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ENAMEL STRUCTURE IN PRIMATES: A REVIEW OF SCANNING ELECTRON MICROSCOPE STUDIES

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Abstract

Comparative studies of dental enamel microstructure have involved three main areas of enquiry, with structural features having been investigated in relation to developmental mechanisms, function and/or phylogeny. The phylogenetic, or taxonomic aspect has been emphasized in the majority of studies involving the Order Primates, where efforts have focused upon attempts to recognise structural differences among various hierarchical groups.

Studies of primate enamel microstructure by SEM are reviewed here, with emphasis on what has been learned concerning the most suitable preparative techniques that can be employed, and with particular emphasis to the relevance of enamel microstructure in taxonomic analyses of living and fossil primates.

No one technique of enamel preparation can be held to be the most suitable for all types of material (e.g., fresh developing, wet mature, dry mature, and fossil enamel) but experience to date allows us to make some recommendations.

Two aspects of enamel structure have been shown to possess considerable potential in taxonomic analyses: the enamel prism packing patterns, and the enamel formation rates as documented from prism cross-striation repeat intervals. Although the distribution of enamel prism packing patterns among primates suggests considerable homoplasy of this character, this feature does have considerable taxonomic interest at certain hierarchical levels in Primates. The study of rates of enamel secretion coupled with analyses of enamel thickness has considerable potential in resolving taxonomic and phylogenetic questions.

KEY WORDS: Primates, systematics, haplorhine, strepsirhine, evolution, Scanning electron microscopy, enamel prism packing patterns, enamel prism cross-striations, enamel thickness.

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Introduction

There are two major fields of interest in relation to comparative studies of enamel microstructure: function and taxonomy. There is still considerable debate as to which aspect is most strongly reflected in enamel structure. In some mammalian orders, such as Rodentia and Perissodactyla, some investigators have concentrated on providing functional interpretations of the observed microstructural patterns (Koenigswald, 1980; Rensberger and Koenigswald, 1980). In these instances, structural features of enamel are viewed in terms of an adaptationist paradigm, with various structures being seen as having been selected for in relation to their presumed functional advantages. Other studies of enamel structure, e.g., in the Order Primates, have tended to focus upon questions concerning taxonomy or phylogeny (Gantt et al., 1977; Vrba and Grine, 1978a, 1978b; Gantt 1980, 1983; Boyde and Martin, 1982, 1983, 1984a, 1984b; Shellis and Poole, 1977; Shellis, 1984). In other words, efforts have been directed to the characterization of a particular hierarchical group (e.g., families, superfamilies, infra-orders, etc.) of primates by a particular kind of enamel structure in the hope that such features may prove to be useful in resolving questions pertaining to the phylogenetic relationships of problematic living and fossil taxa. In this approach, the differences between groups are taken as evidence of evolutionary distance rather than functional differences. The resolution of this thorny issue is well beyond the scope of this paper but it is important that **both** possible interpretations of similarity and/or dissimilarity are considered before drawing conclusions about the possible taxonomic value of enamel structure.

Interest in primate enamel microstructure can be traced back to papers by Carter (1922), Korvenkontio (1934-35), Shobusawa (1952) and Kawai (1955). For the purposes of this review, we will address studies utilizing scanning electron microscopy as these have been the major contributions in recent years, even though such studies may owe much debt to previous light microscopic analyses. As a prelude to reviewing previous studies and attempting to incorporate

them into a current description of enamel structural diversity in the primate Order, mention should be made of the two principal factors which have motivated people to attempt such studies, and which may also have influenced their findings.

Studies of primate enamel structure by SEM have concentrated, with minor exceptions, on the systematic or taxonomic utility of enamel prism packing patterns. This emphasis is attributable to the great interest in the fossil record for primate and especially human evolution. The systematic relationships of most living primates were already largely resolved by the mid 1960s and while describing enamel structure in relation to these would be a useful exercise in Natural History, it would not of itself have provided the impetus for the studies which became relatively abundant in the late 1970s and 1980s. In many ways it would have been logical to expect that functional studies would have dominated analyses of primate enamel as a supplement to the already considerable knowledge about dental and dietary adaptations. However, the relatively rich fossil record pertaining to primate and, especially, human evolution shifted the course of SEM studies of primate enamel towards taxonomy. Fossils are much less well known than modern animals, not only in terms of numbers of individuals sampled from any species, but also in numbers of taxa sampled from any particular group, and in the parts of animals which are preserved in the fossil record. Despite very considerable field efforts this century, and especially since the 1960s, many extinct primates are known only from parts of jaws and teeth, and in a number of cases only from isolated, that is single, teeth. This applies to the fossil record which pertains most closely to human origins. As we have to accept the present limitations of the fossil record in reconstructing the course of primate evolution, it is obviously necessary to attempt to incorporate even such limited remains into a picture of primate history. Consequently, the notion that enamel prism packing patterns, which can be observed even in fragments of teeth, might provide clear-cut evidence concerning the taxonomic position of a particular fossil, and possibly also contribute to the dating of a particular evolutionary branching event, provided the impetus for the main direction which studies of primate enamel have taken.

Another factor that has become apparent in attempts to resolve the structural details of extant and extinct primate enamels relates to the methods and techniques employed in the preparation of enamel specimens for SEM study of enamel microstructure. Preparation techniques are a factor in the interpretation of details of enamel structure as different methods have been shown to be responsible for different results in some cases. Thus it is appropriate to review these various methods before we review the results obtained during the course of the last twenty-two years of (mainly SEM based) research on primate enamel structure.

Review of methods

Studies of primate enamel by SEM have utilized two kinds of material: i) the mature tissue, and ii) developing enamel. Moreover, analyses of mature enamel have relied variously upon fresh (wet) material, dried specimens (e.g., dried museum collections) and fossil teeth. For intact, mature teeth it is necessary to remove the outer layer of the enamel, which is prism free, in order to reveal the underlying enamel structure. This will have been done in any natural attritional wear facet. However, the wear produces a smeared layer in which it is difficult to resolve enamel structural detail. The only occasions when the microstructure of mature enamel may be observed directly by SEM without any preparation is when naturally fractured surfaces are present, though these usually must be recent fractures to have any real value as the information may have been obscured by further wear, or through various taphonomic processes in the case of fossils (Beynon and Wood, 1987).

Mature enamel

Studies of mature enamel have principally utilized acid etching techniques to reveal enamel structure beneath a smeared surface, whether the smear results from *in vivo* wear or from specimen preparation. Early studies, such as Gantt et al. (1977), used acid to remove the outer, prism free layer from the teeth and to simultaneously erode the underlying enamel to enhance the prismatic detail. The etching regime used, viz., 10% HCl for 150 seconds, was subsequently shown to remove about 70 µm of wet, fresh human enamel (Boyde et al., 1978). The results of this systematic investigation of the effects of acid etchants on fresh human enamel also showed that any acid etching regime that cuts deep enough into the enamel to remove the prism free layer would also result in etching artefacts which would render accurate interpretation of the observed morphology problematic.

The problem was to develop a technique which produced consistent results and which minimised the artefacts introduced while still enabling the structure to be observed. The study by Boyde et al. (1978) showed that the use of a 0.5% by volume phosphoric acid (H_3PO_4) etching regime applied for between 45 and 60 seconds would suffice to remove smeared enamel and lightly etch up the enamel structure in fresh enamel. This recommendation would not necessarily apply to studies using fossil enamel whose characteristics will differ from recent enamel, as well as from one fossiliferous situation to another. To date, however, no study has been undertaken to analyse the amount of dried, mature, or fossilized enamel that is removed under controlled conditions such as those defined by Boyde et al. (1978). Recent studies by Lester and Hand (1987) have shown that the same strength solution of H_3PO_4 may be applied for as little as 2-3 seconds to etch up enamel prism boundaries on natural wear facets. A recent study by Grine (1986) indicates that while dilute H_3PO_4 may be the preferred etchant for fresh (wet) enamel, dilute HCl for short periods (e.g. 10 seconds) may be preferable for dried,

and perhaps also fossilized, mature enamel. Etching regimes for fossils need to be determined in relation to specific geological deposits as was done by Carlson and Krause (1985). Many such studies will be required to determine whether general rules can be formulated for acid etching fossils.

The major consequence of the study of acid etchants by Boyde et al. (1978) was to show that chemical etchants should not be used to expose deep layers of enamel by dissolving away substantial thicknesses of surface enamel. Two responses to this finding were possible, and both have been taken. Firstly, mechanical removal of the outer layer of material, usually by grinding and polishing the tooth surface with fine grades of wet silicon carbide paper (Grine et al., 1985; Grine, 1986) or by diamond polishing, the use of diamond wafering blades, or ideally by diamond micromilling. Mechanical removal of enamel tissue to expose the underlying structure results in the presence of a "smeared layer" which itself must be removed through the use of acid or mechanical etchants in order to resolve the exposed structural details by secondary electron SEM. Acid or chelating etching agents, which will be discussed below, also result in the enhancement of structural detail through the exploitation of natural discontinuities in the structural fabric - they may, however, also result in unwanted artefacts. The second development has been used by Boyde and Martin (1982, 1984a) and particularly by Lester and Hand (1987) and involves very light etching of natural wear facets in which attrition has removed the prism free surface layer. This has some advantages over polishing facets as it involves less tissue removal, but the disadvantage of the loss of dental microwear features and that the depth below the orginal tooth surface of the exposed enamel structure is not known. However, microwear features can easily be replicated prior to etching so that the use of natural wear facets is very close to the minimally destructive, but useful, method sought by, for example, Boyde and Martin (1982). These recent studies on the effects of etching agents would appear to indicate that there is no single best agent for all types of preserved enamel, and that the etchant utilized should be chosen according to the state of the enamel and an understanding of the chemistry of the etching effect which is produced.

Recently, Boyde (1984) and Boyde and Fortelius (1986) have shown that mechanical means, rather than chemical etchants, may also be employed to remove the smeared enamel layer resulting from cutting or polishing, thus avoiding the problem of artefact production. They have employed Airpolishing (TM) (with gas propelled $NaHCO_3$ powder shrouded by a concentric water jet) and neutral ion beams. Both methods have the advantages of precise control of the regions affected, and that the etching exploits the relevant discontinuities in the structure, and leaves the underlying bulk tissue unaffected. This should be contrasted with the results of wet chemical etchants (both acids and chelating agents) which cause considerable damage in depth below the surface which is created.

When very flat surfaces are prepared, either by diamond polishing of polymethyl methacrylate (PMMA) embedded tissue (Boyde and Tamarin, 1984) or, preferably, by diamond micromilling (Boyde and Jones, 1983), the smeared layer may be left intact and the immediate subsurface layer imaged by utilizing higher energy backscattered electrons (BSE). This technique is useful as it enables prism packing patterns to be imaged without any artefacts. The resultant density dependent (atomic number) images may be especially important in imaging enamel prism cross-striations and incremental lines (Brown Striae of Retzius) (Boyde, 1979). Mature enamel structure may also be examined without any preparation in the study of naturally, or accidentally, fractured surfaces of teeth - a technique that has been shown to be of considerable potential in recent reports on fossil hominid specimens (Beynon and Wood, 1987). These surfaces usually need to be the result of recent fractures to have any real value, as structural details may easily be obscured by in vivo wear or by taphonomic factors that affect the preservation of fossils.

Developing enamel

Developing enamel may be studied in an attempt to understand developmental mechanisms and constraints, as well as to obtain a more perfect 3-D concept of the tissue structure. Methods for the preparation of developing enamel material involve the removal of cells and cell debris from the developing surface and tissue drying. Preparative methods have been summarised by Boyde and Martin (1982), who are the only workers yet to have used developing enamel surfaces for studies of enamel prism packing patterns in primate species. Critical point or freeze drying of anorganic specimens has been used with considerable success, but the best preparative method for developing material has developed from a previously unreported combination of techniques.

According to this recently developed procedure, tooth germs are dissected from jaws and are refluxed in a chloroform/methanol mixture at about 48°C for between 5 and 7 days. The enamel organ may be left adhering to the developing tooth, or it may be peeled away; the cell debris remaining after this action may be ignored. After refluxing according to the procedure described by Boyde and Tamarin (1984), the specimens will be completely dehydrated (and defatted). The developing tooth may then be immersed in flash distilled, or otherwise de-inhibited methyl methacrylate monomer (MMA) and left in a cool dark place for 24 h. After 24 h the MMA is drained away and fresh MMA is substituted. A total of three such changes is needed to ensure that all of the chloroform and methanol has been replaced by MMA. After three changes a further substitution with MMA with a destabilizing agent (or "catalyst") is made, and this MMA is allowed to polymerize to polymethyl methacrylate (PMMA) at 32°C. The polymerization may be accelerated by exposing the monomer to UV light at room temperature. This method has the great advantage

that the tissue is never dried and the delicate developing enamel surface is therefore not subjected to surface tension forces. Following complete polymerization, the delicate tissue is fully supported by the PMMA so that it may be cut or polished with little distortion. We have found it most useful to cut into the developing enamel surface by diamond polishing so as to expose the enamel deep to the developing surface. After this procedure, the embedded tooth is oxygen plasma ashed to remove some plastic as well as any cell debris from the developing enamel surface surrounding the polished facet. The end result is a specimen in which the developing enamel surface morphology is exposed, but has never been subjected to drying, with the deeper, mature enamel structure exposed in adjacent regions. This permits a direct correlation to be made between the morphology and arrangement of the Tomes' process pits and the resulting prismatic structure in the mature tissue.

Review of SEM studies of primate enamel

Prism patterns

In his initial work on mammalian tooth enamel structure, Boyde (1964) named three basic categories for the arrangement of the Tomes' process pits seen in the developing enamel surface. These corresponded with the prism cross sectional shapes seen by light microscopy (LM) by earlier authors (e.g., Shobusawa, 1952). Boyde formulated a developmental model which explained the nature of the prismatic appearance in terms of crystallite orientation discontinuities. The three categories are simply descriptions which allow one to divide up the spectrum of enamel prism packing patterns. Comparative analyses of mammalian dentitions have demonstrated that while all three types may commonly be found in localized areas of any tooth of any species, one of these patterns usually predominates, at least at any one depth into the enamel. It is in that light that we employ these descriptive categories.

The three major arrangements of Tomes' process pits and therefore of enamel "prisms" are shown in Figure 1.

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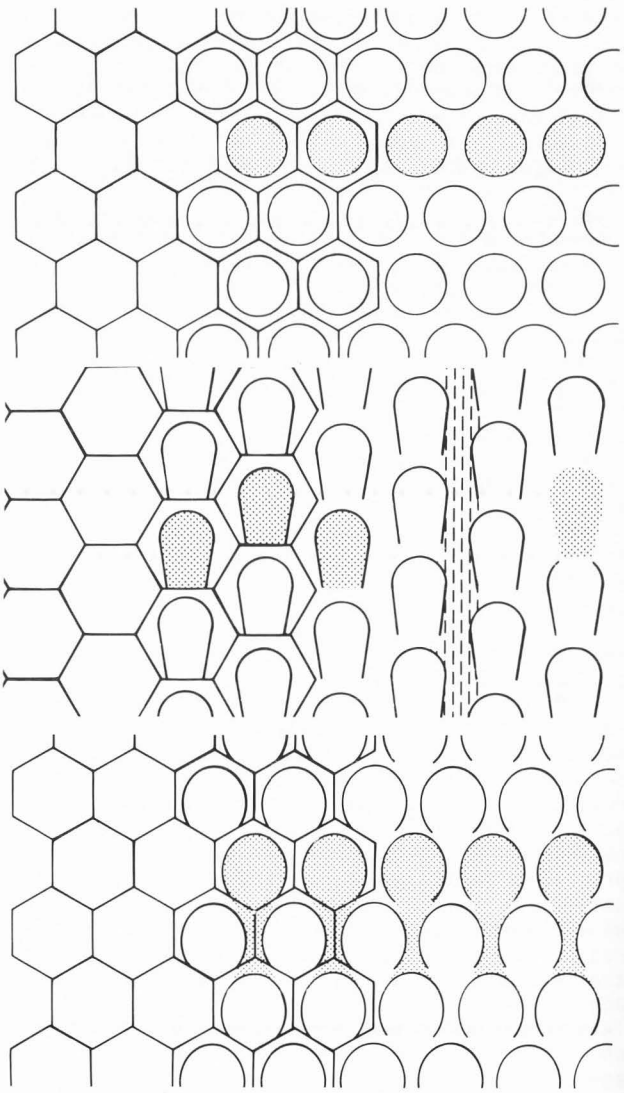
Figure 1. This diagram, modified from Boyde's (1964) thesis (Figure 1), introduces the terminology for the three major prism packing patterns referred to in this review. The lines in the diagram represent (sectioned) **boundary planes** of abrupt change in crystallite orientation. There is only a gradual change in crystallite orientation between any two points which can be connected by a line which does not pass through such a (prism) boundary plane.
Pattern 1: Cylindrical (circular in transverse section) prism boundaries with separate "interprismatic regions".
Pattern 2: "horseshoe" (cross-sectional shaped) prism boundaries: prisms arranged in longitudinal rows with no regions which can be defined as interprismatic between prisms in the same row. In some variations there may be greater separation between the rows of prisms and inter-row sheets can be defined.
Pattern 3: Arcade arrangement of prism cross sections. The cervical, open side of the prism boundary faces a "gap" between two prism boundaries cervically. This means that there is no abrupt change in crystallite orientation from the center of the prism to the narrow region situated between the two prisms on its cervical side - the "gap" is the "winged process"; there is no region which should be called interprismatic. In some variations, the prism boundaries are more complete, i.e. they extend through considerably more than a half circle as appreciated in transverse section. This may be found in conjunction with wider regions separating the boundary planes. In such cases it is difficult to conceive of these regions as "winged processes" - one would call them "interprismatic", but they are still continuous with the regions "within the prisms" via the open side of the prism boundary. This pattern obviously approaches that called Pattern 1 above.



Shobusawa (1952) had previously referred to what Boyde (1964) termed Pattern 3 enamel as the "primate type". However, Boyde (1964) found that while Pattern 3 did indeed characterize human enamel, a representative cercopithecoid (Old World) monkey, Macaca mulatta, exhibited a high frequency of Pattern 2 enamel. Moreover, the "primate type" is also common in non primates. Further evidence of the diversity of enamel prism packing arrangements in primates came when Boyde (1966) reported that a strepsirhine primate, Lemur catta, exhibited a predominance of Pattern 1 prism packing. Despite the limited nature of these early samples in terms of numbers of taxa and also specimens, the discovery that the three major prism packing patterns known for Mammalia were present within the Order Primates, and the fact that different patterns appeared to characterize species in different taxonomic categories within that Order suggested that enamel prism packing patterns might prove useful for primate systematics, and especially for the interpretation of fragmentary fossil specimens.

Hominoidea

Hominoid interrelationships. Despite the fact that the Hominoidea is one the least taxonomically diverse primate superfamilies, more effort has been expended in analyses of enamel structure exhibited by the taxa comprising it than for any comparable primate group. Indeed, comparatively little attention has even been paid to documenting enamel structure in the lesser apes (the gibbon species comprising the family Hylobatidae) in contrast to the attention that has been paid to the extant and extinct members of the great ape and human clade. To set the scene for the subsequent development of such studies, it is useful to review briefly the state of our knowledge during the last century about the relationships of humans with other hominoid primates.

Much of the argument that has gone on concerning the phylogenetic and systematic relationships amongst the hominoid primates has centered around the question as to which of the great apes represent(s) humans closest living relative(s), and at what time in the past did these lineages diverge. Some early evolutionary taxonomists (e.g. Darwin, 1871; Huxley, 1863) believed that humans were most closely related to the African apes (Pan troglodytes [the chimpanzee] and Gorilla gorilla). An alternative view was that the Asian apes, the orang-utan (Pongo pygmaeus) and gibbons (Hylobates) were man's closest living relatives. This view was particularly espoused by Haeckel (1866), and more recently in modified form by Schwartz (1984) who has argued that the orang-utan alone is the closest living relative of humans. For most of the present century, however, the view has prevailed that the four living great apes are each others closest relatives (Pilgrim, 1927). This dichotomy, that large hominoids are either apes ("pongids") or humans ("hominids") had a considerable influence on the way in which fossil hominoids were analysed and interpreted. Fossil teeth which appeared essentially human-like in dental form were interpreted as evidence for the great antiquity of the uniquely human line, while any with ape-like features were dismissed as belonging to less interesting side branches.

In the early 1960s, studies on the molecular biology of extant hominoids began to have an increasing impact on the question of ape - human relationships and the timing of the divergence of the human lineage largely as the result of the work of Goodman (1963) and Sarich and Wilson (1967). These workers proposed that the African apes and humans formed a clade (i.e., monophyletic unit) which excluded the orang-utan. In addition, Sarich and Wilson (1967) argued for a very recent divergence of the human line from the African ape line: between 3.5 and 5 myr BP.

These dates were in marked conflict with the interpretation of the fossil record prevailing at that time. In particular, Ramapithecus from the Miocene of Indo-Pakistan was believed to represent the earliest hominid (Simons, 1961) (this genus has subsequently been synonymised with Sivapithecus). Since Ramapithecus dated to at least 12 myr BP it was central to the question of the antiquity of the human lineage. Sarich and Wilson argued that Ramapithecus was too old to be a hominid, no matter what it looked like, particularly as no strong morphological evidence had been advanced to demonstrate its hominid affinities. Attempts to provide morphological evidence of the hominid status of Ramapithecus were hampered by the fragmentary nature of the fossils assigned to that taxon; even at the present time, Ramapithecus is known almost exclusively from a few jaws and, mainly isolated teeth. The ability to resolve phylogeny based on dental elements was therefore crucial to the determination of the antiquity of the human line.

SEM studies directed at hominoid taxonomic questions. In 1977, Gantt et al. reported on the SEM analysis of enamel structure in modern large hominoids and in Ramapithecus. Intact teeth were prepared for SEM study by immersing them in 10% HCl for 150 seconds. These authors found that humans had Pattern 3 enamel, as reported by Boyde (1964) and all early accounts back to Nasmyth (1839). They also reported that all of the great apes examined (Pan troglodytes, Gorilla gorilla, and Pongo pygmaeus) had Pattern 1 enamel, and concluded that there was an ape/human dichotomy for enamel prism packing patterns. They advanced no evidence that the pattern seen in humans was the derived condition with respect to the hypothetical common ancestral condition for great apes and humans, but nevertheless assumed this to be the case. A specimen of Ramapithecus from Indo-Pakistan was also examined and was found to have Pattern 3 enamel. This result was interpreted to confirm that Ramapithecus was a hominid and that the human line had diverged from all ape lines during the Miocene, and well before the dates suggested by the molecular evidence. However, this work ignored the findings of Boyde (1964) that Pattern 1 prisms are common near the surface of (human) teeth as well as close to the enamel dentine junction - see below. An important paper by Shellis and Poole (1977) also indirectly cast doubt on these results. They reported that Pan had Pattern 1 enamel towards the outside of its teeth, but that it was Pattern 3 deeper into

the tooth, again implying that control of the depth at which enamel prism packing patterns were being sampled was essential. The same authors also reported that Gorilla had **only** Pattern 1 enamel and that this showed no prism decussation, as evidenced by Hunter-Schreger band formation, but these latter findings were later contradicted by Boyde and Martin (1982, 1983, 1984a).

Vrba and Grine (1978a, b) attempted to confirm the results of Gantt et al. (1977) using the same preparative method, but they obtained quite different results. These workers found that although regions of Pattern 1 enamel could be found on extant hominoid teeth, especially at cuspal apices, Pattern 3 enamel predominated in Pan troglodytes, Gorilla gorilla, and Pongo pygmaeus, as well as in humans and undoubted fossil hominids, the australopithecines. As a result, Vrba and Grine (1978a, 1978b) argued that enamel prism packing patterns were of little value for resolving hominoid relationships, and especially that the presence of Pattern 3 enamel in Ramapithecus could not be used to support its hominid status.

Systematic studies on the effects of different acid etchants on enamel by Boyde et al. (1978) showed that the regime used by Gantt et al. (1977) and repeated by Vrba and Grine (1978a, 1978b) would produce results which would render interpretation of the observed morphology problematic (but not so as to affect the diagnosis of clear areas of Pattern 1 or 3 enamel). Gantt (1979) used these findings concerning possible "artefactual images" to argue that Vrba and Grine's (1978a, b) results should be ignored, presumably together with his own earlier work (Gantt et al., 1977). Gantt did not address the discrepancy between his results and the finding by Shellis and Poole (1977) of Pattern 3 enamel in Pan troglodytes. He repeated his 1977 study using the etching regime recommended by Boyde et al. (1978) (viz., 0.5% H_3PO_4 for 45-60 seconds) on diamond polished facets and reproduced his earlier findings of a Pattern 1/Pattern 3 dichotomy corresponding with an ape/human dichotomy.

As much debate had revolved around mature enamel etching techniques and the depth at which enamel structure was being analysed, Boyde and Martin (1982) undertook a study of the morphology of the developing enamel surface, as well as mature enamel in hominoids. A developing enamel surface samples a layer which will represent a variety of depths into the mature enamel when the tooth is completed. For the hominoids, most of the specimens utilized were early tooth germs in which only occlusal and cuspal enamel was formed (see Boyde and Martin, 1984a, Table 15.1 for details). They found that the non-human hominoids all exhibited a predominance of Pattern 3 arrangement of the Tomes' process pits at all (developmental horizon) depths into the enamel sampled in the specimens available to them as developing teeth. These samples did not include outer, lateral crown developing surface. From studies of mature teeth, they found that Pattern 1 was common close to the tooth surface in all great apes (see Boyde and Martin, 1982, Table 1). In chimpanzees (Figures 2 & 3) the developing enamel was strongly decussating Pattern 3, as was the case in human material (Figures 4, 5, 6). Gorilla material showed somewhat less dramatic decussation although (contra Shellis and Poole, 1977) changes in prism orientation from one group of prisms to another were clearly apparent (Figure 7). Gorilla enamel was found to be characterized by a relatively large amount of interpit phase enamel (interprismatic substance or prism "tails" as against "heads") compared with the other large hominoids (Figure 8), but this again depends on the depth of sampling within the tissue - in the case of developing material, relative proximity to the non-secretory, completed and maturing enamel surface. Orang-utan enamel was difficult to distinguish from chimpanzee enamel (Figures 9, 10, 11) being Pattern 3 and strongly decussating. In all species some localized areas of Pattern 2 enamel were also encountered (Figure 12) but, by surveying many fields of enamel, sampling a variety of positions on the tooth and depths into the enamel, the predominant arrangement was found

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Figure 2. Pan troglodytes (common chimpanzee) developing enamel surface showing well marked zones of ameloblastic pits associated with the development of prism decussation (Hunter-Schreger bands). Developing upper first permanent molar critical point dried and then oxygen plasma ashed to remove cell debris. Secondary electron image, 10 kV. Field width = 125 µm.

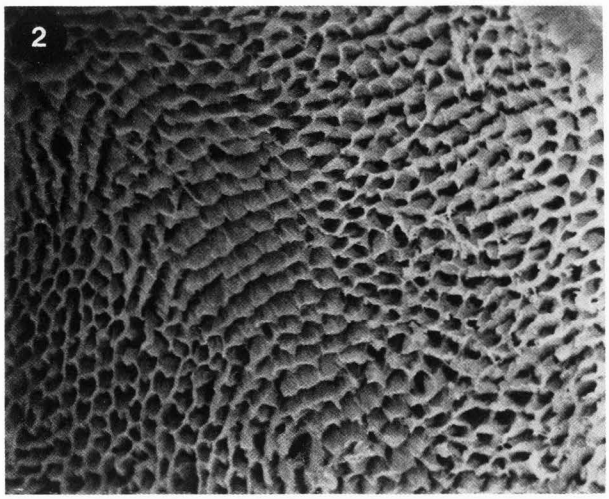
Figure 3. Hunter-Schreger bands in etched mature enamel of P. troglodytes upper first permanent molar, the more transversely sectioned prisms are referred to as a diazone and the more longitudinally sectioned as a parazone. Longitudinal section, diamond polished, H_3PO_4 etched for 30 seconds. BSE image, 20 kV. Field width = 377 µm.

Figure 4. H. sapiens developing enamel surface shows different entry direction of pits running in bands obliquely across the field of view associated with the development of prism decussation (Hunter-Schreger bands). Developing lower first deciduous molar, critical point dried, oxygen plasma ashed. Secondary electron image, 10 kV. Field width = 91 µm.

Figure 5. Hunter-Schreger bands in a longitudinal section of H. sapiens canine tooth enamel. Diamond polished, K2EDTA etched, BSE image 20 kV. Field width = 257 µm.

Figure 6. Oblique cross sectional shape of H. sapiens enamel prisms as seen in an en face view from the tooth surface ground parallel to the enamel dentine junction. The interest in this specimen is that it is **not coated**. The specimen has been made conductive by extensive infiltration with silver nitrate (10% $AgNO_3$ for 4 days) following 0.5% H_3PO_4 etching for 30 seconds. This is a negative BSE image so that the denser, silver impregnated prism boundaries appear black. 20 kV. Fieldwidth = 84 µm.

Figure 7. Gorilla gorilla longitudinal section of lower first permanent molar showing strong prism decussation (Hunter-Schreger bands) in mid lateral enamel 0.5% H_3PO_4 etched for 30 seconds. BSE image, 20 kV. Field width = 370 µm.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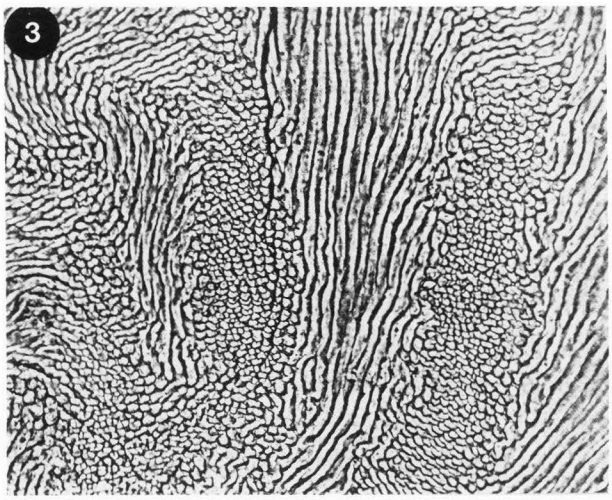
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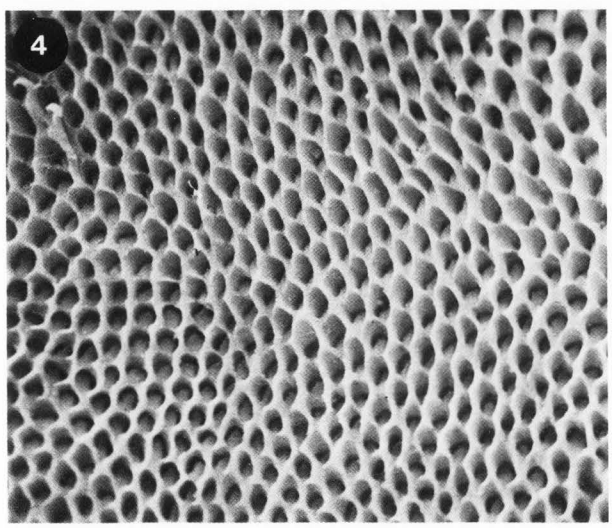
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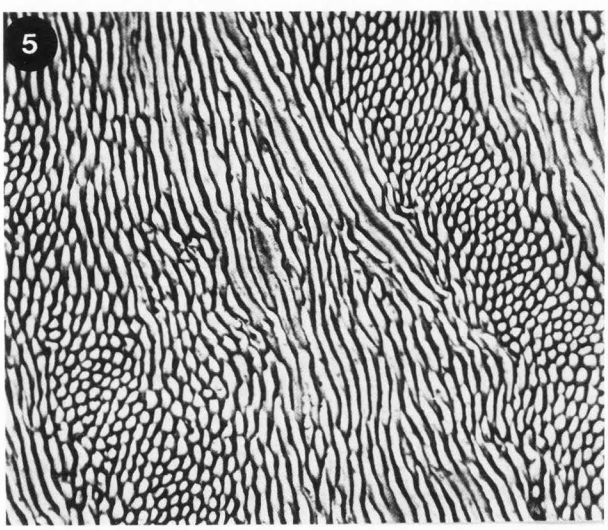
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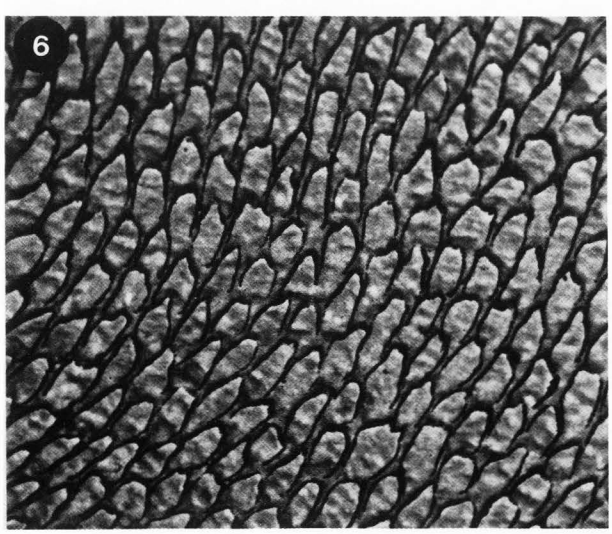
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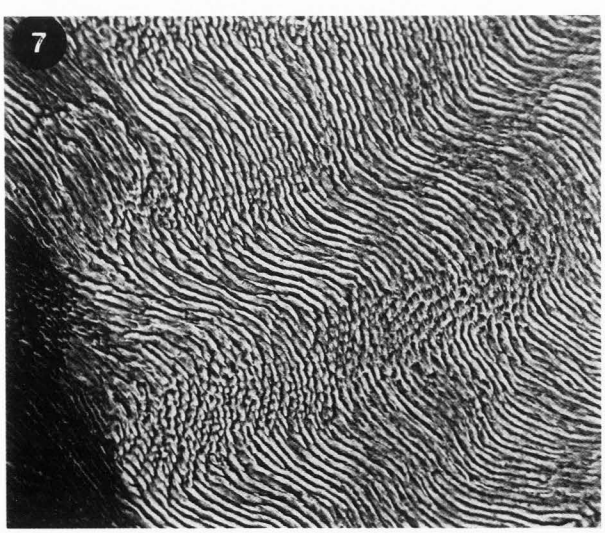
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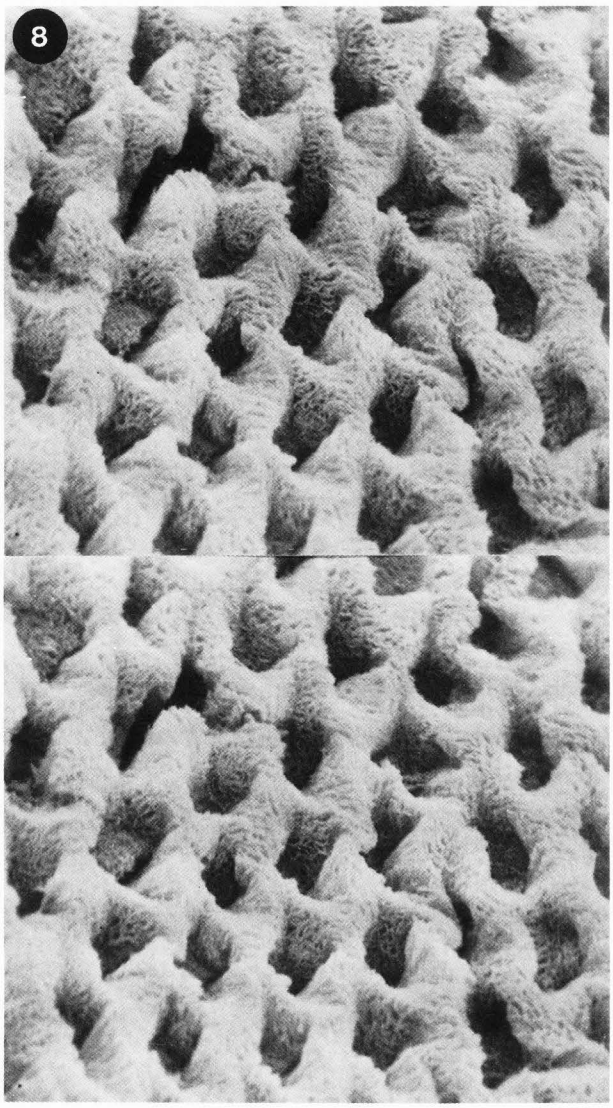
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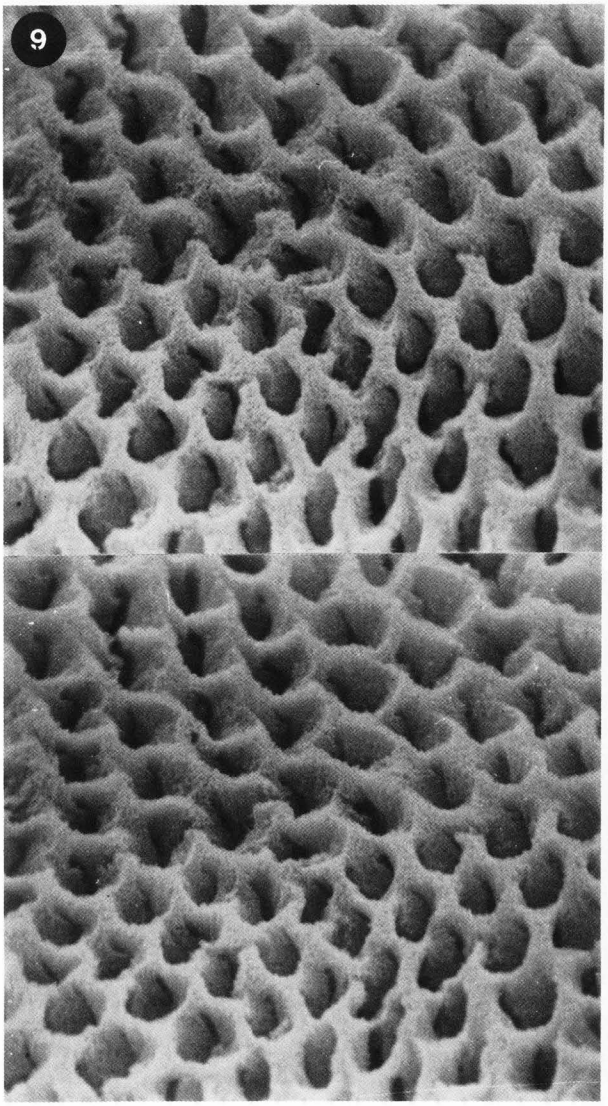
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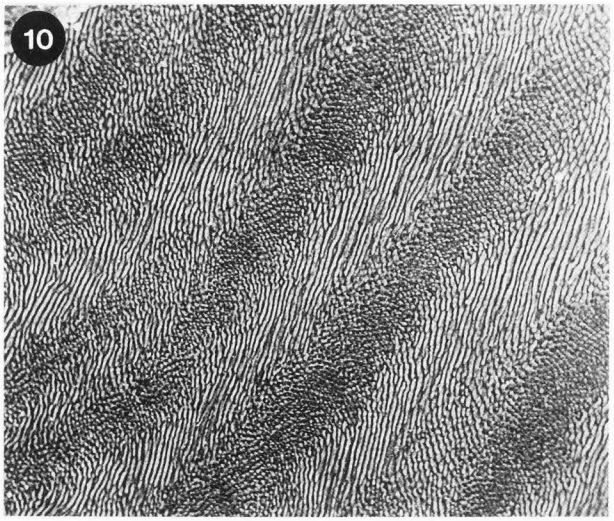
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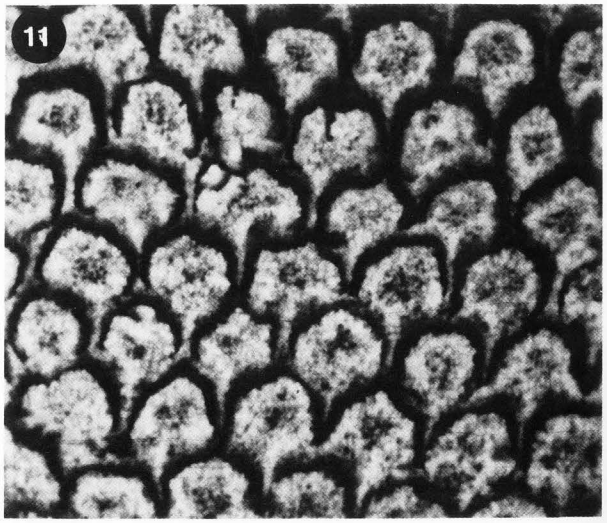
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Figure 8. Gorilla gorilla developing enamel surface of lower first deciduous molar cervical to top, stereopair, tilt angle difference 10°. To view in stereo, turn the page through 90°. Note in particular the width of the interpit phase enamel. Specimen treated with 4% sodium hypochlorite (NaOCl) for 30 minutes, then critical point dried from CO_2 via Freon 113. Secondary electron image, 10 kV, cervical to top. Field width = 30 µm.

Figure 9. Pongo pygmaeus (orang-utan) developing enamel surface of lower second deciduous molar hypoconid. Stereopair, tilt angle difference 10°, showing border between two decussating zones, i.e., ameloblastic pits are entering surface in different directions at top and bottom of the field when viewing the page the right way up. To view in stereo turn the page through 90°. Specimen treated with 4% NaOCl for 30 minutes prior to critical point drying. Secondary electron image, 10 kV. Field width = 43 µm.

Figure 10. Longitudinal section through the mesial cusps of a P. pygmaeus lower first permanent molar showing diazones and parazones of the Hunter-Schreger bands. Note that the more longitudinally sectioned prisms in the parazones apparently obliquely cross the Hunter-Schreger band axis: proof that one prism does not remain in one zone. In other words, prisms undergo several changes in orientation from side to side across the thickness of the enamel. 0.5% H_3PO_4 etched for 30 seconds, back scattered electron image, 20 kV. Field width = 627 µm.

Figure 11. Cross sectional shape of enamel prism boundaries which appear dark in this BSE image of H_3PO_4 (phosphoric acid) etched P. pygmaeus enamel. Diamond polished facet on metaconid of lower first permanent molar, 2% H_3PO_4 etched for 60 seconds. It should be remembered that in an acid or EDTA etched preparation the extent of the prism boundaries is much greater than reality so if they appear to contact each other this is very likely to be an artifact of etching [see Boyde et al. (1978); cf. Tandem Scanning Reflected Light Microscope images, see Boyde and Martin (1988)]. BSE image, 20 kV. Field width = 45 µm.

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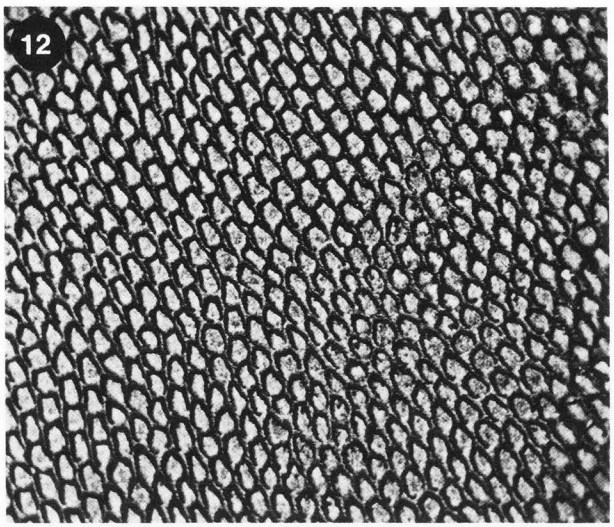
to be Pattern 3 in all cases. Hylobates (gibbon) enamel was also found to be Pattern 3 (Figure 13) (Boyde and Martin, 1983) suggesting that Pattern 3 enamel characterizes at least the Hominoidea, although larger areas of Pattern 2 enamel than had been encountered in other hominoids were found in this taxon. The Pattern 1: Pattern 3 dichotomy argued by Gantt et al. (1977); Gantt, (1979) was therefore contradicted. The results of this study were, rather, in accord with the findings of Vrba and Grine (1978a & b). In a later paper, Gantt (1983) accepted that all hominoids were characterized by a predominance of Pattern 3 enamel but claimed to have discovered a new criterion which maintained the hominid/pongid dichotomy for enamel structure, viz., hominids have Pattern 3B enamel while pongids have Pattern 3A. Neither Boyde and Martin (1982, 1983, 1984a) nor any other workers have found any evidence to support this. In fact, Shellis (1984) reported that Pan, Gorilla and Homo had been found to have Pattern 3A enamel while cercopithecine monkeys had Pattern 3B - see below.

Enamel thickness. Another area of interest in relation to the question of recognising Miocene members of the human lineage is enamel thickness. Most studies have treated enamel thickness separately from enamel structure although these features are strongly interdependent. Some recent studies have related enamel thickness to developmental rates determined from enamel structural features (Martin, 1983, 1985; Martin and Boyde, 1984). Homo and the middle Miocene hominoid Sivapithecus (including Ramapithecus) were found to have thick enamel, Pongo was found to have enamel of intermediate thickness, while Pan and Gorilla were both found to have thin enamel. Gibbons and cercopithecoid monkeys were reported to have thin enamel, which would imply that this condition is ancestral for the catarrhines. Even well defined metrical data do not permit any definitive statements to be made concerning the evolution of enamel thickness as several alternative assessments of ancestral conditions within the Hominoidea are equally parsimonious. However, the combination of these data with an SEM study of microstructural features of enamel which relate to enamel formation rates allowed a more complete analysis of the evolution of hominoid enamel.

Use of incremental lines in interpreting thickness variants. Longitudinal sections used for enamel thickness measurements were diamond polished and a solid state back scattered electron detector was used to image incremental lines in the enamel (Figure 14) which correspond with the Brown Striae of Retzius seen in light microscopy of ground sections (Boyde and Martin, 1982). The configuration of these lines reveals that the rates of enamel secretion vary in different portions of the tooth crown (Figures 14-18). The distance between a given pair of neighbouring incremental lines represents the same time interval over the entire crown, irrespective of changes in the separation between them. Between the incremental lines, finer markings may be seen (Figure 14) which are known as prism varicosities and/or prism cross-striations (Figures 15 & 16). These exist in various numbers between adjacent incremental lines, and very probably represent a circadian pattern in enamel secretion (Gysi, 1931; Boyde, 1963, 1971). Thus the distance between adjacent prism cross-striations may be taken to represent 24 h of enamel secretion. The temporal distance between successive Brown Striae, and their surface expressions - perikymata (Figure 18), is not a constant for all hominoid species and it may range from 4 to 14 days in Pongo (Boyde and Martin, 1982, 1984a). The normal human range is narrower with a mean of 7-8 days (Asper, 1916; Bromage and Dean 1985; Gysi, 1931).

When the spacing of prism cross-striations is studied according to their position in the tooth crown, certain patterns emerge (Martin, 1983; Martin and Boyde, 1984). Enamel close to the enamel dentine junction forms slowly with a cross-striation repeat interval of < 2 µm and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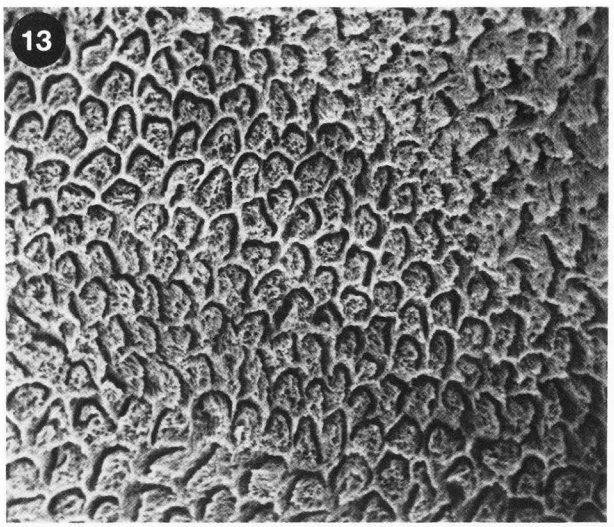
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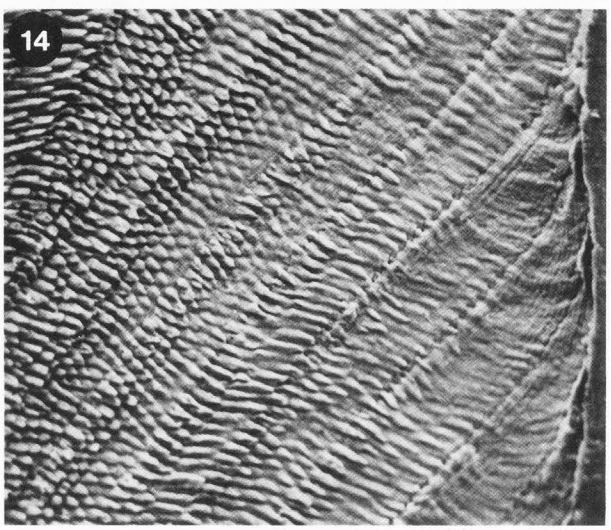
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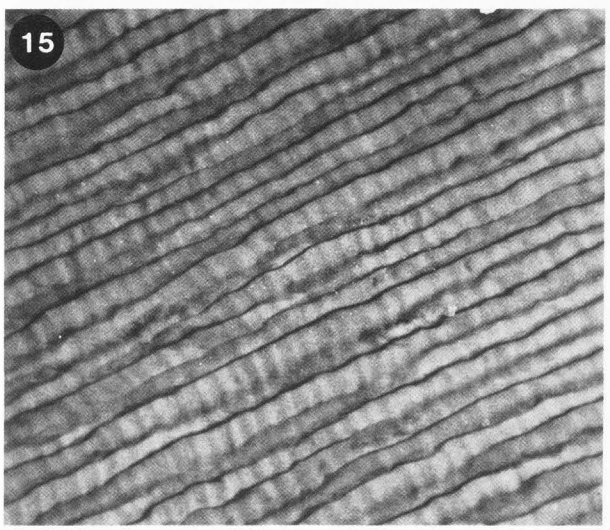
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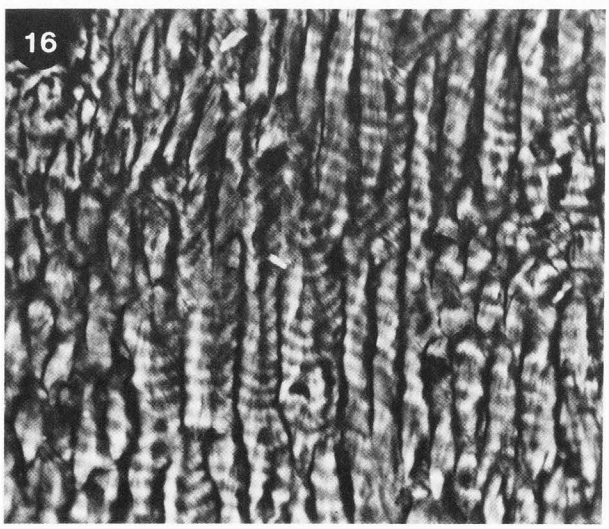
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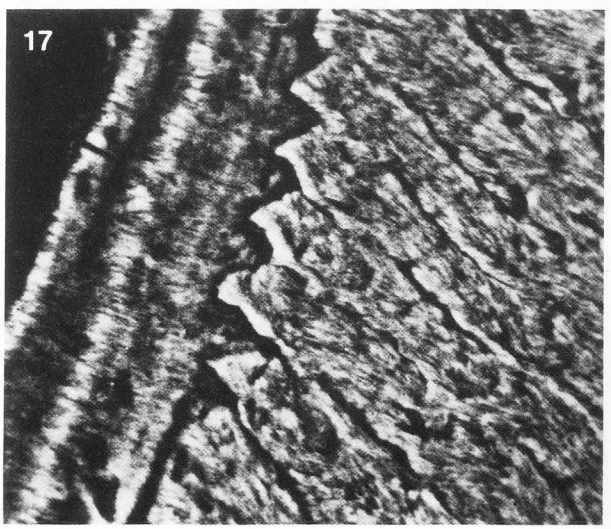
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Figure 12. Diamond polished facet on protoconid of lower first permanent molar of P. troglodytes, 2% H_3PO_4 etched for 120 seconds. Transversely sectioned prisms showing an area of mixed Pattern 2 and Pattern 3 fields. In both cases there is an unusual signature to the cross section of the prisms which we have previously noted for this species in developing enamel preparations (Boyde and Martin, 1982). BSE, 20 kV. Field width = 152 µm.

Figure 13. Diamond polished facet on lower first permanent molar of Hylobates sp, 0.5% H_3PO_4 etched for 60 seconds. Transverse section of Pattern 3 prisms. SE, 10 kV. Field width = 91 µm.

Figure 14. Longitudinal section of P. pygmaeus lower molar mounted on glass. Etched with EDTA ph 7.2 for 18 h to emphasise Brown Striae of Retzius and finer incremental lines, presumed to be daily incremental lines. A number of 14 such striations can be counted between these increments in this particular case. The tooth surface is to the right of the field of view and the cervix towards the bottom. CBSE image obtained by biasing the specimen to +200 V to prevent the escape of low energy secondary electrons (Boyde and Cowham, 1980), 20 kV. Field width = 334 µm.

Figure 15. Homo sapiens lower third permanent molar embedded in PMMA before cutting to provide a longitudinal section. The block surface was finished by diamond micromilling to produce an ultra-flat surface which was not etched. This BSE image therefore shows density dependent contrast as there is no topography, thus prism boundaries are particularly dark and cross striations of the enamel prisms are brought into prominence. This phenomenon was first shown by Boyde (1979). BSE, 20 kV. Field width = 118 µm.

Figure 16. H. sapiens longitudinal section of upper second permanent molar. LS was diamond polished to a 1 µm finish and etched with 0.5% H_3PO_4 for 30 seconds. This image shows very fine periodicity of cross striations near the tip of the dentine horn (visible at top left), i.e., the cross striation repeat interval is a smaller fraction of the prism width than in Figure 15. Some prisms are exposed in the head to tail direction so that they appear particularly wide and some in the side to side direction so they appear narrower. The cross striations follow the rules of orientation of the developing enamel surface and thus appear bent or kinked in the wider prisms and oblique across the prism axis in the narrower ones. BSE image of acid etched specimen so contrast could be due to both topography and density variation, 20 kV. Field width = 93 µm.

Figure 17. Longitudinal section through the mesial cusps of right upper first permanent molar of Oreopithecus bambolii (M 11565) a middle Miocene catarrhine from Tuscany, Italy. The specimen was refluxed in chloroform/methanol for 24 h prior to embedding in PMMA. Specimen was cut using Cambridge Microslice diamond saw, diamond polished to a 1 µm finish and etched for 45 seconds in 0.5% H_3PO_4. The tooth surface at top left is just cervical to the lingual cingulum. Enamel formation has proceeded relatively normally from southeast to northwest (bottom right to top left) until reaching the very prominent incremental line visible with the picket fence profile. Enamel formation stopped at that level and when it recommenced to make the final surface layer of the enamel it did so without prisms being formed, i.e., prism free surface zone enamel was formed on the surface of the previously prismatic enamel. This situation is as close as we have yet come to observing a fossilized developing enamel surface. BSE, 20 kV. Field width = 53 µm.

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this corresponds with a region of enamel in which the prism packing pattern is of Pattern 1 type. In great ape teeth, there is an outer layer of variable, but considerable thickness in which the cross-striation repeat interval is reduced, i.e., the enamel is formed slowly, which is also associated with Pattern 1 prism packing. The bulk of the enamel (deep and mid thickness) is formed quickly (5-7 µm) per day and is of Pattern 3 type in all hominoids (Martin 1983; Martin and Boyde, 1984).

This finding of a considerable thickness of slowly formed enamel near to the tooth surface in great apes may serve to explain Gantt et al.'s (1977) finding Pattern 1 enamel in these taxa. The particulars of the distributions of slow formed (Pattern 1) and fast formed (Pattern 3) enamel in the molar teeth of various hominoid species provided the key to understanding enamel thickness.

The great apes all have a considerable thickness of slowly formed enamel on the outside of their teeth while humans, gibbons and Sivapithecus form almost all of their enamel at the fast rate. This means that the thin enamel seen in gibbons, and probably also that seen in cercopithecoids, is not developmentally homologous with the thin enamel seen in the African apes. Martin (1983, 1985) and Martin and Boyde (1984) proposed that all of the great apes and humans have the potential (in terms of time devoted to tooth formation) to form thick enamel, but that the layer of slowly formed (Pattern 1) enamel reflects a secondary reduction of enamel thickness. The consequences of this conclusion are that thick enamel is the condition expected in the common ancestor of the great ape and human clade and that thin and intermediate enamels would be of more value in deducing relationships for fossil hominoids (Andrews & Martin, 1987a).

On the basis of available data for the hominoids, SEM studies of enamel structure in relation to developmental rates and enamel thickness have great potential for improving our knowledge and understanding of many aspects of primate evolution and morphology. As a caveat, we should mention the desirability for such studies to be based on large samples of specimens, tooth positions in the jaw, individuals within a species, and species used as representatives of higher taxa. In addition, it should be stressed that the correlations between secretory rates and enamel prism packing patterns seen in hominoids may not apply in other primates.

Cercopithecoidea

In spite of the great diversity of species within this family and the availability of material in museum collections, few workers have undertaken even superficial surveys of enamel structure. Few of the 57 or so species have been examined by SEM and any conclusions about enamel structure must be considered preliminary. A potential solution to this sampling problem has been proposed by Boyde and Martin (1988) through the use of Tandem Scanning Reflected Light Microscopy.

Boyde (1964) reported on the structure of enamel in Macaca mulatta which he found to have a high proportion of Pattern 2 enamel. Given that hominoids evince a predominance of Pattern 3 enamel this result would have considerable taxonomic significance if it were found to apply to cercopithecoid monkeys generally (Figures 19-26). Boyde and Martin (1982, 1984a) have reported on enamel prism packing patterns in M. mulatta (Figures 19 & 24) and Erythrocebus patas (Figure 23). They found that M. mulatta had a high frequency of Pattern 2 enamel as had been reported by Boyde (1964) and that E. patas had considerable areas of Pattern 1 (Figure 23) as well as Pattern 2 enamel. New studies of developing enamel from Cercopithecus neglectus reveal a similar pattern to that seen in Macaca with a relatively high frequency of Pattern 2 enamel (Figure 20) interspersed among Pattern 3 enamel (Figures 21 & 22). Recently, however, Shellis (1984) has reported the presence of Pattern 3B enamel, often in rows with marked prism decussation in all of the cercopithecoids which he examined from the genera Cercopithecus, Erythrocebus, Papio, and Macaca. He contrasted this with his finding that all of the hominoids which he studied had Pattern 3A enamel It seems possible that Shellis (1984) is using modified definitions of enamel prism packing patterns as, in contrast to his previous publications (e.g., Shellis and Poole, 1977), he now reports that **all** primates with the exception of Daubentonia have either Pattern 3C, Pattern 3B, or Pattern 3A enamel. Shellis (1984) did not illustrate the enamel prism packing patterns which he reported; thus, we are unable to provide a conclusive explanation for the difference between his results and our own reviewed and reported here.

The most wide-ranging survey of cercopithecoid enamel prism packing patterns that has been reported to date was performed on HCl etched mature enamel at mid-depth in conjunction with an analysis of the enigmatic fossil catarrhine Oreopithecus bambolii from the Middle Miocene of Tuscany, Italy (Grine et al., 1985) and its results are described more fully here. These workers found Pattern 2 enamel to predominate in Papio sphinx (the Mandrill) (Figure 25), but found considerable areas of Pattern 3 enamel in Papio cynocephalus (Figure 26) as well as Pattern 2 enamel. Cercocebus torquatus and Cercocebus albigena were found to have a predominance of Pattern 2 enamel with the development of inter-row sheets in C. torquatus. Cercopithecus neglectus and Cercopithecus mona were both found to have a predominance of Pattern 3 enamel, although we have shown above that at least C. neglectus has considerable Pattern 2 also, on the basis of examination of the developing enamel surface. Many taxa were found to have a mixture of substantial portions of Pattern 2 enamel and Pattern 3 enamel. This is true for the genus Macaca with Macaca nemestrina having a predominance of Pattern 2 enamel, Macaca sylvana having a predominance of Pattern 3, and Macaca mulatta showing both Pattern 2 and Pattern 3. Presbytis entellus and Presbytis cristatus had a predominance of Pattern 3 enamel mixed with considerable amounts of Pattern 1 enamel, while Presbytis obscura had a predominance of Pattern 2 enamel, also mixed with a considerable amount of Pattern 1 enamel. A second colobine genus, Colobus, also showed a mixture of patterns with Colobus polykomos showing a predominance of Pattern 3 and Colobus angolensis some Pattern 3 but a predominance of Pattern 1. These preliminary reports show that cercopithecoids may have any of the three major types of enamel prism packing patterns and that there is a high degree of intra-generic variability. It is clear, however, that Pattern 2 enamels are to be found in much greater quantity than is ever the case in hominoids. Whether this represents a specialized pattern in some Old World monkeys (which might then be useful for determining relationships within this family), or a reflection of the

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Figure 18. Longitudinal section of the same specimen of Oreopithecus bambolii as shown in Figure 17 shows the regular series of incremental Retzius lines associated with surface perikymata which have been used by Bromage and Dean (1985) as seven day incremental markings. The tooth surface is to the left, cervix to bottom. The groove visible on the outside of the tooth is the lingual cingulum. BSE, 20 kV. Field width = 735 µm.

Figure 19. A former TEM block of PMMA embedded Macaca mulatta developing lower second permanent premolar (P_4) treated with Dalton's chrome osmium prior to embedding ex Boyde (1964). The block was oxygen plasma ashed to reveal the mineralizing front at the developing enamel surface (almost synonymous terms) (see Boyde and Martin, 1982). A clear example of Pattern 3 enamel development in an Old World monkey. One or two prisms within prism pits can be identified in this field of view. SE, 10 kV. Field width = 96 µm.

Figure 20. Cercopithecus neglectus developing lower first deciduous molar (dp_3) prepared by refluxing in chloroform/methanol followed by critical point drying from CO_2 via Freon 113 (Boyde and Tamarin, 1984) followed by oxygen plasma ashing to remove cell debris from the mineralizing front of the developing enamel surface. Occlusal to top as oriented on the page, turn through 90° to view this 10° tilt angle difference stereopair in which an area of Pattern 2 arrangement is seen at the center of the field of view. SE, 10 kV. Field width = 50 µm.

Figure 21. Same C. neglectus specimen as Figure 20 showing an area with predominant Pattern 3 arrangement of the prisms. SE, 10 kV. Field width = 91 µm.

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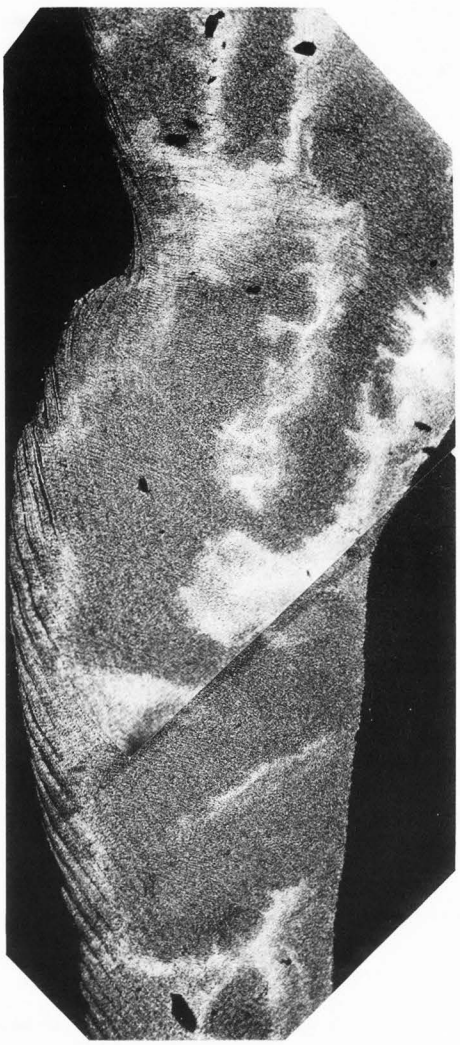

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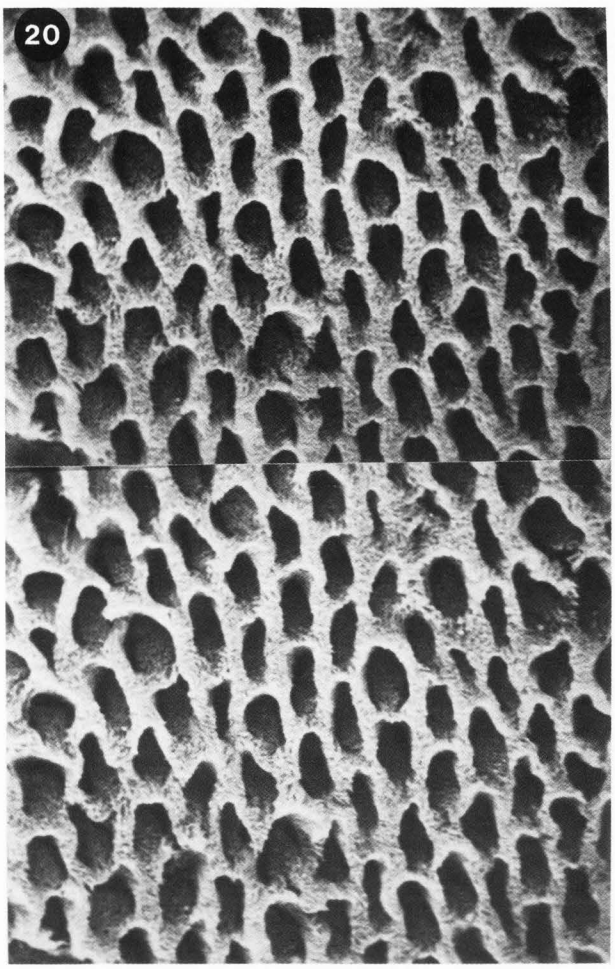

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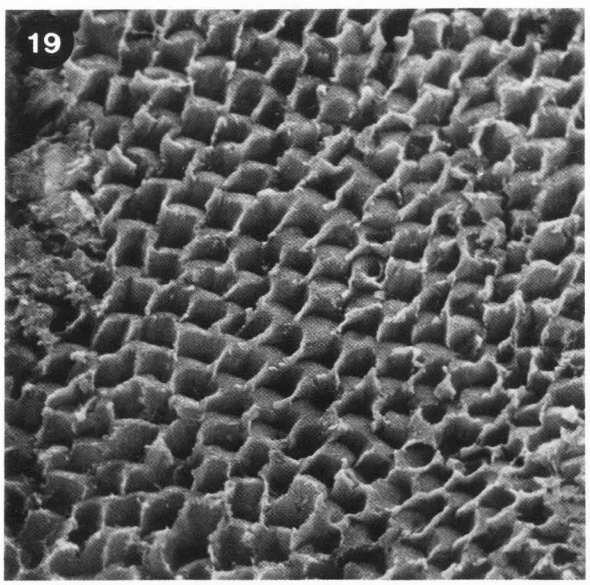

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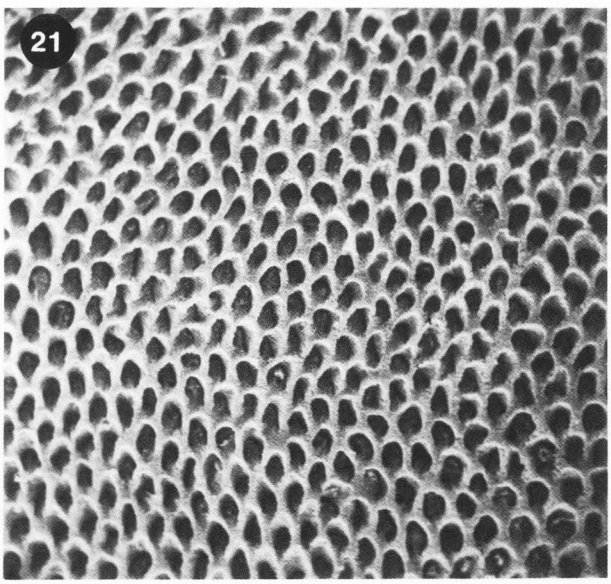

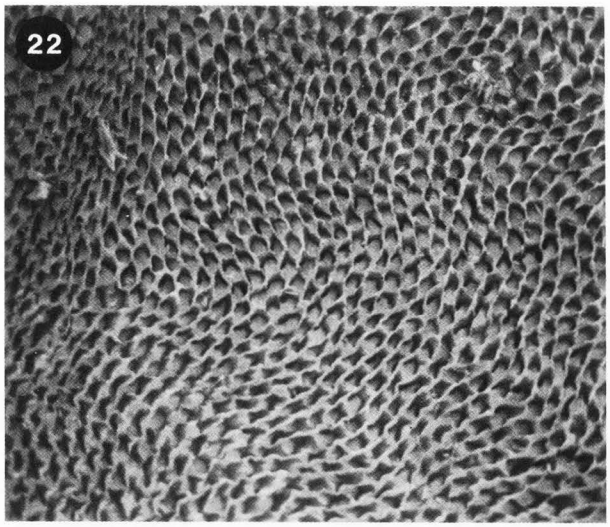
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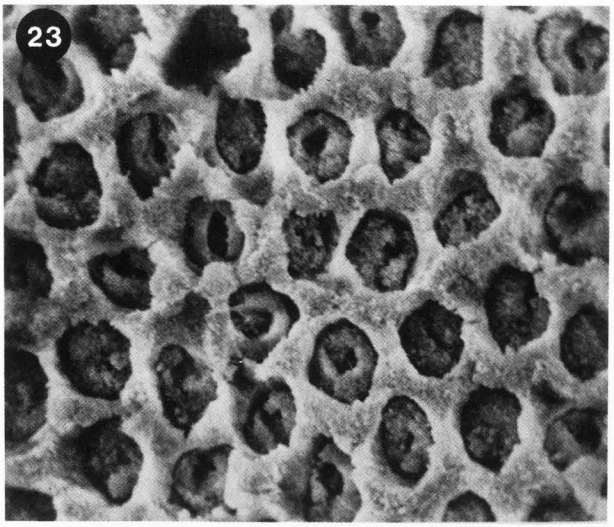
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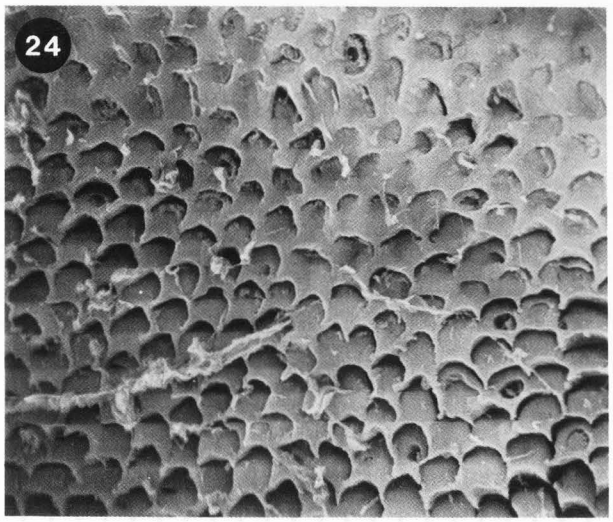
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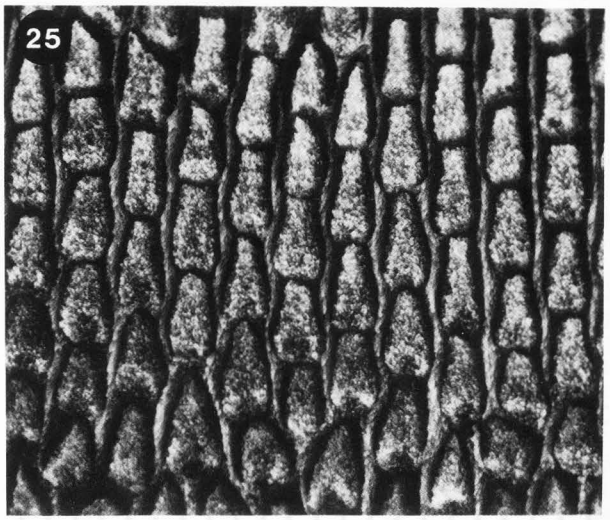
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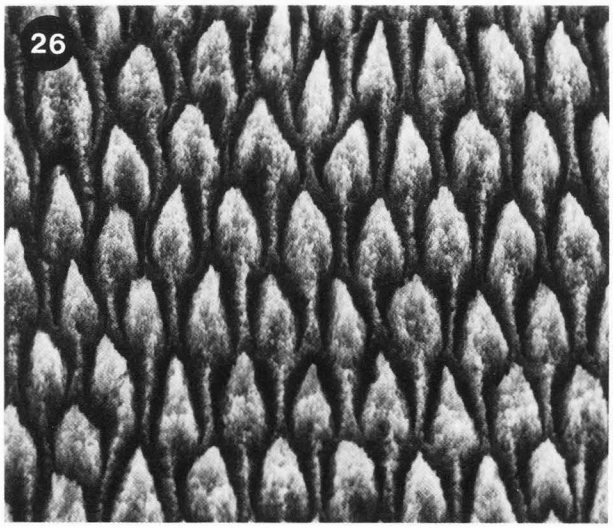
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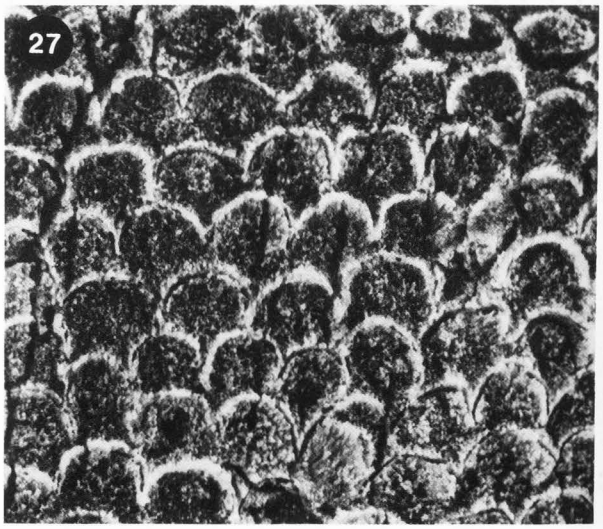
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Figure 22. Another tooth, lower second deciduous premolar (dp4) of the same C. neglectus specimen showing an area of mostly Pattern 3 enamel prism arrangement with decussation, i.e., the ameloblastic pits enter the surface in different directions in different parts of the field of view corresponding to the development of Hunter-Schreger bands. Preparation as for Figs 20 & 21. SE, 10 kV. Field width = 159 µm.

Figure 23. Erythrocebus patas developing lower second permanent molar. Area showing Pattern 1 ameloblastic pits with a high proportion of pits within pits which would be associated with the formation of prisms within prisms. Preparation by treatment with 4% NaOCl for 30 minutes followed by washing in water, dehydration in ethanol, substitution with Freon 113 and critical point drying from CO_2. SE, 10 kV. Field width = 30 µm.

Figure 24. Macaca mulatta plasma ashed PMMA developing enamel surface (former TEM block). Pattern 3 enamel showing clear pits associated with the development of prisms within prisms. The developing enamel surface trends into surface (maturation) zone enamel towards northeast. SE, 10 kV. Field width = 95 µm.

Figure 25. Papio sphinx molar showing Pattern 2 enamel, facet polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV. Field width = 40 µm.

Figure 26. Papio cynocephalus molar showing Pattern 3 enamel. Facet polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV. Field width = 39 µm.

Figure 27. Oreopithecus bambolii molar showing Pattern 3 enamel. Facet polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV. Field width = 40 µm.

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ancestral catarrhine condition is currently unclear as the outgroup for the catarrhines, the platyrrhines, also shows a variety of enamel structures.

In their analysis of the enamel prism packing patterns displayed by catarrhine taxa, Grine et al. (1985) also undertook an initial assessment of prism compression, using the quantitative techniques developed by Fosse (1968) for the description of human enamel configurations, and suggested that the prisms of cercopithecoid monkeys - whether they show Pattern 2 or Pattern 3 packing arrangement - display more apicocervical distension than the prisms of hominoid species.

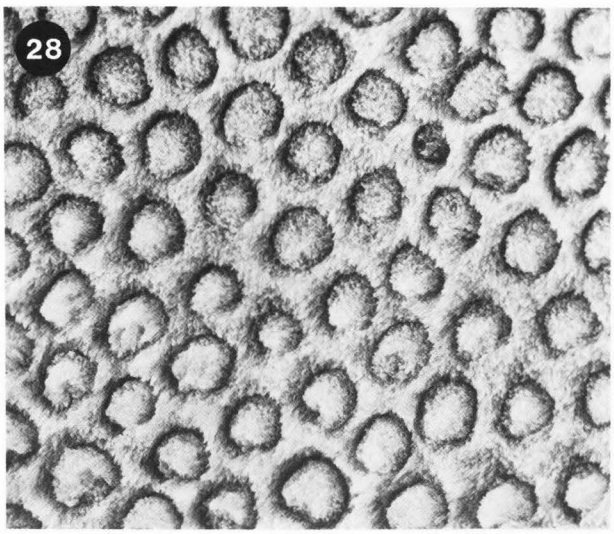
An attempted diagnosis of affinity. Oreopithecus bambolii has been argued to be both a cercopithecoid and a hominoid on alternative morphological characters and it had been hoped that its enamel structure would help to elucidate its phylogenetic affinities. A study of enamel structure in this taxon (Figures 17, 18 & 27) revealed that it had a predominance of Pattern 3 enamel with little or no Pattern 2 (Figure 27) (Grine et al., 1985). At first sight this would suggest affinities with the hominoids, but that interpretation assumes that the hominoid condition with Pattern 3 is derived with respect to the ancestral catarrhine morphotype. There is, however, no a priori reason to consider hominoids derived and cercopithecoids primitive. The best way to resolve the issue is by examination of the pattern of enamel structure in outgroups, in this case the New World monkeys.

Ceboidea

The main published work on ceboid enamel is by Gantt (1980). He reported that all of the large South American primates, traditionally referred to as the Cebidae, exhibited Pattern 2 enamel while the callitrichids (marmosets and tamarins) had entirely Pattern 1 enamel. This might mean that the ancestral catarrhine condition would be likely to have been with Pattern 2 enamel, and the hominoids (and also Oreopithecus) derived in having Pattern 3 enamel. Boyde and Martin (1982, 1984a) reported that a marmoset (Callithrix) had Pattern 1 enamel, but with Hunter-Schreger bands. Shellis (1984) reported the presence of Pattern 3B enamel in Ateles and Pattern 3C enamel with little or no decussation in the cebids Aotus and Saimiri and in the callitrichids Saguinus and Callithrix.

A recent study of New World monkey enamel has thrown further light on the problem and largely contradicts the findings of Gantt (1980). Grine et al. (1986) examined the structure of acid etched, mature enamel in a number of ceboids and found evidence for all three major packing arrangements even though they examined only limited samples of teeth for any taxon. For the platyrrhines traditionally assigned to the family Cebidae, they report a predominance of Pattern 1 enamel in Alouatta fusca and Alouatta seniculus (Figure 28), although both species also showed substantial areas of Pattern 2 and Pattern 3 enamel. Ateles paniscus (Figure 29) had exclusively Pattern 1 enamel, Aotus trivirgatus (Figure 30) displayed both Pattern 1 and some Pattern 3 enamel. Brachyteles arachnoides, Cebus capucinus, Pithecia pithecia and Pithecia monachus were found to have Pattern 3 enamel (Figures 31-33) although in the case of Cebus this was overlain by a relatively thick layer of Pattern 1 enamel (Figure 34). Chiropotes chiropotes and Saimiri sciureus were found to have a predominance of Pattern 2 enamel (Figures 35 & 36) and Lagothrix lagotricha and Callicebus moloch (Figures 37 & 38) a mixture of Patterns 1, 2 and 3 enamel. Thus the cebids display all three prism packing patterns with the subgroup of atelines appearing to display a high frequency of Pattern 1 enamel. This result is in marked contrast to the findings of Gantt (1980). The results reported above indicate that we are unlikely at present to be able to resolve clearly the ancestral condition for the Cebidae, which further complicates determination of the ancestral anthropoid and catarrhine conditions.

Grine et al. (1986) also reported on enamel structure in some callitricids. They found that Saguinus fuscicolis and Callithrix sp. had Pattern 1 enamel which agrees with the reports of Gantt (1980) and Boyde and Martin (1982, 1984a, 1984b) though their data did not address the presence of prism decussation reported on by Boyde and Martin (1982, 1984a) (Figure 39). However, these authors also found Pattern 2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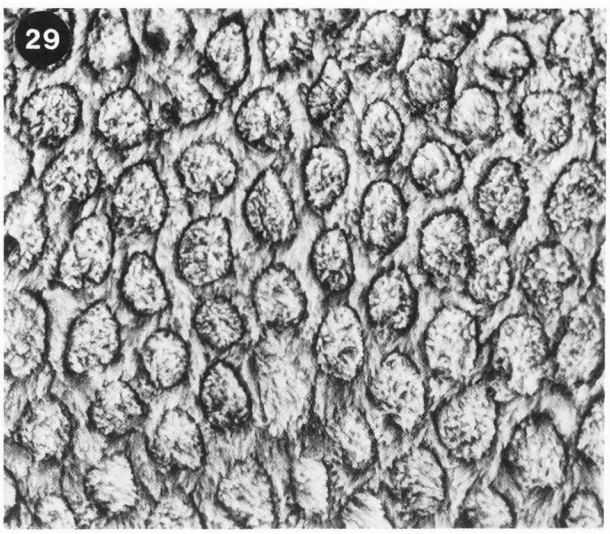
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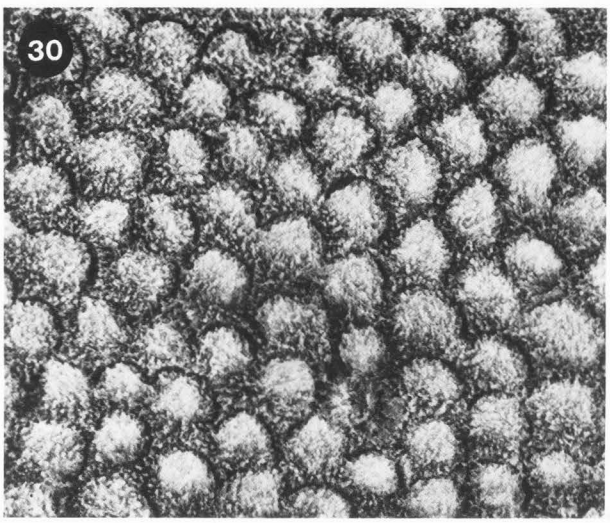
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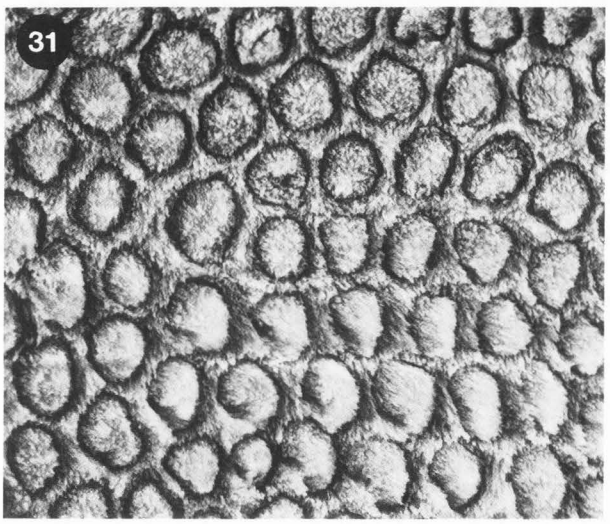
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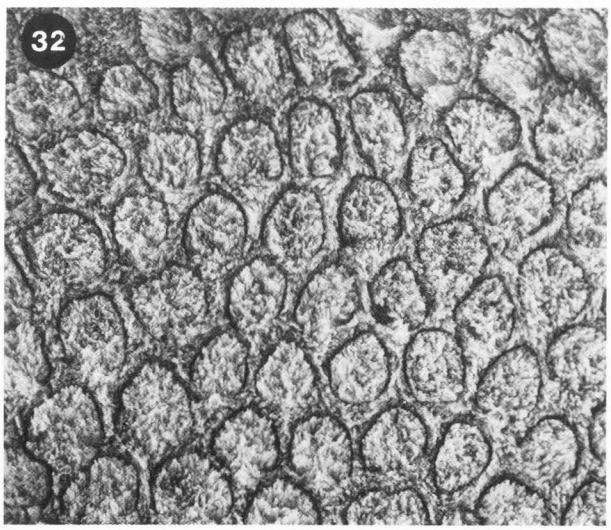
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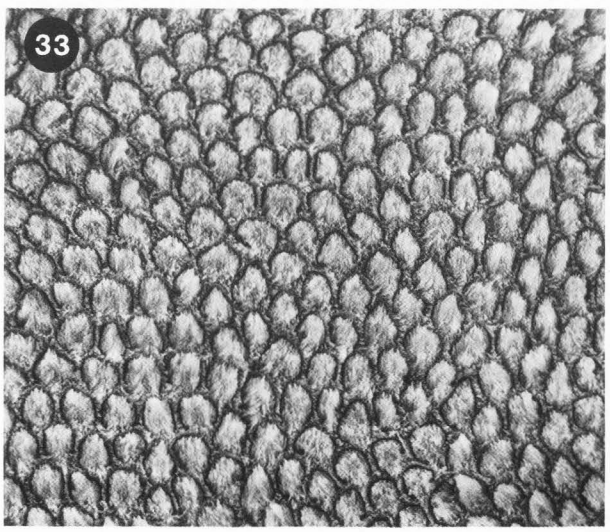
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The specimens shown in figures 28-33 were all prepared in the same way, viz., a facet was polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV.

Figure 28. Alouatta seniculus molar showing Pattern 1 enamel. Field width = 40 µm.

Figure 29. Ateles paniscus molar showing Pattern 1 enamel with the enamel showing a marked tendency to be arranged in longitudinal (cuspal to cervical) rows as seen in Pattern 2 enamel. Field width = 40 µm.

Figure 30. Aotus trivirgatus molar showing Pattern 1 enamel. Field width = 40 µm.

Figure 31. Cebus capucinus molar showing Pattern 1 enamel in the superficial layers of the enamel. Field width = 40 µm.

Figure 32. Cebus capucinus molar showing Pattern 3 enamel in the mid-thickness enamel. Field width = 40 µm.

Figure 33. Pithecia monachus molar showing Pattern 3 enamel. Field width = 81 µm.

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enamel to characterize Leontopithecus rosalia and Saguinus oedipus (Figure 40) and Pattern 3 enamel in Cebuella pygmaea (Figure 41) conditions not previously known for callitrichids.

It seems likely that the common ancestor of ceboids could have had at least Patterns 1 and 2 enamel and the finding of Pattern 3 enamel in Cebuella suggests that any one of the three major prism packing patterns, or some combination there of, could have characterized the last common ancestor of the Ceboidea. This renders the problem of determining the ancestral anthropoid condition beyond present capabilities. All three patterns must be considered equally likely in the absence of clear evidence that one or other pattern must necessarily be the developmental or phylogenetic precursor of any other. Perhaps it would be reasonable to say that it is likely that the ancestor of all anthropoids had ameloblasts capable of secreting Pattern 2 or Pattern 3 enamel in addition to Pattern 1 enamel.

The Haplorhine condition

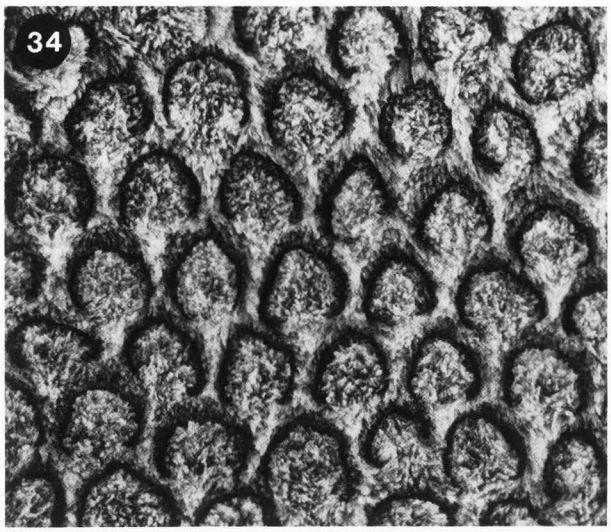
In recent years it has become widely accepted that the Tarsier is more closely related to the anthropoid primates than are any of the strepsirhines (lemurs and lorises) (see Aiello, 1986 for a review of the arguments and literature). Consequently the condition of enamel structure in this genus may be of value in resolving the question of the ancestral anthropoid condition. Two studies have addressed this question. In their work on New World monkey enamel reviewed above, Grine et al (1986) also examined the enamel in a specimen of Tarsius and found Pattern 1 enamel (Figure 42) at the tooth surface underlain by Pattern 2 (Figure 43) enamel. Boyde and Martin (1988), using Tandem Scanning Reflected Light Microscopy, found Pattern 3 enamel in Tarsius spectrum. Consequently, it is presently clear only that the presence of enamel prism packing patterns other than Pattern 1 characterizes Anthropoidea (New World monkeys, Old World monkeys, apes and humans) and Haplorhini (Anthropoidea + Tarsius). Whether an anthropoid condition will be distinguishable within a haplorhine condition must await more detailed analyses. It would be important to clarify this point to aid with the taxonomic interpretation of Eocene fossil primates. The exact determination of the condition of enamel in all known species of Tarsius should be made a high priority for future work. In order to establish whether a Haplorhine condition is derived with respect to strepsirhines and ancestral primates we must examine the limited evidence currently available concerning enamel structure in lemurs and lorises.

Strepsirhines

The strepsirhine primates include the lemurs, indriids, cheirogaleids, lorises and galagos, and comprise what was often called the prosimian primates, but excluding the Tarsier, now usually grouped with the Anthropoidea in the Haplorhini. There have been few studies which have done more than mention enamel structure in any strepsirhines and any conclusions regarding their enamel must be considered as preliminary.

The only detailed study has been of the enamel in a single species, Daubentonia madagascarensis (the aye-aye), by Shellis and Poole (1979). This study concentrated on the ever growing incisor teeth of this highly specialized, rodent-like primate. It is indeed unfortunate that high quality data such as those for the aye-aye are not available for other strepsirhine taxa. The conclusions of Shellis and Poole's study (which relate to the topic under discussion) were that the enamel prisms in the incisor teeth are of Pattern 2 type.

The other strepsirhine taxa which we are aware of having been reported are Lemur catta (Boyde and Martin, 1982, 1984a), Lemur sp., Propithecus sp., Perodicticus potto, and Galago senegalensis (Shellis and Poole, 1977), Nycticebus sp. (Shellis, 1984) and Galago sp. (Grine et al., 1986). Boyde and Martin (1982, 1984a) found that L.catta showed Pattern 1 prisms with no prism decussation (Figure 44), in contrast to the situation observed in some callitrichids, but Shellis and Poole (1977) reported the presence of Pattern 3 prisms in a specimen of Lemur sp. These latter authors, however, reported Pattern 1 enamel to predominate in Galago and in Perodicticus. Subsequently, Shellis (1984) has concluded that Pattern 1 enamel is not the predominant prism packing pattern in any primate and that Lemur, Galago, Nycticebus and Perodicticus are characterized by Pattern 3C enamel with little or no decussation. No explanation is given for the change in interpretation from the reports of Shellis and Poole (1977) and unfortunately the paper does not contain illustrations. Grine et al. (1986) also reported Pattern 1 enamel in Galago (Figure 45). Shellis and Poole (1977) found Pattern 1 enamel in Propithecus (see, however, Boyde and Martin, 1988) and made the interesting LM observation that enamel tubules were common in that taxon, confirming the much earlier studies of Carter (1922). Shellis (1984) has modified this position and now interprets the enamel in Propithecus as Pattern 3B. Shellis and Poole (1977) illustrated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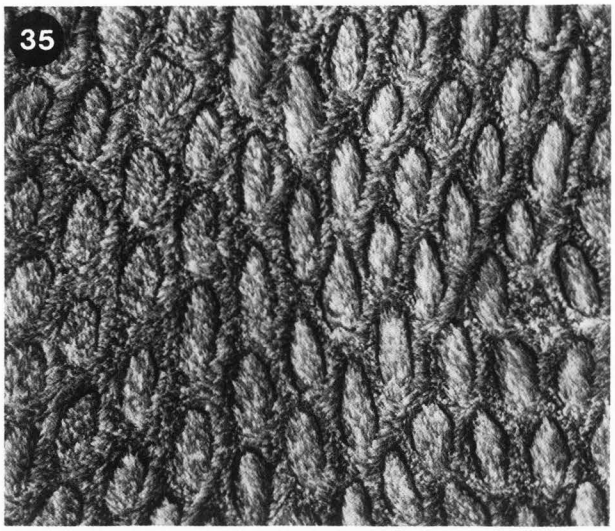
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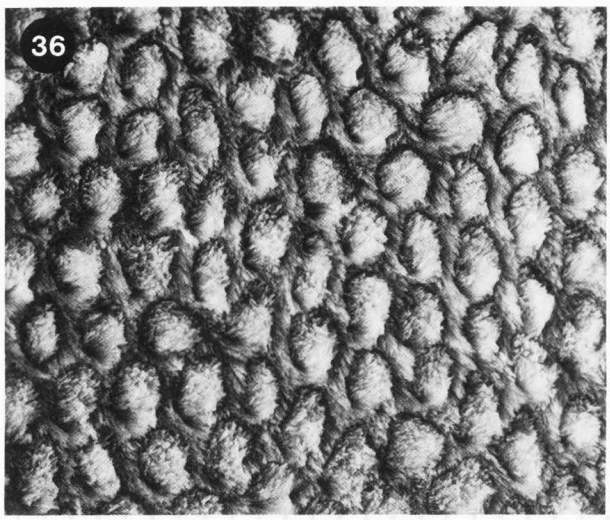
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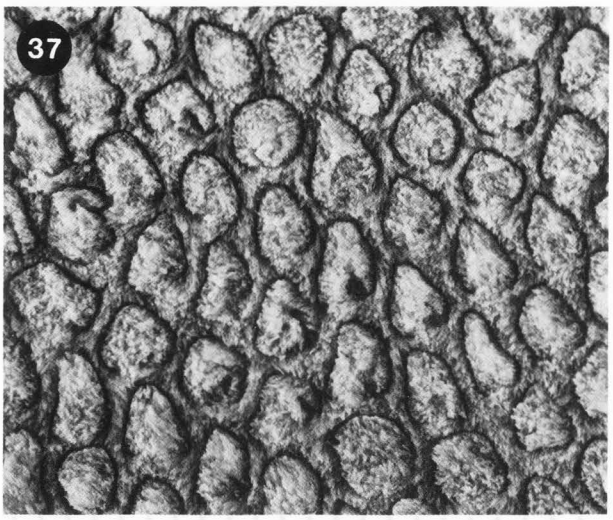
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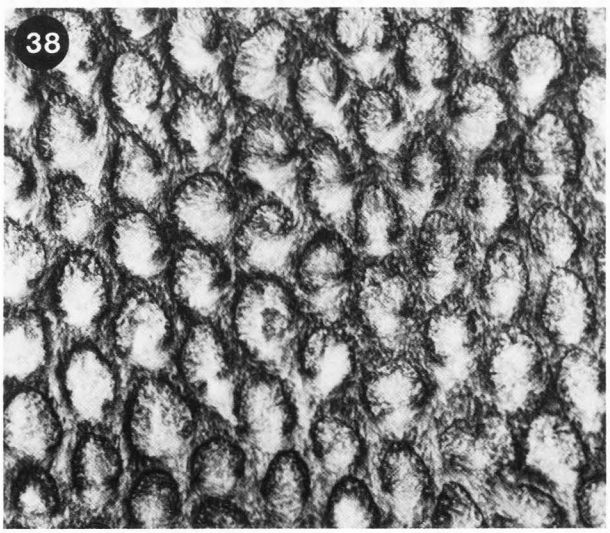
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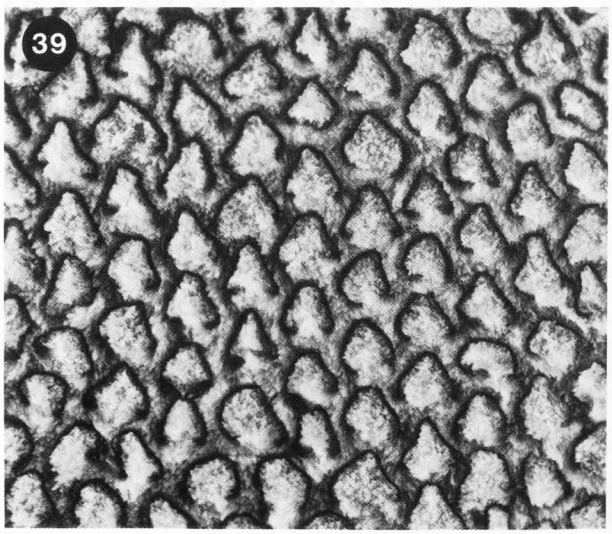
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The specimens shown in figures 34-39 were all prepared in the same way, viz., a facet was polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV.

Figure 34. Brachyteles arachnoides molar showing Pattern 3 enamel. Field width = 41 µm.

Figure 35. Chiropotes chiropotes molar showing Pattern 2 enamel. Field width = 40 µm.

Figure 36. Saimiri sciureus molar showing Pattern 2 enamel. Field width = 40 µm.

Figure 37. Lagothrix lagotricha molar showing Patterns 2 and 3 enamel. Field width = 40 µm.

Figure 38. Callicebus moloch molar showing Pattern 3 enamel. Field width = 41 µm.

Figure 39. Leontopithecus rosalia molar showing Pattern 2 enamel, with a characteristic and so far unique inverted V shape to the prism outline. Field width = 40 µm.

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that Pattern 1 prisms may be associated with marked prism decussation in, e.g., Propithecus, but concluded that prosimians generally have Pattern 1 prisms with limited decussation. Shellis (1984) has since changed his interpretation, he now believes that all prosimians, indeed all Primates, have Pattern 3 enamel except Daubentonia which has Pattern 2 enamel. Shellis argues that all other prosimians have Pattern 3C enamel with little decussation except for Propithecus which he reports as having Pattern 3B enamel with marked decussation. In the absence of an illustration showing Pattern 3 prisms in Lemur and Shellis and Poole's (1977) own summary table giving Pattern 1 prisms as characteristic for prosimians, the present authors feel that the presence of Pattern 3 enamel in strepsirhine primates, other than in Propithecus (see Boyde and Martin, 1988) has yet to be established. We do not feel that Shellis (1984) has demonstrated a case for interpreting enamel previously read as Pattern 1 as being Pattern 3C, and may in fact be using modified definitions of enamel prism packing patterns. The presence of Pattern 2 enamel in the incisor teeth of the aye-aye conceivably may be in some way related to the functional specialization of the strongly decussating enamel of these ever growing incisors. Thus it is likely to be an independent development in that taxon. Shellis (pers. comm.) has also found Pattern 2 in the molar enamel of Daubentonia.

We would therefore conclude that strepsirhine primates, with the probable exceptions of Daubentonia and Propithecus, are characterized by Pattern 1 enamel which may be associated with prism decussation in some taxa. This modifies the position taken by Boyde and Martin (1984a) who reported an absence of decussation in prosimian enamels (other than Daubentonia).

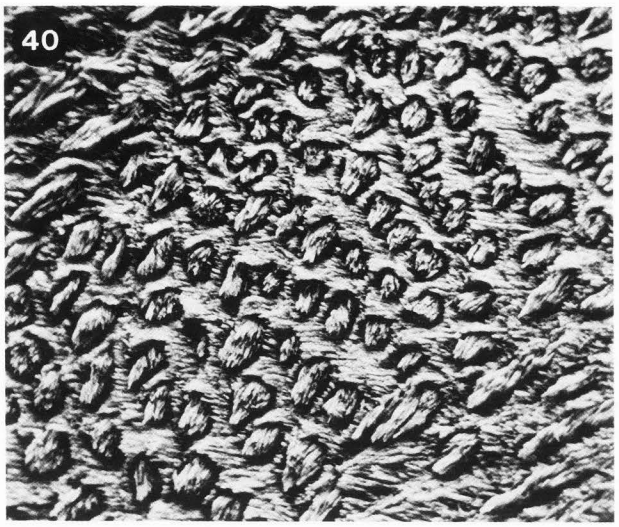
Discussion and Conclusions

Studies of primate enamel microstructure by scanning electron microscopy have added greatly to our knowledge of the distribution of enamel prism packing patterns in the Order. The range of variation already known is difficult to interpret phylogenetically but indicates that when more complete surveys have been completed, particularly those using developing material and other preparations whose interpretation is not complicated by acid etching, enamel prism packing patterns will have something useful to contribute to considerations of primate phylogeny and especially to the interpretation of fossil taxa. That a great deal of parallelism will be found is not in question, particularly when making comparisons among higher taxonomic categories, but within relatively restricted taxonomic groups we feel that a knowledge of enamel structure will considerably increase our ability to resolve phylogenetic relationships.

The current knowledge of structural diversity in primate enamels might be summarised as follows. Where several alternative descriptions have been offered for taxa, we have adopted those findings supported by illustrative material.

Hominoidea

All modern, and presumably extinct, hominoids are characterized by the presence of Pattern 3 enamel and only very localized patches of Pattern 2 enamel if this is found at all. This is an unusual situation in Primates, as no other superfamily shows a predominance of Pattern 3 enamel, and no other anthropoid superfamily is characterized by a single major prism packing type. All of the species have a layer of slowly formed (Pattern 1) enamel close to the tooth surface which is of variable thickness. In Hylobates and Homo this layer is only a few microns thick; in Pongo it accounts for 20% of the completed enamel thickness, while in African apes it accounts for almost 50% of the completed enamel thickness. This is interpreted as evidence for a secondary reduction of enamel thickness in great apes. The African apes share this feature in terms of both extent and mechanism of reduction. Pongo has reduced enamel thickness to a lesser degree and also achieved it by a stepwise slowing down process in contrast to the single step seen in African apes. This is interpreted as evidence that orang-utans have secondarily reduced enamel thickness in parallel with the reduction in African apes. Contrary to recent molecular studies supporting the interpretation of humans and chimpanzees as sister taxa (Sibley & Ahlquist, 1984: see Andrews, 1986 for details), this secondary reduction of enamel appears to support the existence of an African ape clade, or in other words that chimpanzees and gorillas shared a period of common ancestry not shared with humans. In light of the fact that orang-utans have secondarily reduced their enamel thickness in parallel with African apes, supporters of a chimpanzee/human clade might argue that the same is likely to be true for secondary reduction in chimpanzees and gorillas. It seems unlikely that this is the case because parallelism can be shown between orangutans and African apes, not only due to the degree of secondary reduction, but to the pattern of the slowing down process. The fact that chimpanzees and gorillas have secondarily reduced their enamel thickness by the same amount and by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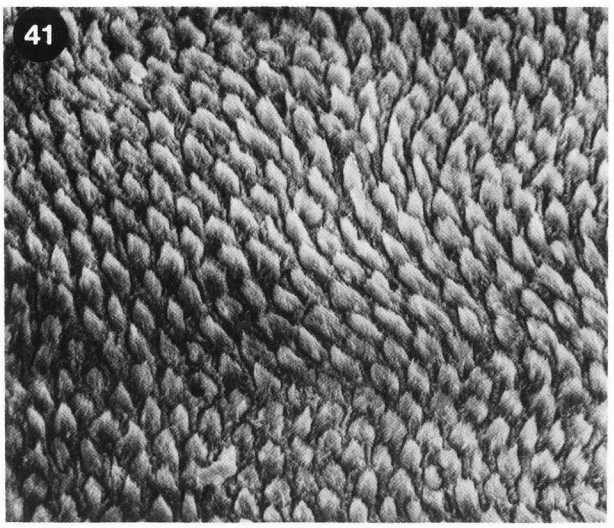
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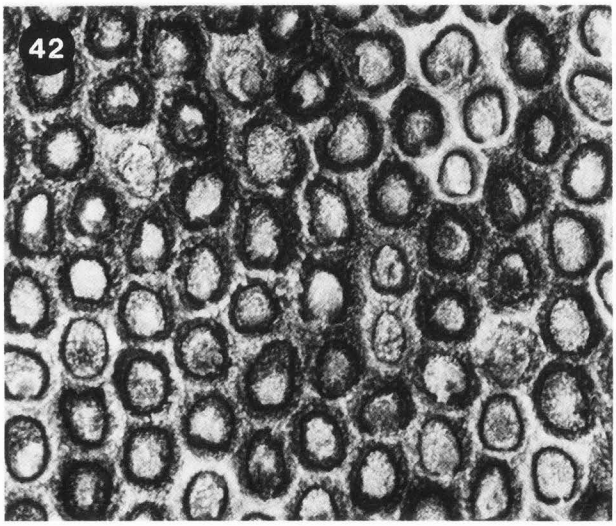
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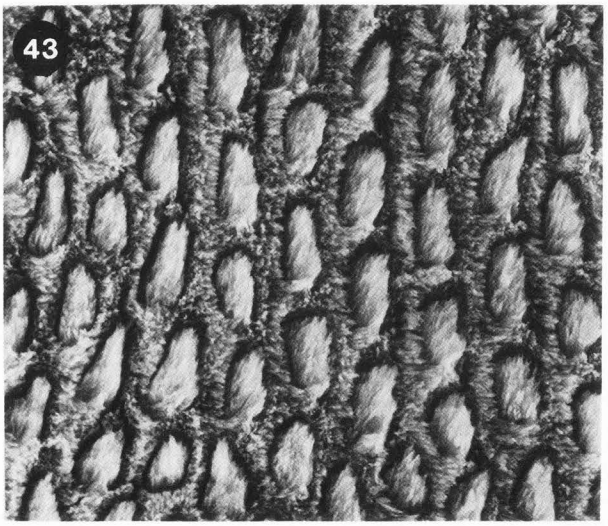
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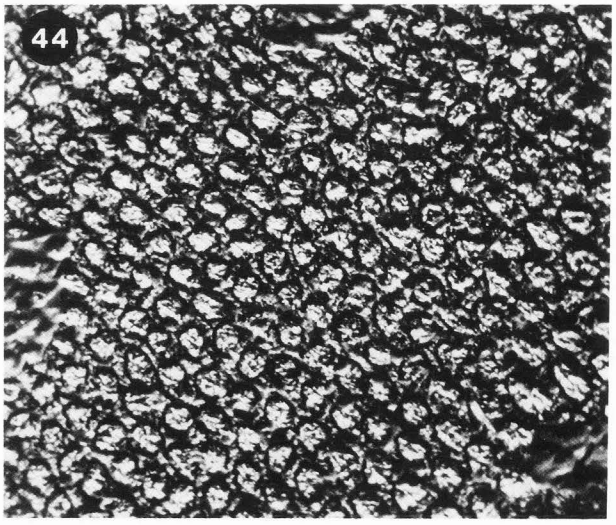
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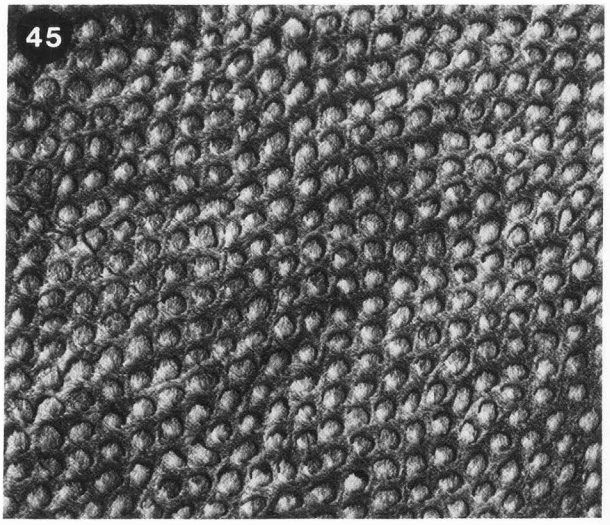
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Figure 40. Callithrix sp. Marmoset. H_3PO_4 etched diamond polished longitudinal section. CBSE, i.e., specimen was biased to +200 V to prevent the escape of low energy secondary electrons (Boyde and Cowham, 1980) giving rise to this high topographic contrast image in which we have portions of parazones (longitudinally sectioned prisms) of Pattern 1 prisms northwest and southeast and a diazone of more transversely sectioned prisms with the intervening interpit phase interprismatic substance at the center of the field of view. CBSE, 20 kV. Field width = 78 µm.

The specimens in Figures 41-43, and 45 were all prepared in the same way, viz., a facet was polished with 1200 grit silicon carbide paper and etched with 0.5% HCl for 30 seconds. SE, 20 kV.

Figure 41. Cebuella pygmaea molar showing strongly decussating Pattern 3 enamel. Field width = 78 µm.

Figure 42. Tarsius sp. molar showing Pattern 1 enamel in this particular region. In this case the Pattern 1 shows a marked tendency to be aligned into longitudinal (cuspal to cervical) rows. In other areas on the same tooth extensive regions of Pattern 2 and more localized regions of Pattern 3 enamel were also encountered. Field width = 40 µm.

Figure 43. Tarsius sp. molar showing an area of Pattern 2 enamel. Field width = 41 µm.

Figure 44. Lemur sp. lower molar enamel prepared by diamond polishing a facet parallel to the surface of the tooth and etching with 0.5% H_3PO_4 for 30 seconds. Only Pattern 1 enamel was encountered in this specimen. CBSE, specimen biased to +200 V, 20 kV. Field width = 72 µm.

Figure 45. Galago sp. molar showing Pattern 1 enamel. Field width = 81 µm.

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apparently identical developmental pathways (Martin, 1983) renders parallel evolution an unlikely explanation (Andrews and Martin, 1987b).

Cercopithecoidea

The material reviewed here demonstrates that some cercopithecoids may display a predominance of Pattern 3 or Pattern 1 enamel although a predominance of Pattern 2 enamel appears to be more common from current data. In all cercopithecoid species, Pattern 2 enamel is found at a much higher frequency than is ever seen in hominoids and might represent a derived condition within the Catarrhini. However, Pattern 2 enamel is also found in ceboids so it is possible also that the lack of Pattern 2 enamel is a derived condition of hominoids which would then support the interpretation of Oreopithecus as a hominoid. Some species of cercopithecoid show a high proportion of Pattern 1 enamel but unfortunately data are not yet available to relate such differences to developmental rates and enamel thickness.

Ceboidea

Cebids display Patterns 1, 2 and 3 enamels with only the Atelinae appearing to have a particularly high frequency of one pattern (i.e., Pattern 1, although Brachyteles and Lagothrix differ from the other three genera in having Pattern 3 enamel). Until the frequency and distribution of the alternative patterns is more fully known it is difficult to deduce the pattern which might have been seen in the ancestral cebid.

The callitrichids previously have been reported (Gantt 1980) to have entirely Pattern 1 enamel. Recent studies have shown this to be an incomplete description, as some species have been found to have Pattern 2 and others Pattern 3 enamel. It seems probable that the ancestral platyrrhine would have had Pattern 1 mixed with either Pattern 2 or Pattern 3 enamel. It is therefore presently unclear whether Pattern 3 enamel, where it occurs in ceboids, represents an independently derived condition from that seen in some cercopithecoids and in hominoids.

Tarsier

Pattern 1 and Pattern 2 enamel are reported by Grine et al. (1986) and Pattern 3 enamel by (Boyde and Martin, 1988) which makes interpretation difficult. It seems at least likely that haplorhines as a group may be characterized by ameloblasts which can secrete Pattern 2 or Pattern 3 enamel in addition to Pattern 1 enamel, which appears to largely characterize strepsirhines. The resolution of the condition of enamel in Tarsiers is a priority for future work as it pertains to the identification of the ancestral condition for the Haplorhini and for Anthropoidea.

Strepsirhines

In the absence of clear evidence to the contrary, we conclude that strepsirhines are characterised by the presence of Pattern 1 enamel, with or without decussation, the only exceptions being the indriids (Propithecus), which have Pattern 3 enamel, and the aye-aye (Daubentonia), which has Pattern 2 enamel. If this interpretation is correct (i.e., that the ancestral condition for strepsirhine enamel was Pattern 1) then we have evidence for either a strepsirhine/haplorhine dichotomy (Pattern 1 against Patterns 2 and/or 3) if Tarsiers have Pattern 3 enamel, or for a prosimian/anthropoid dichotomy if Tarsiers have Pattern 1 enamel. In either case, the enamel prism packing type would appear to have great potential for addressing the affinities of certain fossil specimens: for example, the omomyid primates from the Eocene of Europe and North America, thought to be early Tarsier relatives, and fossils thought to be early anthropoids such as Pondaungia, Amphipithecus, Apidium and Parapithecus.

Conclusion

A great deal has been learned about primate enamel structure through SEM studies and a good deal learned about primates and their relationships. Perhaps the major lesson from studies to date is that we need to collect much more numeric, descriptive data before developing too elaborate schemes to explain the data we have at present. In every case, the initial description

of the enamel type which characterizes any higher taxonomic group has required modification in the light of subsequent studies of larger samples of taxa from within that group. We recommend the survey of more tooth types per individual at a range of depths within the enamel, and of more individuals per species, as well as more species representative of higher taxa.

Acknowledgements

The work reported and reviewed here would be impossible without the support and encouragement of museum curators who were and are prepared to make material available for enamel structure research. While we continue to strive to develop less destructive techniques to obtain these undoubtedly valuable data, we would not be where we are today in our knowledge were it not for those people. We do not know the names of curators who provided material to workers other than the authors of this paper, but we thank them none the less.

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Discussion with Reviewers

C.P. Groves: Dostal and Zapfe (1986; see also Dostal et al., 1985) have recently claimed to find differences in enamel prism shape between various taxa of Cercopithecoidea; in particular, they claim that prism shape in Mesopithecus pentelicus can be allied to that in an Asian colobine group.

The authors of the present paper note that the HCl method, the one used by Dostal et al., produces "etching arfefacts" which "render accurate interpretation...problematic". On the other hand, Dostal et al.'s results seem consistent and comprehensible, and one would like to think that they are soundly based. A comment on this matter would be helpful.

Authors: We thank you for drawing our attention to the work of Dostal and Zapfe. Our concerns about the use of HCl as an etching agent are based upon studies using fresh enamel. We remark in the text that etching reagents for fossils need to be evaluated separately. For their specimen preparation, Dostal and Zapfe used normal HCl for about three minutes. Assuming the same rate of etching for cercopithecoid and human enamel they would probably be looking about 150 µm deep into the enamel. This etching regime would have resulted in a surface contour of several microns. The exact cross-sectional form of the enamel prisms would depend upon the direction of view of the surface. It should be remembered that these views will represent a view perpendicular to the tooth surface, roughly said, not a view perpendicular to the prism long axis. There is no information given as to what level on the tooth's surface one is looking at, nor that the authors have assessed the angle that the prisms make with the enamel-dentine junction. So, we have no way of knowing, or assessing, the difference between the cross-section seen perpendicular to the tooth surface, and the cross-section seen perpendicular to the prism.

We have analysed Dorsal and Zapfe's SEM images as follows:- Plate 1 - Macaca mulatta - 6 part illustrations all show Pattern 3; Plate 2 - Papio anubis - 6 part illustrations - Patterns 3, 2, 3, 2, 3, 2; Plate 3 - Papio hamadryas - 6 part illustrations - Pattern 3; Plate 4 - Cercopithecus aethiops - 6 part illustrations - Patterns 2, 2, 2, 2, 3, 2; Plate 5 - Presbytis entellus - 6 part illustrations - Patterns 3, 2, (rather than 2A, but some 3), 3, 3, 3, 3; Plate 6 - N. larvatus - 6 part illustrations - Pattern 3; Plate 7 - Colobus polykomos - Patterns 1, 3, 1, 3, 1, 3; Plate 8 - Mesopithecus pentelicus - 6 part illustrations - Pattern 3.

R.W. Fearnhead: This paper uses some terminology which may not be familiar to all readers and which should be defined. The word "taphonomic" is new to me; also the use of the word "clade" to mean monophyletic unit.

Authors: Taphonomy is a term coined by a Russian paleontologist Efremov (1940) to describe the study of the transition (in all its details) of animal remains from the biosphere into the lithosphere. The major foci of taphonomy are the events that intervene between death and fossilization and their effects on the retrieval of information about the past (Shipman, 1981).

The term clade is commonly used by taxonomists practising phylogenetic systematics (Hennig, 1966). Its use to mean a monophyletic unit dates to the work of Huxley (1958).

D.G. Gantt: In general this is an excellent paper but there are two points which the authors should address. Firstly, the statement by Vrba and Grine that prism morphology contains no information on phylogenetic relationships is shown by this paper to be **false** as I have stated previously. Secondly, the only point of disagreement with my findings is that I have accepted Pattern 3 to be replaced by two variants within the hominoids, Pattern 3A in the living apes and extinct Miocene hominoids; while Pattern 3B is present in the hominids (both living human and extinct forms). Studies using image analysis procedures have documented a 25% increase in the "tail" section of Pattern 3B prisms when compared to Pattern 3A prisms.

Authors: Vrba and Grine (1978a, pp. 891-892) concluded that "gross prism morphology contains no information on phylogenetic relationships of hominoid species. ....The occurrence by itself of a prismatic keyhole pattern in Ramapithecus suggests no closer kinship of this taxon to H. sapiens than to the extant apes". We look forward to the publication of a quantitative study of enamel prisms in three dimensions that demonstrates the greater "tail" section of Pattern 3B prisms. For the present, our conclusion is that all hominoids share a predominance of Pattern 3 enamel in the deeper layers which supports Vrba and Grine's assertion that prism morphology contains little information on the phylogenetic relationships of **hominoid species**. In particular, we agree with their conclusion that the presence of Pattern 3 enamel in Ramapithecus does not ally it with H. sapiens. Their conclusions should perhaps be amended slightly to allow that the differing incidence of Pattern 1 enamel in great ape teeth has phylogenetic information.

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