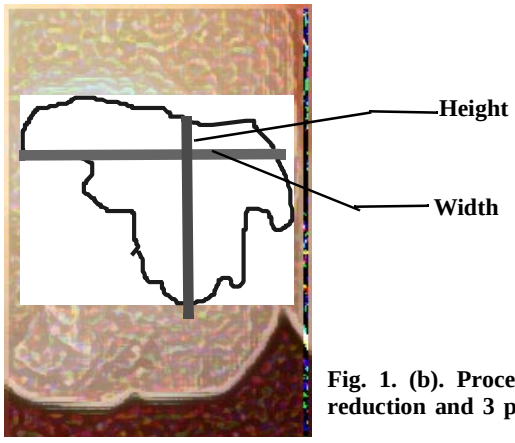


Fig. 1. (b). Processed Image following linear contrast reduction and 3 passes of a Laplace Edge filter showing

The software is capable of a wide range of enhancement techniques to enable the morphology of the lesions to be studied. Noise reduction, gradation, histogram equalisation, thresholding and edge detection are the most frequently used image processing techniques.

Standardised photographs of post-orthodontic patients' teeth showing thirty white enamel lesions associated with the orthodontic brackets were acquired in the above manner. Following image processing with a 3 x 3 noise reduction filter only, the demineralised white lesions were outlined on the computer screen and subjected to image analysis. Similarly, standardised clinical photographs taken before orthodontic treatment of patients showing 18 developmental white opacities were subjected to similar image processing and analysis. Before measurements were taken, the software was calibrated using a standard slide taken using the standard setting.

The following measurements were made:

1. Area of the white lesion. This was calculated in square millimetres as the area bounded by the outline of the lesion.
2. Circularity of the lesion. This is the ratio of the lesion's perimeter length squared, divided by the lesion's area for a simple polygon where the boundary does not cross itself. It is a dimensionless number within a minimum value of 4pi (12.57) achieved only for circular boundaries (the value is 16 for square boundaries and 20.78 for equilateral triangular boundaries), thus the higher the circularity value, the less like a circular shape is the lesion. It is a useful numerical measure for indicating the degree of circularity or irregularity of a lesion.
3. Mean grey of scattered white light The average of the pixel grey values interior to the area. Zero represents total lack of any reflected light (black) and 255 would represent total reflection of all white light (white). The grey level value for each pixel seen on the TIFF image, is computed and a mean value for the whole outlined image derived.
4. Longest axis of the lesion. The length of the longest possible line that is completely interior to the area boundary.
5. Width of the lesion. The sum of the maximum distance of the boundary from either side of the Longest Axis

RESULTS

The results are shown in Table 1. For the area measurement of 30 demineralised white lesions in post-orthodontic cases there was a mean lesion area of 2.06 sq.mm (SD $^{+}$/- 3.93). The mean area measured from the 30 developmental white opacities in the pre-orthodontic cases was of 5.41 sq.mm (SD $^{+}$/- 5.34). The longest axis of the 30 post-orthodontic demineralised white lesions was 2.56mm (SD $^{+}$/- 1.15) and that of the developmental white capacities was 3.42mm (SD $^{+}$/- 1.56). The mean width of the post-orthodontic demineralised white lesions was 1.37mm (SD $^{+}$/- 0.78) and for the developmental enamel opacities was 2.19mm (SD $^{+}$/- 0.85). Thus the developmental enamel opacities were larger in each dimension.

Table 1. Results of image analysis of post-orthodontic demineralised white lesions and developmental white opacities seen prior to orthodontic treatment.

	Demineralized Lesions			Developmental Lesions			
	n	Mean	S.D.	n	Mean	S.D.	P
Area (sq.mm)	30	2.06	3.93	30	5.41	5.34	*
Perimeter (mm)	30	7.33	3.16	30	9.48	4.01	*
Circularity	30	41.51	17.22	30	19.60	4.30	NS
Long Axis (mm)	30	2.56	1.15	30	3.42	1.56	*
Width (mm)	30	1.37	0.78	30	2.19	0.85	NS
Grey Level	30	227	20.24	30	160	27.1	NS
Normalised Grey Level	30	24.31	13.90	30	16.57	12.90	*

*P< 0.05

The mean circularity value of the post-orthodontic demineralised white lesions was 41.51 (SD $^{+}$/- 17.22) whereas the mean for the developmental white opacities was 19.60 (SD $^{+}$/- 4.30). This indicated the more regular outline of the developmental white opacities and their closer approximation to a circular shape. Statistical analysis using paired two sample t-Tests showed that measurements except width and circularity were significantly different at the 5 per cent level.

The mean grey level value of pixels of the post-orthodontic demineralised white lesions was 227 (SD $^{+}$/- 20.24) whereas the mean for the developmental white opacities was 160 (SD $^{+}$/- 27.10). In order to correct for the differing light levels of the various acquired images, the differences in grey level from adjacent normal enamel value for each lesion were calculated and termed the normalised grey level. The mean normalised grey level between normal enamel and demineralised white lesions was 24.31(SD^{+}/- 213.90) and that for developmental white opacities was 16.57 (SD $^{+}$/- 12.90). Statistical analysis using paired two sample t-Tests showed that normalised grey levels were significantly different at the 5 per cent level.

Line profile analysis measurements of the grey levels of a line passing through a white post-orthodontic enamel lesion showed a peak with a gradual increase and decrease of grey level from the margin to the centre of the lesion (Fig 2) for all the components of white light. Line profile analysis through developmental white opacities showed a degree of whiteness which was generally consistent throughout the lesion giving a “plateau” affect (Fig. 3).

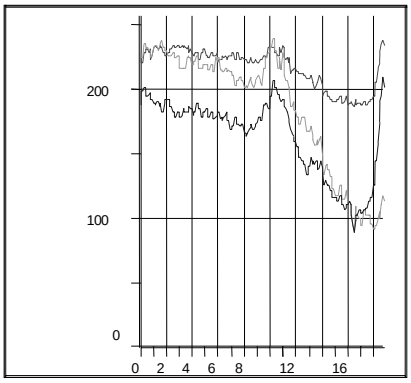


Fig. 2. Graphs of line profile (3 colours) showing grey levels along the line and a rising peak effect passing through an area of white enamel demineralisation (unprocessed image).

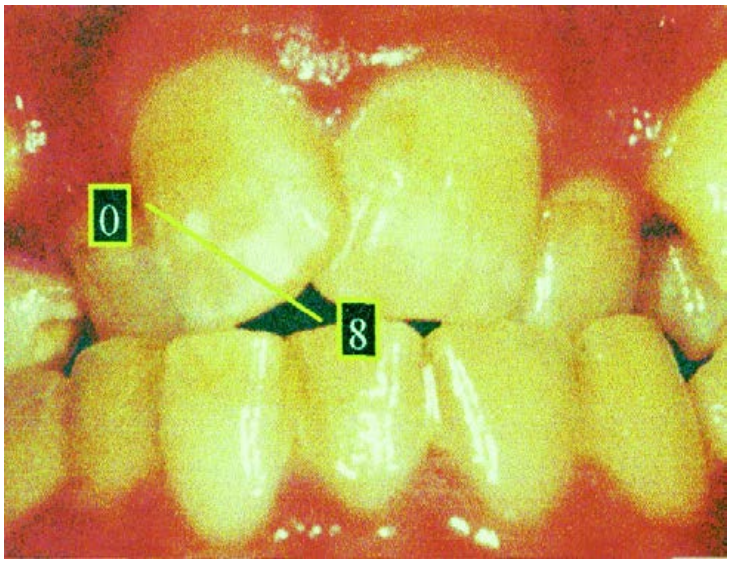


Fig. 3. (a). Line profile passing through two developmental white opacities on the labial surface of 1/ (unprocessed image).

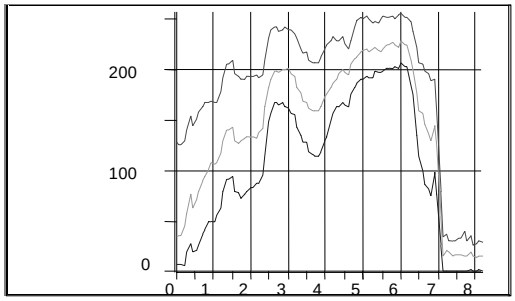


Fig. 3. (b). Graphs of line profile (3 colours) showing grey levels along the line and a plateau effect.

DISCUSSION

Measurements of lesion area showed a wide variation in the size of post-orthodontic demineralisation with a mean of the order of 2 square millimetres and a standard deviation of 4. Such variation probably reflects the differing sites affected and the differing severity of the lesions. These findings may well be related to the patients variation in oral hygiene or diet. In contrast the developmental enamel opacities had a mean area of 5.41 square. A similar contrast between the demineralised and developmental lesion was found, in length and in width.

The shapes of the post-orthodontic demineralised white lesions were varied and irregular with a mean circularity value of 41.50. Figure 4 shows a range of shapes seen in

the post-orthodontic demineralised white lesions which generally reflect the margins of the orthodontic brackets on the labial surface of the teeth compared with typical shapes seen in developmental white opacities. Measurements of the shapes of the developmental white opacity lesions gave a circularity value of 19.60 indicating that on average many of these defects approximated not to true circles (value 12.57) but rather to a polyhedral structure. Thus it was possible to distinguish using this system the differing morphologies which were typical of the two different types of white enamel lesions.

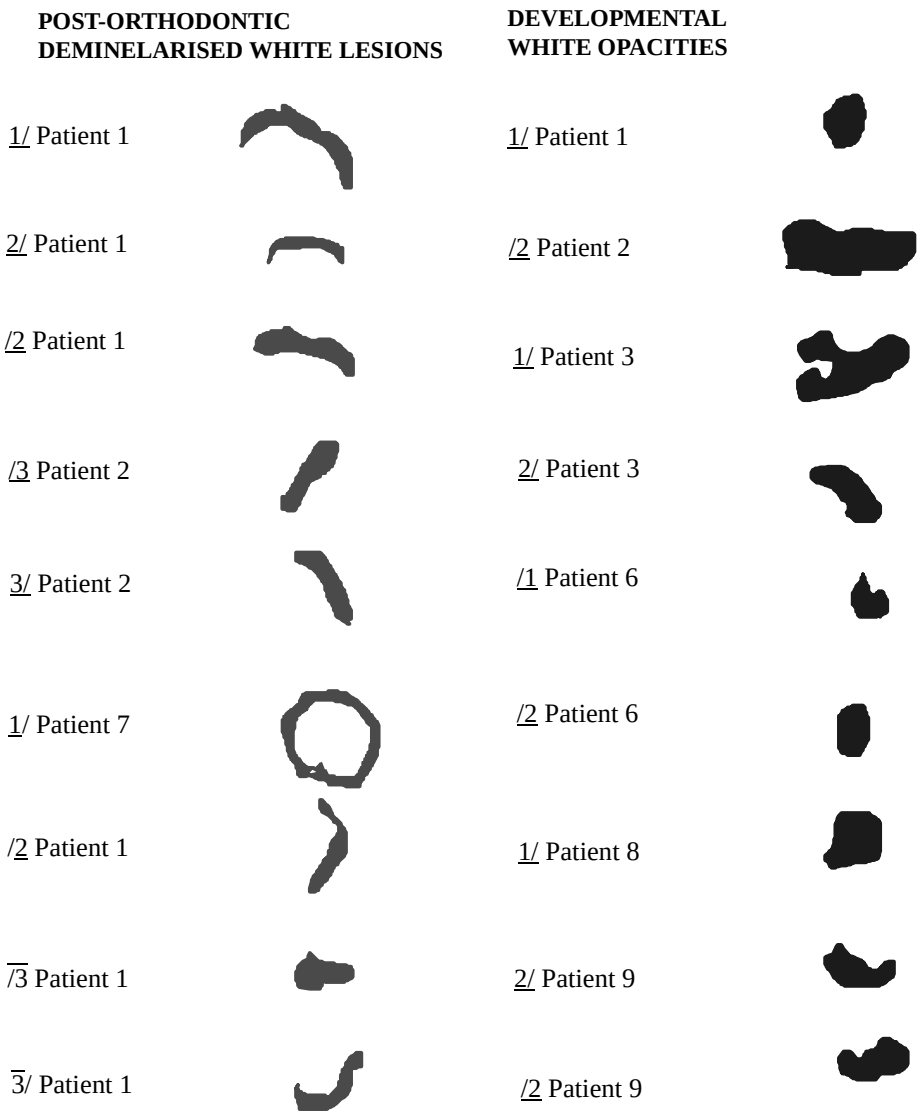


Fig. 4. A range of shapes seen in post-orthodontic demineralised white lesions which generally reflect the margins of the orthodontic brackets on the labial surface of the teeth compared with typical shapes seen in developmental white opacities.

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Grey level measurements showed demineralised white lesions to be on average whiter (227) than developmental white opacities (160). There was however a wide variation between the various acquired lesions and this was judged to be due to a variation in light intensity of the different images used. This was corrected by calculating the difference between the grey level of the lesion and an adjacent area of normal enamel for each lesion. The resulting normalised grey levels represented the true difference in whiteness for each individual lesion. It was found that these differences were statistically significant ($p<0.05$) indicating that in general demineralised white lesions were whiter than developmental white opacities.

Line profile analysis measurements of the scattered white light passing through lesions (Fig. 2 and 3) show the distinct difference between a developmental white opacity defect and post-orthodontic demineralised white lesions showing the plateau seen in the trace of developmental white opacities but not of the demineralised white lesions. Thus the presence or absence of such a plateau is a further useful marker to help differentiate between enamel demineralisation and developmental opacities.

CONCLUSIONS

1. It is possible to acquire and process images of labial white areas of enamel by computerised image processing equipment.
2. Developmental white opacities tend to be larger than post-orthodontic demineralised white lesions.
3. Developmental enamel opacities tend to be more regular in outline and closer in shape to a circle than post-orthodontic demineralised white lesions.
4. Developmental enamel opacities show plateau effect on a trace of grey levels (light intensity) across the surface whereas post-orthodontic demineralised white lesions tend to demonstrate sharp peaks.
5. Demineralised white lesions were whiter than developmental white opacities.
6. By combining these findings it is possible to discriminate between many of these two types of white lesions.

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A New Method for Measuring Three-Dimensional Dental Morphology

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INTRODUCTION

In dental morphology research there has been a trend to go into deeper and more detailed analysis of occlusal morphology in order to gain a better understanding of different developmental pathways. Obtaining a real three-dimensional image of occlusal surfaces has thus been the goal for many projects whose aim has been to develop measuring methods for dental anthropology (Corruccini and Potter, 1981; Saunders and Mayhall, 1982; Kanazawa et al., 1983; Mayhall and Kanazawa, 1989).

There are various possibilities to measure real three-dimensional dental occlusal morphology. One of the most commonly used systems has been the Moiré method, where the beam is guided through a granting to the object where contour lines are formed, which in turn can be registered optically or by a video image. Recently an application of the Moiré method was introduced to gain a more detailed view of the inspected occlusal surfaces. In this application a modern image analysis technique was used for handling the basic data produced by Moiré contourography (Mayhall and Kanazawa, 1989; Mayhall and Alvesalo, 1992; Mayhall and Kageyama, 1997) The Moiré method has to some degree been applied for measuring dental cuspal morphology and intercuspal distances. One specific application of this method has been the measurement of intercuspal heights and distances (Kanazawa et al., 1983).

To overcome difficulties and inaccuracy in three-dimensional registration of dental morphology a method was developed where the real three-dimensional morphology of tooth surfaces could be registered with the aid of a projected grid with which a contour of the tooth surface could be obtained. For further analyses, these images can be digitized and thereafter analyzed by a computer program. With this photogrammetric method the distances between points, angles, and number of cusps can be measured (Taverne et al., 1979).

The morphometric method differs in many respects from other three-dimensional measurement systems, as it is very invasive in character. The measured tooth is cut into slices and the 3D-structure of the tooth can be created by the summary data that are derived from the separate slices. The advantage of the method is, naturally, that real data of inner dental structures like pulpa cavum and enamel thickness can be obtained (Fedjeskov et al., 1973; Ekstrand et al., 1990).

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The latest application of computerised tomography (CT) in dental morphology research is X-ray microtomography which is a miniaturized version of computerized axial tomography. From this, the mineral concentration can be computed and three-dimensional images with high resolution of teeth and bones can be gained (Davis and Wong, 1996.

When the analyses of fissure and fossa morphologies in particular are concerned, the use of scanning electron microscopy has certain advantages when compared to other available methods. Very detailed information of morphological microstructures can be registered, since the method is relatively non-invasive when compared to the morphometric method (Juhl, 1983).

A very accurate measurement system for dental purposes has been presented, based on two separate laser displacement meters. The use of two laser meters instead of one gives the advantage that shades in measurements are avoided and the measurement process is quicker (Kimura et al., 1990). The system is further improved for accurate tooth model manufacturing by incorporating the CAD/CAM technique. A recent commercial application of the CAD/CAM technique is used in a system where the view created by a digital scanner is converted to a three-dimensional model of the tooth surface, which can then be used for prosthetic manufacturing purposes (Krejci et al., 1994).

It can be concluded that a great variety of methods have been developed for tooth surface examination. The general trend in these methods is their increasing accuracy and the high level of digital processing of the records aimed at facilitating the analyzing process.

The purpose of the present project was to develop a 3-D measurement system for professional use in dental research. The system should be suitable for measuring, with high accuracy, any distances and angles between morphologically interesting points on dental crowns and dental arches.

METHOD

Orthometrics is a 3-D measurement system designed for professional use in dental research. It is suitable for measuring distances and angles between morphologically interesting points on dental crowns. The measurements are performed using dental casts where the points to be measured are marked by small dots. The distances and angles are obtained in three-dimensional domain which also allows the depth information to be exploited.

The measurement system consists of one video camera, frame grabber, stand and a PC. The stand holds the camera in a fixed position, and allows the plaster model to be turned in five different orientations (Fig. 1). The camera used is a standard off-the-shelf CCD camera, which is calibrated with high precision.



Fig. 1. The CCD-camera and the stand that allows five different views of the cast to be digitized.

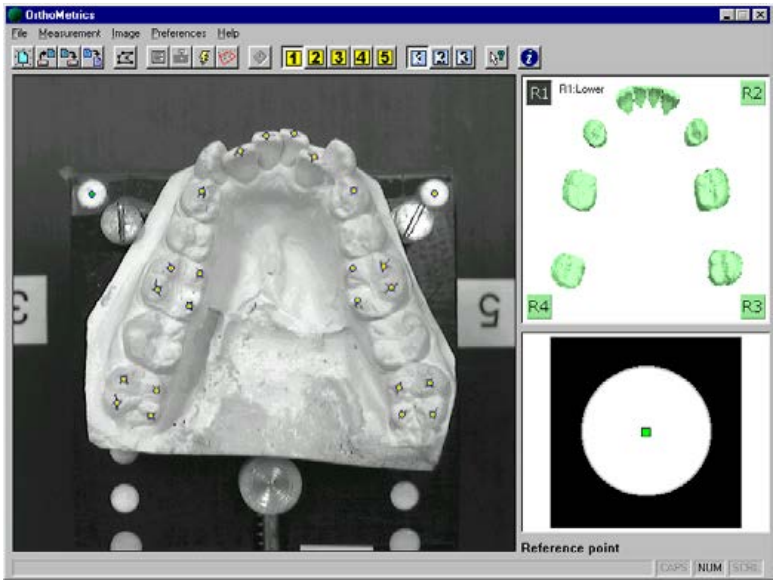


Fig. 2. The user interface of the program during the digitizing procedure.

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The measurement session is started by capturing five digital images from the target. The locations and the correspondences of the measurement points are then determined by picking them manually from the images (Fig. 2.). The amount of laborious human intervention is minimized by an easy-to-use graphical user interface. After the manual stage, the measurement software takes care of the rest of the processing.

The 3-D structure, distances and angles are now computed automatically based on the measurement plan. The results are visualized in the user interface (Fig. 3) and they can be saved as an ASCII file, which is accessible by Microsoft Excel, for example. Also, the camera calibration procedure included in the system is fully automatic.

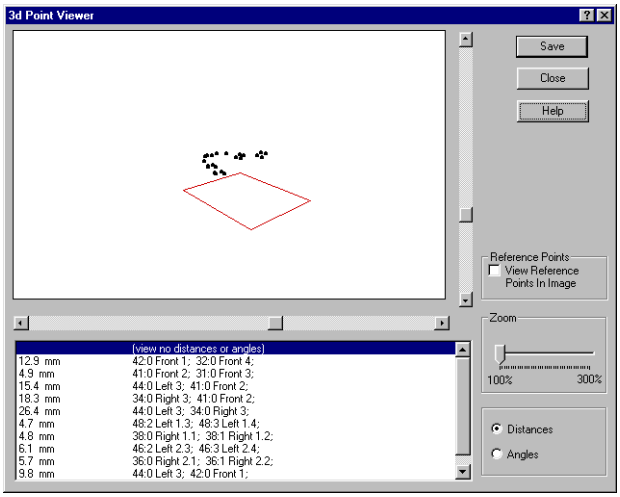


Fig. 3. The results window of the user interface with the 3D-view of the computer-created model.

