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# Genotype, Phenotype, and Developmental Biology of Molar Tooth Characters

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*ABSTRACT* Primate molar shapes reflect developmental and ecological processes. Development may constrain as well as facilitate evolution of new tooth shapes, affecting how reliable dental characters are in phylogenetic studies. Much of the genetic machinery of development uses the same genes among different organs, including teeth, limbs, and feathers. Furthermore, within a tooth, the development of individual cusps repeatedly uses the same set of developmental genes, forming a "developmental module." The repeated activation of the developmental module can explain the cumulative variation in later-developing cusps. Therefore short, later-developing cusps may be evolvable but also more homoplastic. This patterning cascade mode of cusp development can be used to explain the variational properties of dental characters and character states related to cusp initiation. The developmental basis and variational properties of crown termination, cusp shape, and cusp configuration characters are currently less well understood. It is unlikely that there is a simple "gene to phenotype" map for dental characters. Rather, the whole cusp pattern is a product of a dynamic developmental program manifested in the activation of the developmental modules. Yrbk Phys Anthropol 43:171–190, 2000. © 2000 Wiley-Liss, Inc.

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## INTRODUCTION

Were it not for teeth, anthropology would be a different subject. Teeth, living and dead, have much to contribute in the study of ecology, paleontology, functional morphology, and systematics. Teeth seem to be a good, and durable, source of characters for phylogenetic analysis. These characters include the surface features of tooth crowns, most notably cusps and crests, structural details of enamel, and measures of size and shape. Moreover, these dental characters often appear discrete and heritable, and they have different degrees of expression, i.e., different character states. The two most prevalent uses of teeth are studies of phylogeny and ecological adaptation. Tooth characters have a role in phylogenetic reconstructions of primates in general and hominid interrelations in particular. Moreover, detailed dental characters have a wide use in comparisons of human populations. Different aspects of teeth can also be associated with diet, allowing the estimation of dietary specializations in extinct species. Thus, both dental micro- and macrostructure are correlated experimentally or analytically to different foods. However, like any biological structure, dental characters reflect not only functional demands and dietary adaptations in the context of phylogeny, but also developmental processes controlling morphogenesis. This latter is more apparent in teeth than in other skeletal elements because after eruption crown shape changes only by wear, and not through remodeling as in bones.

Since the previous *Yearbook* articles addressing tooth development (Weiss, 1990; Keene, 1991), there has been a renaissance of developmental biology, mostly propelled by new discoveries from developmental genetics. Indeed, enough new investigators have begun to examine development in the context of evolution that a new field of evolutionary developmental biology (evo-devo) is in the making. The sequencing of the entire human genome may transform the field of developmental biology even further. Currently, most attempts to understand development stem from research on individual gene pathways, but this approach is much like trying to describe the ecology of a whole forest from studying a single primate species. The advent of genome-wide data will allow population ecology-like approaches on developmental biology, as large groups of genes can be analyzed using new techniques like microarrays.

Acknowledging the role of development in morphological evolution can quickly become a practical problem if we want to estimate how much and what kinds of effects development has on frequently used dental characters. Do dental characters really possess the properties of being discrete and heritable, having biologically meaningful character states, and can they really change character states independently from each other? All these properties are considered important for a reliable character in reconstructing phylogenies (Lieberman, 1999). Moreover, once we have a clear idea about tooth development, we can begin to ask questions, e.g., what kinds of *developmental* criteria can be used to define reliable characters? In other words, this review explores whether one should worry about development when considering dietary causality for evolution of new tooth cusps or when choosing dental characters for phylogenetic studies.

## RETURN OF DEVELOPMENTAL BIOLOGY INTO THE MODERN SYNTHESIS

Several researchers have pointed out the absence of developmental biology from the modern synthesis of evolutionary biology (e.g., Gilbert et al., 1996; Hamburger, 1980). Gilbert et al. (1996) state that classical embryology, so central to Darwin's arguments, was later seen as old-fashioned, as genetics was found to be a powerful paradigm for explaining the evolution of characters. Indeed, comparative tooth development was

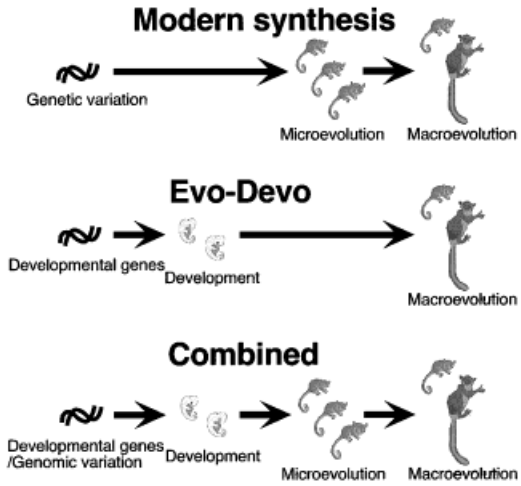



Fig. 1. Changing practices in evolutionary studies. The modern synthesis (top) has largely concerned linking genetic level (as population genetics) to microevolution. Macroevolution is an extension of microevolution; development is not included. Evo-devo studies (middle) link genetic level (as developmental genetics) to development. Changes in development explain macroevolution; microevolution is not necessarily explained. Combined approaches (bottom) use genetic level (as developmental and population genetics) to link development to microevolution and macroevolution.

intensively studied in diverse groups of mammalian species until the 1930s (e.g., Ahrens, 1913; Bolk, 1920, 1921, 1922; Heinick, 1908; Leche, 1915; Ohlin, 1896; von Korff, 1932). After that, research on teeth began to focus on quantifying traits relevant to the genetics of microevolution. One such research focus was the analysis of correlation fields in which tooth measurements were correlated with each other along the tooth row and among the jaws (e.g., Kurtén, 1953). As population genetics became the central model used to explore evolution, development and even phenotypes were mostly considered the ephemeral extension of the genotype (discussed in Dawkins, 1982; Gilbert et al., 1996; Mayr, 1997). Yet, this paradigm relies on the assumption that there is an almost one-to-one relationship between genotype and phenotype. Wagner and Altenberg (1996) called this a "representation problem," or "gene to phenotype mapping" (also see Minelli, 1998), and Weiss and Fullerton (2000) referred to a "phenogenetic" relationship. Essentially, if the phenogenetic relationship is very strong, we can indeed infer phenotype from genotype, and genetic determinism is warranted (Fig. 1). This would mean that natural selection could act directly on genes. However, explaining macroevolution as merely the extension of (gradual) microevolution, and ultimately as the extension of changing genotype, is problematic (Bateson, 1894, 1913; Gilbert, 1994; Gilbert et al., 1996; Mayr, 1959). Large morphological changes, when developmental processes underlying their variation properties were ignored, seemed to be beyond the explanatory power of gradual changes in the genotype. Eventually, Gould (1977) triggered the incorporation of development, in terms of heterochrony and allometry, in the study of morphological evolution.

Interestingly, the reductionist approach of genetic determinism continues to manifest itself in much research on development, but in a slightly transformed way. For a long time, the study of development was the study of cell differentiation, which was directed by differential gene expression. This focus on cell *differentiation* was contrasted in the 1980s by the discovery that homeobox genes were found to direct the differentiation of *whole* body parts. Moreover, as homeobox genes were found to be shared among practically every type of animal, developmental biology underwent a paradigm shift. Gilbert et al. (1996) pointed out that the discovery of shared homeobox genes marked a rediscovery of the concept of homology among developmental biologists (i.e., the concept that many features are shared among organisms due to common ancestry, and that this could be used as a tool in research). As a great deal of the research on tooth development had focused on enamel and dentine differentiation, now "developmental regulatory" genes entered the spotlight. These were genes that were found to be expressed in other organs such as limbs and, moreover, they were usually genes that had homologues across all studied animals. And in the 1990s, as in population genetics in the 1930s, molecular genetics could be used to explain evolution. The most simplistic view of evolution implied that changes in the homologous genes and their regulation resulted in the evolu-

tion of new morphologies. A central point to remember is that the "model" organisms that were behind the discovery of homeobox genes included species as disparate as the mouse and the fruit fly. Therefore, new evolutionary developmental studies were directly involved with macroevolution, because the conserved developmental genes were homologous across classes and phyla. These evo-devo studies, addressing the origin of new organs and tissue-types across higher taxa, are quite different from the studies of the "old" modern synthesis involving genetics and microevolution. In the most simplified terms, changes in the conserved developmental regulatory genes cause changes in development, in turn causing changes in morphology (Fig. 1). Whereas the modern synthesis lacked developmental biology, the evo-devo studies are often lacking microevolution. In practical terms, rather than extrapolating microevolutionary changes to macroevolution, expression domains of homeobox genes account for large changes in morphology directly (e.g., Cohn and Tickle, 1999; Burke et al., 1995).

Without doubt, recent advances in developmental biology have enabled us to add new information on the early evolution of higher taxa previously beyond the resolution, or reach, of the fossil record. Fields like biological anthropology are in the fortunate position of being able to integrate development into evolutionary studies. Detailed organismal-level knowledge of tooth evolution in ecological contexts can be used to link new information on development to micro- and macroevolution (Fig. 1). This may produce better appraisals of the developmental basis for characters used in phylogenetic studies (Holland, 1999). The risk involved in applying molecular-level models of characters directly to macroevolution is that it is difficult to test whether character codings are meaningful when considering natural selection and microevolution. In other words, it is uncertain how variational properties of developmental systems can be inferred by extrapolating from molecules to fossils. Furthermore, examining development in the context of micro- and macroevolution can be used to estimate the extent of phenogenetic drift (Weiss and Fullerton, 2000), defined as how much genotype can evolve without a corresponding change in phenotype. Phenogenetic drift is a crucial issue because if phenogenetic drift is common, simple genetic reductionism is unwarranted and some higher-level developmental principles must be discovered in order to understand the developmental basis of morphological evolution. In this case, epigenotype (the whole developmental complex), rather than genotype, would form the basis for the developmental control of phenotype. Phenogenetic drift will also affect how we approach homologies. Indeed, it is likely that the discovery of homologous developmental genes, interacting in operational groups or *modules* (see Raff, 1996), already demands the reappraisal of character homologies. For example, as recognition of characters depends on their being inherited units (see Lieberman, 1999), the problem becomes, what exactly is the developmental information underlying the characters if their development is controlled by modules that are themselves shared among different organs? A reasonable solution is perhaps to rely on the homology concept of Van Valen (1982, 1994), in which continuity of developmental information, without strict assumptions on the physical nature of that information, causes homology.

## TOOTH SHAPE DEVELOPMENT AND EVOLUTION

### Primate teeth

In this paper we concentrate on molar tooth shapes in order to illustrate a more focused picture of the links between development and evolution. Another reason to limit the discussion to molar crowns is the fact that the standard mammal used in developmental studies, the mouse, has only incisor and molar teeth, and thus practically nothing is known about the molecular aspects of development in adjacent tooth families. The evolution of dentition as a whole, and its resemblance to the evolution of other segmented structures such as the vertebral column, are discussed extensively in Weiss (1990).

First, we will examine the diversity of primate molar teeth themselves. Unlike

common taxonomic diversity measures, morphological diversity measures that cut across phylogenetic boundaries are useful in delineating the minimum range of morphological diversity that is possible to construct. By grouping primate upper molar shapes into discrete crown types, we can first examine just diversity, or the number of morphological types. Crown type scheme is a simple classification system of tooth crown topography, where no phylogenetic relations (i.e., homologies) are implied (Jernvall et al., 1996, 2000). Five variables (characters) are used to define each crown type: cusp shape, number of buccal cusps, number of lingual cusps, number of longitudinal lophs, and number of transverse lophs (Jernvall et al., 1996, 2000). Lophs are well-developed shearing crests, and both lophs and cusps have to be two thirds of the crown height to be tabulated, making the crown type scheme conservative but robust.

Extant primates have seven crown types, which is more diverse than in the ungulate orders Artiodactyla (three) and Perissodactyla (four). Therefore, primates seem to have high molar shape diversity compared to ungulates, whose dentition is traditionally considered diverse, reflecting different dietary specializations to herbivory (Janis and Fortelius, 1988). However, if we include the fossil record, the number of primate crown types (order Primates only) rises to nine, whereas Artiodactyla and Perissodactyla have 13 and 17 crown types, respectively (Jernvall et al., 2000). Moreover, when estimating dental *complexity* in terms of the number of cusps and lophs, primate molars are simpler than ungulate molars (Fig. 2). Indeed, the crown type complexity of primate upper molars is closer to that of Condylarthra, archaic ungulates that have been implicated in the ancestry of perissodactyls and artiodactyls in the Eocene epoch (Fig. 2). Macroevolutionary diversity of primate molar shapes can be thus described as having a relatively high diversity of basic morphotypes with only a moderate range of anatomical design, in that the number of cusps and lophs is generally low and in that the topographies of crown types are quite similar (Fig. 2). This limited range of anatomical design may be in itself a concern in phylogenetic studies (Wagner, 2000), as the chance for convergent evolution of similar dental morphologies can be hypothesized to be higher among primates than, for example, among ungulates. This stresses the need to understand the developmental basis of dental characters.

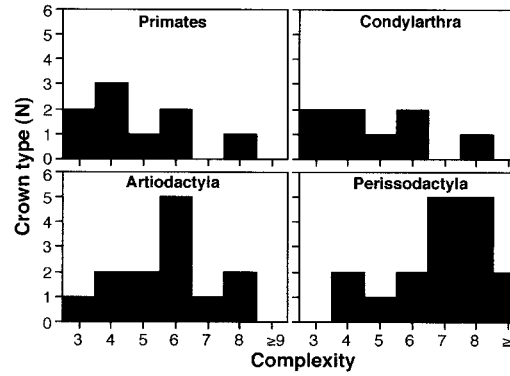



Fig. 2. Complexity of primate upper molars. Frequency distribution of crown types with different numbers of cusps and lophs on second upper molars. Note that complexity (as the summed-up number of cusps and lophs) is lower in primates than in artiodactyls or perissodactyls, but similar to archaic ungulates, the condylarths. The average disparity (morphological difference among crown types) is 2.7, 2.7, 3.3, and 3.6 in primates, condylarths, artiodactyls, and perissodactyls, respectively.

## Development of molar cusps

Of the main characters of tooth shape, the development of cusps is perhaps currently the best understood. Mammalian tooth shape is a product of the folding and growth of the interface between epithelium and neural crest-derived mesenchyme. As folding proceeds, the differentiating inner enamel epithelium facing the mesenchyme gives rise to enamel-forming ameloblasts, while the mesenchyme below gives rise to the dentine-forming odontoblasts (Butler, 1956; Jernvall and Thesleff, 2000). The developmental stage when individual teeth become visible begins by the formation of a tooth bud that can first be seen as a slight outgrowth of the dental lamina (Fig. 3A,B). These earliest stages of tooth development resemble morphologically other epithelial appendages, such as hairs and glands (Jernvall and Thesleff, 2000). Also, in all these organs, development is regulated by interactions between the epithelial and underly-

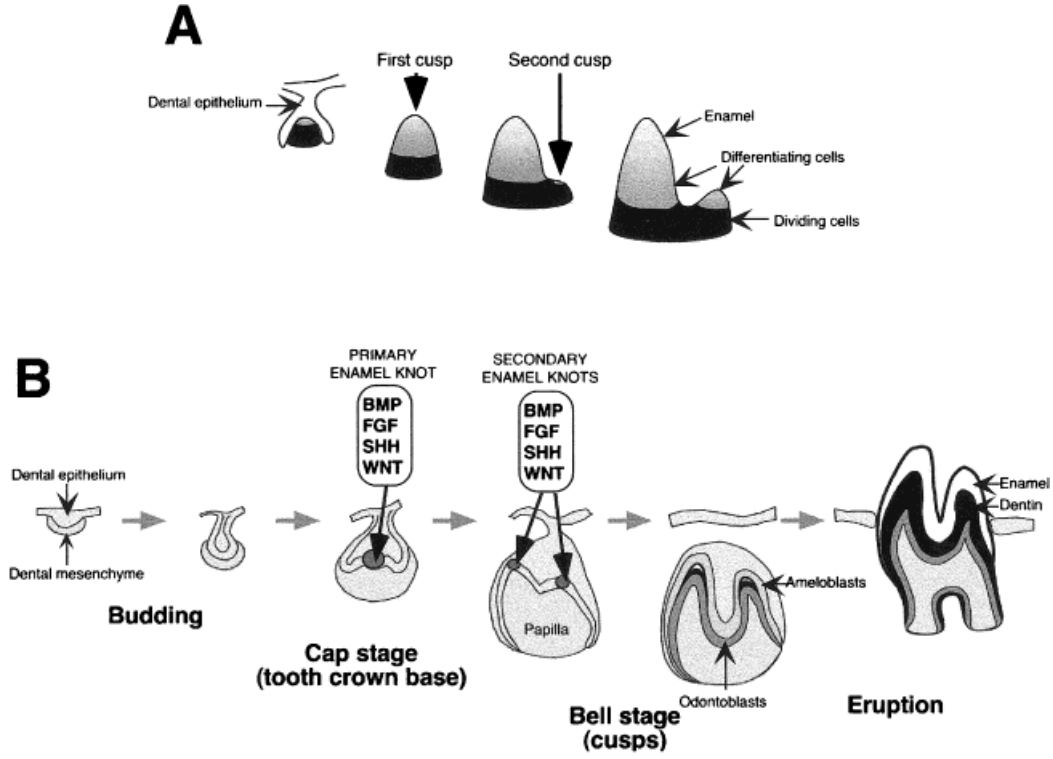



Fig. 3. Schematic representations of tooth development. **A:** Beginning in the cap stage, the tooth crown forms by unequal growth of the inner enamel epithelium (shaded). **B:** Molecular signals considered to be important for particular developmental stages are indicated above the morphology. Note how the same signaling pathways are used repeatedly during advancing tooth development. BMP, bone morphogenetic protein; FGF, fibroblast growth factor; SHH, sonic hedgehog; WNT, wingless-integrated.

ing mesenchymal tissues. The development of molar shape begins by the folding of the bud tip that results in a cap-resembling structure surrounding the mesenchymal dental papilla (Fig. 3A). Unlike the iterative branching morphogenesis of lungs and glands, the tooth bud only forms one set of lateral branches along the anterio-posterior axis of the bud.

***Formation of a signaling center before the cusps.*** The formation of the cap stage marks the future tooth crown base. This developmental stage begins with the formation of the primary enamel knot at the tip of the tooth bud. The primary enamel knot is an epithelial structure that is histologically visible as a cluster of densely packed cells. These cells are nondividing and express several signaling molecules (Vaahtokari et al., 1996a,1996b). The coexpression of over 10 signals (http://honeybee.helsinki.fi/toothexp; Nieminen et al., 1998) manifests the primary enamel knot as an embryonic signaling center. Signaling centers interact with surrounding tissues by secreting molecular signals that are required for the progression of morphogenesis and they are understood, in developmental biology terms, to "regulate" or "control" different aspects of morphogenesis. These views stem from classic grafting experiments showing that certain regions of the embryo (i.e., signaling centers) are able to reorganize embryogenesis (Tickle et al., 1975; Spemann and Mangold, 1924). Other comparable embryonic signaling centers include the zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER), which together participate in the patterning and growth of limbs (Niswander and Martin, 1993; Tickle et al., 1975). The notochord is another signaling center in the patterning of the neural tube

and somites (Echelard et al., 1993, Fan and Tessier-Lavigne, 1994, Liem et al., 1995), and the tips of the lung buds have been proposed to function as signaling centers in lung branching (Hogan, 1999; Metzger and Krasnow, 1999). While the developmental context and outcome differ among signaling centers, the genes used in molecular signaling are usually the same. Indeed, discoveries where signaling centers could be reciprocally replaced among organs and organisms had already pointed toward a common basis for embryonic signaling (Hornbruch and Wolpert, 1986). For example, the Sonic hedgehog (*Shh*) gene is expressed in the developing limb in the ZPA, in the lung bud, notochord, and enamel knot (Echelard et al., 1993; Riddle et al., 1993; Roelink et al., 1994). The anterior-posterior limb patterning requiring ZPA can be mimicked by implanting a bead releasing SHH-protein (Riddle et al., 1993) or a tooth germ (Koyama et al., 1996) into limbs. These discoveries make it obvious that one gene does not produce one structure.

***Embryonic role of the enamel knot.*** The formation of the tooth cap is basically all the epithelial folding that is needed in a unicusped tooth, e.g., in a canine. The tip of the tooth cap becomes the tip of the cusp, and the sides of the cap continue to grow down, forming the cervical loop and eventually the root (Fig. 3B). The formation of tooth cap and enamel knot is accompanied by unequal growth in different regions of the inner enamel epithelium. Only epithelium has been found to have a distinct pattern in cell proliferation during early crown morphogenesis, suggesting that the formation of the crown base is essentially a folding of a two-dimensional sheet. The role of the cells of the enamel knot as the first area of mitotically quiescent cells has been intensely studied in the folding and unequal growth of the tooth cap. Indeed, it is curious that the nondividing cells of the primary enamel knot express fibroblast growth factor (*Fgf*) genes, which are known mitogens.

At least four *Fgf*s are expressed in a developing tooth, of which all are found to promote cell division in cultured dental tissues. Two of the *Fgf*s, *Fgf4* and *Fgf9*, are only expressed in the enamel knot cells, and *Fgf3* is expressed in the enamel knot and the underlying mesenchyme. *Fgf10*, the fourth *Fgf* transcribed in teeth, is expressed during the cap stage only in the mesenchyme (Kettunen and Thesleff, 1998). Moreover, the different FGF proteins (we follow the practice of marking genes in italicized letters and protein products in capital letters) do not stimulate all dental tissues equally. FGF4 and FGF9 have been shown to stimulate cell proliferation of both dental epithelium and mesenchyme, whereas FGF10 stimulates cell division only in dental epithelium (Kettunen et al., 1998, 2000). Thus it appears that the primary enamel knot promotes tooth germ growth around itself, which may play a role in the folding of dental epithelia during the formation of the tooth cap and crown base (Jernvall et al., 1994).

While the embryological role of the enamel knot may be related to epithelial folding, the molecular-level process of the enamel knot expressing growth-stimulating *Fgf*s while not growing itself has been a puzzle. Partly, the nonproliferation of enamel knot cells can be linked to the dearth of FGF receptors in the enamel knot area (Kettunen et al., 1998). Thus, the enamel knot appears to be "deaf" to the stimulatory signals of FGFs. Additionally, an early marker for the enamel knot is cyclin-dependent kinase inhibitor *p21* expression in the enamel knot. *p21* expression is associated with cells stopping to divide and beginning terminal differentiation. Therefore, as in differentiating muscle cells (Parker et al., 1995), *p21* may be involved in the differentiation and withdrawal of enamel knot cells from the cell cycle (Bloch-Zupan et al., 1998; Jernvall et al., 1998). In the case of the enamel knot, the differentiated stage involves the expression of growth-stimulatory genes. *p21* is also expressed in the limb AER that also expresses FGFs while remaining nonproliferative (Parker et al., 1995). It is worthwhile to point out that experimental knockout mice lacking functional *p21* had no reported dental (or limb) defects (Deng et al., 1995). This illustrates the possibility that *p21* may have functional redundancy with other inhibitors

of cell proliferation as a part of a developmental "insurance policy" against loss of normal cell cycle control. The biology of redundancy, however, is not fully understood.

The primary enamel knot begins to disappear soon after the bud has branched out to form the cap (Fig. 3). The disappearance of signaling centers is in itself a subject of research. It is now known that the cells of the enamel knot disappear via apoptosis, or programmed cell death. This apoptosis is associated with the expression of another growth factor, bone morphogenetic protein 4 (*Bmp4*), in the enamel knot (Jernvall et al., 1998). While *Bmp*s have multiple roles in development, they appear also to be generally involved in apoptosis in other organs, such as rhombomeres and digits (Graham et al., 1994; Pizette and Niswander, 1999). Biologically, apoptosis has been suggested to be a mechanism involved in the control of duration of molecular signaling of the enamel knot (Vaahtokari et al., 1996b; Jernvall et al., 1998), and this has been suggested to be the case in the apoptotic removal of the AER in the limb bud as well (Pizette and Niswander, 1999).

***Cusps are enamel knot replays.*** In teeth that have many cusps, i.e., usually molar teeth, new enamel knots appear soon after the primary enamel knot disappears. The new enamel knots, called secondary enamel knots (Jernvall et al., 1994), appear at the places of future cusp tips. As for the primary enamel knot, secondary enamel knots are also nonproliferative, express *Fgf4*, and are removed apoptotically (Vaahtokari et al., 1996b; Coin et al., 1999).

The secondary enamel knots are the first signs of cusps (Fig. 4), and using *Fgf4* as an enamel knot marker, the onset of species-specific cusp patterns was recently analyzed in developing mouse and vole lower molars (Keränen et al., 1998). Cusp patterning was estimated to begin as early as a day after the formation of the tooth cap, thus before cusps are visible morphologically. This result confirms the notion of Butler (1982) that tooth germ presumptive domains exist in the early tooth germ from which cusps develop.

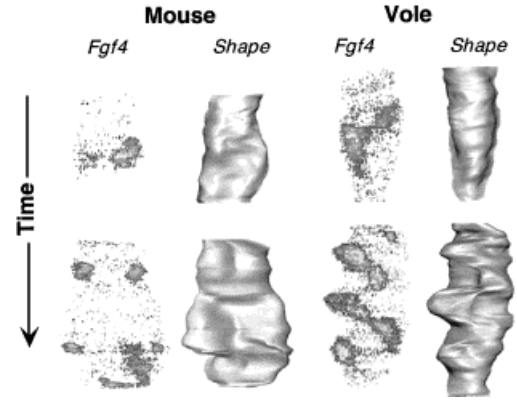



Fig. 4. Occlusal view (three-dimensional reconstructions of inner enamel epithelium viewed from the above) of developing mouse and vole lower molar. Note how *Fgf4* expression marks the adult cusp patterns.

Unfortunately, experimental inactivations of genes affecting tooth development result in the total lack of some of or all of the teeth (Chen et al., 1996; Ferguson et al., 1998; van Genderen et al., 1994; Hardcastle et al., 1998; Kratochwil et al., 1996; Peters et al., 1998; Thomas et al., 1997), an obvious limitation if one wants to study changes in cusp patterns. Thus knockout mice are not necessarily informative for considering cusp development per se. However, a natural mouse mutant, *Tabby*, has defects in molar cusps that are informative about development. The *Tabby* gene has a corresponding human gene, the anhidrotic ectodermal dysplasia (*EDA*) gene (Ferguson et al., 1997; Srivastava et al., 1997), and both EDA patients and *Tabby* mice have abnormal development of epithelial appendages such as teeth, hair, and sweat glands. In *Tabby* mice, molar cusps are compressed, as the tips of buccal and lingual cusps are close to each other or completely united. In addition, the last developing cusps are often missing. This altered cusp pattern is already visible at the level of secondary enamel knots, which are often fused (Pispa et al., 1999). The altered cusp pattern in *Tabby* molars appears to stem from an affected primary enamel knot that is small, while still expressing all the known enamel knot genes. Therefore, a general retardation of growth may cause the delayed formation of cusps and the lack of the later developing molars

in *Tabby* mice. Indeed, cusp development in *Tabby* molars is partially corrected when *Tabby* tooth germs are cultured in vitro with FGF4 and -10 present in culture medium (Pispa et al., 1999). This effect suggests that *Fgf*s may indeed have a role in cusp development in vivo.

Considering the development of individual cusps, no cusp seems to differ from another in terms of secondary enamel knots. Every cusp studied expresses genes of the families *Fgf*, *Shh*, *Wnt*, and *Bmp*. Indeed, there are currently no genes that would, individually or in a combination, mark the identity of, for example, the metaconid. However, while several signals appear to be active in the initiation of cusp development, only *Fgf4* expression remains strictly restricted to the cusp tips. For example, *p21* expression begins to spread around the enamel knots, and this spreading is associated with cessation of cell proliferation and subsequently, together with other signals, including *Fgf9* and *Shh*, with the differentiation of enamel-forming ameloblasts. The primary enamel knot, however, seems to possess slightly different gene activity from the secondary enamel knots, as it also expresses *Bmp2*, the transcription of which quickly becomes undetectable as the first secondary enamel knots are activated (Åberg at al., 1997). Also, as the primary knot disappears apoptotically, its gene activity is silenced.

The iterative activation of the secondary enamel knots allows us to rephrase the problem of tooth development in slightly new terms. The development of tooth shape may require the spatial and temporal control of the secondary enamel knots that are no different from each other genetically. The spatial pattern of the enamel knot activation influences the relative position of cusps to each other, while the temporal spacing of the knots influences cusp size. While phylogenetically ancient cusps have been documented to begin their development earlier than phylogenetically recent cusps, the main determinant of initiation of cusp development is the relative size of each cusp (Butler, 1956). Tooth development starts from the formation of the tallest cusps, and the growth and folding proceed so that the smallest cusps are the last to form (Butler, 1956). Thus, for example, the phylogenetically recent but large metacone forms prior to the phylogenetically older paracone in opossums (Berkowitz, 1967). This kind of development, where there is no single gene for a single cusp, requires us to explain the evolution of cusps using dynamic patterning mechanisms (Jernvall, 2000; Weiss et al., 1998; Zhao et al., 2000a).

***Cusps as feathers.*** In teeth, an accurate control of secondary enamel knot spacing is likely to exist during morphogenesis, as this process results in correct cusp position and size. Both these characters (position and size) are critical for a functioning occlusion and feeding. The iterative activation of the secondary enamel knots resembles that of feather primordia development. Feather primordia patterning is currently perhaps the best characterized system of iterative patterning. Feather primordia are arranged in specific tracts over the avian body. Feathers, like teeth, are formed by interactions between the epithelium and mesenchyme. In feathers, the spacing between feather primordia can be altered by affecting molecular signaling in vitro. Using small beads releasing BMP4 protein, Jung et al. (1998) inhibited feather formation at the distance of the protein-releasing bead. This regional inhibition effect, or field, strictly depended on the concentration of BMP protein. In contrast, FGF4 and SHH protein stimulated the formation of feathers nearby, by producing ectopic feather primordia around the protein-releasing bead. Jung et al. (1998) concluded that *Fgf* and *Shh* may be the activators, while *Bmp* is the inhibitor involved in the spacing of feathers. Furthermore, both FGF4 and SHH protein induced the expression of *Bmp4*, while BMP4 protein inhibited the expression of the two former genes (Jung et al., 1998). These experiments are compatible with a reaction-diffusion model of morphogenesis (Jung et al., 1998). In this model, an initial point (i.e., signaling center) expressing an activator (e.g., *Fgf4*) will cause local growth and also the expression of an inhibitor (e.g., *Bmp4*) to prevent the formation of new signaling centers nearby. However, as the inhibitor will also

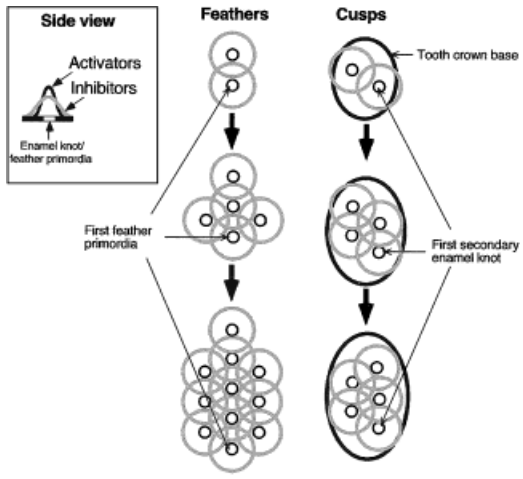



Fig. 5. