

Why tribosphenic? On variation and constraint in developmental dynamics of chiropteran molars*

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* Dedicated to Professor Dr. Vladimír Hanák on the occasion of his 80th birthday

12.1 Introduction

Teeth and dentitions are key evolutionary novelties of vertebrates – much of the success of that clade can be traced to just these structures. The extreme ecological efficiency, rapid rate of adaptive rearrangement and growth dynamics, and large body size, as well as the finely tuned developmental mechanisms characterizing the vertebrates have one commonality: all are closely linked to a very high rate of energetic turnover. The core of the circuit lies in the rate of energetic flux from outside to inside vertebrate bodies. Teeth and dentitions act as its powerful amplifiers, the appearance of which may have played a decisive role in triggering a great deal of the current scope of vertebrate adaptations.

The dentition is not only a physical interface between the exterior and interior of an organism, but also a very complex interface between the energetic demands of the body, characteristics of diet, food availability and foraging. The form of dentition is influenced by selection related to these factors, as well as the phylogenetic history of a taxon and pathways of its past adaptive efforts. Theoretically, the state of dental characters may provide condensed and relevant information on any of these variables.

The core agent of the interface is the highly mineralized enamel cap, which covers the dentine body of the tooth crown with a rigid layer of hydroxyapatite crystallites. It is the most pertinent attribute of any tooth and the ultimate source of the extraordinary resistance of teeth to external influences, either chemical or mechanical. The exclusive role of teeth in vertebrate evolution is directly linked to this enamel crown, the resistance of which is, alongside

others, also responsible for excellent preservation of teeth in sediments and, hence, for the particular richness of the vertebrate fossil record.

Dental characters present the essential (and often the only) source of information available from the vertebrate fossil record. Supplemented with comparative information on contextual variables accompanying the respective character states in extant forms (for which detailed ecological or phylogenetic information often is available), these characters can provide information about the phylogenetic and ecological attributes of fossil forms. Yet, to extract such information necessitates not only a careful descriptive screening of the states of particular dental characters, but also an understanding of their variation and the functional, structural and developmental constraints that modify the pattern of their variation (Gould, 1989) and which may essentially influence the phenotypic effects of phylogenetic rearrangements (Maynard Smith *et al.*, 1985).

The variation dynamics of dental characters and the developmental mechanisms producing teeth are constrained by spatial limitations (including spatial demands of neighboring structures) and the structural integrity requirements of the whole orofacial complex. In addition, the life-history traits (essentially unrelated to them) may impose temporal constraints on development. Thus, the implicit demand to maximize the size and functional versatility of the dentition has been moderated by the capacity of available developmental mechanisms, by spatial competition, the developmental requirements of the morphological structures surrounding it and by competition of the factors contributing to biomechanical and structural integrity of all its elements (such as shape and size of particular teeth, modes of their articulation, construction morphology of jaws and jaw musculature etc.). The stronger the constraint upon variation of dental characters, the more acute the pressure to evolve innovations that would release the constraining effects and enlarge the variation. The inherent feedback control of the respective innovations by developmental constraints and functional demands is perhaps the major path of dental phylogeny and source of the conspicuous continuity of dental evolution.

All these factors somehow influence the dental phenotype and should be taken into account when analyzing the phylogenetic and ecological information that dental morphology provides. This chapter is intended to explore some of these influences and to discuss the basic setting of developmental and construction constraint upon chiropteran molars, the patterns of innovation and the methodological consequences for study of phylogenetic transformations of dental characters and bat evolution.

The objectives of this work are to: (1) analyze structural and functional specificities of tribosphenic molars, including their developmental background and a possible mechanism leading to precise occlusion (2) document patterns of

variation in chiropteran molars and relate this to taxon-specific developmental differences; and (3) argue that the current state of knowledge of dental characters in bats is insufficient for their direct use in phylogenetic studies, unless detailed information on their developmental dynamics is taken in account.

12.2 The essentials

Probably the most influential constraint upon dental variation arises from the essential functional quality of a tooth, i.e., the extreme rigidity of the enamel due to its complete mineralization, which provides the tooth with enormous resistance to mechanical stress. Of course, for obvious reasons: (1) the enamel must resist mechanical stress of any act of occlusion and hence be completely hardened even prior to the first use; (2) the completely hardened enamel does not allow any plastic changes, reshaping etc.; (3) any rearrangement, however minute, in tooth shape and size can be thus achieved exclusively via rearrangements of the developmental processes forming the tooth prior to its first use; and (4) once the tooth is mineralized and appears above the gingiva no changes in its size and shape appear (except by wear caused by usage).

The state of dental characters is strictly controlled by capacity of dentition to respond to functional demands appearing during the course of an individual's life (such as actual efficiency of food processing, correspondences between dietary preferences and capacity of respective food resources, and ad hoc variations in their availability). Such a *prospective regulation* would undoubtedly promote enlargement of capacity to modify the state of particular characters in response to ad hoc variation of the respective contextual variables, the enlargement of variation and plasticity of dental characters. Yet, everything in that respect must be produced prior to the first occlusion event, which would be beyond the direct effect of the prospective regulation. In other words, the ultimate effects responsible for all fine dental variations arise from a domain of *retrospective regulation* via the rigorous developmental machinery that produces the teeth. More strictly than in any other systems of the vertebrate body, in the dentition any influences of prospective regulation can be imposed only in the form of a phylogenetic event, i.e., via rearrangements of the inherited programs of retrospective regulation. For the practice of odontologic analysis this means the following: the state of a particular dental character, while undoubtedly having something to do with foraging specificities of a given taxon, does not reflect this directly, nor does it illustrate the best response to prospective regulation – rather it expresses the capacity of taxon-specific retrospective regulation to respond to the respective cues. A dental character is produced by a superposition of developmental rearrangements implemented

into the recurrent pathways of dental ontogeny. It is a palimpsest rather than a message easily readable with the help of an odontologic dictionary.

Nevertheless the information provided by dental characters is undoubtedly quite important, and in some cases, such as often in fossil taxa, the only one which is available. To exploit it means to adapt our reading in regards to what its direct phylogenetic or functional meaning might be – the fact that the proper triggers of the phylogenetic divergences and/or the factors responsible for taxon-specific variation or constraints on particular dental characters are situated somewhere in the developmental machinery producing teeth and any rearrangements in dental phenotype (regardless of whether it is significant for a phylogenetic or functional point of view) are the epiphenomena of developmental processes. Yet, to support these claims is, for more than one reason, still a bit complicated.

Teeth are produced by specifically regulated interactions of the ectodermal epithelium and the neural crest mesenchyme, the most progressive cell populations of the vertebrate body. Recurrent steps of the interaction are triggered by cascades of signaling proteins specifically expressed either in the epithelium or in mesenchyme, which mostly effect growth rate and metabolic profiles in neighboring cell populations (Thesleff and Sharpe, 1997; Thesleff, 2003; Tucker and Sharpe, 2004). A signaling function was established for about 130 of *c.* 280 proteins for which genes were identified to express in tooth primordia (comp. <http://bite-it.helsinki.fi> for a detailed account). Despite extensive redundancy in signaling effects, even a minute variation in expression of a single factor may cause extensive phenotypic effects (Kassai *et al.*, 2005; Peterková *et al.*, 2005). In that respect, the genocentric optics of contemporary biology would orient our search for the triggering factors of phylogenetic divergences into the sphere of comparative molecular biology of dental development – a field both conceptually and methodically very distant from the scope of the primary interest of this book. Nevertheless, as demonstrated below, relevant information on the developmental background of dental variation in bats can be obtained beyond molecular optics.

The process of odontogenesis starts with the initiation stage by local thickening of the odontogenetic epithelium and local condensation of mesenchyme, and continues by extension of an epithelial bud into the mesenchyme. A dramatically increased growth rate of the epithelium in the next stage results in lateral expansion of the epithelial bud which subsequently encapsulates the mesenchymatic condensation in the form of the bilaminar epithelial cap. The outer epithelial layer forms (in interaction with the neighboring mesenchyme) the dental sac separating the spatial domain of the tooth primordium from outside effects, while the inner layer shows a deep evagination of the three-dimensional growth of the encapsulated mesenchymatic papilla. The accelerated growth of that stage results in the spatial interplay between two-dimensional growth of the inner epithelium

and three-dimensional growth of mesenchyme, which characterizes the next step of dental development – the stage of the dental bell.

In the bell stage, enlargement of the tooth primordium and shaping of the future tooth by locally specifying folding of the inner enamel epithelium (tooth morphogenesis) is later accompanied by histogenetic changes in the cells interacting along the basal membrane of the inner enamel epithelium: epithelial cells change into cylindrical ameloblasts, mesenchymal cells into odontoblasts equipped with protruding, densely branched odontoblastic processes. Both ameloblasts and odontoblasts present a mineral compound into the bordering zone. With their secretory processes the ameloblasts increase concentration of the mineral matrix and preform hydroxyapatite crystallites, which subsequently aggregate into a highly mineralized enamel, while the odontoblasts organize a complex redistribution of primary crystallites along the collagen scaffold by which they produce a tubular, highly organized, but less mineralized dentin. The mineralization begins from the inflexion points of the deepest epithelial evagination – tip of the adult tooth cusp – and subsequently spreads down to the cervical loop separating the inner and outer enamel epithelium. Following complete enamel mineralization, the morphodifferentiation of the tooth terminates and the completely mineralized tooth erupts (notwithstanding derived conditions in some mammals as discussed below). The above described pattern presents an invariant phenomenon common to all gnathostomes (Reif, 1982; Smith and Hall, 1990; Stock, 2001; Huyseune *et al.*, 2009), and this holds also for the proximate developmental machinery and the signaling cascades specific for particular stages (Stock, 2001; Thesleff, 2003; Tucker and Sharpe, 2004).

The essential qualities of adult teeth, such as size or thickness and rigidity of enamel depend upon the time available for tooth development (and the particular steps through which it proceeds e.g., morphogenesis, histogenesis and mineralization). Prospective control (e.g., deficiencies in these qualities in adult teeth) can quite strongly promote any innovation prolonging the period of dental development or establishing specific heterochronies accelerating the performance of the respective processes or heterotopies acting in a similar way (e.g., simultaneous development of different dental generations or synchronous appearance of different odontogenetic processes at divergent loci of a single tooth primordium).

The odontogenesis of mammals, both in diversity of teeth types and their structural or functional complexity, is characterized by rigorous exploitation of all these possibilities. It is particularly well illustrated by the major dental apomorphies of mammals: large monophyodont multicuspidate posterior teeth (molars) and prismatic enamel. The prolonged early tooth development and accelerated growth of the oral region in mammals is the outcome of accelerated

epithelial growth that results in the morphogenesis of mammalian teeth starting with very large cell populations, both epithelial and mesenchymal. Then, thanks to a long gestation period, mammalian teeth can grow large, are disposed to intricate shapes and their enamel coat can be quite thick, at least at tooth tips where amelogenesis starts first. For teeth of the permanent generation and monophyodont molars, the developmental time is even further prolonged, with a postnatal lactation period that allows these teeth to grow very large and respond to requirements of large adult body sizes and/or specialized foraging and longevity.

12.3 Tribosphenic molar: an enigmatic phenomenon

The vast majority of the information on dental development has been obtained from just a single mammalian model, unfortunately one that is extremely derived in almost all dental characters, the mouse. Which of the odontogenetic mechanisms described in detail for mice (e.g., Caton and Tucker, 2009) operate in dental ontogeny of other mammals cannot be answered completely due to scarcity of relevant comparative data. Unfortunately, surprisingly little embryological information (except perhaps for Marshall and Butler, 1966) is available on development of the tribosphenic molar – the molar type uniformly distributed in bats (except for Pteropodidae and some Phyllostomidae). This fact is noteworthy in particular because the tribosphenic molar is generally considered to represent the phylotypic stage of mammalian dental evolution, the molar type common to ancestors of all extant clades of mammals. Such a view was proposed first by Cope (1874, 1883) and Osborn (1888) within the framework of the trituberculate theory of mammalian dental evolution. In its final form (Osborn, 1897, reprinted in Osborn, 1907) the theory provided an integrated concept of the evolutionary history of mammalian molar teeth and a paradigm for comparative study of mammalian dentitions. The theory declared the pathways of phylogenetic transformation from a presumed ancestral condition – a single-cone tooth of reptiles to derived multicuspidate molars of extant groups – predicted a set of homologous relations among teeth of mammals and among the structural elements of mammalian teeth in phylogenetic, structural, developmental and functional respects. These assumed homologies were further expressed the form of a detailed nomenclature of structural elements of mammalian molars – cusps, crests, cingula, styles etc. The identical terms applied for particular elements of the upper and lower molars (with the suffix *-id* used for the latter) stressed a complete homology between them. The theory also explained the obvious difference in design of the upper and lower molars, which it stressed even by different terms denoting the respective designs, i.e., trituberculate for the former and tuberculosectorial for the latter.

The term *tribosphenic* replacing the Cope–Osborn term trituberculate-tuberculosectorial was introduced for the respective molar type by Simpson (1936), perhaps in response to criticism of verbal confusions of the Osbornian nomenclature by Friant (1933, see Simpson, 1933). The term (*tribein* = to rub, *sphenic* = wedge) stressed integrity of upper and lower molar design in their complex function, combining effects of shearing and compression at every single occlusion event, suggestive of a mortar and pestle (with talonids of the lower molars as mortar and protocone of the upper ones as pestle).

It should be remembered that particular issues of the Cope–Osborn concept were almost immediately criticized, first using embryogenetic data (Woodward, 1896), and later by fossils as well (Gidley, 1906), both demonstrating convincingly that the primary cusp supposedly homologous to a single-cone tooth of reptiles is not the protocone (in the upper molars) but the antero-external cusp, i.e., paracone or parastyle. The condition in lower molars (i.e., protoconid as the primary cusp), in contrast, fits well with the prediction, which seems to disprove the predicted model of upper-lower molar homology. In his later survey, Osborn (1907) partly accepted the criticism, but did not respond to it with any changes in dental nomenclature, apparently in order to prevent possible confusion.

Subsequently, of course, nearly all particular conceptual issues and predictions of the Cope–Osborn concept (reviewed in detail by Hershkovitz, 1971) were refuted and the multiple homologies among all elements of mammalian teeth disproved (Butler, 1956; Vandebroek, 1961; Butler, 1978; Van Valen, 1982, 1994). Several attempts were made to innovate the nomenclature of dental elements in response to changes in the concept of dental homology, the most conclusive by Vandebroek (1961, 1967), who proposed a radical departure from the Osbornian terminology. Perhaps due to deeply ingrained usage of the Osbornian terms in practice of odontologic descriptions and doubts about alternative homology predictions, the nomenclatural innovations were not widely accepted, except perhaps for the terms eoconus (= paraconus)/eoconid (= protoconid) that are alternatively used for the Osbornian paraconus/protoconid.

Despite all doubts and refutations, the basic idea and key assumption of Cope–Osborn theory, i.e., that the trituberculate (tribosphenic) molar represents the phylotypic stage of mammalian dental organization – a platform from which the diversification of molar teeth began, retained its role in the paradigmatic framework of mammalian comparative odontology and in recent decades it has received even more robust empirical support. The earliest mammals (Flynn *et al.*, 1999; Luo *et al.*, 2002; Rauhut *et al.*, 2002) all show the same structurally basic pattern of molar shape: V-shaped crest centered with major

cusps positionally identical with those characterizing tribosphenic molars in extant mammals. In other words, despite the possible dual origin of tribosphenic design (Australosphenida vs. Boreosphenida – Luo *et al.*, 2001) or variations in pretribosphenic stages exceeding states in extant mammals (Luo *et al.*, 2007), tribosphenic molars and the structural design characterizing them presents a robust common feature of mammalian organization and perhaps the most influential triggering factor in the radiation of mammalians.

Both with respect to taxonomic distribution and in terms of number of species, the tribosphenic molar is also the most common molar type among the extant mammals. An important question that (particularly concerning the enormous divergence of derived tooth types in mammals) necessarily arises is why just tribosphenic? What makes that design so successful, and at the same time so progressive and so constrained that it survived nearly unchanged for almost 180 million years of mammalian evolution?

A default answer to these questions would perhaps first address the functional qualities of that tooth type. A combination of shearing, grinding and transporting of food particles, simultaneously during a single occlusal action, provides nearly ideal condition for rapid food processing and enhancement of mastication effort. The geometry of shearing blades that contribute to these effects was recently analyzed in detail by Evans and Sanson (1998, 2003, 2006) and Evans (2005, 2006) in terms of bio-engineering models of optimal tools. The chiropteran tribosphenic molar was found to be a “tooth of perfection” because its shearing blades and the arrangement of its leading and rake angles correspond exactly to the functionally most effective design. The study was criticized for underestimation of the role of crushing in occlusal effort (Weil, 2003), but this objection, in the case of tribosphenic molars, is not well supported. The tribosphenic molar is obviously designed for insectivory or microfaunivory, i.e., feeding on tissues of animals, the diet that is essentially easily digestible without any mechanical treatment prior to the digestion (e.g., reduction of molars and secodont rearrangements in the specialized flesh-eaters or complete disappearance of teeth in specialized myrmecophagous mammals). Feeding on insects is constrained by the fact that the easily digestible food is covered by a chitinous cuticula that must be mechanically broken apart. Not compression, but shearing of the cuticula is the most efficient way to proceed, and correspondingly, the shearing tools (the crests) are by far the most essential attributes of the tribosphenic design. Insectivory further differs from macrofaunivory in that insect food resources are quite common, widely available, but also in that insects are rather small, and variegated in spatial distribution, which promotes tactics of opportunistic feeding with a need for rapid processing of large amounts of a small-sized vagile prey.

The basic design of tribosphenic molars is quite uniform and remains generally unchanged except in clades that abandoned insectivory. Nevertheless, taxon-specific arrangements of particular dental elements (e.g., proportions of molars and their crests) show considerable variation, although some common trends can be easily identified. For instance, teeth are higher and more robust when larger and more heavily armored prey is consumed the arrangement often correlated with enlargement of temporalis musculature and its insertion areas on the mandibular ramus (Storch, 1968; Freeman, 1981a, 1981b, 1984, 1988). Strait (1993) argued that dietary choice inversely scales with total length of molar crests. Accordingly, soft-object feeders exhibit more shearing development than do closely related taxa that have more generalized feeding patterns and/or those feeding on hard-bodied insects (but see Evans and Sanson, 1998).

The phylogenetic rearrangements *within* the frame of generalized micro-faunivory and tribosphenic molar design are characterized by little selection for crushing and food compression, but very strong selection for efficiency of shearing and rapid food processing. Correspondingly, relatively large variation in compression surfaces (protoconal fossa, talonid basin) can be expected with rather constrained variation in the components contributing to shearing (i.e., crest sharpness, resistance of the crest edges to mechanical stress of attrition and the total length of the crest system of the dentition). Indeed, the crests present the most obvious component of the tribosphenic molar and are true agents of its function. At least from the functional point of view, tribosphenic molars can be viewed as mere *bases for promoting shearing crests*. Then, of course, the morphology of the tooth would present a balance of two structural and functional units quite different in their structural and developmental demands: (1) the crest system requiring extensive enlargement and potential flexibility (at least from a phylogenetic perspective) and (2) a tooth base requiring reliable physical integration of all components of the tooth into a rigid framework of spatial organization (ensuring an even redistribution of the residual energy of shearing and counterbalancing local strain). Yet, these partly opposing requirements and the structural elements disposed to respond to them share the same limited space of the tooth primordium and the same limited time available for tooth development.

In addition there is still another factor that may play a role in ultimate constraint on tribosphenic design, the factor that scales efficiency of shearing and strictly controls functionality of the whole dentition: the pattern of interlocking among occluding blades and, hence, among all occluding elements of the dentition – crests, cusps, walls, fossae etc. To achieve a proper shearing effect, the interlocking must be quite precise, and the smaller the prey the more precise interlocking is required. The interlocking precision also acts as a

key factor in the domain of life-history traits. It can considerably reduce the effects of attrition of occluding structures (i.e., a predominant component of tooth wear in insectivore's insect food is essentially non-abrasive), prolong functionality of dentition and promote longevity.

The functional complexity of the crest system and its precision interlocking requirements integrate the whole dentition into a single synergistic functional unit. This arrangement is highly advantageous and increases the functional effect of every single occlusal action. Nevertheless, at the same time it is extremely risky because any small misalignment in cusp position, crest angle or cusp height may dramatically reduce the functionality of the whole dentition and can potentially be lethal. For that reason, answering how interlocking is achieved and which developmental mechanisms prevent malocclusion is the paramount step in answering, Why tribosphenic?

Marshall and Butler (1966), who specifically searched for answers to that question, demonstrated that at every stage of embryonic development the lower and upper molars develop in perfect correlation and are potentially occludable. They concluded that "the mechanism by which this unity is brought about remains mysterious." The contemporary paradigm of odontogenetic mechanics suggests that there is a fine-tuning of respective signaling cascades and synchronous timing in both upper and lower molars.

Yet, the developmental context of upper and lower molars is very different and the differences in shape and size of potentially occluding crests are also different. Indeed, it is very hard to imagine a molecular mechanism capable of tuning the interlocking pattern over the whole dentition with precision at a micrometer level. In any case, despite certain variation (Polly *et al.*, 2005), respective interlocking precision is the essential quality of tribosphenic dentition. This precision necessarily must be produced by developmental mechanisms and we are obliged to look for its explanation there.

12.4 Mammalian molars and developmental traits

Salazar-Ciudad and Jernvall (2004) proposed two models of regulation of tooth development: morphodynamic (with the appearance of apomorphic regulatory cues, the establishment of an autonomous regulation domain etc.) and morphostatic (where the morphogenetic variation is exclusively due to heterotopic or heterochronic modulation of growth). Morphodynamic regulation characterizes the early stages of molar development, while later development falls entirely in the domain of morphostatic regulation. The former is particularly linked to the specific apomorphy appearing during development of multicuspidate mammalian teeth: the temporary condensations

of cells at the outer surface of inner enamel epithelium, first beginning at cap stage, called enamel knots. Since Ahrens (1913), the enamel knot is considered as the primary source of morphogenetic information or, more strictly, an organizing agent of the morphogenetic field of cusp formation (Butler, 1956, 1995).

Histogenetic techniques demonstrate that the enamel knot plays the role of an autonomous signaling organ controlling initiation of cusp formation via expression of signaling proteins. In turn this influences mitotic activity of cell populations that surround the knot resulting in specific folding of the basal membrane of the enamel epithelium and shaping the future enamel–dentine junction (Jernvall *et al.*, 1994; Thesleff *et al.*, 2001; Tucker and Sharpe, 2004). In molar primordia, the primary enamel knot activates infoldings of the epithelium into mesenchyme papilla, then disappears. Later, at the beginning of the bell stage, the secondary knots appear *de novo* (Matalova *et al.*, 2005), at the tips of the cusps. Butler (1956) proposed and Jernvall *et al.* (1994), Jernvall (1995) and Salazar-Ciudad and Jernvall (2002, 2004) demonstrated in detail that additional cusps within tooth primordium are produced by heterotopic repetition of one and the same signaling module characterizing the enamel knot (e.g., Shh, Bmp-4 signals from mesenchyme, mass production of Fgf-4 by cells of the enamel knot, besides a large number of other signaling molecules). Correspondingly, the phylogenetic transformations in tooth shape or in the proportions of its structural components are established by specific spatial and temporal changes in triggering and switching of particular modules of dental development (and, last but not least, a different response of epithelial cells to these signals at different stages of their histogenesis). A high resolution three-dimensional map of these heterotopies and heterochronies overlain on a presumptive design of molar tooth (say a phylotypic blueprint of the mammalian molar) would be the best way to express taxon-specific dental characteristics. Unfortunately, this is beyond the scope of available methods to achieve directly via embryogenetic studies.

Nevertheless, a source of indirect but finely scaled information on that subject is available and it seems to be relatively easy (at least in comparison to methodical requirements of embryogenetic studies) to gather it from a fairly broad spectrum of taxa. The source of this information can be found in spatial variation of enamel microarchitecture (e.g., enamel thickness or prism orientation). In this context it is important to remember that: (1) enamel production is enormously time consuming and has to cover a considerable part of the developmental time; (2) when an enamel crystallite and/or enamel rod is established it becomes an invariant component of a morphospace and preserves, by its physical qualities, the information on conditions under which it

originated; and (iii) local differences in crystallite and/or enamel rod size reflect local differences in amelogenetic activity (similarly, local variations in orientation of enamel rods with respect to enamel–dentine junctions or another plane of comparison inform on the variations in orientation of the secretory front of ameloblasts and on local variations in morphogenetic dynamics within the tooth primordium). In short, a detailed three-dimensional map of the inner architecture of the enamel cap of a molar tooth can provide an informative reference on heterotopic variations in tooth morphogenesis during the mineralization stage and a reliable source of comparative information.

12.5 Enamel architecture of some chiropteran tribosphenic molars

Perhaps Loher (1929) was the first to study enamel microstructure in bats (*Myotis myotis*) in detail – then as a model taxon of primitive mammalian enamel. He provided detailed data on prism and crystallite size and relevant histological evidence suggesting the essential role of “Tomes’ fibers” (odontoblast processes) in the organization of prismatic enamel. Other than descriptive notes by Friant (1964) and Boyde (1965), further data appeared much later. Lester and Boyde (1987) and Lester and Hand (1987) examined the basic enamel patterns in *Hipposideros diadema* and compared its occlusal surface enamel patterns to *Rhinolophus euryotis*, *Macroderma gigas*, *Taphozous georgianus*, *Nycteris* sp., *Artibeus* sp., *Mystacina tuberculata*, *Chalinolobus morio*, *Macroglossus minimus*, *Pteropus scapulatus*, *Dobsonia* sp. and two fossil species, *Palaeochiropteryx tupaiodon* and *Hipposideros nooraleebus*. Lester *et al.* (1988) supplemented that information with several species of phyllostomid bats and discussed certain specificities of that group.

Since then the topic has been addressed only rarely. Koenigswald (1997a), who provided a comprehensive survey of diversity in enamel patterns and “schmelzmuster” (Koenigswald and Clemens, 1992) in mammals, summarized the situation in bats (both with respect to previous studies and his own examinations) by noting that mainly radial enamel (prism orientation perpendicular to the enamel–dentine junction) is present with a thin prismless outer enamel, and that the crystallites of interprismatic matrix are mostly parallel to the prisms. In contrast to most other mammalian clades, neither any derived state of these characters (such as prism decussation, Hunter–Schreger bands, tangential or zipper enamel) nor any pronounced variability of enamel structure at the dentition level (Koenigswald, 1997b) was found in any bat. Considering the enormous evolutionary radiation in enamel structure among mammals typically accompanied by modification from simple to more complex structures

within each particular clade and the uni-directionality of the trends in enamel differentiation (Koenigswald, 1997b), the situation in bats is quite exceptional. Bats are one of the few high-ranking clades that retain a number of dental plesiomorphies, including characters like an incomplete sheath of enamel prisms, otherwise only found in early mammals (Wood and Rougier, 2005).

The concluding textbook summary on bat molars (and tribosphenic molars in general) is that, in contrast to derived molar types with thick and heterotopically divergent enamel, chiropteran molars are characterized by a primitive mammalian condition that includes thin and evenly distributed radial enamel with a cingular rim. This rim presumably compensates for the functional scantiness of the enamel cover by redistribution of mechanical stress to the crown base (Rensberger, 2000; Lucas *et al.*, 2008).

To re-examine these conclusions we developed a simple technique of serial sectioning of molar teeth and post hoc quantitative analyses of SEM images and applied it in several model species of vespertilionid, rhinolophid and molossid bats. Particularly detailed records enabling us to reconstruct three-dimensional patterns of enamel architecture for all teeth were obtained from *Myotis myotis*, a large-sized vespertilionid bearing a complete generalized dentition. In that species we also studied details of enamel maturation and eruptional dynamics of molars using a postnatal series of 89 individuals of known age from 0 to 65 days (previously reported, mostly in regard to external and postcranial characters by Sklenar, 1962, Sigmund, 1964, and Krátký, 1970).

12.5.1 Adult molars

In all species under study the results show that: (1) there is a greatly pronounced spatial heterogeneity in enamel thickness with a markedly thickened layer at shearing (i.e., the convex) walls of major crests, both in the upper and lower molars (i.e., palatal side of para-, meta- and protocristas in upper molars, labial side of protocristid, cristid obliqua and hypocristid in lower molars, Figures 12.1, 12.3); (2) in contrast, the reverse (concave) sides of shearing walls (i.e., paraconal and metaconal foveae, and the bottom of protocone fossa in the upper, trigonid fovea and talonid fossid in the lower molars) are covered by a very thin enamel layer; (3) while in the shearing walls the enamel thickness increases towards the cusp tip, the reverse is true for the reverse sides, so that the most contrasting differences in enamel thickness are just along the crest edges; (4) the enamel coat is built of three types of enamel, prismatic, interprismatic (IPM) and aprismatic (AP), which differ not only in mode of spatial integration of structural crystallites, but also in their mean length and volume (Figure 12.2); (5) aprismatic enamel forms a surface of the

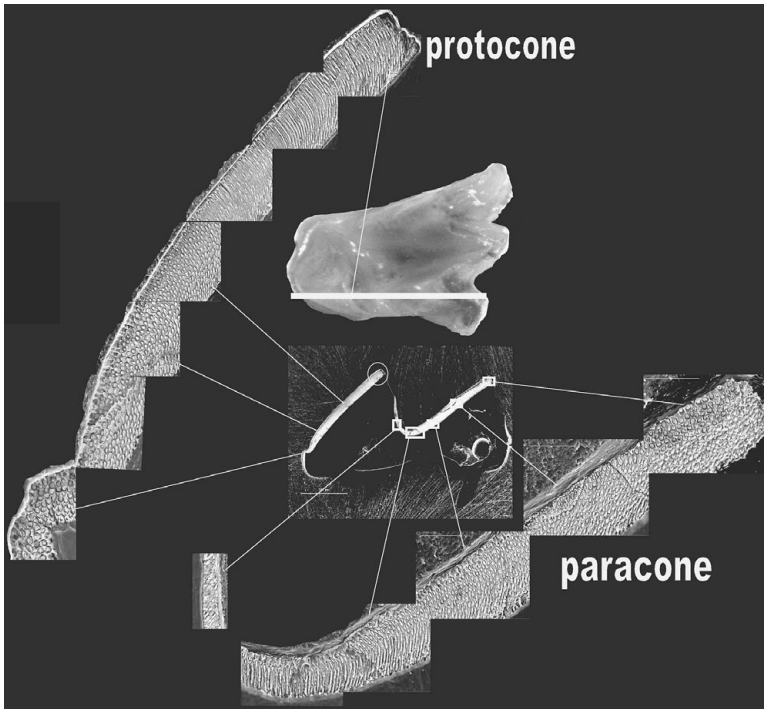


Figure 12.1 Cross section of an adult M1 (*Myotis myotis*). Note spatial heterogeneity of enamel thickness and differences between protocone and paracone in enamel microarchitecture at their convex walls.

enamel coat and it appears as a denser layer in the labial cingular ridge of the upper molars and at the lingual base of the lower molars; (6) prismatic enamel is the dominant component of the enamel layer throughout the tooth – its relative volume (to that of IPM and AP) is consistently the highest around the middle of the column thickness; (7) despite the prismatic enamel being of the radial type only, it is far from being structurally homogeneous – namely (8) the patterns of deviations of prismatic axes from the plane perpendicular to the enamel–dentine junction (declination) and/or the vertical section plane (inclination) show conspicuous differences between spatial domains of particular major cusps (Figure 12.3); (9) at the same time, declination angles of the enamel prisms exhibit clear altitudinal changes along cusp walls from bottom to cusp tips, and the differences in these characteristics between particular cusps are even more conspicuous (Figure 12.1, the detailed quantitative data are published elsewhere – Špoutil *et al.*, 2010); (10) in contrast to differences among the major cusps of the upper molars in these characteristics, the shearing walls of lower molars, both trigonid and talonid, show a uniform

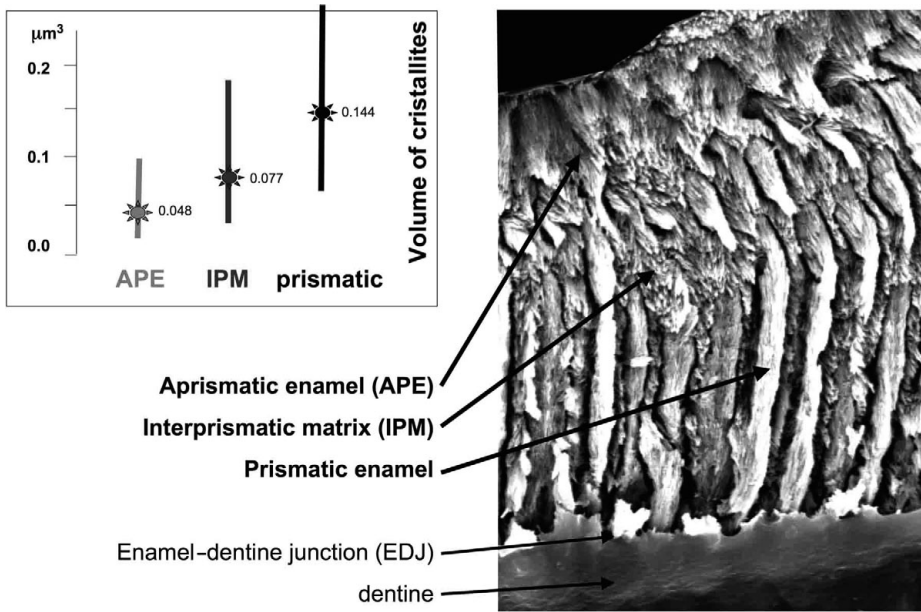


Figure 12.2 Cross section of the enamel layer (*Myotis myotis*: slope of adult M₁ fossa) illustrating differences among three enamel types in size and spatial arrangements of crystallites. Histogram shows differences among major enamel types in two-dimensional volume of crystallites (estimated as $s = \text{length} \times \text{width}$), stars = mean values, bars = span of recorded values ($n = 1367$). For further characteristics see Špoutil *et al.* (2010).

pattern, and consequently, it seems that there is no massive heterotopy in lateral expansion of particular structures of the lower molars during the late morphogenesis and mineralization of their enamel coat.

12.5.2 Ontogenetic aspect

The above-mentioned spatial heterogeneity in declination or inclination dynamics of enamel rods undoubtedly has something to do with the responses of the enamel epithelium to the morphogenetic movements of the tooth primordium. An essential question therefore is which part of tooth morphogenesis is recorded by these characteristics. To answer this question requires first to explain at which stage of mineralization the enamel prisms are formed. This is no easy task, of course, because the histological techniques applied in the study of earlier stages of tooth morphogenesis include decalcification, by which the enamel crystallites are removed. Nevertheless, a microdissection of embryonic tooth primordia (Figure 12.4) reveals that from the

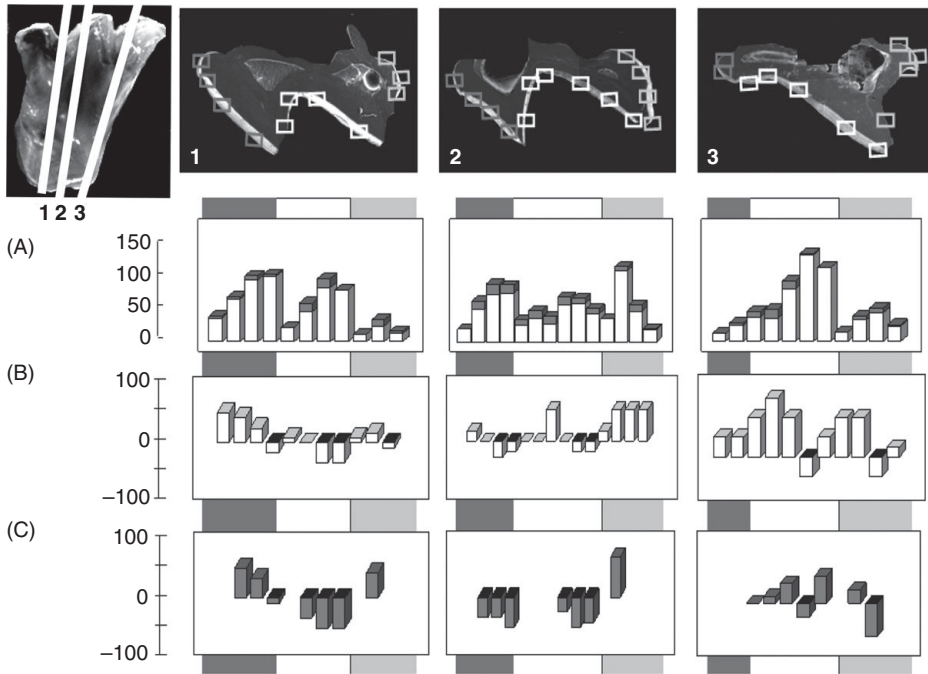


Figure 12.3 Enamel thickness (A), declination (B) and inclination (C) of enamel prisms at three vertical sections of adult M1 of *Myotis myotis*. Note considerable differences between cusps in combination of these characteristics.

beginning of mineralization the hydroxyapatite is primarily organized into prismatic form and also shows that the volume of embryonic enamel crystallites corresponds to that of mature prismatic enamel (see [Figure 12.2](#)). Consequently, the spatial rearrangements mapped by inclination/declination dynamics refer to the whole period of mineralization that, in main cusp tips, begins synchronously with the onset of major morphogenetic activity during the first half of the bell stage. During most of the tooth development the enamel rods remain unconsolidated and take the form of a loose bundle of hydroxyapatite nodules, the surface of which may resemble shrinkage cracks when dehydrated ([Figure 12.5](#)). The infilling of interprismatic matrix which consolidates them into a compact layer takes place much later – just shortly prior to eruption, and the surface aprismatic enamel (with the smallest and least spatially coordinated crystallites) is produced just below the gingiva immediately prior to eruption perhaps by the end of life of individual ameloblasts and under a reduced effect of organic intervention ([Figure 12.4B](#)).

Until consolidation by interprismatic matrix and the surface aprismatic layer, the prismatic enamel cover of the crown is flexible, the rods are disposed to

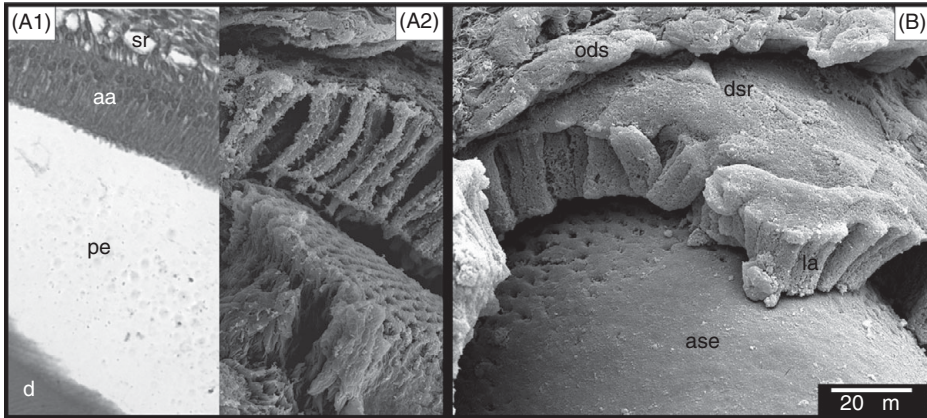


Figure 12.4 Histological section of enamel formation at the late bell stage (A₁) and an SEM view of the corresponding situation at M₁ primordium (*Myotis nattereri*, embryo 14 mm) sectioned by a micro-dissection (A₂). Note thick layer of unconsolidated prismatic enamel (removed in A₁ by decalcification). (B): Surface of tooth tip (P₄) immediately prior to eruption – note changed form of ameloblasts and secretion of surface aprismatic enamel. aa, active ameloblasts, ase, aprismatic surface enamel, d, dentine, dsr, disintegrated stellate reticulum, la, late ameloblasts ods, outer dental sac, pe, prismatic enamel (removed in A₁), sr, stellate reticulum.

plastic deformations and the enamel cover still does not present an ultimate mechanical constraint upon growth and shaping movements of the underlying mesenchyme–dentine core of the tooth. The completely matured enamel first appears at crest edges of the paracone, metacone and parastyle of upper molars, followed by the edge of the protocrista, and mesial and buccal cingulum. The enamel of the protocone complex (including its cingulum) matures much later, while the distal margin of the protocone complex, together with the bottom of the protocone fossa, are the zones where the enamel matures last. In the lower molars, the enamel maturation begins from the edge of the protocristid (including para- and metaconids) and trigonid cingulum, followed by the entoconid and lingual base of the tooth, while enamel maturation in the talonid is apparently delayed (it starts with the entocristid, followed by the tip of hypocristid, while the bottom of the talonid basin and cristid obliqua mature later).

Even during the time when the crest edges and mesial cingula are completely matured and performing initial occlusion actions, the deeper zones of the crown covered by unconsolidated enamel, as well as the foveae of major crests and bottom of the fossa, remain flexible and capable of responding to three-dimensional growth of the dentine core of the tooth. At that stage, the mineralized segments of the tooth crown can be looked upon as autonomous

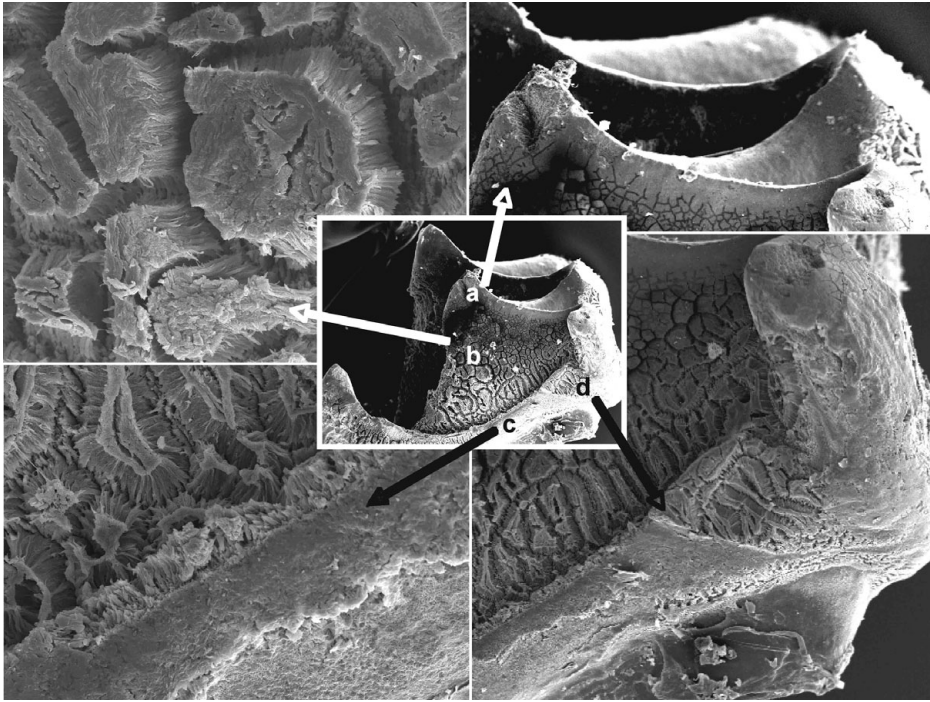


Figure 12.5 Mesial view of the enamel surface of M₁ (*Myotis myotis*) at the onset of peri-eruption (postnatal stage 3, *c.* 14 days): note (a) the completely hardened enamel at crest edges and at (c) cingula, (b) thick layer of still unconsolidated prismatic enamel at walls of crown, still lacking interprismatic matrix and surface aprismatic enamel, and (d) suture zones between cingular elements (here mesial cingulum and parastyle).

structural units at least in the context that their mutual position and size are further modified during tooth eruption and the zones interconnecting them are until then almost without enamel cover.

The period from the first appearance of mineralized cusp tips above the gingiva to the complete mineralization of whole crown is rather long. In *Myotis myotis*, the first tips of molars appear by the end of the first week of life, while the bottom of the fossa is not completely mineralized until after weaning. The duration of molar eruption is about 65 days. During that time the size of molars more than doubles (Figure 12.6).

The detailed analysis of the eruption dynamics (46 metrical and non-metrical variables in a series of 89 juveniles covering that period) further demonstrated that: (1) the rate of (peri)eruptional growth of a particular dental element is neither isometric nor isochronous; (2) M₁/m₁ are the first permanent teeth which appear in occlusion (first with protoconids of the lower and paracones of

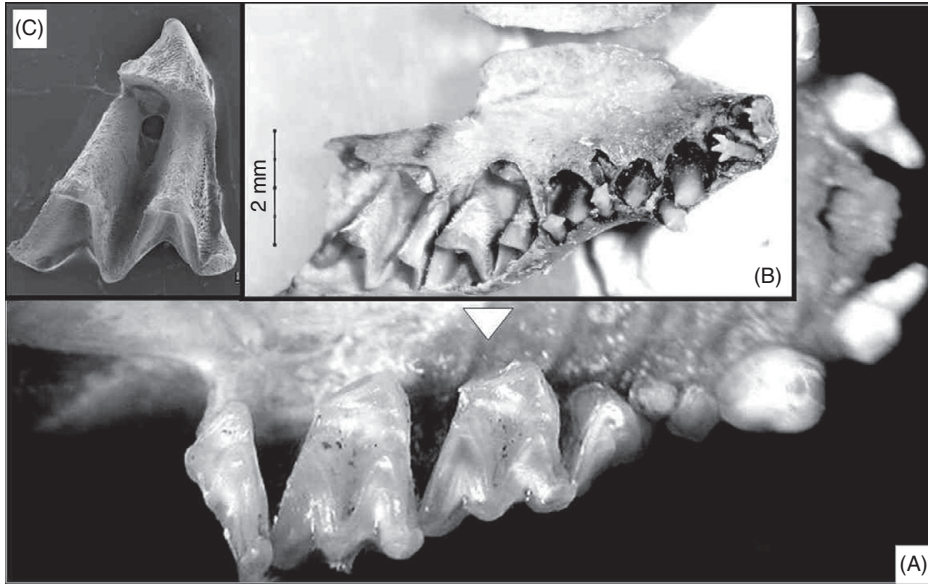


Figure 12.6 An upper jaw of adult *Myotis myotis* (A) compared to (B) a juvenile (postnatal stage 2, c. 12 days, same scale as in A). (C) M2 at roughly same stage showing considerable differences from the adult shape.

the upper molars); (3) the tips of the teeth of the unicuspid row appear after the eruption of the major cusps of M₁/m₁ and M₂/m₂ are complete. This is synchronous with the disappearance of deciduous teeth accompanying an accelerated growth of the I–P₄ length; (4) the lingual axis of lower molars (paraconid–entoconid length, established in m₁) appears in nearly an adult dimension at eruption and shows a minimum growth rate after that; (5) the trigonid width growth rate is nearly constant (particularly in m₁) and relatively low, while the growth of the width of talonid shows a clear acceleration, both at the beginning and at the terminal stage of eruption (Figure 12.7); (6) the longitudinal dimensions of upper molars show an accelerated growth, particularly during the first half of the eruption period, with a certain delay to acceleration of lower molar growth (see Figures 12.7–12.8); (7) the asymmetric growth of paracone–protocone and metacone–protocone distances and the fluctuations in their growth rate (Figure 12.8) suggest a dynamic growth of the protocone complex and changes in its relative position during the second half of the eruption period; (8) despite the asymmetries, the angle between the postparacrista and premetacrista steadily increases, as does the areas of the paraconal and metaconal foveae; (9) nearly all variables show a constant decrease in variation during the eruption period, with minimum variation at

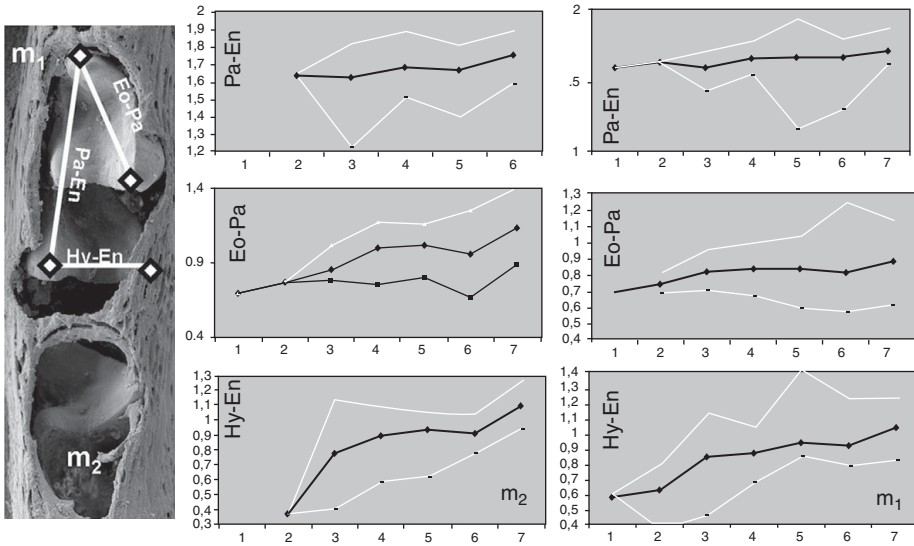


Figure 12.7 Biometry of the postnatal development of m_1 and m_2 in *Myotis myotis* ($n = 89$, 1–65 days): mean and min-max values of three metric variables (ordinates) for postnatal stages 1–7 of dental development (abscissa). In terms of postnatal days the stages can be roughly delimited as follows: stage 0 = day 0–5, stage 1 = 6–9, stage 2 = 9–12, stage 3 = 12–16, stage 4 = 16–21, stage 5 = 21–31, stage 6 = 32–45, stage 7 = 45–65.

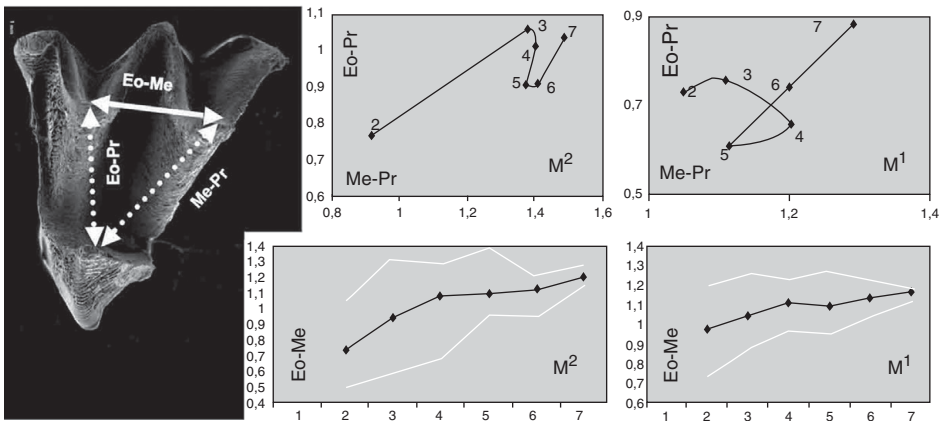


Figure 12.8 Biometry of the postnatal development of M_1 and M_2 in *Myotis myotis* ($n = 89$, 1–65 days). Upper: eo (= para) cone to protocone distance vs. metacone to protocone distances in individual stages of dental development (1–7) in M_2 and M_1 . Lower: mean and min-max values of eo (= para) cone to metacone distance at postnatal stages 1–7. In terms of postnatal days the stages can be roughly delimited as follows: stage 0 = day 0–5, stage 1 = 6–9, stage 2 = 9–12, stage 3 = 12–16, stage 4 = 16–21, stage 5 = 21–31, stage 6 = 32–45, stage 7 = 45–65.

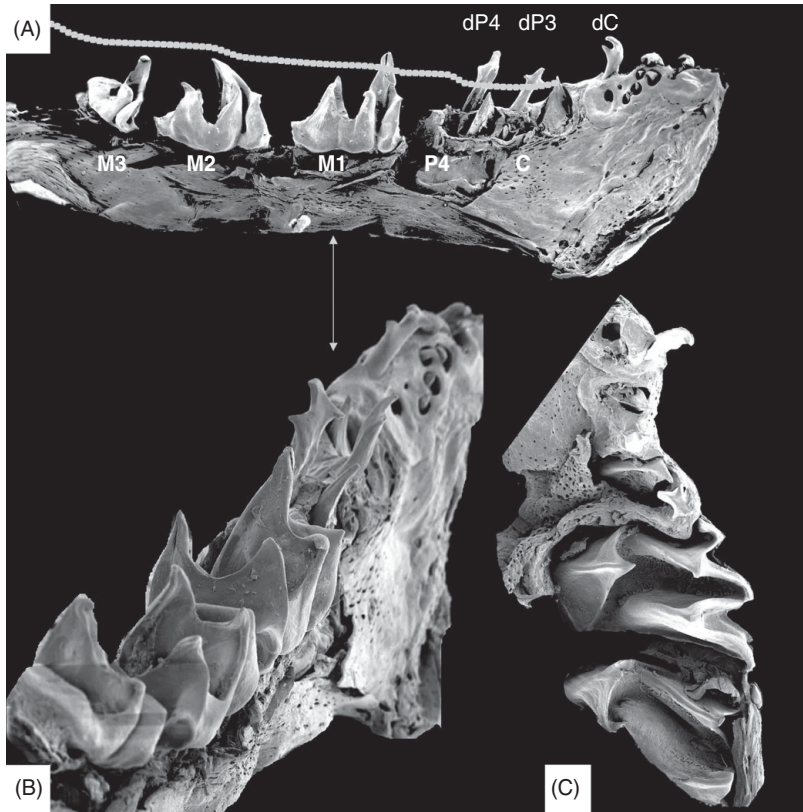


Figure 12.9 Totally decalcified jaws of *Myotis myotis* (stage 1 of postnatal development, i.e., beginning of m_1 eruption): (A) lower jaw, lingual view (bone removed), (B) same specimen as A in distal occlusal view, (C) upper jaw of same individual with M_1-2 . Note continuous collagen surface of the teeth even at loci not calcified at this stage of development and not covered by enamel, even in later stages (see [Figure 12.6](#)).

the stage when adult tooth size and shape are attained; (10) the results of multivariate analyses demonstrated a concerted growth of the components of the protocone complex (including the area of fossa), but only faint relation to longitudinal variables of the upper molars, either the paracone and metacone – also the lateral enlargement of talonid exhibiting the fastest growth rate during the eruption period (particularly in m_2) is only faintly related to growth of the trigonid (which is, conversely, almost isometric to growth of the lingual base of lower molars) – the lingual axis of the lower molars (m_1 in particular) takes a central position of the factor space (in CCA representation), suggesting its indexing role in the geometry of the growth field and indicating that it may be

an ordinal factor of peri-eruptional reshaping of the molariform row; (11) the application of the newly developed technique of total decalcification (which removes all enamel cover on the teeth) to embryonic dentitions (Figure 12.9) demonstrated that all teeth (even those in which the enamel cover is still not completely established and that split into separate cusps in a dry native preparation—such as m_3 , M_3) are completely designed with all fine details of their mature crowns in dentine collagen. The collagen surface of dentin visible in SEM images of completely decalcified teeth shows in detail even those structures still not established in the enamel (deep roots of crests, cingula etc.); (12) against expectation, the collagen blueprints of teeth do not bear any vestigial or ancient structures (such as unresolved position of postcrisid of lower molars, form of mesostyle or presence of para- and metalophs in the upper molars). Rather than the expected role of enamel formation, this suggests that the mesenchymatic papilla preforms design of the adult crown as a whole, without leaving the unresolved alternatives to be accomplished by enamel formation.

12.6 Conclusions

In fact, we are unable (because of scarcity of comparative data) to distinguish exactly which of the above-mentioned observations are specific just for the model species, which refer to specificities of bats and which refer to constitutional qualities of the tribosphenic molar organization in terms of essential apomorphy of mammals. Nevertheless, we believe that at least some of them may address topics of general significance. Here we will focus just on four of them.

12.6.1 Crest sharpness

An essential precondition of shearing efficiency results not only from occlusal sharpening and the geometric design of blade edges, as proposed by Popowics and Fortelius (1997) and Evans (2005), but also from constitutional asymmetry of enamel thickness along the crest edges, with very thick enamel at the convex and a very thin enamel at the concave sides. Inversely, in terms of developmental causality of the adult phenotype, the crests as the constitutional elements of tribosphenic design are preformed by extensive epithelial-mesenchymal evagination associated with heterotopic histo-differentiation of inner enamel epithelium along presumptive crest edges. The process starts at the bell stage and apparently represents an essential part of the developmental program that produces tribosphenic molars (Figure 12.10A). Hypothetically, this can appear as a result of strong lateral strain during early growth of a cusp, which then will produce convex ridges rather than straight vertical cones. Then

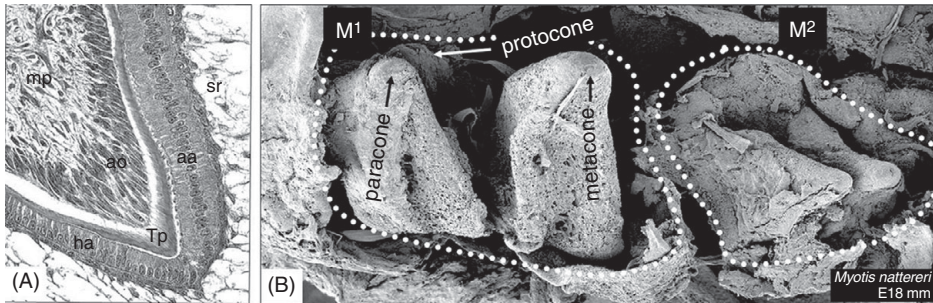


Figure 12.10 (A) Heterotopic histo-differentiation along presumptive crest edges at early bell stage is likely common to all tribosphenic teeth (here M₁ *Monodelphis domestica*, postnatal day 4). (B) Topology of major cusps in embryonic primordia of M₁ and M₂ (*Myotis nattereri*, embryo length 18 mm): note position of protocone different from adult arrangement. ha, unmaturred ameloblast, Tp, odontoblast processes, other abbreviations as in Figure 12.4.

the above-mentioned differences in size and histo-differentiation of ameloblasts can simply respond to heterotopy in the growth of their spatial domains, with the expansion of the surface of the convex side and compression of that on the concave side. We can further hypothesize that the geometry of contextual rearrangements may produce a steady effect upon the shaping of the tooth primordia (cf. dorsoventral growth of the lateral wall of dentale vs. medial growth of palatal extension of maxilles towards palatal fissure, and palatal enlargement of the upper molars).

12.6.2 Modular structure of molar teeth

Although the common pattern of enamel thickness along major crests suggests that all are produced by the same developmental machinery, the greatly pronounced differences among the cusps in the angularity pattern of the prisms (at least in the upper molars) suggest considerable differences in their positional history during the formation of enamel prisms, i.e., movements of the enamel organs within the tooth primordium relative to vertical growth of the tooth. Uneven distribution of enamel thickness with the zones of thick enamel at cingula and shearing walls (convex sides of crests of major cusps) mutually separated by the zones uncovered by enamel (or covered by a very thin enamel layer with incomplete or delayed mineralization) provides to the tooth primordium considerable flexibility. Until the enamel of the latter zones is completely mineralized, the former can develop more or less independently and (as also confirmed by dissections of embryonic teeth) in positions different from the adult arrangement (Figure 12.10B). In the form of densely compressed

structural modules, the major structures of a tooth can grow large in the relatively small spaces available in the embryonic jaw. The positional competition among primordial centers of the tribosphenic crown during early morphogenesis is perhaps responsible both for the excessive vertical growth of cusps and the lateral strain producing the V-shaped crest pattern. The lateral elements of the crown base may take the key role in that respect, i.e., parastyle and mesostyle in the upper molars and lingual base of the tooth (paraconid–metaconid–entoconid) in the lower molars.

In that respect the tribosphenic molar seems to be composed of several more or less *autonomous developmental units* whose mutual integration is postponed to the later stages of tooth morphogenesis. The large potential variation provided by that arrangement is, of course, strictly constrained by the demands of the functional integrity of the adult tooth and perfect interlocking of occluding crests over the entire dentition. The integrity is achieved by refined interaction of growth dynamics of these structural modules. The particular modules can be subdivided by their functional and developmental characteristics into three distinct groups: (1) modules of the crown base (providing a socket for the crest system); (2) modules of shearing crests; and (3) modules of the spaces “in between,” including suppressed enamel formation. The modules composing these groups are, in the *upper* molars: (1a) parastyle, (1b) mesostyle, (1c) palatal cingulum, (2a) paracone, (2b) metacone, (2c) protocone, (3a) paracone fovea, (3b) protocone fovea and fossa, and, in the *lower* molars: (1a) metaconid, (1b) entoconid, (1c) hypoconulid, (1d) labial cingulum, (2a) protoconid, (2b) paraconid, (2c) hypoconid, (2d) cristid obliqua, (3a) trigonid fovea, (3b) talonid fossid.

12.6.3 Delayed enamel maturation, peri-eruption growth and reshaping of teeth

We demonstrated that the enamel prisms (at shearing walls) remain unconsolidated (with soft enamel) until eruption when the fine crystallites of interprismatic matrix and surface aprismatic enamel are inserted among them. Until then the respective zones of the tooth remain flexible and can be reshaped by heterotopic growth of the dentine core of the crown. In full it holds true for the enamel-less zones “in between” which can expand and redistribute the neighboring structures to distant adult positions. Thanks to that and further aspects of modular structure (see above), the tribosphenic molar can expand its size during eruption and the prolonged eruption period in bats allows an increase in rate of expansion to as much as 200% of initial size. The cusp tips and the crest edges, which first appear in eruption, are completely hardened

(as are the major socket elements which fix the tooth position in the jaw – the mesiolabial base of the tooth and palatal cingulum in the upper molars and lingual axis in the lower molars) and can act in real occlusion events. The soft coated surface of the crown allows, at the same time, the tooth primordium to respond sensitively to the contextual signals, such as improper crest interlocking or ad hoc cues of actual morphogenetic and ecological contexts. Stress of imperfect peri-eruptional occlusion responded to by mesenchymatic papilla with heterotopic growth and apposition of dentine provides then a simple, yet apparently quite reliable and precise, mechanism of interactive fine-tuning of the crest interlocking pattern. The reshaped tooth resulting from this process is subsequently fixed into the adult tooth form by peri-eruptional enamel hardening.

In agreement with previous observations (e.g., Phillips, 2000), the eruption dynamics of the permanent dentition follows a hard-wired scheme of sequence and timing of particular events. M1/m1 are the first elements that start the process and establish the functional center of the dentition. The lingual axis of lower molars delimits the space of interaction – further steps include the interaction of the postprotocristid/preparacrista, while the talonid/protocone interaction and reshaping terminate the peri-eruptional reshaping of the molar.

12.6.4 Cope–Osborn concept of dental homology exhumed?

In correspondence with earlier discussions on the primary cusp of tribosphenic molars (Woodward, 1896; Osborn, 1907 etc.) and consensual opinion on that subject, Marshall and Butler (1966) centered their projections of growing stages of upper molars on the paracone as the singular point remaining apart from the lateral morphogenetic movements within the tooth primordium. Above we demonstrated dramatic changes in prism declination (Figures 12.1 and 12.3), which suggest massive lateral movements of the paracone during early morphogenesis, indicating that the paracone is not the true singular point of the upper molar primordium. In contrast, the expected characteristics seem to appear in the parastyle, the cusp that occupies exactly the most anterolateral position predicted for the primary cusp by Woodward (1896). In the lower molars, the invariant axial element of the lingual tooth socket, the metaconid, seems to represent the probable candidate for the most ancient structure. These conclusions address the conceptual issues of dental homology and should be, perhaps, briefly discussed in that context.

The traditional Cope–Osborn concept of dental homology can be summarized as follows (see Hershkovitz, 1971; Butler, 1978; Van Valen, 1982, 1994 for details): (i) all mammalian teeth are homologous to the single

cone tooth of reptiles supplemented by (2) the additional structures which appeared first as secondary cusps at mesial and distal sides of the major cusp; (3) the additional structures (cusps, cingula, crests etc.) arise within a single tooth primordium by budding, not by concrescence; (4) the enlarged secondary cusps (mesial and distal) push the primary cusp out of the axis of occlusion, which results in a triangular arrangement of the three basic cusps; (5) the position of the primary cusp is then palatal in the upper molars, and labial in the lower molars, the basic cusp triangles of maxillary and mandibular molars (trigon and trigonid) are then inversely homologous; (6) the primary cusps retain their essential functional significance and structural dominance; (7) the further derived elements arise by budding on the buccal side of the tooth in the upper molars (styles) and distal to the principal cusp triangle, i.e., (7) talon and hypocone in the upper molars is homologous to talonid of the lower molars. The paradigmatic statements (1)–(4) refer to rather hypothetical situations, however, they present a default core framework of the dental homology concept and as such they can be accepted by definition. The others already have been refuted by empirical evidence to the contrary (Van Valen, 1982). Here we reconsider them with respect to the above conclusions. Statement (5) could be in a good accord with our findings if statements (6), (7) and (8) are replaced with the following: (6) the primary cusps are subsequently reduced, though they may play an essential polarizing role in early stages of tooth morphogenesis (e.g., as the agents contributing to development of convexity of crests of the secondary cusp), while (7) the dominant functional role is taken by the apomorphic elements (i.e., those situated palatally in the upper and labially in the lower molars). In that respect, (8) the paracone is homologous to the protoconid, and the talonid (or hypoconid) to the protocone of upper molars, while the talon and hypocone represent an apomorphic structure that appeared independently in various clades (Hunter and Jernvall, 1995) and cannot be directly homologized with the structures of lower molars. In any case, the developmental processing of these relations falls in the domain of morphodynamic regulation and for the above-mentioned reasons hypotheses on that matter can hardly be tested by direct morphological comparisons.

12.7 Implications

With the appearance of the tribosphenic molar and the apomorphies constituting its developmental background, mammals were released from the essential constraints forming the barriers of dental evolution in other vertebrates.

The structural modularity of the tribosphenic molar, including the possibility of peri-eruptional growth and late reshaping, provides a sensitive substrate for considerable variation – but within the framework of the tribosphenic design and insectivory, the feeding specialization constrains the life-trait pattern in many respects. Except for specialized foraging for social insects, feeding on insects requires agile locomotion and the ability to search for spatially dispersed food that is seldom abundant. Consequently, small body size, excessive spatial activity and high metabolic rate are directly related to insectivory, and apparently promote the *r*-selection effects upon the life-history traits, including shortening of the gestation and lactation period (the trend just the reverse of the demands of complex tooth development). Nevertheless, thanks to their capability for powered flight, bats, at least partly, succeeded in escaping from these constraints and evolved a pronounced *K*-strategy life-history pattern. The associated rearrangements of life-history traits, such as extensively enlarged parental investment, including prolonged gestation and lactation, also provided a developmental release for bat tooth morphogenesis.

Thus, the basic setting of dental morphogeny of bats (redirections of certain constraints, and specific heterochronies and heterotopies modifying the original developmental program) was most probably established within the frame of the complex constitutional rearrangements composing the chiropteran organization. The release of some developmental constraints (provided by prolongation of developmental time) could at the same time become an essential driving factor for increased variation in dental characters and adaptive radiation of the early stages of chiropteran evolution. Yet, as any rearrangement in domain of the retrospective regulation of dental morphogeny can be fixed only when passed through a rigorous control of the prospective regulation (by the efficiency of respective dental rearrangement under actual ecological context) we are obliged to supplement the above hypothesis on the early dental evolution of bats with a brief comment on its contextual factors.

The environmental conditions of the Early Paleogene were undoubtedly much different from modern analogies and the contextual factors that promoted a transition from a non-volant insectivore to bats and the abrupt diversification of the night aerial insectivory niche were probably quite unique. In any case it seems obvious that the night sky at the time of chiropteran early evolution must have been enormously rich in flying insects (say, the effect of pressure by diverse groups of daytime insectivorous birds, including aerial foragers and passeriforms – for the corresponding fossil record and/or time setting see, e.g., Mlíkovský, 2002; Beresford *et al.*, 2005; Mayr, 2009). A large capacity of such a food resource – dense clouds of insects swarming at crepuscular and night hours – could eventually play the role of a sufficient driving factor

promoting capability of flight and the rearrangements in sensory systems characterizing the earliest stages of bat evolution. Yet specialization for just such a kind of food resource also necessarily had an influence on the setting of the dental rearrangements. The clouds of swarming insects are characterized by (1) enormous abundance and high density and (2) small body size and thin cuticula of their members. These qualities would strongly select (1) large capacity and rapid performance of each occluding effort and (2) an extremely precise shearing effect. No wonder that the basic dental design of bats is characterized by the enlarged molariform part of the dentition, prolonging crests and refining their complex interlocking pattern.

12.7.1 Tribosphenic molars: developmental sources of variation and phylogenetic signal of dental characters

As demonstrated above, the perfection of interlocking occlusion, the key prerequisite for proper functioning of the tribosphenic dentition, is achieved neither by genetic regulation or by any other “mysterious” mechanism of intricate retrospective regulation, but is achieved exclusively within the domain of prospective and morphostatic (in the sense of Salazar–Ciudad and Jernvall, 2004) regulation. The regulation proceeds via the mechanical forces exerted from physical contact between the upper and lower teeth after initial eruption. Since the enamel on the non-erupted parts of the tooth is not hardened, the shape of the tooth can be modified – most probably under active contribution by the mesenchymatic papilla responding to uneven mechanical stress by heterotopic production of dentin (for the respective capabilities of dental papilla and included mechanisms see Yoshida *et al.*, 2006, 2007; Lee *et al.*, 2010). The essential factors that make it possible are these: (1) “simple” radial prismatic enamel combined with (2) delayed enamel maturation via last minute apposition of IPM/APE; (3) constituent heterotopy of enamel thickness closely linked to appearance of crests; (4) modular structure of the tooth primordium and (5) positional flexibility of structural modules; and (6) a prolonged period of tooth eruption accompanied by (7) peri-eruptional growth and (8) late reshaping of the tooth crown by heterotopic activity of mesenchymatic papilla and apposition of dentine, subsequently fixed by peri-eruptional enamel hardening. We believe that the combination of these factors (or at least some of them) characterizes the developmental background of tribosphenic molars in general, and together with the morphodynamic mechanisms accelerating developmental heterotopy of the tooth primordium (enamel knots), it can be looked upon as the most essential apomorphy of the mammalian dental organization.

Yet, it seems well substantiated to expect considerable variation in each of these factors and to hypothesize that a combination of specific states presents the most pertinent odontologic characteristics of particular clades. Accordingly, particular grades of dental traits can be expressed in terms of differences in spatial and temporal settings of these factors and/or heterochronies in growth dynamics of particular structural units, and it could be hypothesized that the bifurcation points or transitional states between the respective grades of adult organization can be found in earlier stages of dental development. For instance, the essential grades of organization of bat lower molar, nyctalodonty and myotodonty (Menu and Sigé, 1971; Menu, 1985) can be, in terms of the developmental background, looked upon as a difference in growth rate of the modules composing the distal margin of the tooth, i.e., posthypocristid (= postcristid), entoconid, hypoconulid and talonid fossid. The early enlargement of the talonid fossid, delayed growth of the entoconid with early catchment of the distal posthypocristid at the hypoconulid margin of the tooth will produce nyctalodonty, while a delayed lateral to vertical growth of the hypoconid, delayed lateral to longitudinal enlargement of the fossid and rapid vertical growth of the entoconid with early catchment of the hypocristid will produce myotodonty. Yet, according to the standard view of dental development (e.g., Bhaskar, 1991), the morpho- and histogenesis of inner enamel epithelium and enamel mineralization are to be looked on as the true agents of the differences in tooth morphology and ultimate processing of heterochronies should appear just in that domain. Against that expectation, the analysis of completely decalcified teeth in various stages of development demonstrates that the adult design of crown surface (as later visualized in its enamel coat) is entirely preformed in a collagenous blueprint produced by the mesenchymatic papilla. The expected transitional states may possibly appear as virtual steps of morpho- and histogenesis of mesenchymatic papilla, which finally produces the tooth as a single, completely integrated unit. In accordance with the conclusions of Loher (1929), this suggests that the dental papilla is the major agent of molar morphogenesis and tooth design, while the actual role of enamel epithelium in tooth morphogenesis can be much less than commonly proposed, a fact also stressed by further recent studies (see, e.g., Soukup *et al.*, 2008; Koentges, 2008).

12.7.2 Common trends of phylogenetic morphoclines

The tribosphenic molar and heterodont dentition are designed for a generalized faunivory and generalized aerial insectivory is a plesiomorphic and widespread state in foraging biology of bats (Jones and Rydell, 2003).

Nevertheless, as the local diversity of bats is promoted by the diversity of their diet and foraging preferences (Findley, 1993; Patterson *et al.*, 2003), it might be expected that this factor could have played a key role in the driving mechanism of early phylogenetic divergence in bats (Habersetzer and Storch, 1989). Specialization within faunivory is typically associated with an elementary dilemma: to forage larger or smaller (poorer but widespread) prey? Larger prey provides high energy intake per foraging action, but necessitates appropriate morphological and behavioral adaptations for immobilization and mechanical processing of the large prey. The dentition typically responds to this with increased size of canines, increased height of dentition and robustness of individual teeth combined with shortening of the postcanine elements and relative jaw length (which increases transposition of the power of mandibular adduction to canines, see Figure 12.13). Smaller prey, in contrast, provides less energy intake per foraging action, but compensates for this by requiring less energy expenditure, which can be further reduced with specialized foraging for mass prey aggregations (as is the case in fast aerial hawkers). The dentition is then required to increase the mean rate of food processing, which is typically achieved by enlargement of total occlusion space and maximizing the length of crests via enlargement of the molariform part of the dentition, including molarization of P₄/p₄. In both the cases, each specialization is accompanied by a reduction in premolar number and, particularly in those taxa that specialize on larger prey, a reduction in distal dental elements (M₃/talonid of m₃).

The fine molar traits controlled by a sophisticated set of constraining factors probably respond more slowly to specialization tendencies than do unicuspid teeth. Nevertheless, under selection for larger prey, which promotes high and robust teeth, the selection for the crest length can be suppressed (also the rate of “cuticula to be sheared relative to energy intake” is here much lower than in microfaunivory). In contrast, the specialization for smaller prey will undoubtedly further enlarge crest length, either via relative enlargement of molar size or by specific enlargement of major crests by reshaping molar teeth or by enlargement of crest-like structures within the connecting spaces (as para- and metaloph).

The reduction of premolars and M₃/m₃ obviously has contributed to phylogenetic morphoclines in almost all clades of bats. The responses of the molar traits are, of course, quite diverse, far less uniform and not directly linked to degree of premolar reduction. A vast majority of chiropteran families show radical reduction of unicuspid (often with complete disappearance of P₂/p₂ and/or P₃/p₃) and a surprisingly high degree of premolar size reduction is well pronounced, even in the earliest and most primitive known bat, *Onychonycteris finneyi* (Simmons *et al.*, 2008). Nevertheless, several clades of extant bats

(Natalidae, Thyropteridae, Myzopodidae) show opposite trends: enlargement of premolars, prolongation of the unicuspid row, evenly sized molars with unreduced M_3/m_3 , and the state of these characters exceeds even those of the earliest bats. One of them (Kerivoulini – *Phoniscus* in particular) appears in the largest family of bats, Vespertilionidae, for which the generalized state of dental characters is particularly characteristic (Simmons and Geisler, 1998). All three premolars in both the upper and lower jaw are retained in *Cistugo*, Kerivoulinae and its sister clade, Myotinae, with the most diverse genus of the order, *Myotis*, showing broad variations in degree of premolar size reduction. The other genera of Vespertilionidae are characterized by further reduction terminating with the disappearance of P_3/p_3 and/or P_2/p_2 or both, often in specific combinations for particular genera (Miller, 1907). A random distribution of loss of premolars over all branches of the family tree (Hooper and Van Den Bussche, 2003; Hooper *et al.*, 2006; Stadelmann *et al.*, 2006) suggests that the process has developed convergently in different lineages of the clade, in a manner similar to that of other characters such as myotodonty/nyctalodonty (Horáček and Hanák, 1985). It seems quite probable that the respective rearrangements of dental system in each particular clade passed through a phylotypic stage of a complete but moderately reduced *Myotis*-like dentition. Correspondingly, the Oligocene and Early Miocene record consists almost exclusively of the forms bearing *Myotis*-like dentitions (Horáček, 2001; Ziegler, 2003) though robust molecular evidence (Stadelmann *et al.*, 2006) suggests a much younger date of about 15 Ma for basal divergence of *Myotis sensu stricto*.

Accordingly, despite common trends in premolar and M_3/m_3 reduction, the fine molar characters show considerable divergence. None of the molar characters is directly linked to degree of premolar reduction and the variation patterns of particular molar traits are mostly mutually independent (at least in a sample of 65 species of extant vespertilionid bats – see Figure 12.11). Despite absence of any significant correlation among particular dental characters, the clusters of species produced by the same analysis are quite consistent with standard generic and suprageneric affiliations. This suggests that the particular combinations of these dental characters are obviously clade specific, and may be relevant for phylogenetic analyses.

12.7.3 Grades, clades and dental phylogeny of bats

It is beyond the scope of this chapter to discuss dental phylogeny of particular chiropteran clades in detail. Nevertheless, it should be remembered that this was the primary motivation for our study and such a step should be taken in order to properly integrate the fossil record into chiropteran

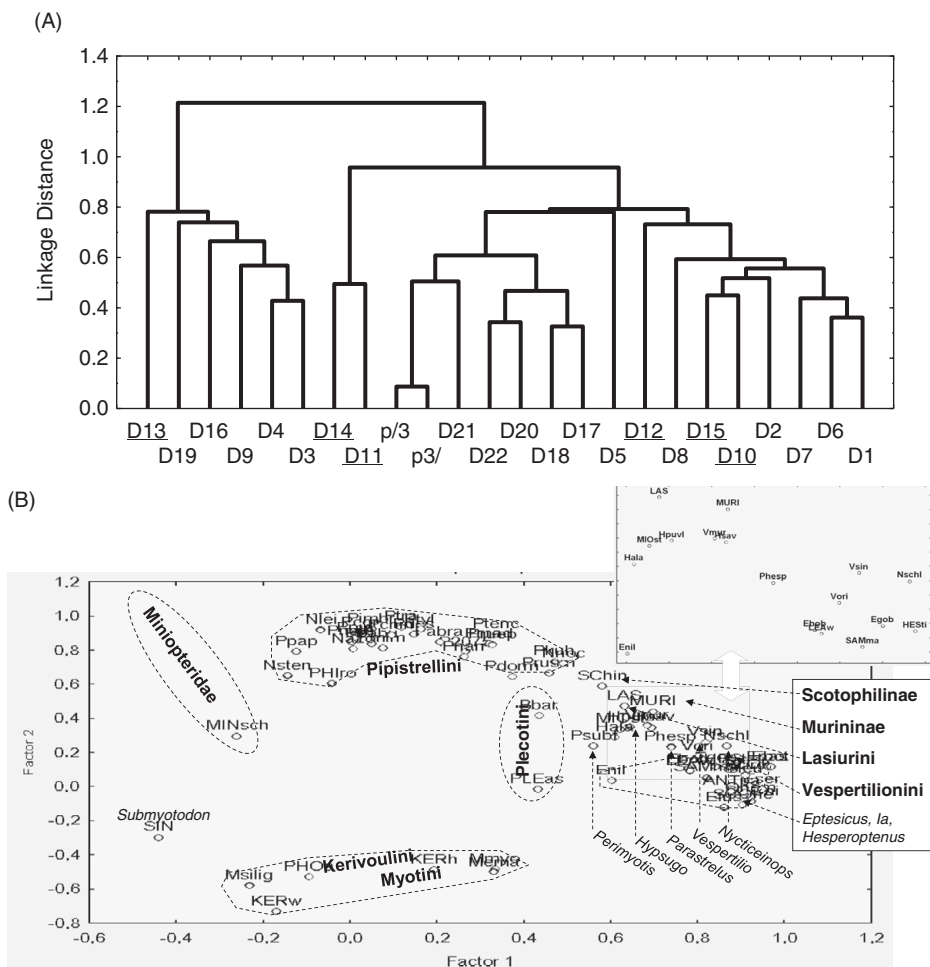


Figure 12.11 (A) UPGMA clustering of 24 non-metric dental characters in 65 species of vespertilionid bats based on the 1 - r distance (dynamic coding of character states scaled to categories 1 to 5). Note: absence of strong correlation among state of particular dental characters (except for P₃/p₃ reduction) and conspicuous independence among molar characters (underlined). (B) Factors I and II scores resulting from PCA based on state of respective dental characters. Note good correspondence with current taxonomic grouping (Hoofer *et al.*, 2006). For list of species and sample specimen see appendix in Hoofer *et al.* (2006). Variables, postcristid lingual termination (D₁); lower incisor spatial integration (D₂); I/3 relative size (D₃); I/3 cusp number (D₄); C/1 relative height (D₅); P/2 mesio-distal compression (D₆); P/2 height (D₇); M/3 talonid size (D₈); coronoid height (D₉); Msup/paralophus reduction (D₁₀); Msup/metalophus reduction (D₁₁); Msup/transcrista reduction (D₁₂); Msup/hypoconal extension of protocone complex (D₁₃); M sup/distocrista reduction (D₁₄); M₃/reduction (D₁₅); I₁/distal cusp size (D₁₆); I₂/relative height (D₁₇); I₂/position, serial/displaced (D₁₈); C₁/shape (D₁₉); P₂/reduction (D₂₀); P₄/mesiolateral cingulum shape (D₂₁); dentition elongate or compressed (D₂₂); P₃ reduction (p₃/); p₃ reduction (p₃/).

phylogeny, complementary to analyses based on completely different sources of phylogenetic information, such as molecular phylogenetics.

Delineating phylogenetic morphoclines for particular dental characters should be here perhaps the first step. Yet, the straightforward trends are apparently quite exceptional in bat dental characters, and this concerns even the characters closely linked to foraging specialization. Rather typical is a variegated mosaic of derived characters often conforming to divergent specializations, illustrating a complex pathway of adaptive history oscillating around the centroid of the aerial insectivory niche.

Despite all of that, there are certain molar traits for which the orientation of phylogenetic morphoclines common to most clades can be determined. Molars of early bats appear to be more compact, proportional in respect to the structural roles of particular functional modules (see above), with markedly robust elements composing the socket of the crown (such as the lingual axis and cingula in the lower molars or parastyle, buccal styles, cingula and talon-like sweep of protocone postvallum in the upper molars). In contrast, the molars of extant bats show a considerable divergence in these characters and, on average, have higher and more prolonged crests, enlarged volume of protocone fossa and talonid fossid and more delicate walls of the crest system (Figures 12.14–12.16).

The morphocline suggested by that comparison would hence include the developmental suppression of structures contributing to the socket of the crown, while the mechanisms contributing to prolonging crests and increase of developmental flexibility of the crest system are promoted. In regard to the above conclusions, the respective mechanisms may include accelerated growth of the undifferentiated primordium prior to definite morphogenetic differentiation of particular structural elements, prolonged autonomy of modern structures, i.e., major crests and their walls (modules 2a–2c) and, in particular, the zones “in between them” not covered by enamel (modules 3) for which late enlargement allows an extension of the overall size of the tooth and fine tuning of its shape during the peri-eruptional period. In terms of developmental dynamics it means a redirection of developmental energy to derived components of the tooth crown and enlargement of the domain of the prospective regulation in the latest stage of tooth development. As a result, the effects of modularity of molar structure (and its consequences for dynamics of dental adaptations) increases the developmental freedom for increased variation in pathways of mutual integration of particular structural modules.

Thus, on a phylogenetic scale, variation in structures interconnecting the basic modules and/or extending the essential tribosphenic design (e.g., mesostyle, profossa, para- and metalophs, hypocone and talon, hypoconid fossid, mesial end of cristid obliqua, distal postcristid, entoconid crest) may extensively

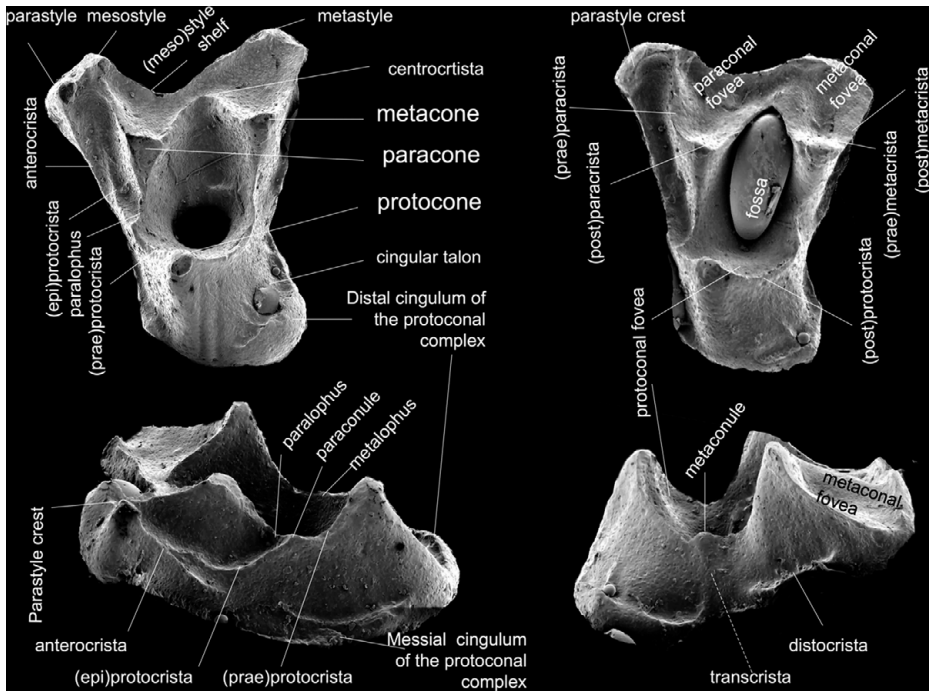


Figure 12.12 Upper molars of archaic bat *Ageina tobieni* (MP7 Mutigny, France) and topology of cusp and crests: M1 occlusal and mesial view (left, UCMP Louis 409Mu), M2 occlusal and distal view (right, UCMP Louis 375Mu), not to scale. Note roughly proportional volume of major modules, including excessively large parastyle with a separate parastylar crest, small volume of protocone fossa and shallow loop of centrocrista.

increase. Clade-specific divergences in the states of these characters illustrate this phenomenon quite convincingly and confirm that these characters and their combinations may be the best candidates for dental traits that provide the most robust phylogenetic signals (see [Figures 12.12–12.16](#) for examples).

12.8 Concluding remarks

As illustrated by the above discussions, an immediate application of dental characters in phylogenetic analyses is not yet feasible for many groups. This is particularly true for studies of the relationship among distant clades (say at the family level), where the dental phenotype is typically characterized by a large number of autapomorphies or pleisomorphies, while the synapomorphies are often limited to just a few characters, often those for which a detailed taxonomic mapping of their character states is not available. Establishment of

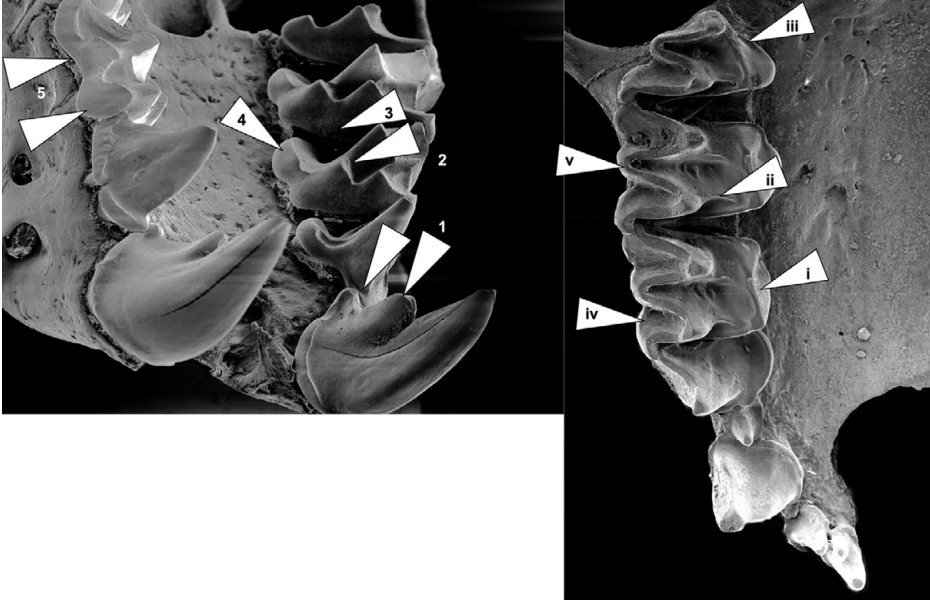


Figure 12.13 Innovations in upper molars related to specialization for larger (left: Megadermatidae: *Cardioderma cor*) and smaller prey (right: Vespertilionidae: *Pipistrellus pipistrellus bactrianus* feeding on Chironomidae). (1) Canine with supplementary distal cusps, (2) reduction of protocone and protocrista, (3) upper molar cingulum absent, (4) emancipation of a low distal talon extension, not related to crest system, (5) extensive increase in height of ectoflexus via enlargement of para- and mesostyle. (i) High and extended protocrista, mesio-distally prolonged, (ii) fossa broad and spacious, subdivided by para- and metalophs (often absent in derived dentitions), but (iii) closed with fusion of protocrista and metalophus at M₃, (iv) crest high and delicate, fovea deeply incised, and (v) centrocrista tapered above mesostyle catchment.

higher taxa and/or inferences on interfamilial relations based on such weak evidence may start controversies. The current discussion accompanying the description of the fossil chiropteran family Mixopterygiidae Maitre, 2008 can illustrate such a situation quite well. The particular source of this controversy is the proposed position of the new family near the root of divergence of emballonurids and Rhinolophoidea, suggesting (corresponding to the traditional view – e.g., Van Valen, 1979) a sister group relationship between these clades (Maitre *et al.*, 2008). We do not wish to discuss here the actual content of the new clade nor will we comment on the relevance of its familial status. However, we feel it important to mention that this case is an example of a phylogenetic conclusion entirely based on odontologic evidence (certain similarities in dental characters among *Vespertiliavus*, Emballonuridae, some of the forms composing the new taxon and rhinolophoids) that necessarily comes in

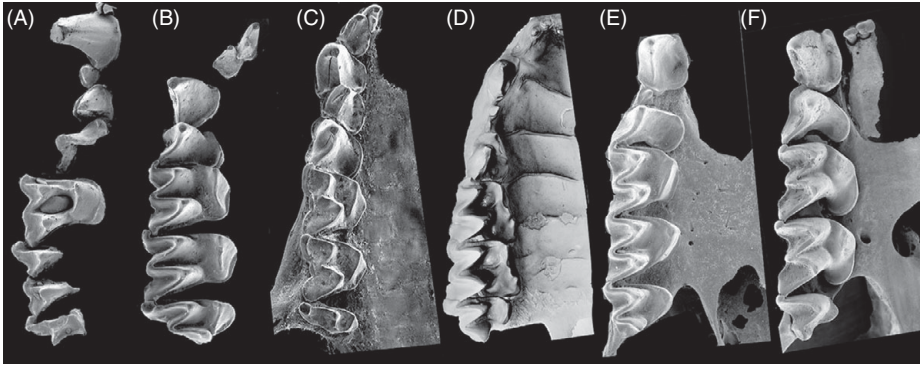


Figure 12.14 Primitive vs. derived upper dentition in selected examples (not to scale). (A) *Palaeochiropteryx spiegelii* (Palaeochiropterygidae): primitive with molar row not enlarged (in comparison to anterior teeth), protocone complex occupies most of tooth volume, para- and metacone crests relatively small, centrocrista do not reach buccal margin, complete anterior tooth row with unusual reduction of P₂ and small unicuspid incisors. (B) *Hypsugo savii* (Vespertilionidae): modern with unicuspid row reduced, moderately large C and P₄, relatively large incisors (I₁ bicuspid) and unreduced molar row exhibiting derived characters (molar row occupies greater than half toothrow length, crest system prolonged, para- and metacone fovea spacious and deep, centrocrista tapered behind buccal margin of tooth, protocone complex enlarged distally, transcrista integrated to postprotocrista, paraloph and metaloph absent). (C) *Murina leucogaster* (Vespertilionidae): unique features include: very robust teeth, extremely reduced M₃, heavy mesial premolar (?P₃), very large M₁ and M₂ with narrow protocone, flat fovea without para- and metaloph, no transcrista, broad and fused paracone-metacone fovea distinctly separated from buccal crown base, centrocrista shallow, not reaching buccal margin, mesostyles absent. (D) *Emballonura alecto* (Emballonuridae): combination of primitive and unique derived states including: unicuspid section longer than molar row, but unicuspid relatively small, incisors conic and very small, canine mesio-distally tapered, with incomplete cingulum, mesial premolar reduced, P₄ molarization modest, molars prolonged but para- and metacones remain relatively narrow while protocones are enlarged with extensive talons bearing distinct cingular cusps, all crests present, conules and transcrista divide flat talon basin. (E) *Rhinolophus* aff. *lepidus* (“kirgisorum” *sensu* Horáček *et al.*, 2000) (Rhinolophidae): derived rhinolophoid arrangement with large canines, reduced premolars, distinct mesio-distal compression of molarized P₄, enlarged molars, molar crests elongate, unreduced M₃, para- and metalophs and transcrista absent. (F) *Nycteris thebaica* (Nycteridae): derived as in (E), but exhibiting differences: M₃ reduced, P₄ less compressed and with a different protocone crest pattern (extensive disto-palatal extension of protocone base, small protocone fossa and incomplete postprotocrista).

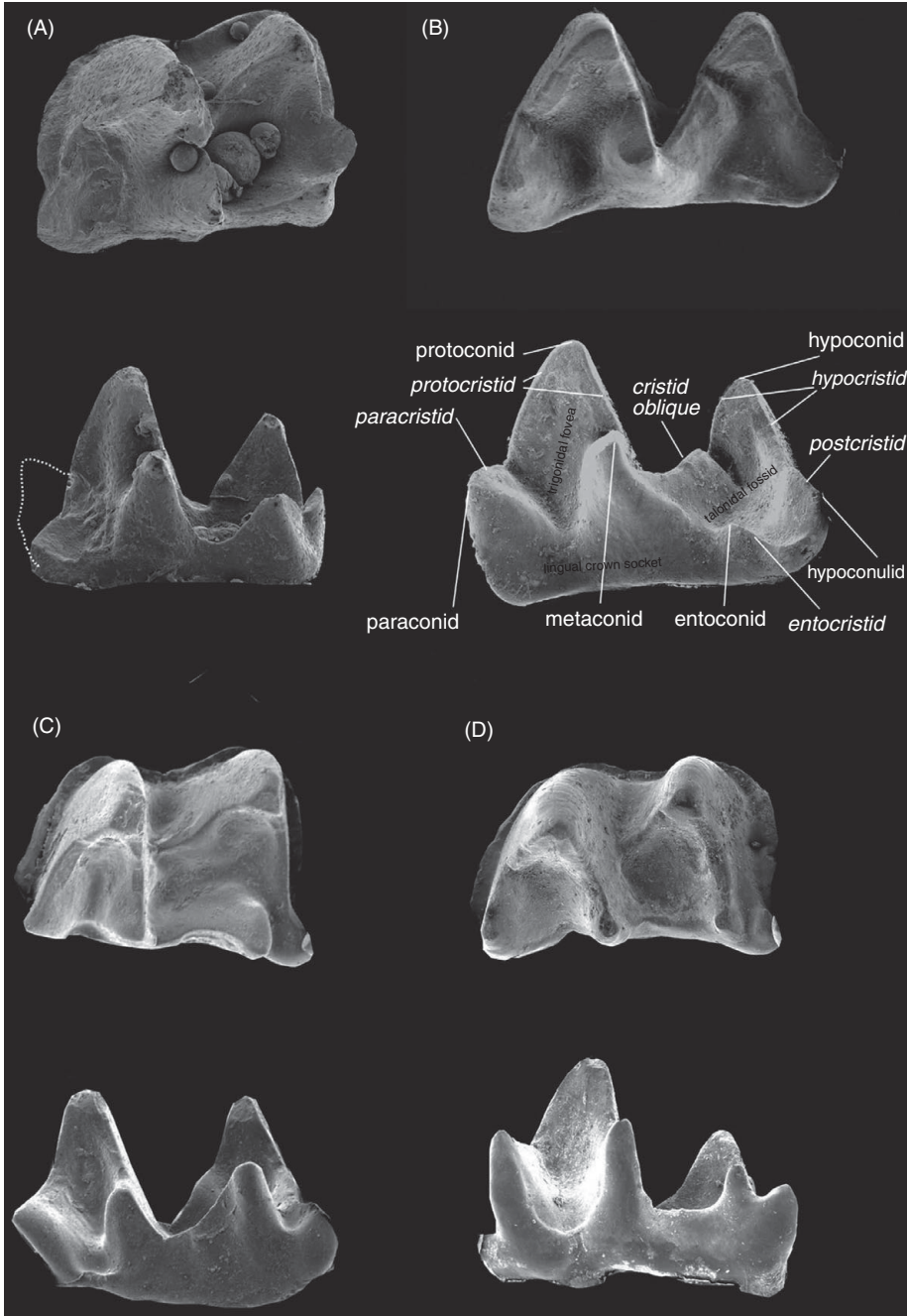


Figure 12.15 Primitive and derived states in *mr*: examples of topology of basic structures in occlusal and lingual views. (A) *Ageina tobieni* (MNHN-Mu5112) – note robustness, almost square in outline, massive labial cingulid, continuous lingual wall,

direct confrontation with the current phylogenetic hypotheses of the order based on robust support from molecular studies (e.g., Teeling *et al.*, 2000, 2002, 2005; Hulva and Horáček, 2002). Regardless of the factual validity of the newly proposed family, a formal comparison of the supporting arguments will clearly indicate the inferiority of odontological evidence compared to molecular information. Though we can believe in the relevance of the odontologic information and point out the empirical arguments of its importance for phylogenetic studies, we must admit that the immediate output of odontologic comparisons often fails to establish a sufficient platform for rigorous testing of alternative phylogenetic hypotheses corresponding to implicit usage of molecular data. Simply put, we need more robust odontologic information. To achieve this requires refining the definition of dental characters in regard to both their developmental setting and actual variation, identifying the orientation of their phylogenetic morphoclines and gathering a large amount of comparative data on dental character states in all clades of bats, both fossil and extant. We hope that the present chapter illustrates not only that such a task is unfortunately a bit complicated, but that – at the same time – there are ways to manage it.

Caption for Figure 12.15 (*cont.*) narrow trigonid basin, relatively low crowned, pre-entocristid not contacting base of metaconid, highly medially situated hypoconulid (necromantodonty see Sigé *et al.*, Chapter 13, this volume), rounded postcristid separated from postentocristid and posthypocristid, cristid obliqua labial and short, joins postvallid almost at right angle. (B) *Nycteris thebaica*: mostly the apomorphies, nyctalodonty (*sensu* Menu and Sigé, 1972) combined with reduction of entoconid, absence of pre-entocristid due to enlargement of metaconid, cristid obliqua joins high on distal wall of metaconid and not on postvallid (rare characters in bats but common in Didelphimorphia), disproportional enlargement of trigonid, particularly the paraconid and metaconid, talonid relatively reduced with low hypoconid, all suggesting adaptation to larger prey and carnivore-like food processing. (C) *Pipistrellus pipistrellus*: nyctalodonty with broad talonid, deep and wide trigonid fossa with narrow paracristid and metacristid, high entoconid, cristid obliqua joins postvallid labial of center, hypoconid high, distal position of hypoconulid along with integration of postcristid and (post)hypocristid results in enlargement of talonid basin (characteristic of molossids and vespertilionids that utilize small aerial prey). (D) *Otonycteris hemprichii*: myotodonty (*sensu* Menu and Sigé, 1972) and light construction (narrow crests) combined with adaptations for larger prey and carnivore-like food processing such as relatively enlarged and broadened trigonids, high para- and metaconids, and low hypoconid with sharp and high (post)hypocristid and indistinct (pre-) entocristid.

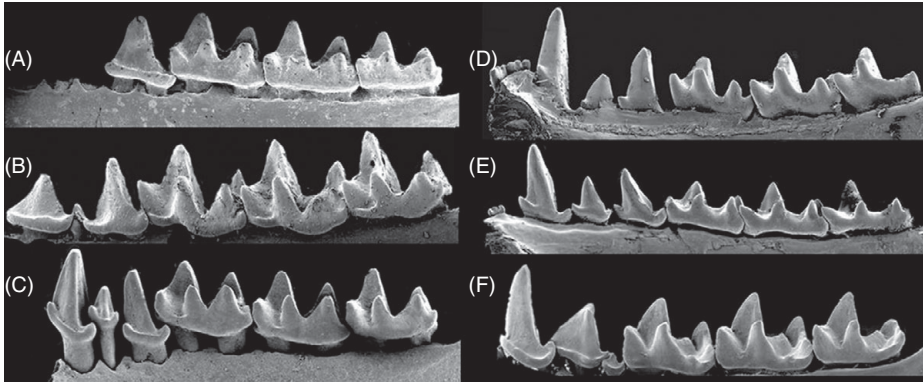


Figure 12.16 Lingual view of lower dentition illustrating some clade-specific rearrangements. (A) *Steblynia* sp. (Eocene, Coll. IPHG Munich): generalized state dentition with large unreduced premolars, nyctalodont molars with heavily built lingual crown base and a massive high wall. (B) *Myzopoda aurita* (Recent, BM (NH)): a unique vertical tapering of molar crests, open trigonids, paraconids oriented mesially and not completely integrated into lingual crown base, which is formed by high and lingually protruding metaconid and entoconid separated by a deep incision, lingual wall continues as low cingular strip along lingual base of paraconid. (C) *Vespertilio murinus* (Recent, ISZ Prague): derived dentition with extreme premolar reduction and mesio-distal compression of the unicuspid row, large molars reducing in size from m1 to m3, major cusps of lingual crown edge equally developed and integrated into common wall. (D) *Rhinopoma cystops* (Recent, NM Prague): robust unicuspid teeth with incomplete cingulids, large, unreduced m3, heavily built lingual base of trigonids and relatively small entoconids lacking well-marked entoconid crests. (E) *Emballonura alecto* (Recent, ISZ Prague): similar to *Rhinopoma* but differs in having molars with enlarged trigonids, relatively small para-, meta- and entoconids that are integrated into a moderately high lingual wall with a well developed entoconid crest (see [Figure 12.14D](#)). (F) *Nycteris thebaica* (Recent, NM Prague): unique mode of premolar reduction: with minute p4 displaced from tooth row, larger p3 not molarized but with sharp mesial shearing edge (for details of unique molar morphology see [Figure 12.15B](#)).

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