The software package is completely free and it can be downloaded on the Internet. It runs under Windows NT 4.0 and Windows 95. The GUI is compiled with Borland C++ 5.0, the computation and calibration modules are generated from MATLAB (The Math Works Inc) code with MATCOM (Math Tools Ltd) translator. Testing the software does not require any special equipment, because there are some example images included in the package.

Camera calibration procedure

Camera calibration in context of three-dimensional machine vision is the process of determining the internal camera geometric and optical characteristics (intrinsic parameters) and/or the 3-D position and orientation of the camera frame relative to a certain world co-ordinate system (extrinsic parameters).

Camera projection is often modelled with a simple pinhole camera model. In reality, the camera is a much more complicated device, and if it is used as a measurement instrument, a proper calibration procedure should be performed. Various methods for

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calibrating cameras can be found in the literature. Most of these methods may be subdivided into linear, non-linear and multi-step techniques. The linear techniques are fast, because no iterations are required. However, their accuracy is quite poor, since some simplifications to the camera model have been introduced. The most common techniques in this category are Tsai's method (Tsai, 1987) and the direct linear transformation (DLT) (Faugeras and Toscani, 1987). On the other hand, non-linear methods, often used in photogrammetry, are not restricted by selection of the camera model, and therefore they can be more accurate. The disadvantage in the non-linear approach is the increased computational cost. Minimizing a non-linear cost function requires an iterative optimization technique and a good initial guess. Therefore, in multi-step methods the camera parameters are first computed with linear technique and then the result is improved by using the existing parameter estimates as an initial guess for the optimization step (Weng et al., 1993; Heikkilä and Silvén, 1997).

Matlab is a very powerful and commonly used tool for numerical computation and data visualization. Therefore it is also a suitable platform for performing camera calibration experiments. The camera calibration toolbox developed in the University of Oulu consists of several Matlab functions, also called m-files. These ASCII files can be easily modified to correspond to user requirements.

The calibration toolbox supports: radial and tangential correction (two parameters for each), multiple frames captured from different viewpoints, coplanar and non-coplanar targets, correction for the asymmetric projection of the circular control points and correction of the image co-ordinates.

There are a total of eight intrinsic parameters to be estimated: scale factor, effective focal length, principal point co-ordinates, two radial and two tangential distortion coefficients. These parameters are solved either in two or three stages, depending on the control point geometry. Image correction is then performed by using a special inverse distortion model.

For using the toolbox a calibration object is needed with a set of visible control points whose mutual locations are known accurately. As input data the calibration procedure requires the 3-D co-ordinates of the control points and the corresponding co-ordinates of the image observations. In order to achieve better calibration accuracy there can be observations from several camera locations. Moreover, not all the control points are required to be visible in each image. This makes it possible to capture the images from different locations without constraining the distance between the object and the camera.

Error of method analysis

The error of method was tested by doing ten repeated measurements on the first permanent molars on three dental casts (Fig. 4.). The measured parameters included linear measurements (L1-L11) and angular measurements (A1-A7). The total error, including systematic and random error, was determined.

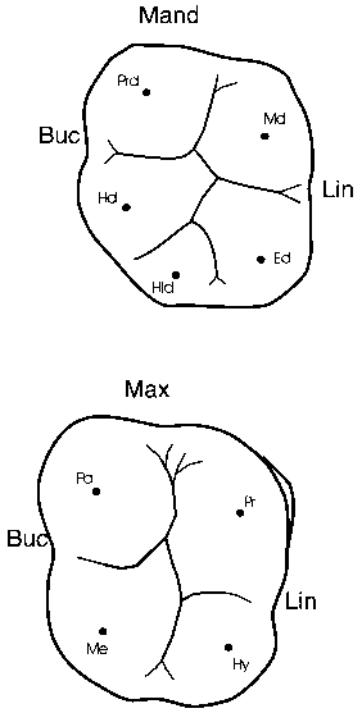


Fig. 4. The cusps that were marked in the first maxillary and mandibular permanent molars. The marks were placed to the level or nearest to the level tangenting the three most prominent tips of cusps of the molars. Maxillary points: Paracone (Pa); Protocone (Pr); Hypocone (Hy); Metacone (Me); Mandibular points: Protoconid (Prd) Metaconid (Md); Entoconid (Ed) Hypoconulid (Hld) Hypoconid (Hd).
The following angular (A1-A7) variables of the first molars were used in computerized 3D measurements. The second given point indicates the tip of the angle and the first and last given points indicate the lateral points of the angle. Maxillary angular variables; A1: Me-Pa-Pr; A2: Me-Hy-Pr; A3; Hy-Pa-Pr. Mandibular angular variables; A4: Hd-Prd-Md; A5: Hld-Ed-Me; A6: Hy-Me-Pr; A7: Hld-Ed-Hd.
Linear variables (L1-L9); Maxilla: L1: Me-Hy; L2: Pr-Me; L3: Pr-Hy; L4: Pa-Pr; L5: Pa-Me. Mandible: L6: Hld-Ed; L7: Md-Hld; L8: Md-Ed; L9: Prd-Md; L10: Prd-Hd; L11: Hd-Hld.

RESULTS

The measurement error with 95% confidence intervals calculated separately in eleven linear and seven angular parameters is shown in Table 1. The mean measurement error ranged from 0.00 to 0.06 mm, depending on the measured dimension. The mean measurement error in angular measurements ranged from 0.03 to 0.20 degrees.

Table 1. The total measurement error and 95% confidence intervals for means for variable.

Variable	N	Mean	SD	95% Conf. Intervals	
L1 (Me-Hy)	30	0.02	0.16	-0.0422	0.0790
L2 (Pr-Me)	30	-0.01	0.11	-0.0491	0.0343
L3 (Pr-Hy)	30	-0.01	0.06	-0.0312	0.0157
L4 (Pa-Pr)	30	0.03	0.16	-0.0330	0.0853
L5 (Pa-Me)	30	0.01	0.07	-0.0165	0.0327
L6 (Hld-Ed)	30	0.02	0.12	-0.0271	0.0613
L7 (Md-Hld)	30	0.03	0.15	-0.0280	0.0842
L8 (Md-Ed)	30	-0.00	0.07	-0.0274	0.0217
L9 (Prd-Md)	30	0.06	0.33	-0.0680	0.1819
L10 (Prd-Hd)	30	-0.00	0.09	-0.0345	0.0311
L11 (Hd-Hld)	30	-0.01	0.13	-0.0617	0.0381
A1 (Me-Pa-Pr)	30	-0.07	1.26	-0.0536	0.4017
A2 (Me-Hy-Pr)	30	0.03	1.80	-0.6406	0.7039
A3 (Hy-Pa-Pr)	30	-0.20	0.72	-0.4724	0.0657
A4 (Hy-Me-Pr)	30	0.14	0.69	-0.1158	0.3971
A5 (Hd-Prd-Md)	30	-0.38	2.30	-1.2409	0.4743
A6 (Hld-Ed-Md)	30	0.05	1.63	-0.5556	0.6649
A7 (Hld-Ed-Hd)	30	-0.12	0.57	-0.3306	0.0966

Linear measurements (L1-L11) = mm; angular measurements (A1-A7) = ^{o}.

DISCUSSION

The error of method analysis shows that the method developed for occlusal morphology measurements is capable of making all the planned measurements with sufficient accuracy. The highest range of variation was found in the angular parameters, which result can be explained by the fact that each angular measurement comprises three digitizing procedures instead of the two needed in linear measurements. Therefore the possibility for random errors increases in these variables. On the other hand, the error level even in these angular parameters remains low when compared to the inter-individual biologic variance of these parameters, when dental cuspal morphology is concerned (Pirttiniemi et al., 1998).

The accuracy level of the current method could be further increased by many different procedures. However, in this case the versatility of the method would be diminished. One way to gain more sharpness and thereby higher accuracy is to take even more closer views of tooth surfaces. This could easily be done by a close-up lens with the similar procedures as in the present method. In that case a complete view of the dental arch could not be achieved with the same settings. A more detailed three-dimensional image could also be created by increasing the number of digitized images. The current number is, however, practical due to the manual marking procedure. This matter could easily be solved if the digitizing procedure were done fully automatically. The techniques used would allow this automation, but for general versatility purposes this was not developed further in the current version of the software.

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In conclusion, the present examination method for occlusal morphology appears to be accurate and versatile as it facilitates precise 3D measurements, whose results can be stored digitally. By combining a calculating program to the software, direct angular results of measurements can be achieved and the application can easily be modified further to give shape information by mathematical modelling.

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MORPHOLOGICAL INTEGRATION WITHIN THE DENTAL AND CRANIOFACIAL COMPLEX

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Craniofacial and Dental Variation in Caucasians with Population Comparisons to Australian Aborigines: I. The Study Material

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INTRODUCTION

The existence of orderly though variable interrelationships between the size and shape of the body and its parts with both the environment and bodily functions have been postulated since Aristotle. Bergmann's mathematically sophisticated formulation of the reason for a functional relationship between body size and thermoregulation dates from 1847. Thirty years later, in 1877, Allan added the relative proportions of body parts to Bergmann's formulation.

Observations not dissimilar to those of Bergmann's and Allan's have continued to accumulate to the present day. Some have demonstrated that specific phenotypic changes take place as a response to changing environmental conditions or events as in secular trends. Genetically based morphological changes accompanying functional relationships have also been described. Some of which have been occurring progressively since the very beginnings of our species. One example, relevant to the subject of this symposium, is an apparent overall increase in cranial size as the size of the teeth have decreased in the course of human evolution (Hooton, 1947; Howells, 1959; Hulse, 1971).

The increase in average cranial size that seems to have slowly taken place through human history has been toward brachycephalization (roundheadedness), greater head breadth relative to head length. It has therefore been reasoned that with an overall decrease in tooth size, individuals with greater head circumferences, and greater head breadths in particular, will have smaller teeth while individuals with long heads (the dolichocephals) should on average have larger teeth. Ancient skeletal material would seem to support this conclusion, but surprisingly little living material that includes both cranial measurements and tooth measurements have been available for analysis to test this hypothesis.

In today's ever increasing hi-tech world compartimentalization, specialization if you will, that has been taking place within the life sciences (medical and academic), has resulted in the human body being divided into ever smaller bits and pieces. While this has contributed to more detailed information about each piece, it appears to have made it increasingly difficult to put these bits and pieces back together again, and to relate them to each other and to function. In fact, the ultimate importance of the functional interrelationships of the body's parts is far too frequently forgotten about. An exception may be in orthodontics where the size of the teeth and the size and shape of their

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supporting bony structures comprises the central focus of this specialty. But even here concern for actual function is frequently in second place to socially dictated physiognomic appearance.

Our objective in these three presentations on Craniofacial and Dental Morphological Integration is to take advantage of present technological advances in data analysis to put the dentition back into place in the craniofacial complex; advances significantly beyond those available to Olsen and Miller in their 1958 publication on Morphological Integration.

This is a partial follow through from the concerns that I expressed at the First International Symposium on Dental Morphology at Fredensborg, Denmark, September 27-29, 1965 when I wrote,

> Evolutionary interpretations based on dental characteristics must take into account the fact that the teeth, though the best preserved fossil remains, are only part of a complex organism and, by virtue of their preservation, are not necessarily the primary adaptive element.
>
> Human evolution must be viewed as more than a mere sequence of dentitions through time, and it must be recognized that the teeth and jaws may have importance beyond the basic function of mastication.
>
> More fruitful than speculations as to possible effects of changing patterns of food preference and preparation on evolutionary modification of tooth character might well be investigations of the genetic mechanisms underlying dental characteristics as a part of broader studies that include consideration of other aspects of the organism" (Osborne, 1967, p. 947).

STUDY MATERIALS

Beginning in the 1930s, before the prerequisite analytic tools needed for the job had been developed, there was a recognition of the need to view the human body, even including the psyche, as an integrated system. An example of this recognition was the Sheldonian system for describing and classifying observable variation in both mind and body. This consisted of categorization of body types, with psychic and functional sequelae.

In 1952 collection of the Columbia adult twin and sibling data was begun to provide a genetic test of Sheldonian somatotype (Osborne and DeGeorge, 1959). It is this body of data that is being utilized for today's study of morphological integration.

As the criteria for the somatotyping were almost totally subjective, the only exception being the ponderal index (stature/$\sqrt[3]{}$ of weight), extensive anthropometric measurements were obtained for all study subjects. For maximum return from this investment and labor, and to look for specific functional relationships with either somatotypes or anthropometric measurements, cardiovascular parameters (Mather et al., 1961; Osborne et al., 1963) and serum lipid determinations (Osborne et al., 1959) were also obtained.

Because of the investigator's interest in pre-natal environmental events and ontogenic vs. specific genetic effects, a study of the teeth was included with the help of Sidney L. Horowitz (Horowitz, et al., 1958; Osborne, et al., 1958). The University of Wisconsin

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twin study subjects have been added to the Columbia series to obtain more advantageous numbers for the anthropometric measurements in the analysis to be presented here. These Wisconsin subjects were measured in 1967 as part of a later twin study.

Ascertainment of Columbia study subjects

Because specific entities were not under investigation a total ascertainment of twins in a prescribed population was not called for. What was desired, however, was a representative sampling of the adult population of New York City between the ages of 18 and 55, of European descent, and in good general health.

As this was not an institutionalized twin population, volunteer participation in study was necessary. Every effort was made to draw twin pairs from a broad spectrum of New York City. The study was advertised at colleges and universities within the four boroughs of the city of New York and to blood donors at the various blood banks within the city. Referrals by the twins already participating in study were an important source of additional subjects.

University of Wisconsin, Madison Twins

In 1966 a question as to twin-born status was added to the health questionnaire applicants completed for admission to the University of Wisconsin. This twin poll was carried out until 1968. The 33 Wisconsin twin pairs and their siblings were volunteers from that Twin Roster, and added for this analysis.

Because we are only presenting on phenotypic associations at this time as the genetic analysis has not been completed, I will not discuss the zygosity diagnosis. We are using our twin born subjects simply as an adult population screened for their health status, to examine phenotypic associations.

MEASUREMENTS

All measurements were obtained by a single observer to eliminate inter-observer measurement errors which can greatly reduce the power of the analysis for certain dimensions. Sidney L. Horowitz, D.D.S., obtained all of the dental casts and took all of the dental measurements. All anthropometric measurements for both twin populations were taken by the same anthropometrist, Richard H. Osborne, using the same anthropometric instruments throughout.

All of the mesio-distal tooth measurements obtained for the 12 anterior teeth are included in the present analysis. Measurements for the remainder of the dentition can be analyzed in the future. A total of 67 measurements were obtained for the adult twins and their siblings, 21 of the cranium and face and 46 of the post cranial body. Of these, six craniofacial measurements are being used at present. Selection of measurements was based upon their reproducibility (low measurement error) common usage, and in earlier analyses had defined a statistically significant genetic component of variability (Osborne and DeGeorge, 1959).

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Population Comparisons

Different ethnic populations in radically different environments commonly exhibit strikingly different physiognomies. Two of the most different from each other and from people of European descent are Eskimos and Australian aborigines. In the course of the circumpolar projects under the U.S. International Biological Programs, Albert A. Dahlberg, Paul Jamison and Stephen Zegura, obtained dental casts and anthropometric measurements of virtually the entire adult Eskimo (Inuit) population of Wainwright, Alaska. These data are located at three different institutions and could not be brought together in time to be analyzed for presentation at this time. This will hopefully be accomplished in the near future.

The Australian Aborigines

The Australian Aborigines are a fairly diverse people. The important thing for our analysis here was to obtain a representative sample of adults for whom the same dental measurements and anthropometric measurements were obtained as for our Caucasian sample. Grant Townsend, University of Adelaide will describe the aboriginal data when this comparison is made (Kohn et al, this volume).

The distribution of our New York City Caucasian subjects deviate markedly by sex and zygosity from the theoretical, as has been true for all major twin studies (Osborne and DeGeorge, 1959, p. 51, Table 3). Our numbers are 113 female and 81 male pairs, for 226 and 162 individuals respectively.

As Karl Pearson wrote in 1903, “It is far more difficult to get measurements on two adult brothers than on two sisters.” This was at least as true at the time of this study. Why are these forty year old data of value today? There are two very good reasons. First is the rarity with which descriptive data are obtained for two or more systems in the same individuals. A consequence, as we have already noted, of the ever increasing specialization’s taking place at all levels in the life sciences.

Second is a fact, that will not have escaped many from personal research experience; the restrictions now being placed upon the kinds of descriptive data that can be collected for human subjects. I do not believe there is any medical research center still in existence in the United States at which subjects could be brought in free of any medical problems for admission to the hospital or its clinics, put through institutionally endorsed full medical examinations, poked and prodded in the name of research, and have all of this institutional input compensated by moneys from outside sources, even with signed and witnessed consent forms by all the study subjects, as obtained here. What may be favorable for lawyers has become seriously regressive for researchers.

What was done with these data forty years ago that can be done better today? At the time these data were collected the simplistic 1950’s analytical model that was followed and adapted for the investigation of what we are now calling morphological integration came out of agriculture (Lerner, 1950, 1954, 1955).

In animal breeding where the purpose is to combine two or more economically valuable characteristics in the same strain the study method is a cross-character analysis between individuals of known relationship (Lerner, 1950). This cross-character method was translated to a cross-twin analysis by Osborne and DeGeorge (1959, p. 64) and applied to dental and anthropometric measurements by Osborne, Horowitz and DeGeorge

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(1958). At that time our computer technology and statistical methods did not permit the simultaneous analysis of more than two variables at a time so this simplistic method was the best that could be done.

This method, good for no more than two phenotypic traits at a time, did provide a statistical test of whether these two traits were under a common genetic influence, or possibly by linked loci. Today's more sophisticated analytical methods and technological tools make it possible to work with multiple measurements and expand tremendously our capacity to study morphological integration across multiple body systems. It is now actually possible to try and put the bits and pieces back together again! In the classic work, *On Growth and Form*, D'Arcy Wentworth Thompson quotes Karl Pearson's 1901 prediction "… the day must come when the biologist will – without being a mathematician – not hesitate to use mathematical analysis when he requires it" (Thompson, 1952).

To achieve an integration of the dental measurements, and of different dimensions of the cranial vault and face requires more than biological expertise and insight. Today we have far more sophisticated methods than those available to Olsen and Miller at that time of their monumental 1958 effort on *Morphological Integration*; certainly more than Karl Pearson visualized 97 years ago.

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Craniofacial and Dental Morphological Integration. II. Data from North American Twins

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INTRODUCTION

The integration of morphological measurements has been a focus of biologists for over a half a century. Butler (1939) introduced the concept of "dental fields", describing the inter-relationships of molar morphology. Later, Olson and Miller (1958) presented a theory of morphological integration, where functionally or developmentally related structures should be expected to be closely associated. Analyses of morphological integration have been limited until recently, when advances in computing technology have allowed the theories of morphological integration to be tested.

When we ask if morphological traits are integrated, we ask whether functionally or developmentally related traits are more highly associated or correlated than traits that are not related. The traits that are functionally or developmentally related are said to make up a morphological unit. We expect relatively high correlations between traits within a morphological unit, and relatively low correlations between traits that are not within a morphological unit (Olson and Miller, 1958; Cheverud, 1982, 1989, 1995, 1996; Cheverud et al., 1989).

Morphological integration has been examined in human and nonhuman primates. Functional integration of craniofacial dimensions was found by Kohn and coworkers (Cheverud et al., 1992; Kohn et al., 1993, 1994, 1995) when examining the effects of cranial vault shape modification by prehistoric populations. A change in form of the cranial vault was accompanied by associated changes in the cranial base and face. Significant morphological integration in facial morphology has been found by Cheverud (Cheverud, 1982, 1989, 1995, 1996) in several species of nonhuman primates, including tamarins and macaques.

In this paper we test for significant morphological integration of craniofacial and dental morphology in a sample of Caucasian twins. This paper is the first examination of morphological integration on living human material, and is part of an ongoing study of genetic and phenotypic morphological integration in modern populations.

MATERIALS AND METHODS

The data for this study consist of subsample of twins recruited for studies of the genetic basis of morphological variation (Osborne et al., 1958; Horowitz et al., 1958; Osborne and DeGeorge, 1959), as described elsewhere in this volume (Osborne et al., 1999). Our

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sample consists of 130 twin pairs (60 dizygotic, 70 monozygotic) recruited from the New York City area between 1952 and 1956. An additional sample of 33 twin pairs (9 MZ, 24 DZ) were recruited from the student body of University of Wisconsin in 1968 (Table 1). All individuals were between 18 and 65 years of age. In our sample, craniofacial data were available for 126 males and 192 females. A subset of these data, 36 males and 72 females, also had dental data available for inclusion. Only the phenotypic data will be used in this study, as our genetic analyses are ongoing.

Table 1. Number of individuals included in craniofacial and dental samples.

	Craniofacial	Traits	
	MZ Twins	DZ Twins	Total
Male	70	56	126
Female	84	108	192
	Dental	Traits	
	MZ Twins	DZ Twins	Total
Male	22	14	36
Female	40	32	72

The craniofacial data for this study consists of: 1) head length; 2) head breadth; 3) head circumference; 4) bizygomatic breadth; 5) bigonial breadth; 6) total facial height. The dental measurements used in this study include: 1) maxillary and mandibular right and left central incisor mesiodistal diameter; 2) maxillary and mandibular right and left lateral incisor mesiodistal diameter; 3) maxillary and mandibular right and left mesiodistal canine diameter.

Males were larger than females for all traits. To enlarge sample sizes, all data were corrected for differences due to gender prior to further analysis. The mean ($\overline{X}$) and standard deviation (σ) of each trait in females (F) was arithmetically adjusted to equal that of males (M) using the formula

$$\mathbf{X}_{Fi} = (\mathbf{X}_{Fi} - \overline{X}_F)(\sigma_M/\sigma_F) + \overline{X}_M$$

where X_i refers to an individual female's measurement of a trait. These sex-adjusted data was used in all further analyses.

Six morphological units were defined. These include the 1) craniofacial unit, including all craniofacial dimensions; 2) dental unit, including all dental dimensions; 3) maxillary unit, including all maxillary dental dimensions; 4) mandibular unit, including all mandibular dental dimensions; 5) incisor, including all incisor dimensions; 6) canine, including all canine dimensions.

Pearson product-moment correlations were calculated between all traits. The expectation is that traits that are closely associated will be highly correlated, while traits that have little association can be expected to show a low correlation.

In testing for significant integration of these morphological units, we test whether traits within a morphological unit, for example within the dental unit, are more highly correlated with each other than they are with other traits and more highly correlated than the other traits are with each other (Cheverud, 1989, Cheverud et al., 1989). To test for significant morphological integration, we calculate a matrix correlation between the matrix of the observed correlations among traits and a "hypothesis matrix" which

contains 1 in the matrix elements of traits within a functional unit, and 0 in the matrix elements of traits not within a functional unit. The significance of this matrix by matrix correlation (the measure of integration) is tested by a nonparametric permutation test in which a permuted distribution of correlations is generated (Cheverud, 1989, Cheverud et al., 1989). A significant result is one in which the observed correlation is greater than or less than 95% of the permuted correlations. These analyses of morphological integration used 1000 permutations to estimate significance.

RESULTS

The average correlation of dental and craniofacial traits are presented in Table 2. In general, craniofacial dimensions are not highly correlated with each other (average correlation is 0.38, range 0.14 - 0.78), and they are not highly correlated with dental dimensions (average correlation is 0.07, range -0.14 - 0.24), and dental dimensions were uniformly highly intercorrelated (average correlation is 0.68, range 0.47 - 0.95). There were no significant correlations of craniofacial traits with dental dimensions.

Table 2. Average correlation of dental and craniofacial dimensions in caucasian twins.

	Tooth Size
Head Length	0.03
Head Breath	0.02
Head Circumference	0.05
Bizygomatic Breadth	0.12
Bigonial Breadth	0.15
Total Facial Height	0.00

The average correlation of traits within morphological units, the average correlation of traits not within a morphological unit, the test of morphological integration, and the probability of integration are presented in Table 3. The craniofacial dimensions are not highly integrated. The average correlation among traits within the craniofacial unit is 0.38. Comparable results are obtained if cranial vault and facial traits are analyzed separately. Each of the dental morphological units are significantly integrated. The average correlation within each dental morphological unit is high, and is much higher than the average correlation between all remaining traits.

Table 3. Test of morphological integration (MI), average correlation within a morphological unit (AVGIN), average correlation of traits not within a morphological unit (AVGOUT), and associated probability of integration (P).

	MI	AVGIN	AVGOUT	P
Craniofacial	0.20	0.38	0.36	0.320
Dental	0.89	0.68	0.12	0.001
Maxillary	0.34	0.70	0.33	0.020
Mandibular	0.39	0.73	0.32	0.002
Incisor	0.57	0.69	0.32	0.001
Canine	0.30	0.81	0.34	0.001

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DISCUSSION

In conclusion, overall craniofacial and dental morphology appear to be relatively independent based, at least in the current data. These data show the included craniofacial dimensions have a relatively low level of integration and are not significantly integrated. While earlier studies found that cranial vault morphology was associated with morphology of the facial region, these results are not reflected in the correlation among broad craniofacial dimensions in this sample. Smaller measurements within discrete regions of the craniofacial complex may be necessary to show a greater integration within this region. In contrast, all dental units are highly integrated. This supports an integrated, coordinated basis for observed dental morphology.

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Craniofacial and Dental Morphological Integration. III. Comparison of North American and Australian Aboriginal Populations

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INTRODUCTION

An issue in morphometrics is whether the observed patterns of correlation among traits and morphological integration are unique to the observed population, or whether this pattern can be generalized to other populations as well. Morphological traits are controlled by genetic and environmental factors. Genetic differences in populations, due to differences in allele frequencies, dominance deviations and epistasis, may be expected cause differences in observed morphology, and correlations among traits (Cheverud, 1995, 1996).

As discussed elsewhere in this volume (Kohn and Osborne, 1999), when we ask if morphological traits are integrated, we ask whether functionally or developmentally related traits are more highly associated or correlated than traits that are not related. A set of functionally or developmentally related traits make up a morphological unit. We expect relatively high correlations between traits within a morphological unit, and relatively low correlations between traits that are not within a morphological unit (Olson and Miller, 1958; Cheverud, 1982, 1989, 1995, 1996; Cheverud et al., 1989).

In this paper, we examine the intercorrelation and morphological integration of craniofacial and dental traits in a sample of Australian aborigines. We test whether the observed patterns of correlation in Australian aborigines is similar to that observed in the Caucasians. Finally, we compare the observed morphological integration in Australian aborigines to that observed in a sample of Caucasians.

MATERIALS AND METHODS

The data for this study consist of a subsample of Australian Aborigines recruited for a study of growth and morphology of Aborigines living in Yeundumu in the Northern Territory of Australia between 1961 and 1971 (Barrett et al., 1965; Brown and Barrett, 1971, 1972; Brown, 1976; Townsend and Brown, 1979). The subsample of individuals between 18 and 35 years of age included 40 males and 66 females. The sample includes data from full- and half-siblings, and is part of ongoing genetic analyses of morphological integration. Morphological integration in these data are compared to the level of morphological integration in Caucasian twins, reported elsewhere in this volume (Kohn

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and Osborne, 1999). Briefly, this sample consists of 70 MZ and 60 DZ twin pairs recruited from New York City area between 1952 and 1956 (Osborne et al., 1958; Horowitz et al., 1958; Osborne and DeGeorge, 1959; Osborne et al., 1999), and 9 MZ and 24 DZ twin pairs recruited from University of Wisconsin students in 1968. All Caucasian twins were between 18 and 65 years of age.

The craniofacial data for this study include: 1) head length; 2) head breadth; 3) head circumference; 4) bizygomatic breadth; 5) bigonial breadth; 6) total facial height. The dental measurements used in this study include: 1) maxillary and mandibular right and left central incisor mesiodistal diameter; 2) maxillary and mandibular right and left lateral incisor mesiodistal diameter; 3) maxillary and mandibular right and left canine mesiodistal diameter.

Males were larger than females for all traits. To enlarge sample sizes, all data were corrected for differences due to gender prior to further analysis. The mean ($\overline{X}$) and standard deviation (σ) of each trait in females (F) was arithmetically adjusted to equal that of males (M) using the formula

$$\mathbf{X}_{Fi} = (\mathbf{X}_{Fi} - \overline{X}_F)(\sigma_M/\sigma_F) + \overline{X}_M$$

where X_i refers to an individual female's measurement of a trait. This sex-adjusted data was used in all further analyses.

The methods for estimating morphological integration are also discussed elsewhere in this volume (Kohn and Osborne, 1999). As in the previous study, six morphological units were defined, including 1) craniofacial unit, including all craniofacial dimensions; 2) dental unit, including all dental dimensions; 3) maxillary unit, including all maxillary dental dimensions; 4) mandibular unit, including all mandibular dental dimensions; 5) incisor unit, including all incisor dimensions; 6) canine unit, including all canine dimensions.

Pearson product-moment correlations were calculated between all traits. The expectation is that traits that are closely associated will be highly correlated, while traits that have little association can be expected to show a low correlation.

In testing for significant integration of these morphological units, we test whether traits within a morphological unit, for example within the dental unit, are more highly correlated with each other than they are with other traits and more highly correlated than the other traits are with each other (Cheverud, 1989, Cheverud et al., 1989). To test for significant morphological integration, we calculate a matrix correlation between observed correlations among traits and a "hypothesis matrix" which contains the value 1 for the matrix elements of traits within a functional unit, and 0 for the matrix elements of traits not within a functional unit. The significance of this integration is tested by a nonparametric permutation test in which a permuted distribution of correlations is generated. A significant result is one in which the observed correlation between the correlation matrix and the hypothesis matrix is greater than or less than 95% of the permuted correlations. These analyses of morphological integration used 1000 permutations to estimate significance.

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RESULTS

The average correlation of craniofacial traits with tooth size in the Australian aborigine populations is presented in Table 1. In general, the correlations of dental traits with craniofacial traits is low (average correlation is 0.18, range -0.07 to 0.41), however, the level of correlation is higher than that observed in the Caucasian twin populations. There are three significant correlations of dental traits with head length (left maxillary canine, right mandibular canine, and left mandibular canine), two significant correlations of dental traits with head circumference (left mandibular lateral incisor, and left mandibular canine), and one significant correlation of dental traits with total facial height (left maxillary canine). The remaining traits do not have significant correlations between them and the dental dimensions. As was observed in the Caucasian twin populations, the dental dimensions were always highly correlated (average 0.57, range 0.22 to 0.92).

Table 1. Average correlation of cranial dimensions with tooth dimensions in Australian Aborigine data.

Average	Correlation
	Tooth Size
Head Length	0.25
Head Breadth	0.07
Head Circumference	0.26
Bizygomatic Breadth	0.11
Bigonial Breadth	0.12
Total Facial height	0.23

The average correlation of traits within morphological units, the average correlation of traits not within a morphological unit, the test of morphological integration and the probability of integration are presented in Table 2. The craniofacial dimensions are not highly integrated at the P = 0.05 level. The average correlation with the craniofacial unit are slightly more correlated than traits not within the unit. We obtain comparable results when cranial vault and facial traits are analyzed separately. The level of correlation among cranial vault or craniofacial traits is not high. The other morphological units within the dental complex are significantly integrated at the P=0.05 level. For each morphological unit, the average bivariate correlation within the morphological unit are much higher than the average correlation between remaining traits. These results are comparable to those observed with Caucasian data presented elsewhere (Kohn and Osborne, 1999).

Table 2. Test of morphological integration (MI), average correlation within a morphological unit (AVGIN), average correlation of traits not within a morphological unit (AVGOUT) and associated probability of integration (P).

	MI	AVGIN	AVGOUT	P
Craniofacial	0.05	0.40	0.37	0.28
Dental	0.75	0.57	0.21	0.001
Maxillary	0.16	0.61	0.34	0.12
Mandibular	0.34	0.59	0.35	0.02
Incisor	0.47	0.63	0.32	0.005
Canine	0.34	0.77	0.35	0.001

We observe the similarity of patterns of correlations in Caucasians and in Australian aborigines. The matrix correlation of the Caucasian twin bivariate correlations and the Australian aborigine bivariate correlations is 0.82. The observed patterns of correlation are significantly similar between the two populations. That is, traits that are more highly correlated in Caucasian twins are also more highly correlated in Australian aborigines.

DISCUSSION

The dental morphological units are all significantly integrated, and their average correlations are higher than those of the anthropometric measurements of the head and face for both American Caucasian twins and Australian Aborigines. The highest within morphological unit correlation is for canines. In his adaptation of Butler's (1939) "dental field" concept, Dahlberg hypothesized that canines had been the most stable tooth throughout human evolution (Dahlberg, 1949). The results from this study are compatible with Dahlberg's hypothesis, especially with canines showing high correlations with craniofacial dimensions.

The anthropometric measurements of facial and cranial dimensions used in this study include more than a single growth center and therefore must encompass more than a single morphological unit. Few, if any, smaller anthropometrically measured cranial or facial dimensions will totally escape this problem. Modern two- and three-dimensional anthropometric measurement devices may provide a way to measure smaller traits within smaller growth regions (Kohn et al., 1995)

Despite genetic differences between these populations, including differences in genes, allele frequencies, dominance deviations and epistasis, similar morphological patterns are observed in Caucasian twins and Australian aborigines. In both populations, craniofacial and dental morphology appear to be relatively independent. The dimensions in the craniofacial complex are not highly correlated and integrated, while the dimensions within the dental complex are significantly integrated.

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Associations between Spinal Morphology and Dental Occlusion

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INTRODUCTION

Natural head posture has for a quite long time been of great interest to orthodontists. Solow and Tallgren (1976) found a connection between head posture and craniofacial morphology in adult males. The vertical jaw relationship was associated with the position of the head in relation to the cervical column and the sagittal jaw relationship had some weak correlations with the craniocervical angulation. However, the same authors, in a later study including the same sample (Solow and Tallgren, 1977) did not find any statistically significant associations between dentoalveolar morphology in terms of alveolar prognathism, incisor inclination and overjet and craniocervical posture.

Even though a widely used orthodontic textbook points out the importance of the body posture for optimal development of the dental occlusion (Hotz, 1974) this has not been focused on in later research. In a study by Gresham and Smithells (1954) on the relationship between neck posture and occlusion in children, those with a ”poor neck posture” manifested as increased lordosis of the cervical spine, were found to have Angle Class II occlusion more frequently than children with good posture. Later, Huggare and Harkness (1993) did a cephalometric investigation concluding that a backward bend of the upper part of the spine was associated with postnormal occlusion and Solow and Sonnesen (1998) reported increased anterior dental crowding associated with a large craniocervical angle.

The aim of the present study was to examine dental intra- and interarch relationship and their associations with morphology of the vertebral column.

SUBJECTS AND METHODS

The material comprised 122 children including 65 boys and 57 girls divided into three age groups of 8, 11 and 15 years of age, respectively. They were of normal health and free from signs or symptoms of back pain or any apparent deviation or malformation of the spinal column. They showed a variety of malocclusions but had not received any orthodontic treatment. The sample is the same as reported in two earlier studies made by Hellsing et al. (1987 a,b).

Lordosis of the cervical spine was measured on the lateral skull radiographs in natural head and body posture. A line (CVT) was drawn from a tangent point located at the superior posterior extremity of the odontoid process of the second cervical vertebra (cv2tg) to the most inferioposterior point on the body of the fourth cervical vertebra (cv4ip). Another line (EVT) was drawn from cv4ip to the most inferio-posterior point on

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the body of the sixth cervical vertebra (cv6ip). Lordosis (CVT/EVT) was defined as the downward opening angle between these two lines. The odontoid line (OPT) connected cv2tg and the most inferio-posterior point on the body of the second cervical vertebra (cv2ip). The inclinations of CVT, OPT and EVT lines were also measured as downward opening angles in relation to a true vertical (CVT/VER, OPT/ VER, EVT/ VER) using the convention, angles formed behind the true vertical (VER) are negative whereas angles formed in front are positive (Fig. 1).

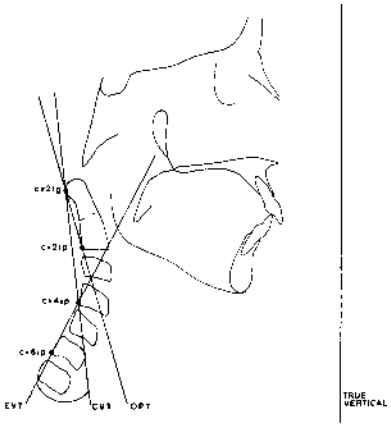


Fig. 1. Reference points and lines measured on lateral skull radiographs. (From Hellsing et al., 1987).

Kyphosis of the thoracic spine and lordosis of the lumbar spine were registered by an orthopaedic surgeon at the same time as the lateral skull examination was made. The degree of curvature was measured using a kyphometer. The curve of the thoracic column (KTPOS) was measured as the angle between thoracic vertebra 2-3 to thoracic vertebra 11-12. The curvature of the lumbar spine (KLPOS) was registered in a similar fashion as the angle between thoracic vertebra 11-12 to sacral vertebra 1-2 (Figs. 2-3).



Fig. 2. Kyphometer for measuring thoracic kyphosis (KTPOS; from Hellsing et al., 1987).



Fig. 3. Kyphometer for measuring lumbar lordosis (KLPOS; from Hellsing et al., 1987).

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Dental casts from upper and lower jaw were used together with a wax index from the intercuspal position. The casts were made of dental stone plaster and produced from impressions made by alginate.

The intermaxillary sagittal relationship was measured bilaterally as the distance between the most prominent parts on the mesial surfaces of the first permanent molars. The measurements were registered as 0 if the mesial surfaces were in a straight line to each other, negative if the occlusion was postnormal and positive if the occlusion was more normal or prenormal. Subjects missing one premolar, molar or more were excluded from these measurements.

The distance between the lingual surfaces of the canines (intercanine distance) and the lingual surfaces of the first permanent molars (intermolar distance) were measured in both upper and lower jaw. These measurements were made in the middle of the lingual part of the teeth and exactly at the level of the gingival margin. The distance between the most buccal part of the incisal papillae perpendicular to a line connecting the mesial surfaces of the first permanent molars was measured in the upper jaw (maxillary arch length). The distance between the approximal surfaces on the lower central incisors and the mesial surfaces on the first permanent molars was measured in the lower jaw (mandibular arch length). Overjet and overbite were measured according to conventional methods. The irregularity index described by Little (1975) was used to measure the anterior dental crowding in both upper and lower jaw but modified here by using the sum of the distances divided with the number of fully erupted front teeth.

The depth in the middle part of the hard palate was measured with a three-point calliper according to the method applied by Lundström (1948). Two measurements were made, one at the molar and another at the premolar region. These measurements were made with a precision of 0,5 millimetres. All the other measurements were made with a slide-calliper calibrated at a tenth of a millimetre. All measurements were made at least twice by the same examiner.

The children were divided into groups presenting Class I or Class II occlusion, based on the following criteria: Children aged 8 and 11 years old were classified as Class I if the distance between the mesial surfaces of the first permanent molars were ≥ 1 mm and as Class II if the distance was ≥ -1,5 mm. Children aged 15 years old were classified as Class I if the distance between the mesial parts of the first permanent molars was ≥ 1 mm and as Class II if the distance was ≥ -1 mm. Mean values of the left and the right side were used. Subjects who did not fit to these groups were excluded from this analysis.

For the statistical analyses Class I subjects were compared with Class II subjects by means of t-test for independent samples. Associations between postural variables depicting the curvature of the cervical, thoracal and lumbar parts of the spine and variables describing dental occlusion were evaluated using the whole sample by a multiple regression analysis considering age, gender and posture as independent variables.

RESULTS

Mean values, standard deviations and differences between boys and girls for the different age groups are presented in tables 1-3. In Class II subjects the upper dental arch was

narrower than in Class I subjects. This was statistically significant for the intermolar distance ($p<0.01$). Their cervical spine showed a more prominent lordosis (CVT/EVT; $p<0.05$), while their lumbar spine was more straight than in Class I subjects (KLPOS; $p< 0.01$; Table 4).

The multiple regression analysis revealed a strong association between the sagittal relation of the dental occlusion and the lumbar lordosis in that an accentuated lordosis was related to a less postnormal relation of the first permanent molars ($p<0.01$; Table 5).

Table 1. Number of subjects (N), mean values (X), standard deviations (SD) and differences (Diff) in spinal and dental variables between boys and girls 8-year of age.

	Boys			Girls			
	N	X	SD	N	X X	SD	Diff.
Cervical spine:							
CVT/EVT	19	17.5	5.85	18	16.4	10.09	1.1
Thoracal spine:							
KTPOS	21	25.7	5.98	18	25	7.28	0.7
Lumbar spine:							
KLPOS	21	29.5	6.10	18	28.6	5.64	0.9
Dental features:							
Sagittal molar relation	22	-0.2	1.36	18	0.3	1.45	-0.5
Overbite	23	1.2	2.79	18	1.6	2.25	-0.4
Overjet	23	4.1	2.29	18	3.7	1.98	0.4
Intercanine distance, max	23	22.5	3.01	16	23.6	1.32	-1.1
Intercanine distance, mand	22	19.6	1.80	14	20.6	1.10	-1.0
Intermolar distance, max	23	30.1	3.49	18	30.0	3.13	0.1
Intermolar distance, mand	20	31.9	2.10	16	30.9	2.23	1.0
Arch length, max	23	26.5	2.30	18	26.5	2.67	0
Arch length, mand	22	22.0	2.03	16	21.7	2.51	0.3
Palatal depth regio 6-5	22	12.0	1.52	18	11.8	1.87	0.2
Palatal depth regio 5-4	22	11.5	1.31	18	11.1	1.86	0.4
Irreg tooth index, max	22	0.9	0.51	16	1.0	0.65	-0.1
Irreg tooth index, mand	22	0.7	0.68	18	0.5	0.35	0.2

Table 2. Number of subjects' (N), mean values (X), standard deviations (SD) and differences (Diff) in spinal and dental variables between boys and girls 11-year of age.

	Boys			Girls			Diff.
	N	X	SD	N	X X	SD	
Cervical spine:							
CVT/EVT	17	14.1	9.18	21	9.4	8.26	4.7
Thoracal spine:							
KTPOS	22	30.9	5.90	22	25.9	5.26	5.0**
Lumbar spine:							
KLPOS	22	33.9	6.71	22	31.6	5.21	2.3
Dental features:							
Sagittal molar relation	22	0-4	1.57	22	0.5	1.66	-0.1
Overbite	22	3.0	1.01	22	2.4	2.29	0.6
Overjet	22	4.1	1.14	22	3.4	1.59	0.7
Intercanine distance, max	16	25.5	1.95	18	23.7	3.07	1.8
Intercanine distance, mand	14	21.7	1.58	19	20.8	2.10	0.9
Intermolar distance, max	22	34.0	2.30	22	32.4	1.89	1.6*
Intermolar distance, mand	22	34.1	1.95	22	31.4	1.83	2.7***
Arch length, max	22	25.9	2.49	22	25.5	2.34	0.4
Arch length, mand	22	22.0	2.29	22	21.4	2.19	0.6
Palatal depth regio 6-5	20	13.6	1.44	22	13.3	1.16	0.3
Palatal depth regio 5-4	21	10.9	1.48	22	11.0	1.35	-0.1
Irreg tooth index, max	22	0.7	0.60	22	0.6	0.29	0.1
Irreg tooth index, mand	21	0.3	0.23	21	0.4	0.33	-0.1

* p>0.05, ** p<0.01, *** p<0.001 by t-test

Table 3. Number of subjects'(N), mean values (X), standard deviations (SD) and differences (Diff) in spinal and dental variables between boys and girls 15-year of age.

	Boys			Girls			Diff.
	N	X	SD	N	X X	SD	
Cervical spine:							
CVT/EVT	16	8.9	7.11	14	1.7	5.90	1.65**
Thoracal spine:							
KTPOS	20	32.3	6.38	17	25.6	6.82	6.7**
Lumbar spine:							
KLPOS	20	31.8	5.2	17	33.1	7.09	-1.3
Dental features:							
Sagittal molar relation	18	1.3	1.48	16	0.8	2.12	0.5
Overbite	20	2.6	1.71	17	2.2	1.40	0.4
Overjet	20	3.1	1.44	17	4.0	1.96	-0.9
Intercanine distance, max	17	25.2	2.55	17	23.9	1.45	1.3
Intercanine distance, mand	19	20.4	1.96	17	19.9	2.53	0.5
Intermolar distance, max	19	32.7	3.98	17	32.6	2.27	0.1
Intermolar distance, mand	19	33.5	2.28	17	32.2	2.57	1.3
Arch length, max	19	23.6	4.00	17	23.9	3.66	-0.3
Arch length, mand	19	19.7	2.63	17	19.6	2.86	0.1
Palatal depth regio 6-5	20	14.8	2.29	16	15.3	2.98	-0.5
Palatal depth regio 5-4	19	12.1	2.79	16	12.1	3.02	0
Irreg tooth index, max	20	0.6	0.35	17	0.6	0.57	0
Irreg tooth index, mand	20	0.4	0.34	17	0.4	0.29	0

** p<0.01, by t-test

Table 4. Number of subjects (N), mean values (X), standard deviations (SD) and difference (Diff) in spinal and occlusal characteristics between Class I and Class II subjects.

	Class I			Class II			
	N	X	SD	N	X	SD	Diff
Cervical spine:							
CVT/EVT	43	10.8	8.83	16	16.5	11.63	-5.7*
CVT/VER	49	-2.9	7.85	19	-0.2	10.21	-3.1
OPT/VER	49	1.0	8.38	19	5.2	9.57	-4.2
EVT/VER	43	-13.7	6.28	17	-13.3	7.54	-0.4
Thoracal spine:							
KTPOS	50	28.2	7.05	19	25.5	6.65	2.7
Lumbar spine:							
KLPOS	50	32.7	6.32	19	27.6	6.74	5.1 **
Dental features:							
Overbite	50	1.9	1.85	19	3.0	2.16	-1.1
Overjet	50	3.2	1.25	19	4.9	2.20	-1.7***
Intercanine distance, max	42	24.5	2.78	15	23.3	2.32	1.2
Intercanine distance, mand	44	20.8	2.15	18	20.3	1.57	0.5
Intermolar distance, max	49	33.2	2.94	19	31.0	3.32	2.2**
Intermolar distance, mand	48	32.8	2.51	18	32.3	2.01	0.5
Arch length, max	49	25.9	3.04	19	24.4	2.34	1.5
Arch length, mand	49	20.8	2.33	18	21.7	2.09	-0.9
Palatal depth regio 6-5	46	14.0	2.35	19	13.2	2.35	0.8
Palatal depth regio 5-4	47	12.0	2.04	19	11.2	1.86	0.8
Irreg tooth index, max	48	0.6	0.36	19	0.8	0.48	-0.2
Irreg tooth index, mand	49	0.4	0.36	18	0.4	0.31	0

* p>0.05, ** p<0.01, *** p<0.001 by t-test

Table 5. Associations between sagittal molar relation and postural variables by multiple regression analysis for a sample of 112 children aged 8 to 15 years.

	Beta coeff:	Std. Error:	Std coeff:	t- Value:	Probability:
Independent variables:					
Age	0.11	0.07	0.19	1.73	0.87
Gender	0.10	0.32	0.03	0.3	0.77
Cervical lordosis (CVT/EVT)	0.002	0.02	0.01	0.09	0.93
Thoracal kyphosis (KTPOS)	-0.009	0.03	-0.04	0.32	0.75
Lumbar lordosis (KLPOS)	0.09	0.03	0.32	2.85	0.005

DISCUSSION

The subjects comprised 122 healthy children grouped according to sex and age. Since it was not possible to register the sixth cervical vertebra on the lateral skull radiographs of 13 children, calculations based on the EVT- line were limited to 112 subjects. Subjects with any sign of not being suitable for the different criteria when measuring the intra- and interarch relations were excluded from these particular measurements.

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All measurements on the dental casts were registered at least twice to make sure that they were made correct. If the two measurements showed a difference of 0,1 mm or more further two measurements were made until the values were within this limit.

The children were classified in groups presenting Class I or Class II occlusion. This classification was made using different criteria in the three age groups. Children aged 8 and 11 years old were classified as Class I if the distance between the mesial surfaces of the first permanent molars was 1 mm and in Class II if the distance was -1,5 mm. Children aged 15 years old were classified as Class I if the distance was 1 mm and in Class II if the distance was -1 mm. These criteria were made to take the effect of the Leeway space in the two younger age groups, into consideration.

The irregularity index described by Little (1975) was modified in this study to compensate for the fact that the subjects showed different tooth eruption stages. By using the sum of the distances divided with the number of fully erupted front teeth almost the whole sample could be used.

The results from a study by Gresham and Smithells (1954) concluding that children with a ”poor neck posture” defined as a pronounced cervical lordosis had a postnormal occlusion more often than children with a normal neck posture are confirmed in this study. Subjects with a postnormal occlusion showed a tendency for the cervical part (CVT/EVT) of the spine to be more curved ($p< 0.05$). On the other hand, our result is not in agreement with that of Solow and Tallgren (1977). However, their interarch relationship measurement was based on measuring overjet on roentgen cephalograms while ours bases on a strict classification made after measuring the sagittal relation of the first permanent molars. Another reason for the discordance could be the difference in the age of our sample as Hellsing et al., (1987 b) showed that the cervical lordosis decreases with increasing age, especially in girls. The material described by Solow and Tallgren (1977) comprised only young adult males.

In a study comprising New-Zealand youngsters in need of orthodontic treatment Huggare and Harkness (1993) observed that subjects with postnormal dental occlusion have a backward inclination of the odontoid process of the second cervical vertebra. However, their study does not provide information about the position of the rest of the spine, but it is plausible to assume that a backward pointed odontoid process is associated with a more curved rather than with a straight spine. As we also found a more curved spine in the postnormal subjects these studies are in accordance.

The present study was made to correlate the sagittal relation also to the thoracic and lumbar curvatures. We found that a less prominent lumbar lordosis was associated with a more postnormal occlusion. We are not able to provide any explanation for this unexpected result but it is in line with a report by Hirschfelder and Hirschfelder (1997) who found that persons with a flat back have an increased tendency to have postnormal occlusion. Hellsing et al. (1987 a,b) reported on the associations between the cervical, thoracic and lumbar spine curvatures and found a significant correlation between lumbar lordosis (KLPOS) and the odontoid line related to the true vertical (OPT/VER). A more backward pointed odontoid process was associated with less lordosis. It was presumed that this could reflect a more subtle mechanism for maintaining good postural balance but could also be of a purely random nature. Thus we suggest that our results regarding the relationship between lumbar lordosis and sagittal occlusion are a reflection of the postural balance of the total axial skeleton.

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In addition to an association between the sagittal molar relation and lumbar lordosis we found that Class II children had a narrow upper dental arch. This is in accordance with the observations made by Varrela (1992) and Erälinna-Kari et al (1996). They suggest that the early development of Class II occlusion is characterised by impaired transverse growth of the maxillary dental arch.

These results indicate that there is a connection between dental variables and morphology of the vertical column that can be of great importance in the assessment of a patient from a holistic point of view. Not only head posture is interesting as we have shown that even the anatomy of the lower spine seems correlated with sagittal occlusion.

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Pre- and Postnatal Skull and Dentition Development: Computer-Animated Visualisation

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INTRODUCTION

The understanding of different aspects of anatomy (Tillmann 1997), morphogenesis, (Moore 1977, Carlson 1984, Kitamura 1989, Hinrichsen 1990) growth and bone remodelling (Gantz, 1922; Diewert, 1985; Berkovitz et al., 1989; Enlow, 1990; Kjaer, 1990; Radlanski et al., 1996, 1997; Neumann et al., 1997) prenatal (O'Rahilly, 1956; Trenouth, 1979; Radlanski et al., 1994; Berndt, 1997) and postnatal cephalometrics (Van der Linden, 1971; Rakosi, 1989) is facilitated by presenting them in virtual reality. In that way the regions involved and the timing of development become more obvious. The same holds true for the complicated process of development of dentition (Van der Linden and Duterloo, 1976; Van der Linden, 1983).

To generate 3-dimensional pictures on the computer screen a set of digitized data is needed, mostly by computed tomography (Neumann et al., 1997; Virapongse and Shapiro, 1985) or by optical scanning (Landsee et al., 1997; Van der Linden et al., 1998). In this paper we report about the making of a fully computer-generated animation- and morphing sequence of human craniofacial skeletal growth and development of the dentition.

MATERIALS AND METHODS

The skull material consisted of 10 prenatal fetal heads, ranging from the 12th to the 30th week, obtained from legal abortions and were fixated in 10% formalin. In addition, 4 prenatal macerated and 8 postnatal macerated human skulls, ranging from birth to adult stage were available. All specimens represented normal anatomical conditions, and were digitized by computed tomography.

Computed tomography was carried out with a commercially available CT scanner (Somatom Plus S, Siemens/Erlangen). A series of contiguous horizontal sections was taken at a distance of 1 mm between each section, using spiral technique. The tube charge per scan ranged between 165 - 210 mAS at a voltage of 120kV. Image data of all specimens were transferred to a workstation (DRC - Siemens / Erlangen) to generate 3D-

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reconstructions by using the installed software (MagicView 1102). The threshold value for bone ranged from 105 - 135 HU (Hounsfield Units). Hard copies were gained with a Kodak Ectascan laser printer.

The data were changed into a format readable by soft image (Microsoft). To this end the data were copied on optical disk and transferred using a special software programmed by mental images (Berlin). After all of the skull material was available as sets of polygone data, artifacts had to be eliminated by manual interaction. They mainly occurred because of the difficulty in setting the correct threshold for bone (Neumann et al., 1997) all over the skull. Radiographs of the fetal material and the original macerated skulls served as controls. For additional control cephalometric data of fetal skull development (Berndt, 1997) and 3D reconstructions from histological serial sections of selected areas of fetal skull development (Brandt, 1997, van Straelen, 1997) were used.

The digitized dentition was generated from a set of enlarged deciduous and permanent ideal plaster teeth of the collection Van der Linden. These plaster teeth of twice the normal size were scanned optically and polygone data sets consisting initially of ca. 30.000 polygones per tooth were produced (Landsee et al., 1997, Van der Linden et al., 1998). All data processing (image composition, selection of point of view, surface rendering) was performed on a DEC Alpha Workstation computer, using the software Soft Image (Microsoft).

RESULTS

The polygone data sets of the single skulls of different stages from the 3rd month p.c. (125 mm CRL) up to the adult stage were used to produce a morphing sequence showing the growth of the skull (Figs 1-5). During morphing, the skulls were rotated in order to show maximum information.

The complete deciduous and permanent teeth were produced as a virtual dentition (Figs 6, 7). With this dentition any type of dental relation and occlusal situation could be produced (Fig. 8-10). In addition, a morphing sequence, showing the development of the deciduous dentition (Fig. 9), root resorption caused by the developing permanent dentition, and replacement of the deciduous teeth by the teeth of the permanent dentition (Fig. 10) was shown.

The proportional increment of the visceral cranium with the associated vertical development of the dentition becomes obvious during the final sequences of the animation (Figs 3-5).

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Fig. 1. Craniofacial skeleton of a human fetus at the stage of 3 months post conception, frontal view. Surface rendered polygone data set.
Fig. 2. Craniofacial skeleton of a human fetus at the stage of 6 months post conception, frontal view. Surface rendered polygone data set.
Fig. 3. Human craniofacial skeleton at the stage of 6 months after birth, frontal view. Surface rendered polygone data set.
Fig. 4. Human craniofacial skeleton 6 years after birth, frontal view. Surface rendered polygone data set. Berlin
Fig. 5. Human craniofacial skeleton, adult stage, frontal view. Surface rendered polygone data set.

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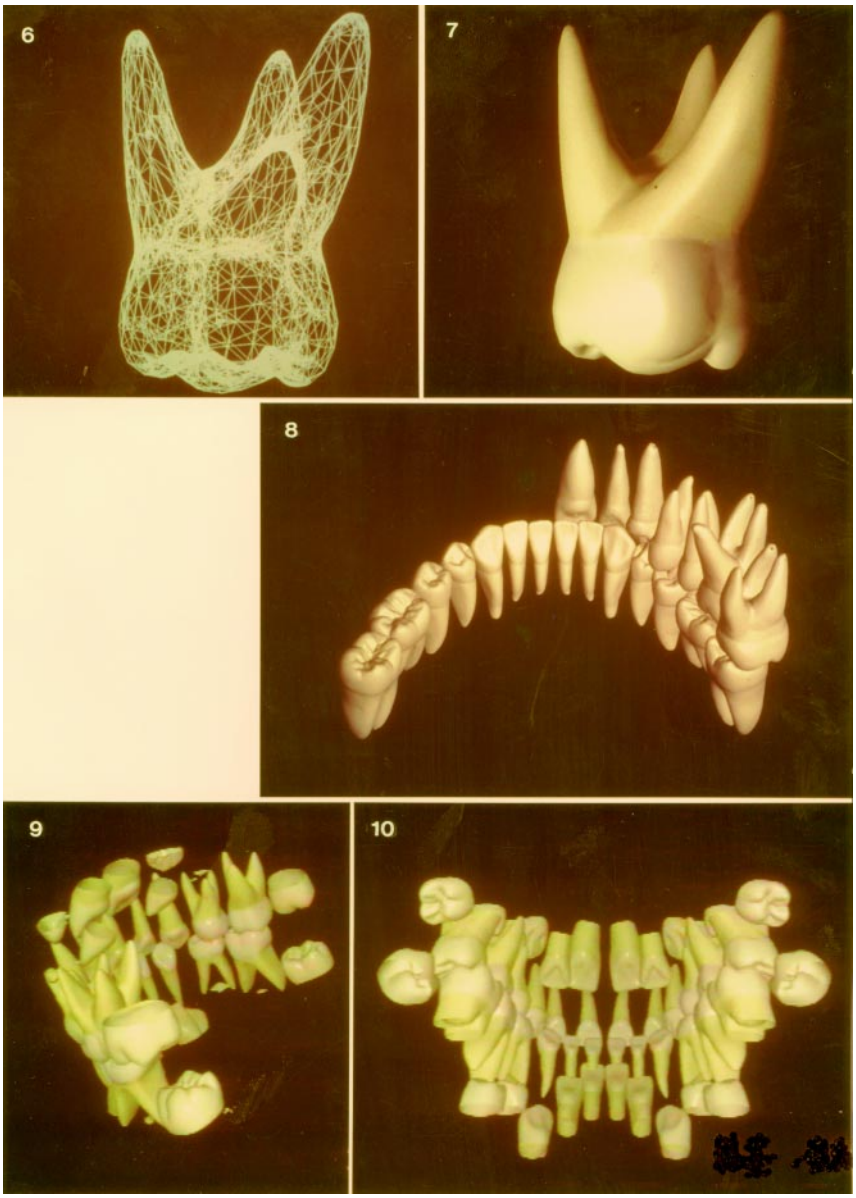


Fig. 6. Polygone data set of an upper right molar on the computer screen, mesial aspect.
Fig. 7. The same polygone data set, surface rendered.
Fig. 8. Arrangement of the permanent human digitized dentition, posterior and cranial view, all third molars and the teeth of the 2nd quadrant omitted. Surface rendered polygone data set.
Fig. 9. Mixed dentition (deciduous and permanent) at the stage of 3 years and 6 months, oblique lateral, dorsal and cranial view. Surface rendered polygone data set.
Fig. 10. Mixed dentition (deciduous and permanent) at the stage of 6 years, oblique dorsal and caudal view. Surface rendered polygone data set.

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DISCUSSION

Gaining insight in morphological changes during prenatal development is difficult, because it can not be watched directly. The same holds true for the development of dentition which is naturally covered by bone and soft tissues.

A video sequence showing computer-animated virtual images based on research data, is a helpful teaching tool (Dahlmann et al. 1994), however, the plot runs only linear, and the velocity of narration is fixed. So in the very near future interactive operation on the personal computer (Schanda, 1995; Research Systems, 1995) can be expected to offer the possibility to navigate through the data stored on the DVD-CD-ROM. In that medium the user can select knowledge of his own choice, rotate the images and observe them from any point of view.

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The Structure of Dentin in the Hooded Seal *(Cystaphora Cristata)*

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INTRODUCTION

The pinnipedes bear many features indicative of a relationship to the carnivora. They formerly were placed in a sub-order of the carnivora, but the trend today is to give them the status of a separate order. The hooded seal *(cystaphora cristata*) is mainly found in the arctic sea. The dentition consists of single-rooted teeth with crowns of simple morphology adapted to a sea-food diet (Colyer, 1990). The deciduous dentition is non-functional and tends to be shed soon after birth; in some seals it does not erupt at all and is resorbed *in utero* (Colyer, 1990). Dentin and pulp constitute the main part of the teeth. The most characteristic microscopic feature of the dentin is evenly spaced dentinal tubules 2-5μm in diameter. Each tubule encloses a cytoplasmic process of an odontoblast. The odontoblast cell body is located in the pulp where it lines the pulpal surface of the dentin in a stratified or pseudostratified layer. In the present study, as part of a comparative investigation, the structure of dentin from the teeth of the hooded seal was studied, since information on this subject is scarce. Particular emphasis was placed on the inorganic phase.

MATERIALS AND METHODS

The material consisted of 3 animals and in addition 3 skulls were examined. The animals were perfused with 0.9 % NaCl followed by 2 % glutaraldehyde at pH 7.3 entering through the carotid artery. After 30 min of perfusion, the tissues were fixed for an additional period of 48 h by immersion in the fixative before processing for light microscopy, microradiography (MR), scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Epon 812 or Vestopal W was used as embedding media. Ground sections parallel to the long axis of the teeth were made. Contact microradiographs were produced on Kodak Spectroscopic plates with a Philips X-ray diffraction unit. For light microscopic viewing 1 μm thick sections were cut and stained with toluidine blue.

Based on light microscopic and microradiographic findings, selected areas were processed for TEM. Ultrathin sections were cut with a diamond knife and collected on formvar- and carbon-coated grids. The sections were examined unstained in a Philips 400T electron microscope operated at 80 kV. In order to study the dentinal tubules in the SEM, some teeth were fractured, dehydrated, critical point dried by CO_2, and

sputtercoated with a 30 nm layer of gold-palladium. The specimens were observed in a Philips SEM 515 microscope operated at 10-15 kV. Some teeth were decalcified in nitric acid, embedded in paraffin, sectioned and stained with hematoxylin/eosin.

RESULTS

The number of teeth was 16 in the upper jaw and 14 in the lower jaw. A small central and a large lateral incisor were noted, followed by a large canine, four premolars and one molar (Figs. 1 and 2). There was little distinction between premolars and molars. The crowns of the premolars and molars were conical with one main cusp and several shallow folds in the enamel surface, while the crowns of the incisors and canines had a smoother enamel surface. An inspection of radiographs and ground sections in transmitted light showed a thin enamel layer and a wide pulp chamber.



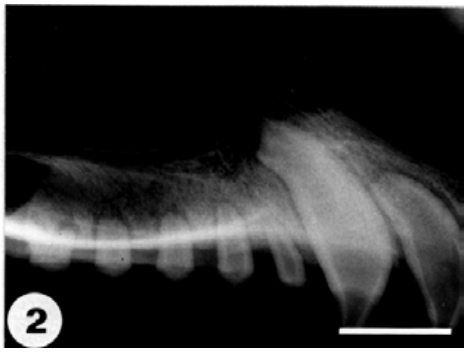


Fig. 1. Photograph of skull from the hooded seal showing the teeth and parts of the maxilla and the mandible. The number of teeth are 16 in the upper jaw and 14 in the lower jaw. A small central and a large lateral are noted, followed by a large canine, four premolars and one molar. The lower jaw has only one incisor. The incisor and the molar of the lower jaw are missing from their sockets in this skull. Bar = 1 cm.
Fig. 2. Radiograph of the upper left jaw showing one molar and four premolars followed by a large canine and lateral incisor. All teeth have a wide pulp chamber. Bar = 1 cm.

Microradiography and light microscopy

The dentin underlying the enamel usually had a lower mineral content than the rest of the dentin (Figs. 3 and 4) and the dentin immediately subjacent to the enamel folds in the premolars was often almost devoid of mineral (Fig. 4). Sometimes large tubules of varying length were also observed (Fig. 4). In several specimens a highly mineralized streak extending from an area close to the dentino-enamel junction to the pulp horn was seen subjacent to the main cusp (Fig. 3). This streak was sometimes divided by a radiolucent slit. Zones with varying mineral content at approximately right angles to the direction of the dentinal tubules were often seen in the dentin. These zones were oriented parallel to the walls of the pulp chamber and their width varied (Figs. 3 to 5). An attempt to measure the distance between the highly mineralized zones was made in areas where they appeared distinct. Exact numbers were difficult to state, but several measurements indicated a distance of 20-50 μm between the highly mineralized zones. In the crown this

pattern of mineralization was most pronounced in the pulpal half of the dentin, while in the root zones of varying mineral content were seen throughout most of the dentin, particularly in the apical area (Figs. 3 and 5). Interglobular dentin was noted in the microradiographs (Fig. 4) as well as in the hematoxylin/eosin stained sections, particularly in the cuspal areas.

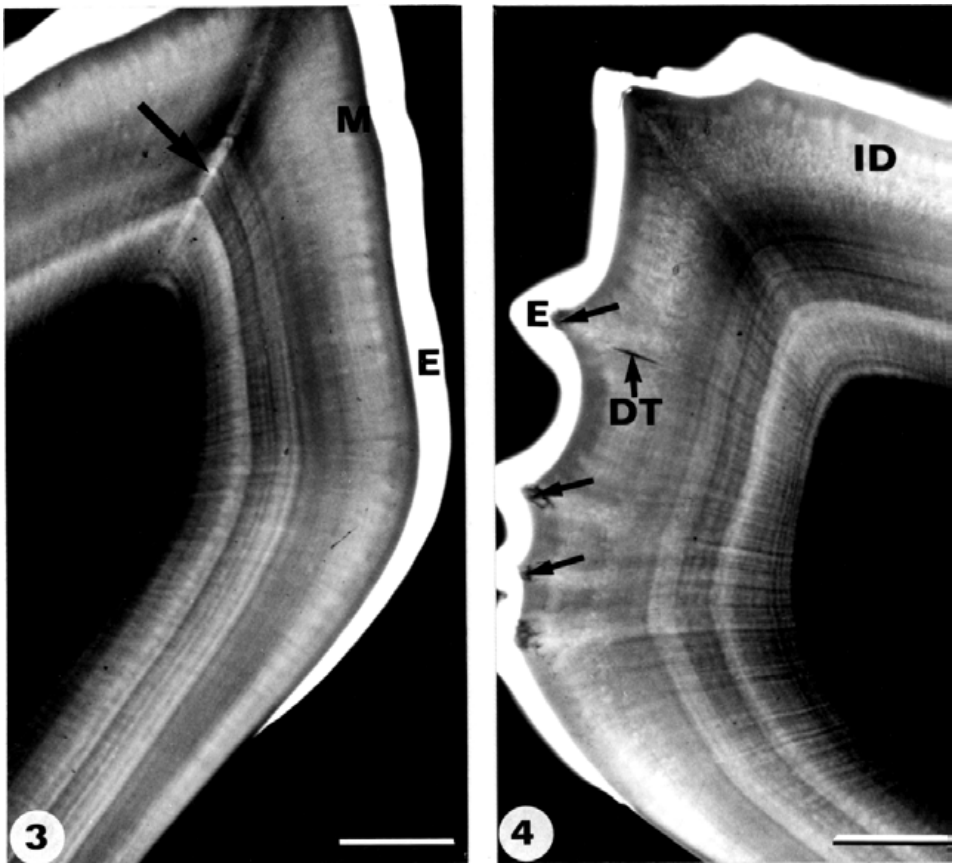


Fig. 3. Microradiograph of ground section from upper third premolar showing dentin with varying degrees of mineralization. The mantle dentin (M) shows a lower degree of mineralization than the rest of the dentin. In the pulpal half of the circumpulpal dentin a zonated pattern of mineralization can be noted. A highly mineralized streak extending from an area close to the dentino-enamel junction to the pulp horn can be noted subjacent to the main cusp (arrow). Enamel (E). x 30. Bar = 0.5 mm.

Fig. 4. Microradiograph of ground section from upper fourth premolar showing zonated pattern of mineralization parallel to the circumference of the pulp. The width of the zones varies. The dentin subjacent to some of the enamel folds (E) has a reduced mineral content (arrows). Interglobular dentin (ID) can be seen in some areas of the tooth. In this section one large dentinal tubule (DT) can be noted. x 30. Bar = 0.5 mm.

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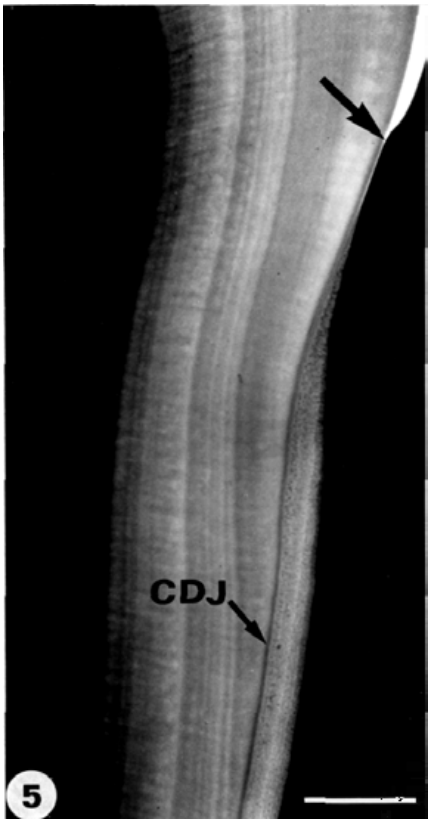


Fig. 5. Microradiograph of ground section from the root of upper third molar showing zonated pattern of mineralization. In the apical part of the root the zonated pattern extends from the cemento-dentinal junction (CDJ) to the pulp. Cemento-enamel junction (arrow). x 32. Bar = 0.5 mm.

Electron microscopy - TEM and SEM

In the peripheral part of the dentin in the root as well as in the crown, the tubules were narrow and located far apart, and a wide layer of peritubular dentin was noted (Fig. 6). Due to a high mineral content and a sparse organic matrix, artifactual cracks were seen in the peritubular dentin. Several cross-sectioned small tubules representing branches of the main tubules could be noted (Fig. 6). Tightly interwoven collagen fibers represent the main part of the organic matrix of the intertubular dentin, and the 64 nm cross-banding of the collagen fibers could be discerned in the mineralized tissue. Close to the pulp the tubules were wider, located closer together and peritubular dentin was usually absent (Fig. 7).

The branching of the tubules was most easily studied in the scanning electron microscope. Not only branches from the main tubules, but also subdivision of the branches was seen (Fig. 8). In the pulpal part of the dentin branching of the tubules was noted as well (Fig. 9), but the branching was not as extensive as in the peripheral part.

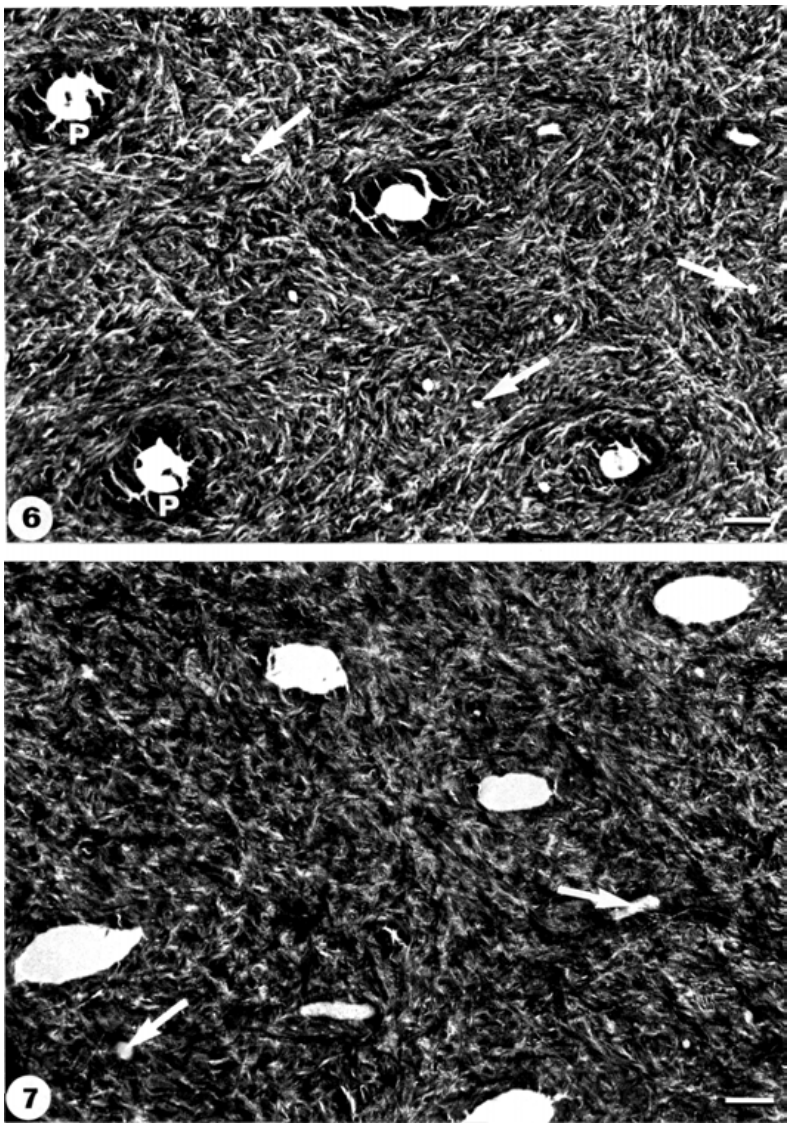


Fig. 6. Electron micrograph of lower canine showing cross-sectioned dentinal tubules from the peripheral part of crown dentin. The tubules are surrounded by a wide zone of highly mineralized peritubular dentin (P). Due to a high mineral content and a sparse organic matrix, several artifactual cracks are seen in the peritubular dentin. Several small cross-sectioned branches can also be noted (arrows). Tightly interwoven collagen fibers represent the main part of the organic matrix of the intertubular dentin, and the 64 nm cross-banding of the collagen fibrils can be seen even after mineralization has taken place. x 6000. Bar = 1 μm.

Fig. 7. Electron micrograph of upper lateral showing cross-sectioned dentinal tubules from the pulpal part of crown dentin. The tubules are evenly distributed, and no peritubular dentin is present in this area. Some cross-sectioned branches from the main tubule can be noted (arrows). x 6000. Bar = 1 μm.

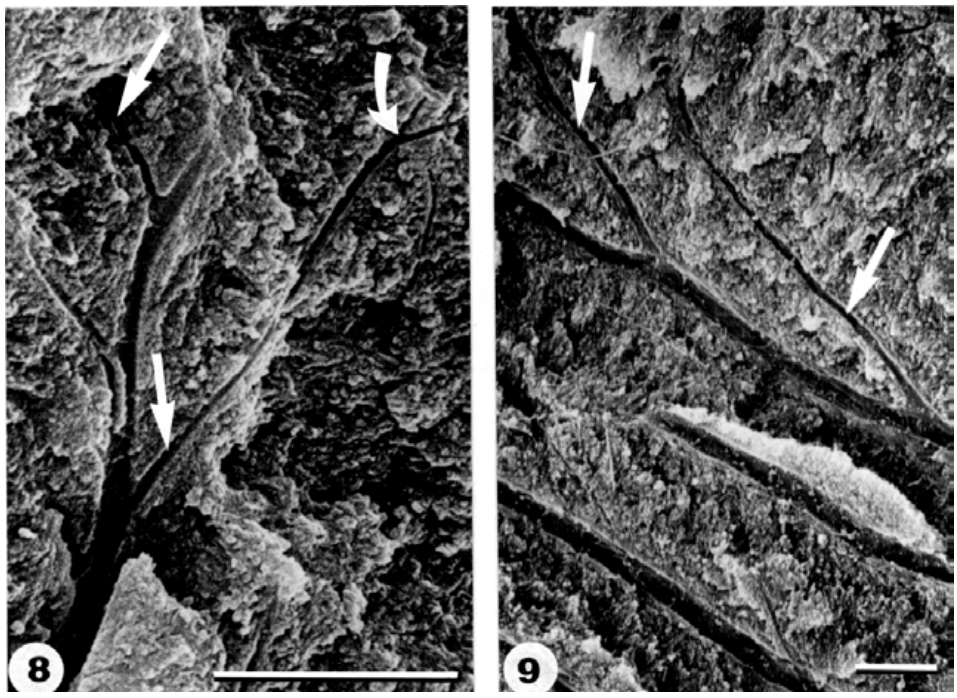


Fig. 8. Scanning electron micrograph showing longitudinally fractured dentinal tubules from the peripheral part of the dentin in lower second premolar. Several branches (arrows) from the main tubule (T) can be noted. A subdivision of one of the branches (curved arrow) can also be seen. x 6300. Bar = 5 μm.

Fig. 9. Scanning electron micrograph showing longitudinally fractured dentinal tubules from the pulpal part of lower first premolar. Two lateral branches (arrows) from the main tubule can be noted. The tubules are wider than in the peripheral part of the dentin. x 2700. Bar = 5 μm.

DISCUSSION

The number of teeth observed in the present study was in accordance with the dental formula, even if the dentition of the seals shows a tendency to reduction in number (Coyler, 1990). The low mineral content observed in the mantle dentin of the seal is in accordance with observations in human teeth (Tronstad, 1972; 1973; Mjör, 1986). The low mineral content observed in the dentin subjacent to the enamel folds, however, does not have a parallel in human teeth. The highly mineralized streak extending from an area close to the dentino-enamel junction to the pulp horn has also been observed in human teeth, and the variation in the degree of mineralization is believed to be due to a higher mineral content in the intertubular dentin (Tronstad, 1972). Large tubules, like the ones observed in the present study, have been described in human coronal dentin (Hals,1983a; Agematsu et al., 1997), in red deer (Hals, 1983b) and is a regularly occurring feature in bovine incisors (Dyngeland and Fosse, 1985). The tubules have been found to communicate with the pulp.

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The zones with varying mineral content were more distinct in some areas than in others and their width also varied, and this may be related to their orientation relative to the plane of sectioning. The zones had a course roughly parallel to the outer surface of the dentin and the walls of the pulp chamber, and are believed to represent incremental lines formed during dentinogenesis. Their course differs from the direction of incremental lines in primary dentin in humans, particularly in the crown, but would be more in accordance with incremental lines in regular secondary dentin. Alternating bands of high and low mineral content have also been observed in human cementum (Furseth and Johansen, 1968). These bands corresponded to incremental lines and were considered to represent alternating periods of activity and quiescence during cementogenesis and thus be a result of biological rhythms.

The daily rate of dentin formation shows a considerable variation ranging from 2-16 μm. The amount varies with the species, the tooth type and the time and stage of formation (Schour and Hoffmann 1939). The daily rate of primary dentin formation in humans has been found to be 4-8 μm a day (Massler and Schour, 1946), and an average daily increment of 6 μm dentin was observed in mouse molars in primary as well as reparative dentinogenesis (Atkinson, 1976). In the rodent incisor the 16 μm increments represent the amount of dentin formed daily (Schour and Hoffmann, 1939). There is some confusion in the literature concerning the nomenclature of incremental lines in dentin, but according to Kawasaki et al.(1980) the incremental lines in human dentin called von Ebner's lines are about 20 μm apart, and within these 20 μm bands 5 further subdivisions approximately 4 μm wide are found. Incremental lines show the rate of mineralization since they are only found in mineralized dentin (Symons, 1967). However, even if matrix production and mineralization are separate processes which can be disassociated experimentally, the rate of mineralization and matrix deposition are the same under normal conditions, even if mineralization is slightly behind the actual deposition of the matrix (Schour and Hoffmann, 1939). The mineralization pattern is therefore a reliable indicator of the growth pattern. In the present study the distance between the highly mineralized zones was found to be approximately 20-50 μm and this is compatible with the distance between the incremental lines of von Ebner. If the daily dentin formation is estimated to be 5 μm daily, the highly mineralized zones would appear every 4-10 days and might represent accentuated incremental lines. This has a parallel in enamel where the daily rate are reflected in the cross-striations and the incremental lines of Retzius represent longer time intervals. The number of cross-striations between the lines is commonly in the range of 7-9 suggesting a rythmicity in enamel formation at an interval of a week or slightly more (Vollrath et al., 1975; Boyde, 1989). The structural explanation for the appearance of the striae of Retzius are highly debatable and several explanations have been suggested. An increase in mineral content, however, are not one of them. The stage of tooth development at the time of birth of the animals is unknown, but one could speculate that the dentin with pronounced highly mineralized incremental lines observed in the pulpal half of the crown dentin and most of the root dentin, was formed after birth. This also has a parallel in enamel formation where incremental lines are virtually absent in prenatal enamel (Fejerskov and Thylstrup, 1986). It has been suggested that this is due to more constant living conditions *in utero*.

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The distribution of highly mineralized peritubular dentin varies in different mammals. In the elephant molar peritubular dentin may form in advance of the mineralization front in intertubular dentin, while it forms synchronously with the mineralization front in the intertubular dentin in the cat and dog (carnivora) and in the cow, sheep and pig (artiodactyla) (Boyde, 1971). In other mammals, including the pulpally located coronal dentin in man (Takuma, 1967), the peritubular dentin forms at some distance from the mineralization front of the intertubular dentin and this was also mostly found to be the case in the dentin of the hooded seal. According to Boyde (1971) the degree of side-branching of the dentinal tubules and the frequency with which these side-branches anastomose is very variable among different mammalian orders. In the present study numerous branches of the dentinal tubules were observed in the root as well as in the crown dentin, and this agrees with previous findings in human material (Furseth, 1971; Jones and Boyde, 1984; Moss-Salentijn and Hendricks-Klyvert, 1990; Mjör and Nordahl, 1996).

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Light-Microscopical Investigations of the Connective Tissue and Calcifications in Human Dental Pulps During Ageing

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INTRODUCTION

The classification of the pulp fibres is based on staining and morphological characteristics, but the nature of the fibrillar materials in the dental pulp remains contentious. Both, type I and III collagen account for the bulk of the tissue collagen (Shuttleworth et al., 1978; 1980). Type III collagen is often found together with type I. However, type III consists of fibrils of much smaller diameter (Lapiere et al., 1977). In human pulp, type III collagen constitutes a large proportion (43 %) of the total collagen (van Amerongen et al., 1983). On the other hand, in cell culture experiments human dental pulp cells produced predominantly (99 %) type I collagen and only trace amounts of type III collagen (Kuo et al., 1992). Collagen type III may contribute to the elasticity of the tissues (Shuttleworth et al., 1980). In contrast, type I collagen is thought to stabilize the tissue architecture (Narayanan and Page, 1976; 1983).

Little data are known about the localization and function of type V and VI collagens in human dental pulps. Type V collagen, found in virtually all organs, seems to play a major role in the formation of granulation tissue (Kurita et al., 1985) and of scars in chronic inflammatory diseases (Narayanan and Engel, 1983). Type VI collagen microfibrils isolated from the dental pulp of fetal calves show long, thin, flexible filaments with a characteristic periodicity of about 100 nm (Shuttleworth et al., 1992). Probably it serves as a flexible anchor interconnecting collagen fibres (Bruns et al., 1986) and collagen fibres with cells (Bonaldo et al., 1990). It has a wide distribution in the connective tissue similar to that observed for type I and III collagens. Collagen fibrils and fibronectin could be identified between the odontoblasts and seem to be part of the von Korff fibres (Higashi and Okamoto, 1996; Whittaker and Adams, 1972; Yoshiba et al., 1994).

Calcified structures like pulp stones and diffuse calcifications may occur in one or all of the teeth of one person, even in unerupted or impacted teeth. A controversy remains whether they should be considered physiological or pathological (Moss-Salentijn and Hendricks-Klyvert, 1988).

In the present study, we investigated the distributional changes of collagen types I, III, V, and VI and of calcifications in the pulp tissue of noncarious human teeth with respect to the age of the donors. The histomorphology of these structures is described by means of different light microscopical techniques.

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MATERIALS AND METHODS

Teeth and age relationships

Most human teeth (332) were molars. They were collected over a period of 3.5 years. The teeth were either noncarious and unfilled or revealed only very small carious defects or minimal restorations. The age of the patients ranged from 11 to 72 years, and 3 age groups were constituted: group I: 10 to 30 years; group II: 31 to 51 years; group III: 52 to 72 years. Informed consent was given prior to surgery.

Histological examinations

Undecalcified teeth were hemisected by a sawing machine and fixed in 4.0 % paraformaldehyde and 0.1 % glutaraldehyde. Tissues were dehydrated through graded glycol methacrylate (GMA) under vacuum at 4^{o} C and embedded in Technovit 7200 VLC (Kulzer, Wehrheim, Germany). Thin slices of about 60 µm were produced, stained with toluidin blue and mounted in Eukitt (Donath and Breuner, 1982; Hillmann et al., 1991).

For paraffin histology teeth were decalcified after hemisectioning and fixation. Routine paraffin sections (5 µm) were prepared and stained with hematoxylin-eosin or Picrosirius Red (Junqueira et al., 1979, 1982; Montes et al., 1980). Sections were examined in transmitted and polarized light in a Nikon light microscope.

For the immunohistological investigation of the collagens type I, III, V, and VI paraffin sections of 46 teeth were examined. In order to enhance the antigenicity of the macromolecules, paraffin sections were prepared with the "Antigen Retrieval System" (Bio Genex, CA, USA). Sections were pre-incubated with non-immune mouse serum and were incubated with primary antibodies for indirect immunohistological characterization. Commercially available monoclonal antibodies (anti-collagen types I, III, IV, and V; Biochrom, Monosan, Berlin, Germany) and polyclonal antibodies (anti-collagen type VI; Serva, Heidelberg, Germany) were used. Their specificity was ascertained by the manufacturer by means of an ELISA. Secondary antibodies coupled with the fluorescent marker FITC were used. Sections were mounted in Mowiol (Dartsch, 1990) and were examined in a Nikon fluorescence microscope. Controls were prepared without using the primary antibodies. Specimens prepared by use of the "Antigen Retrieval System" were carefully compared to appropriate negative controls.

For examination in a confocal laser scanning microscope (CLSM) specimens were prepared in the same manner as for immunohistological investigations. After mounting in Mowiol, samples were examined with a BioRad MRC 600 CLSM equipped with an argon-krypton mixed gas laser using the Comos software (version 6.0; BioRad, München, Germany).

For the qualitative and quantitative histological evaluation 200 teeth were examined. The calcifications were sorted as to whether they were discrete pulp stones or diffuse calcifications. To prepare undecalcified ground sections 141 teeth were used while 59 teeth were decalcified and paraffin sections were prepared. In total, 282 ground sections and 354 paraffin sections were investigated (632 histological specimens). For the statistical analysis (Mantel-Haenszel-test, $p<0.05$) a tooth as rated positively for calcification, when one specimen revealed pulp stones or diffuse calcifications. Additionally, 132 teeth were used for qualitative histological investigations.

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RESULTS

Extracellular matrix. Light- and immunofluorescence microscopy revealed that types I, III, V, and VI collagens were present in all tissues at all ages examined.

Collagen type I. The connective tissue of the dental pulp stained very weakly. Thick fibre bundles, which appeared red in polarized light, were often intensely fluorescent. However, with advancing age thick fibre bundles of collagen type I increased in frequency and thickness (Fig. 1).

Collagen type III. The fibres showed a reticular network throughout the pulp. Thin filaments or small bundles of filaments could be observed. Blood vessels were surrounded by collagen type III. Fibres and bundles of fibres extended between the blood vessels and the connective tissue matrix. At sites of active secondary dentin formation a few specimens revealed a strong staining reaction in the predentin layer. The reticular pattern of fine fibrils disappeared with advancing age. The fibrils were replaced by thick fibre bundles (Fig. 2).

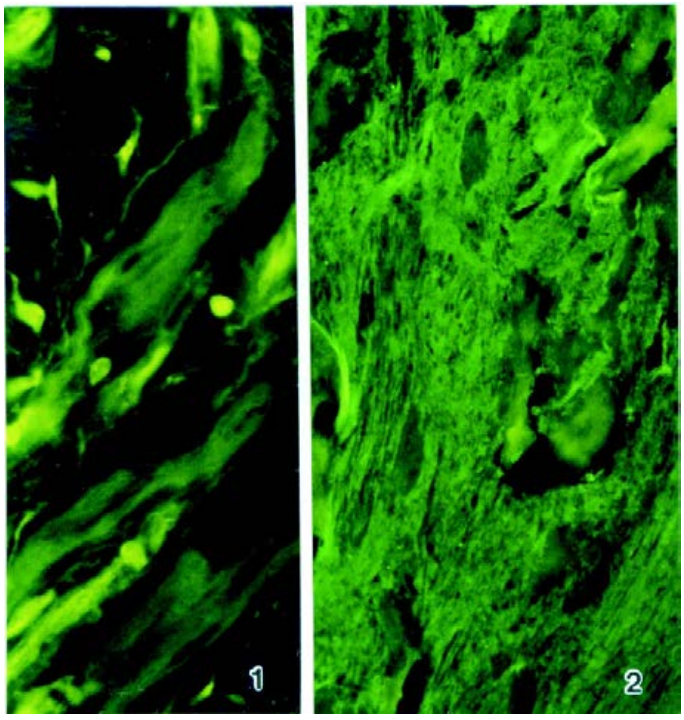


Fig. 1. Collagen type I. Thick fibre bundles increased in frequency with advancing age. 500x.
Fig. 2. Collagen type III. Thick fibre bundles increased in frequency with advancing age. 125x.

Collagen type V. The fibres showed an intricate microfibrillar pattern. They were arranged mainly in small fibre bundles. Blood vessels often were surrounded by concentric layers of fibrils of collagen type V. Adjacent to the layer of odontoblasts

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longitudinally arranged fibres could be observed. Some thin fibres of collagen type V extended between the odontoblasts. With advancing age small fibre bundles were replaced by thick fibre bundles.

Collagen type VI. With antibodies of type VI collagen, a microfibrillar, almost amorphous pattern was observed. Intense staining of blood vessels was noted frequently. In the odontoblast layer of the coronal region, fluorescence revealed corkscrew fibres passing from the pulp between odontoblasts. Some were also seen to penetrate into predentine. No fluorescence was detected in the calcified dentinal matrix. With advancing age collagen type VI revealed thick fibre bundles.

The reticular pattern of fine fibrils of collagen types III, V, and VI disappeared with advancing age and was replaced by thick fibre bundles. So the connective tissue matrix appeared to be condensed and stained homogeniously at higher ages (Table. 1).

Table 1. Immunostaining results.

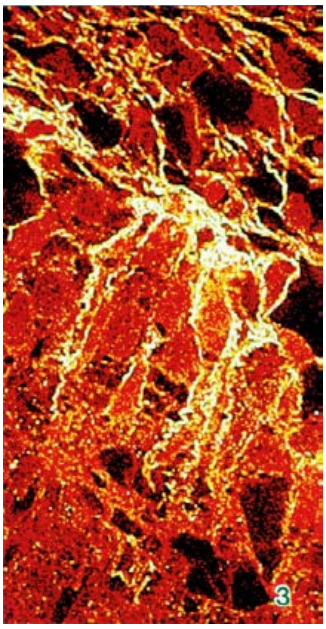
	Thin fibres	
Age (y)	Small fibre bundles	Thick fibre bundles
10 - 30	+ / +	- / -
31 - 51	+ / -	+ / -
52 - 72	+ / -	+ / +

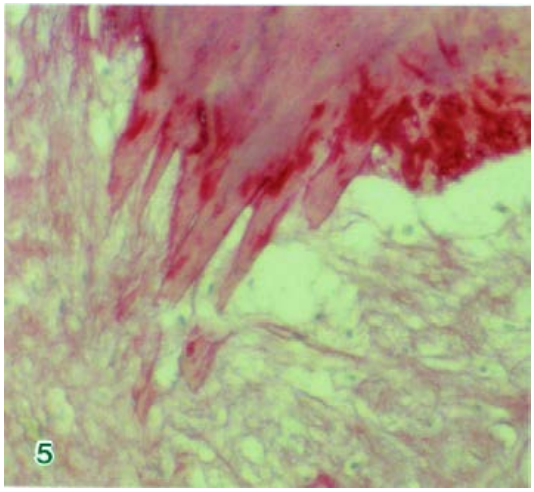
Comparison of immunostaining results using primary antibodies to various extracellular macromolecules (collagen types I, III, V, and VI) at different ages.
Scoring system: +/+= intense staining; +/-=moderate staining; -/-=weak staining.

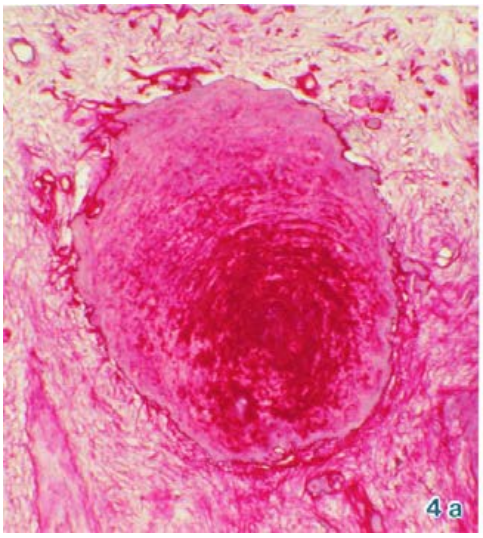
Confocal laser scanning microscopy

Fibres of type VI collagen were observed between the odontoblasts, appearing as corkscrew fibres passing from the pulp into predentin parallel to the long axis of the cells. In the coronal and in the radicular region a similiar distribution of collagen type VI was observed (Fig. 3).

Calcifications. Simultaneously to the condensation of the macromolecules of the connective tissue, pulp stones, and diffuse calcifications increased in size with age. Pulp stones in the coronal part of the pulp of decalcified teeth revealed the presence of different collagen types, which were distributed in concentric layers or in a diffuse manner. Sometimes pulp stones could be identified, which showed different arrangements of collagen bundles (Fig. 4a). In polarized light, the colors red and green revealed the participation of various collagen types (Fig. 4b). Thick fibre bundles of different collagen types inserted from the extracellular matrix into the calcified bodies (Fig. 5). Immunofluorescence microscopy showed a diffuse pattern of collagen type III in pulp stones (Fig. 6). Antibodies of collagen type VI revealed a diffuse distribution with weak staining intensities.







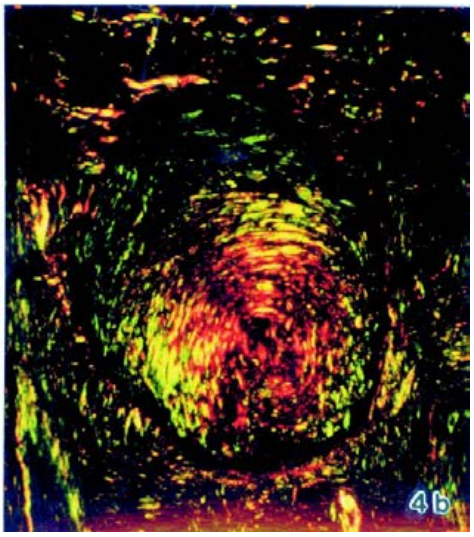


Fig. 3. Collagen type VI. These fibres passed corkscrew-like between the odontoblasts from the pulp into predentin. Confocal laser scanning microscopy. 800x.
Fig. 4. a. Pulp stones revealed different arrangements of collagen bundles. 125x.
Fig. 4. b. In polarized light, the colors red and green revealed the participation of various collagen types.125x.
Fig. 5. Thick fibre bundles of collagen inserted into the calcified bodies. 500x

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The investigation of undecalcified ground sections revealed pulp stones, which were characterized by different kinds of mineralization (Fig. 7). They showed areas of concentric and lamellar calcifications.

Diffuse calcifications could be observed in the root canal (Fig. 8). Needle like and cylindrical shaped calcified material appeared parallel to collagen fibres. Polarization and immunofluorescence microscopy revealed all types of collagens examined, which were equally distributed over the calcified areas.

The quantitative evaluation of the frequency of pulp stones, diffuse calcifications and both in the same tooth yielded different results for the different kinds of the calcifying processes. The number of teeth with diffuse calcifications and the number of teeth with pulp stones and diffuse calcifications increased with advancing age. However, the number of teeth with pulp stones in age groups II and III was comparable (Fig. 9). A statistically significant increase ($p<0.05$) of the quantity of coronal and radicular calcifications could be determined. *In group I* (11 to 30 years) there was a portion of 14.9 % of teeth with calcifications, in *group II* (31 to 51 years) 44.4 %, and in *group III* (52 to 72 years) 65.1 %.

Controls

Control sections were incubated with normal serum instead of the primary antibodies. They showed very limited background staining.

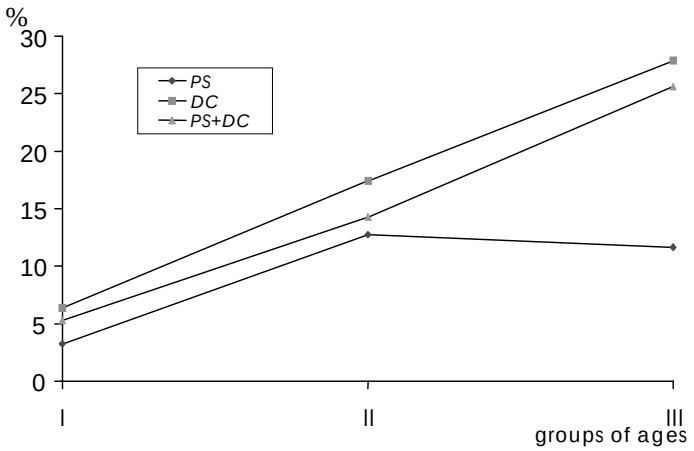


Fig. 9. Quantitative distribution of pulp stones and diffuse calcifications during ageing.

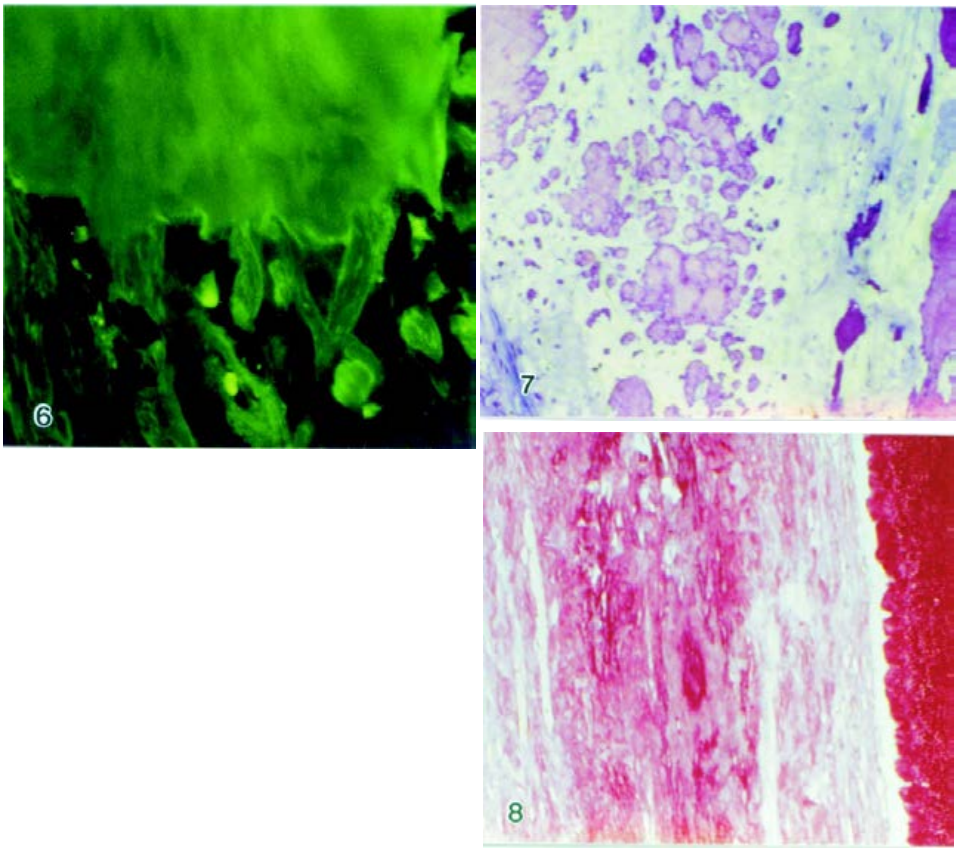


Fig. 6. Collagen type III was arranged in a diffuse pattern in pulp stones.500x.
Fig. 7. In undecalcified ground sections pulp stones showed areas of concentric and lamellar calcifications. 125x.
Fig. 8. In the root canal diffuse calcifications could be observed. 125x.

DISCUSSION

The distinction between pulp calcifications which arise due to caries or traumatic procedures and those which are a result of the process of ageing itself is problematic when pulp calcifications are investigated histologically. In order to exclude the influence of pathological processes, cariesfree teeth or teeth with small carious lesions or fillings only were investigated in our study. The Picrosirius Red staining method revealed the presence of various collagen types in the dental pulp and in the various kinds of calcifications. Polarization microscopy (Junqueira et al., 1978) revealed that collagen type I is the main component of the connective tissue matrix of the dental pulp. Collagen type III was found to be one of the main fibrillar elements. These results were be confirmed by immunohistological methods. Especially type I collagen is found mainly in thick fibrils, while type III collagen is present in thin fibrils in the liver, skin and tendon

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(Nowack et al., 1976) and is scattered throughout the tissue of the human dental pulp without being detected as fibrils passing between the odontoblasts (Magloire et al., 1982). However, investigations of the developing mouse molar revealed collagen type III as a major component of interodontoblastic fibres (Shroff and Thomas, 1992; Ohsaki and Nagata, 1994). The results of our study revealed the presence of collagen type III in the predentin layer but not as component of the interodontoblastic fibres. In correspondence with other authors we could show that types I and III collagen can co-exist in the same fibrils in the pulpal tissue of human teeth (Xu et al., 1992).

The fibres of collagens type V and VI appeared to be enriched in the subodontoblastic layer. Our investigation with CLSM revealed that the fibres of collagen type VI may be one component of the von Korff fibres. These results correspond to the biochemical investigation of Shuttleworth et al. (1992). Scanning- and transmission electronmicroscopical studies revealed the presence of interodontoblastic collagen fibres, which crossed the distal junctional complex of the odontoblasts and penetrated into and across the predentin (Bishop et al., 1991; Salomon et al., 1991; Tabata et al., 1994; Higashi and Okamoto, 1996). Our study showed that collagen types I, III, V, and VI are present in the human dental pulp throughout all ages examined.

The number of calcifications increases with advancing age (Sayegh and Reed, 1968), but the authors did not differentiate between pulp stones and diffuse calcifications. The continous growth of secondary dentin with advancing age and the increase of degenerative calcifications results in a reduction of the pulp cavity (Torneck, 1990).

The customary classification of calcifications includes a differentiation between true and false denticles (Kronfeld, 1933). In accordance with Moss-Salentijn and Hendricks-Klyvert (1988) this classification should no longer be used since most of the calcified structures in our study were composed of concentric collagen fibres and hydroxyapatite crystals. These findings corroborate with other observations (Appleton and Williams, 1973; Le May and Kaqueler, 1991). Our results demonstrate the participation of different collagen types, especially collagens type I, III, and VI, in the composition of pulp stones. The term "denticles" should only be used for calcified bodies, which are formed after an inductive interaction between the pulp tissue and intra-pulpal cell remnants of the Hertwig epithelial root sheath (Moss-Salentijn and Hendricks-Klyvert, 1988). The term "pulp stones" should be used for polymorphous and atypical calcifications located within the pulpal tissue which does not mineralize in normal conditions.

Since the number of calcifications increases with advancing age, structural changes of the organic matrix of the pulpal tissue might participate in the development of pulp stones. Calcifications may occur as a result of a local breakdown of mineralization-inhibitors like proteoglycan complexes (Moss-Salentijn and Hendricks-Klyvert, 1988).

Diffuse calcifications were observed mainly in the radicular area (Bernick, 1967). This type of calcification grows along organic macromolecules and in the periphery of blood vessels. These results correspond with our investigation which revealed different collagen types between the calcified structures in the root canal.

Noncarious teeth showed a smaller amount of calcifications than carious teeth (Sayegh and Reed, 1968). On the other hand, teeth without masticatory function (e.g. impacted molars) and teeth with a lifelong masticatory function may be distinguished (Hillmann et al., 1996). Our investigation revealed a continuous increase in the number of teeth with diffuse calcifications or with pulp stones and diffuse calcifications in noncarious teeth.

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However, the number of teeth with pulp stones only is nearly the same in age groups II and III. This results in a lower number of teeth with pulp stones only at advancing age because of the increase of diffuse calcifications in the root canal in the age group II (31 bis 51 years).

Biochemical investigations of humen teeth revealed a decrease in collagen concentration, which occurs at about the same time as eruption and root closure. But for the rest of the life of the tooth there is no change in collagen content (Nielsen et al., 1983). Our data suggest that all the pulp collagen, types I, III, V, and VI, remains in the pulp during ageing but is compressed into a smaller volume as the pulp cavity shrinks during dentinogenesis (Lechner and Kalnitsky, 1981). The increasing fibril density in the pulp during dentinogenesis confirms the theory of Stanley and Ranney (1962).

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DENTAL GENETICS

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Causal Components of Dental Variation: New Approaches Using Twins

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INTRODUCTION

Following major advances over the past decade in the detection of single genes associated with human diseases, researchers are now focussing on clarifying the etiology of complex multifactorial diseases and disorders where both genetic and environmental factors contribute to observed phenotypic variation. As Martin et al. (1997) point out, the study of twins has much to offer in unraveling developmental genetic mechanisms and helping to identify quantitative trait loci (QTLs) against a background of environmental and polygenic noise.

The use of a variety of twin designs, together with the application of biometrical genetic modelling procedures, is providing new insights into the ontogeny and evolution of the human dentition. Apart from applying the classical twin design, in which comparisons are made between monozygotic (MZ) and dizygotic (DZ) pairs, we have also been studying tooth size in opposite-sexed (OS) twin pairs who provide a natural experiment to test whether hormonal diffusion in utero influences dental development. Another approach is the so-called MZ co-twin analysis, in which comparisons are made of MZ twin pairs who are discordant for various dental features. Studies of twins reared apart (Boraas et al., 1988) and comparisons of the offspring of MZ twins, who are genetically half-siblings (Potter, 1990), can also clarify the influence of genetic and environmental factors on dental variability.

Application of genetic modelling approaches to twin and family data provides a number of advantages over previous methods that often led only to the calculation of broad estimates of heritability. When applied to MZ and DZ twin pairs, biometrical genetic modelling allows testing and estimation of additive genetic, non-additive genetic, common environmental, and unique environmental sources of variation. Hence, both broad and narrow-sense heritabilities can be generated. The process of modelling variances and covariances can be extended to model means as well. Effects such as genotype by environment interaction and a variety of causes of heterogeneity between the sexes can be assessed. If parental data are available, then assortative mating and genotype-environment correlation can also be tested (Neale and Cardon, 1992). Of particular interest is the application of multivariate analytic methods to twin data, thereby enabling the genetic basis of covariation between traits to be explored to clarify possible pleiotropic influences of genes on dento-facial development.

In this paper we present the results of recent studies of dental variation in a large sample of twins enrolled in an ongoing investigation at the University of Adelaide. The findings of univariate and multivariate analyses of permanent tooth size are presented, together with results of analyses of Carabelli trait expression in both deciduous and

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permanent dentitions. In addition, we present comparisons of tooth size between different twin zygosity groups, including opposite-sexed DZ pairs, and also consider MZ twin pairs who are discordant for missing or extra teeth. We discuss our findings in terms of phylogenetic and ontogenetic mechanisms.

MATERIALS AND METHODS

Over 500 pairs of twins are now enrolled in our ongoing investigation of dento-facial variation in Australian twins, together with over 150 other family members. Initially most of the twins examined were teenagers, with most or all of their permanent teeth present. More recently we have extended our study to include children with deciduous dentitions whom we intend to follow up at mixed and permanent dentition stages. A range of observations and records is obtained from subjects including direct oral examinations, dental impressions from which stone models are prepared, photographs of the face and dentition, finger- and palm-prints, as well as information relating to handedness, birthweight and length, and medical histories. Zygosities are confirmed either by comparison of genetic markers in the blood (Townsend and Martin, 1992) or by DNA analysis of buccal cells. The probability of monozygosity, given concordance for all systems analysed, is greater than 99%.

For the analysis of tooth size, maximum mesiodistal and buccolingual crown diameters of all permanent teeth except third molars were recorded from dental models of 596 subjects, including 149 MZ and 149 DZ twin pairs. The method of measurement and its reliability have been described previously (Dempsey et al., 1995). The software packages used for the genetic analysis were PRELIS (Jöreskog and Sörbom, 1986) for the calculation of the variance-covariance matrices, and Mx (Neale, 1995) for the modelling. Various models were fitted to the data to estimate the contributions of additive genetic variation (A), non-additive genetic variation (D) that incorporates both dominance and epistatic interaction variance, common environment (C), and unique environment (E). For the analysis of tooth size in OS DZ twins, permanent crown size dimensions of females from 56 OS DZ twin pairs were compared with those of NonOS females, derived from 83 MZ twins, 49 same-sexed DZ twins and 75 singletons.

Carabelli trait was scored on deciduous maxillary second molars and permanent maxillary first molars using dental models of 1,038 individuals, representing 245 pairs of MZ twins and 274 pairs of DZ twins. The method of Dahlberg (1963) was used to classify Carabelli trait on an eight-grade scale, ranging from absence through seven grades of presence, including single grooves and pits, double and Y-shaped grooves and cusps of increasing size. Comparisons of assessments made for all subjects on two separate occasions confirmed that the trait could be classified with acceptable reliability, agreement ranging from 84%-89%. Polychoric correlations, based on the assumption of an underlying continuous distribution for Carabelli trait expression, were calculated between MZ and DZ twin pairs for both deciduous and permanent dentitions to extend our previous findings (Townsend and Martin, 1992).

Amongst our sample of twins we have noted several instances of MZ twin pairs who display varying expressions of normal, small, peg-shaped or missing maxillary lateral incisors (Townsend et al., 1995). Recently a pair of MZ twin boys have come to our

attention who display supernumerary teeth in the maxillary incisor region, one with a single supernumerary the other with two, providing further opportunity to reflect on the interplay between genes and environment during odontogenesis.

RESULTS

Tooth size - univariate genetic analysis

For the univariate analyses, the simplest model that adequately accounted for the variation observed in the 56 variables was an AE model, that is, one that included additive genetic and unique environmental effects. However, significant improvements in fit were achieved by including non-additive genetic variation (D) for the mesiodistal dimension of the four canines, both of the maxillary first premolars and the mandibular right first premolar, as well as the buccolingual diameter of the maxillary right first premolar. Improvement of fit was also observed when common environment (C) was included in the analyses of mesiodistal and buccolingual dimensions of maxillary first molars.

Additive genetic effects (A), so-called narrow-sense heritability, contributed to more than 80% of observed variation for most dental dimensions, ranging from 59% for the mesiodistal dimension of maxillary first molars to 92% for the buccolingual dimension of maxillary second premolars. Common environment averaged 26% for mesiodistal and 24% for buccolingual dimensions of maxillary first molars. All estimates were similar for right and left dimensions of corresponding teeth.

Tooth size - multivariate genetic analyses

Multivariate analyses performed on the mesiodistal dimensions of the eight incisors, and also on the mesiodistal and buccolingual dimensions of the seven teeth in the maxillary right quadrant, yielded similar estimates of additive genetic variance to those from the univariate analyses. In addition, significant non-additive genetic variation (D) was detected in all maxillary teeth, being centred on the mesiodistal dimensions of the canine and the two neighbouring teeth. Values ranged from 14% to 55% for mesiodistal dimensions and from 7% to 15% for buccolingual dimensions. Common environment was found to be present for the mesiodistal dimension of posterior teeth, and buccolingual dimension of first and second molars, with the strongest effects being displayed by the first premolar and first molar.

Surprisingly, multivariate factors mostly incorporated all teeth examined, or individual teeth and their antimeres. There were very few factors, genetic or environmental, which simultaneously covered all members of a tooth group, be it incisor, premolar, or molar. This would seem to contradict predictions of Butler’s field theory, and Osborne’s clone theory. Genetic and environmental factors appeared to affect the general size of all teeth, or of individual teeth and their antimeres.

Tooth size in OS DZ twins

Table 1 compares permanent mesiodistal crown size dimensions of females from 56 OS DZ twin pairs with those of 207 NonOS females. Although our sample sizes were not

large enough to provide the statistical power to disclose significant differences in univariate comparisons of OS and NonOS female means, all 14 differences between means were positive, indicating that the teeth of OS females were larger generally than those of NonOS females. A similar pattern was observed for buccolingual dimensions, 12 of 14 mean values being larger in females from OS DZ twin pairs. The distribution of positive and negative values was not as expected if there was no systematic difference between OS and NonOS female means, with the sign test for this being highly significant ($p<0.001$). The effect was smallest for maxillary canine. Conversely, there was no significant difference in males, with 16 of the 28 means larger in OS males. Multivariate analyses (MANOVAs), taking into account the inter-correlations between tooth dimensions, were significant, confirming the finding.

Table 1. Statistics for permanent mesiodistal crown size (in mm) in Non-OS and OS DZ females.

	NonOS Females			OS DZ Females		
Tooth	n	Mean	SE	n	Mean	SE
Maxilla						
I1	206	8.47	.04	56	8.67	.08
I2	200	6.56	.04	55	6.61	.08
C	197	7.51	.03	47	7.54	.06
P1	188	6.83	.03	46	6.89	.06
P2	198	6.58	.03	46	6.64	.06
M1	201	10.17	.04	56	10.30	.07
M2	158	9.81	.05	39	10.06	.10
Mandible						
I1	207	5.26	.02	56	5.33	.06
I2	206	5.81	.03	56	5.94	.05
C	203	6.51	.02	54	6.66	.05
P1	199	6.94	.03	50	7.03	.06
P2	197	7.02	.03	47	7.13	.07
M1	203	10.78	.04	54	10.84	.09
M2	151	10.27	.05	40	10.42	.10

Carabelli trait

The degree of expression of Carabelli trait in deciduous and permanent molars of each sex is detailed in Table 2. Although eight grades of Carabelli trait expression were recorded and used for generation of polychoric correlations, only three summary categories are displayed in the tables - absent, concavity and convexity. Around 98% of all subjects showed evidence of the trait in some form in the deciduous dentition, with 16%-18% exhibiting cusps of various sizes. In the permanent dentition, fewer subjects (84%-90%) showed the trait, 32%-39% with some form of cusp. The deciduous dentition showed no evidence of sexual dimorphism, unlike the permanent dentition, in which males had significantly greater expressions of Carabelli trait on the right side ($p<0.05$).

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Table 2. Expression of Carabelli trait in Australian twins.

		Grade of Expression[1]			Sex Difference	
Sex	n	Absent	Concavity	Convexity	χ^2 (2df)	Prob
			Deciduous second molar			
M	245	2	82	16		
F	251	2	80	18	0.62	0.74
			Permanent first molar			
M	324	10	51	39		
F	332	16	53	32	6.35	0.04

[1] percentages for right side

Table 3. Expression of Carabelli trait in the deciduous and permanent dentitions (right side) of the same individuals.

		dm2			
		Absent	Concavity	Convexity	Total
M1	Absent	4	11	0	15
	Concavity	1	75	7	83
	Convexity	0	50	15	65
	Total	5	136	22	163

$\chi^2 = 39.2$ (4 df), $p<0.05$

Table 3 gives data for Carabelli trait in the deciduous and permanent dentitions of the same individuals. Initial analyses of the data for males and females disclosed similar patterns, so combined results are given. There was a strong association between the dentitions for trait expression - if the trait was expressed in the permanent dentition, it was almost always present in the deciduous dentition. The opposite was also true generally, but with a greater number of exceptions.

Polychoric correlation patterns across zygosities were very similar in deciduous and permanent dentitions (Table 4), with values for MZ twins being considerably higher than those for DZ twins. MZ twin correlations, ranging from 0.80-0.92, were very similar to those of right-left comparisons that ranged from 0.78-0.93. For DZ twins, correlations ranged from 0.27-0.50, and were generally higher in DS DZ twins (0.41-0.45) than same-sexed DZ twins (0.27-0.50).

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Table 4. Polychoric correlations (x100) for Carabelli trait in the deciduous and permanent dentitions of twins.

	n[1]	Right vs. Left	Twin 1 vs. Twin 2
	Deciduous second molar		
F MZ	49	93	92
M MZ	46	87	80
F DZ	41	81	50
M DZ	41	91	28
OS DZ	41	78	41
	Permanent first molar		
F MZ	77	88	87
M MZ	71	89	90
F DZ	43	91	31
M DZ	46	85	27
OS DZ	57	91	45

[1]n = number of pairs

MZ twin pair with supernumerary teeth

A pair of nine-year-old twin boys, determined to be monozygotic by DNA analysis of buccal cells, was found to have unerupted supernumerary teeth present. Radiographs disclosed a single, palatally positioned mesiodens in one boy, and two mesiodentes in a similar position in the other. This is an example of an MZ co-twin design in which two individuals assumed to be genetically identical, display a difference in phenotype.

DISCUSSION

Partitioning permanent tooth size variation using Mx disclosed new evidence of non-additive genetic variation centred around the canines and common environmental effects on the maxillary molars. It has been suggested that non-additive genetic variation is displayed by genes that are related to selective fitness (Fisher, 1958; Kacser and Burns, 1981). Therefore the presence of this type of variation may indicate selective pressures acting either currently or sometime in the past. The canine, which shows the most sexual dimorphism in humans, is considered to be a particularly important tooth in evolutionary terms, so it is not surprising that it, along with the adjacent first premolar, shows the strongest evidence for non-additive genetic variation. The presence of common environmental variation in both diameters of the maxillary first molars is also interesting as one would expect these teeth to be the most likely members of the permanent dentition to show signs of uterine environmental effects due to their early formation and calcification.

It is proposed that diffusion of sex hormones from male to female co-twins in utero may account for increased tooth size in females from OS DZ twin pairs. In rodents, the position of a foetus in utero has been shown to be associated with the expression of sexually dimorphic traits and this phenomenon has been explained by prenatal diffusion of sex hormones among litter-mates (Vom Saal, 1989; Miller, 1994), however our finding is one of the first of such an effect for a morphological variable in humans. The fact that

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the maxillary canine, one of the most sexually dimorphic teeth in humans, exhibited the least effect in OS female twins, suggests that prenatal sex hormone levels may have less impact on sexual dimorphism of this tooth than others. The canine seems to be resilient in the presence of severe sex chromosomal anomalies (Townsend et al., 1986) and our analyses in twins suggest that the same is true in the presence of sex hormones. Perhaps there is a mechanism for control of canine development that is independent of, or maybe in addition to, hormonal and sex chromosomal control.

Our findings in relation to Carabelli trait are consistent with a previous study of Australian Aborigines in which significant sexual dimorphism for Carabelli trait was noted in the permanent dentition (Townsend and Brown, 1981). However, several other workers have failed to find evidence of sexual dimorphism in the trait (e.g., Garn et al., 1966; Kieser, 1984) and it seems that sexual dimorphism varies between populations.

Only one example was noted where Carabelli trait was present in the permanent dentition but not in the deciduous dentition. A similar relationship has been reported in Indian children (Joshi, 1975), and Australian Aborigines (Townsend and Brown, 1981). This finding is consistent with Dahlberg's (1963) assertion that the trait will be present on the deciduous teeth if it is present on the permanent. Dahlberg (1950) interpreted this finding in phylogenetic terms, suggesting that primitive characters tend to be retained by the conservative deciduous dentition even when the permanent first molar has lost them. Another explanation for this phenomenon is that genotype may be expressed more faithfully in the deciduous than the permanent dentition (Townsend and Brown, 1981), reduced penetrance in the permanent dentition resulting from environmental disturbances operating during the longer period of development of the permanent teeth.

Correlations between DZ twin pairs varied from around 0.3-0.5, with values between same-sexed pairs, particularly in the permanent dentition, tending to be less than half the corresponding MZ values. This pattern of correlations suggests a possible non-additive genetic contribution to variation, either due to interaction of alleles at the same locus (dominance) or different loci (epistasis). A similar trend was also noted in our earlier study of Carabelli trait in twins (Townsend and Martin, 1992), suggesting that a more extensive model-fitting approach to analysing both deciduous and permanent Carabelli trait data is warranted.

The discovery of a pair of MZ twins discordant for number of mesiodentes, provides an opportunity to consider possible influence of genetic factors on dental development. The occurrence of the supernumerary teeth in both twins suggests a genetic contribution to their presence, while environmental factors would explain the difference in number. Our finding contrasts that of Seddon et al. (1997) who noted that differences in shape and orientation of mesiodentes were common between MZ co-twins but that they were likely to be concordant with respect to number of supernumerary teeth.

We have suggested previously that missing, peg-shaped and small maxillary lateral incisors in twins appear to be related phenomena, being associated with overall tooth size (Townsend et al., 1995). Brook (1984) has proposed a multifactorial model, combining polygenic and environmental influences, that links agenesis and supernumerary teeth with overall tooth size. The model is consistent with observations of greater frequencies of dental agenesis in females whose teeth are smaller on average than those of males. In addition, males exhibit greater frequencies of supernumerary teeth. Brook et al. (1998) have recently reported that tooth size in individuals with supernumerary teeth tends to be

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larger than those of controls. We believe that it is likely that the MZ twins whom we have observed to be discordant either for agenesis of teeth or for supernumeraries may have genotypes that place them near to either the upper or lower thresholds of Brook's model, although the crown sizes of emerged teeth in the pair of MZ twin boys described in this paper were close to the mean values for the entire sample. Further studies of MZ twins discordant for tooth number should help to clarify this possibility.

Molecular genetic studies are now demonstrating how homeobox genes and various signalling molecules and growth factors influence odontogenesis and pattern formation within the dentition (Sharpe, 1995; Vastardis et al., 1996; Stock et al., 1997), however twin studies that apply modern genetic modelling approaches still have an important role in unravelling developmental genetic mechanisms. As Martin et al. (1997) point out, the aim is to determine to what extent combinations of genes pleiotropically influence groups of traits and to what extent there are genetic effects specific to traits. If appropriate models can be developed, the chances of actually detecting QTLs for say, extra or missing teeth, tooth size or even Carabelli trait, will be improved considerably. The next step after the QTLs have been detected by linkage analysis will be to locate and identify the gene products.

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The Effect of Sex and Race on the Symmetry of the Permanent Tooth Clinical Eruption

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INTRODUCTION

Tooth eruption symmetry has rarely been studied in populations and only a few of the contralateral tooth eruption comparisons have given emphasis in the discussions to antimeric differences. Tomes (1859) believed that teeth on the left side erupt earlier, while Bean (1914) was of the opinion that some teeth erupt earlier on the left and others on the right side. Cattel (1928) attempted to verify these facts but found asymmetry only in the maxillary first premolar. Although left-right dental asymmetry has been lately regarded as random (fluctuating) both dimensional asymmetry and temporal (eruption) asymmetry have been suggested as being structured (Garn et al., 1981). Sex and race differencies in eruption timing are well known. Girls are ahead 1 to 6 months in most studies, Caucasoids have later emergence when compared to that of Negroids and American Indians, but the degree of asymmetry in tooth eruption is not that well known (Steggerda and Hill, 1942; Hurme, 1949; Nanda, 1960).

The teeth provide an useful instrument for the study of symmetric/asymmetric development, due to their unique structure and their long period of sequential development. In bilaterally symmetrical structures, such as teeth, the antimeres should develop as mirror images because the genetic information is the same for both sides. Epigenetic processes and environmental factors affect the degree of phenotypic similarity, achieved by paired organs, and a "failure" in this system may result in fluctuating asymmetry, which is frequently used as a measure of individual developmental stability. The ability to buffer against environmental stresses varies by sex and race. Niswander and Chung (1965) note that, among Japanese children, females are more asymmetric than males for upper central incisors, but in a report of DiBennardo and Bailit (1978), the boys are reported to be more asymmetric than girls. Kieser and Groeneveld (1988) have noticed a high degree of asymmetry in South African blacks' dentition, probably due to their high disease and malnutrition level.

The regulation of tooth emergence and the mechanism of eruption have remained unknown in spite of wide research in this area. In 1969, Friedlander and Bailit suggested that their findings and those of Main (1966) and Glasstone (1967) could be interpreted as pointing to an early critical stage in tooth development after which most environmental influences cease to have any effect. This critical stage probably occurs prenatally in those teeth in which mineralization commences before or soon after birth, i.e. the first molars

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and permanent incisors. Many other important aspects of tooth eruption including those involved in the regulation of periodontal growth, blood supply and the innervation of the tooth follicle etc. during the long eruption process certainly cannot be excluded (Davidovitch and Mah, 1998).

We have suggested in earlier papers that the association of some gestational and postnatal variables, such as maternal smoking during pregnancy and reduced head circumference growth in the first years of life, are associated with accelerated eruption in mandibular central incisors and an increased proportion of symmetric eruption in mandibular lateral incisors (Heikkinen, 1996, 1998). Maternal smoking effects on the permanent dentition of a child are probably based on a sensitive time period of the affected tooth crown development reaching from middle gestation until birth, when most of the permanent teeth in sequence have already initiated their development, second premolars and molars and third molars not included. The sensitivity of each tooth in terms of size reduction, crown morphogenesis, asymmetry of antimeres etc. was associated with the sex and the race of the child, and with buffering mechanisms, such as the size of the fetus during critical, sensitive moments of the development (Heikkinen, 1996).

It has generally been assumed that tooth emergence is affected by the environment, such as socio-economic factors, as children from high socio-economic levels are known to have earlier emergence than children living under less favoured socio-economic circumstances, especially since the means of the differences between certain ethnic groups are no greater than those which have been shown to result from socioeconomic differences. The complexity of the interrelationships of race and socio-economic level is illustrated in different populations with respect to race differences in tooth emergence timing, with respect to the secular trend and earlier timing of emergence due to improved overall health and nutritional status. Lee *et al.* (1965), for example, found no significant differences in the time of emergence of the permanent teeth between three social levels among Hong Kong Chinese.

The aim of this study is to explore the effect of race and sex of the child on the symmetry of tooth eruption, and the patterns of that asymmetry/symmetry in various teeth in two populations, using cross-sectional and longitudinally recorded data.

MATERIAL AND METHODS

The U.S. dental study used here includes 2092 children from the approximately 60,000 pregnancies that comprised the Collaborative Perinatal Study of the National Institute of Neurological Disorders and Stroke (NINDS) carried out in the 1960's. The dental examinations were performed at six of the collaborating medical centres (Buffalo, NY; Richmond, VA; Portland, OR; Philadelphia, PA; Providence, RI; and Johns Hopkins, MD) in the early 1970's in a cross-sectional manner at ages varying in 95% of cases from 6.9 to 12.7 years. The mean ages in years at the time of the dental examinations (casts and photographs) were 7.9 for white boys, 7.8 for white girls, 8.9 for black boys and 9.3 for black girls, facilitating the comparison of representative amounts of erupting permanent incisors, first molars, canines, premolars and second molars.

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At each institution alginate impressions were taken and normal dental plaster casts then made as soon as practicable. The casts were checked and compared with oral photographs, taken of every child in study. Teeth with heavy attrition, decay and restorations etc. were not studied.

The eruption of permanent teeth was determined from normal dental casts by one observer (LA), a member of the Genetic-Odontometric Study team at the University of Wisconsin (1972-1974). Within each child the tooth antimeres were compared in terms of a 4-graded eruption scale, each tooth separately. The eruption stages were:

1. Tooth not erupted
2. Occlusal surface of tooth recently emerged
3. Tooth crown half erupted
4. Eruption of tooth complete or nearly complete

This portion of the lifetime clinical eruption for each tooth extends over a period of about one or two years. Each of these stages during this period are easily distinguishable clinically by an experienced observer. The reliability of stages was determined by performing duplicate blind determination on two hundred cases. Interobserver reproducibility (TH) was moderately good, average 85%. The greatest error, 24% of cases, occurred between determinations of stage 3 and stage 4 in the maxillary molars.

When forming the antimeric pairs on dental casts, the total data were used in order to reach the maximum number of antimeric pairs in spite of the biasing fact that occasionally different teeth can be unilaterally missing; in this series it was not possible to ascertain this from X-ray. Casts showing multiple erupting teeth were few, and those were accepted in spite of the presence of the same person many times in comparisons. The only requirement was that the pair, being either a symmetric or an asymmetric pair of contralateral teeth, must have at least one of the two teeth in the active eruption phase (stage 2 or 3) to be included in comparisons, asymmetric pairs being constructed from the teeth in stages 1 to 4, symmetric ones from teeth in stages 2 and 3.

The longitudinal sample of 481 Finnish children, born in the beginning of the 70's, was included for comparison and for the testing of race and sex specific patterns. These children with complete longitudinal tooth eruption data were from three rural communities, Ylivieska, Muurame and Laitila (Virtanen et al., 1994). The teeth were classified as either unerupted or erupted on a 2-degree scale. These records with annual information covered an overall age range from three to 21 years.

The proportions from the trinomial distribution of antimeric tooth pairs, which were right-advanced, left-advanced or symmetric, were calculated in each tooth and by Chi-square statistics testing the hypotheses of trinomial dental equality between boys and girls separately in both populations, as well as between white and black children in the cross-sectional U.S.-population. Directional asymmetry was explored using a binomic test for asymmetric cases being either right or left advanced in eruption. The direction of the laterality of the tooth eruption in each sex and race category was calculated from the %-sum, left-advanced being negative and right-advanced being positive %-values, p-values of 0.05 or less were taken as significant. (Figures 1 and 2).

RESULTS

It appears from Table 1, where all cases are present, that the proportion of symmetrically erupting antimeric pairs is approximately 90% in first erupting mandibular incisors, while the maxillary lateral incisors, which are the last teeth of the first phase of mixed dentition, had this proportion in about 50%. Further, with later teeth this proportion decreased, being the lowest in second premolars. The last erupting permanent teeth in the second phase of mixed dentition appear to be again more symmetric. Population differences are difficult to compare due to methodological differences.

Table 1. The number of symmetrically and asymmetrically erupting antimeric tooth pairs in a cross-sectional sample of 2092 US.White and Black children, and a longitudinal sample of 481 Finnish children.

Tooth	Symmetric Eruption of Antimeres						Asymmetric Eruption											
							Left Advanced						Right Advanced					
	WB#	WG	BB	BG	FB	FG	WB	WG	BB	BG	FB	FG	WB	WG	BB	BG	FB	FG
LM1	81	61	74	59	122	134	25	16	20	12	16	5	12	19	14	7	13	15
UM1	142	134	174	185	129	124	22	22	23	17	11	14	17	17	18	14	10	16
Li1	235	246	184	149	128	142	14	6	2	0	10	9	13	4	3	1	13	3
Li2	63	82	81	73	110	124	30	32	24	18	19	18	24	35	29	18	22	10
Ui1	59	64	59	58	133	129	31	26	20	22	9	12	32	30	25	17	8	12
Ui2	30	57	47	69	107	115	20	19	37	29	14	19	20	22	30	39	27	18
Lc	2	7	40	84	96	99	8	4	19	20	27	35	3	7	27	28	28	19
Up1	2	4	23	16	78	97	6	11	16	26	31	30	11	4	32	27	42	26
Lp1	10	7	13	19	85	98	7	3	21	23	37	28	6	5	21	35	29	28
Uc	6	4	16	29	105	97	4	2	12	21	24	28	2	1	9	21	21	29
Up2	1	1	2	2	77	81	5	5	18	20	43	40	11	4	15	20	26	25
Lp2	1	1	2	2	86	94	6	2	18	12	33	30	5	1	23	24	26	20
LM2	6	4	28	36	93	109	2	2	10	10	25	21	2	0	14	14	32	23
UM2	7	4	39	84	100	105	2	3	12	16	20	23	1	1	9	11	30	24

WB= white boys; BG= black girls; FG= Finnish girls; LM1= lower first molar; Ui1= upper central incisor; Lc= lower canine; etc.

The comparisons of the numbers and proportions of symmetrically and asymmetrically erupting permanent tooth antimeric pairs by sex and race are in Table 2. Significant sex differences in the Collaborative Perinatal Study (girls were more symmetric than boys) appeared in the mandibular central incisor ($p \leq 0.01$) and canine ($p < 0.02$), maxillary 1st premolar ($p < 0.03$) and lateral incisor ($p < 0.03$), and between the races in the mandibular incisor ($p < 0.002$) and canine ($p < 0.006$), where the blacks were more symmetric than the whites. In the mandibular first premolar, however, the whites were more symmetric ($p \leq 0.05$). A sex difference in Finnish children followed the same pattern, girls being more symmetric than boys, in mandibular central incisor ($p < 0.03$) and first molar ($p < 0.04$), and in maxillary first premolar ($p < 0.05$).

Table 2. Sex and race differences in the proportions of symmetric and asymmetric tooth eruption. P-values of the Chi-square comparisons (2x3 cells, Table 1) for each tooth.

Tooth	Sex difference US. Cross-sectional data	Race difference US. Cross-sectional data	Sex difference Finnish longitudinal data
	WB+BB vs. WG+BG	WB+WG vs. BB+BG	FB vs. FG
LM1	0.64	0.22	0.04*
UM1	0.75	0.17	0.41
Li1	0.01**	0.002**	0.03*
Li2	0.72	0.11	0.07
Ui1	0.63	0.29	0.53
Ui2	0.03*	0.53	0.25
Lc	0.02*	0.006**	0.25
Up1	0.03*	0.16	0.05*
Lp1	0.36	0.05*	0.34
Uc	0.69	0.51	0.39
Up2	0.89	0.55	0.89
Lp2	0.62	0.24	0.52
LM2	0.75	0.50	0.21
UM2	0.36	0.48	0.61

Significant ($p \leq 0.05$) directional asymmetry, measured as a sum of left (-%) and right (+%) advanced proportions, appeared in black boys as a right side advantage of the maxillary 1st premolar and left side advantage of the maxillary 2nd premolar in Finnish boys (Fig. 1). Black girls had right side advantage in their mandibular 2nd premolar and Finnish girls in the mandibular 1st molar, significant left side advantage appeared in the mandibular canine in Finnish girls (Fig. 2).

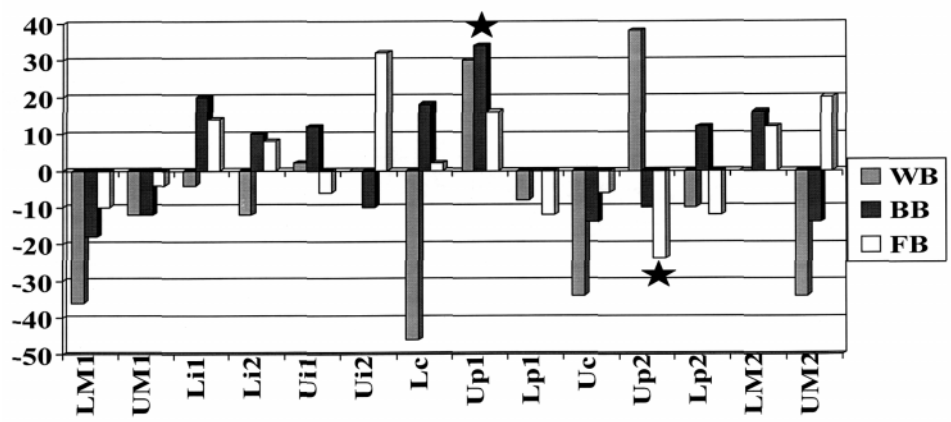


Fig. 1. Comparison of the sum of left (-%) and right (+%) advanced tooth eruption in boys. (WB=US. White, BB=US.Black, FB=Finnish Boys. ★, p<0.05 in binomic test for asymmetry.

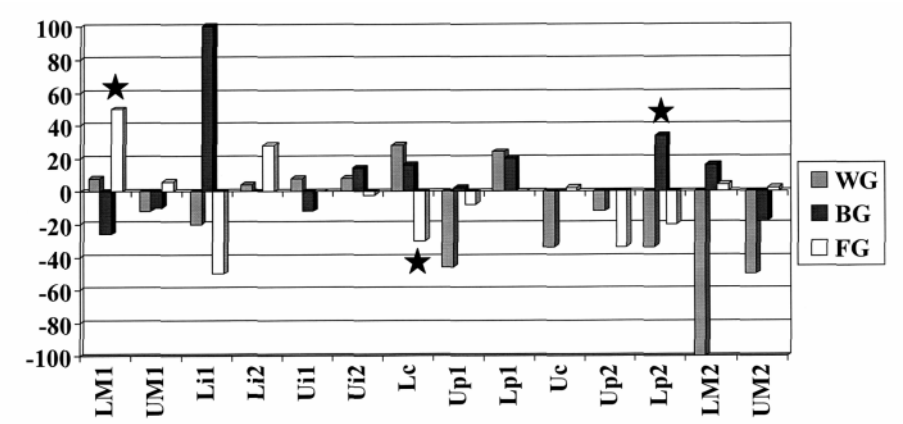


Fig. 2. Comparison of the sum of left (-%) and right (+%) advanced tooth eruption in girls (WG=US.White, BG=US.Black, FG=Finnish Boys. ★, $p<0.05$ in binomic test for asymmetry.

DISCUSSION

The results suggest that tooth eruption symmetry is under the control of structural factors, and the degree of eruptional symmetry in each tooth is more involved with the limited developmental sequence of the field or clone group of teeth, than just with overall tooth developmental sequence (Dahlberg, 1945; Osborn et al., 1983). When comparing the proportions of symmetrical eruption to other odontometric data, such as the size or form variation of the crown in different teeth, the mandibular incisors are here the exception from the Field Theory (Dahlberg, 1945; Townsend and Brown, 1981; Mayhall and Saunders, 1986), the central incisors being more symmetric than the laterals in degrees of eruption.

Dental asymmetries, resulting from differences in the metric dimensions and in the morphology of antimeric teeth, have been used to estimate the effects of genetic or environmental disturbances on developmental homeostasis (Osborne et al., 1958; Sofaer et al., 1971; DiBennardo and Bailit, 1978; Siegel and Mooney, 1987). Asymmetry of teeth is in general randomly distributed with respect to side, being generally called fluctuating asymmetry, and it is a common finding for example in Down syndrome (Barden, 1980; Townsend, 1983). Random discrepancies may indicate an inability of the individual to buffer against developmental disturbances or developmental "noise".

According to a traditional twin study methodology, by Hatton (1950) for example, tooth eruption timing determinants in general are more genetic (78%) than environmental (22%). While such estimates possess low reliability, greater genetic influences on timing than environmental ones may well be the case on average. The symmetric proportions in twins might however be misleading due to their suspected overload in dental symmetry (Boklage, 1987). The number of twins in our study (n=200) might then also be a biasing factor in our results, but it was not considered in the final results due to the relatively small proportion.

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It has been reported that the cerebral hemispheres develop at different rates and ages and a "shift" or a gradient from left to right exists, providing probably the raw material out of which hemisperic laterality of brain function develops (Geschwind and Galaburda, 1985). It has been also suggested that this developmental laterality of the left and right sides is continuous in foetal circumstances and the cyclic changes would continue after birth and throughout development (Thatcher et al., 1987). Such a formula also could provide an efficient mechanism for achieving a balance between the right and left sides of the human dentition, being normally free from directional asymmetry, and representing only "fluctuating" asymmetry in tooth size, morphology, eruption etc., at random (Perzigian, 1977).

The results suggest that the differential proportions of asymmetric tooth eruption are not only fluctuating from one side to another but may have trends to be directional in a temporal manner, which might be characterized by a long term shift from left to right in developing permanent teeth (Cattell, 1928; Hershkovitz et al., 1987). This could be associated also with more individual determinants, such as sex and race of the child (Fig.1), but also for example with the development of the overall functional lateralities of the child. Could an upper 1^{st} premolar right side advantage and the basis of common right sidedness in man have same timing determinants? Based on a low number of statististically significant results in these two samples this result showing temporal dominance of the sides remains unclear, but that variation of laterality, being comparable to some fetal or postnatal rhythms measured earlier in the activity of brain hemispheres, cannot be overruled without further study.

Thatcher et al. (1987), for example, maintain that postnatally the human cerebral hemispheres develop at different rates, which are chronologically comparable to "stages" in perceptual, cognitive development, and it is not overruled in the dental development at least of some individuals. It would be interesting to have more erupting teeth and a more defined eruption scale. However, an increased sample size seems to have a profound effect in negating the existence of fluctuating asymmetry in statistical proposals, as shown by Smith et al. (1982), who used the same Collaborative Perinatal Study data in their calculations.

The common order of dental emergence used in our Tables and Figures may be misleading due to the unknown lengths of the developmentally sensitive phases in the eruption programming of different teeth. In reality the developmental order of teeth may vary greatly between individuals, as varies also the length of mineralization and timing of emergence in sex and race groups (Steggerda and Hill, 1942; Hurme, 1949; Nanda, 1960; Moorrees et al., 1963; Kraus and Jordan, 1965; Sofaer et al., 1971; Harris and McGee, 1990). Symmetry differences between mandibular and maxillary dentitions, as well as the differences between anterior and posterior teeth in each jaw also deserves further interest (Garn and Smith, 1980 a,b,c)

Postnatal dental growth and development in blacks is on average more rapid than that of whites, as well as it is more rapid in girls than in boys, but the prenatal period is not that well known. Mineralization of the permanent teeth is achieved significantly earlier in blacks than in whites (5%) and sexual dimorphism is greater in blacks, 7% vs. 3% in whites (Harris and McKee, 1990). Girls are ahead of boys in the mineralization stages of the crown in both races, as also in the growth of the deciduous cusp distances and in the development of the root and eruption of the tooth. Sexual dimorphism in tooth size is

greater in the labio-lingual dimension than mesio-distally and smallest in the cusp distances. Our earlier findings concerning differences in timing of tooth eruption in the Collaborative Perinatal sample shows whites to be more advanced in tooth eruption than blacks in most teeth, but the mean age differences between the black and white children are a possible complicating factor, as are the socioeconomic differences (Heikkinen et al., 1995).

Sex differences in our results support Garn´s model of X-chromosomal buffering in females, which has been criticized, nevertheless, by Kieser and Groeneveld (1988). More longitudinal data and other methods are probably needed to evaluate tooth eruption symmetry/asymmetry differences in spite of the fact that the results here in two populations, cross-sectional and longitudinal, are parallel and consistent on most levels. Tooth eruption analyses, including odontometrics, dental and occlusal morphology data testing, might be interesting in some of the functional lateralities, such as laterality of the eye, hand or foot, the disorders in speech (aphasia, dyslexia) and vision (strabismus etc.), which may be associated with symmetry/asymmetry related normal/abnormal growth in the head area. Here tooth eruption asymmetry might give a description of a long term developmental instability between the right and left sides originating from several phases of development, ranging from embryonal and foetal to early postnatal. The affecting genetic and environmental factors seem to vary in different proportions between different individuals, and between different teeth, showing differential sensitivity to proportionally increasing environmetal factors during odontogenesis (Garn et al., 1966; Barden, 1980).

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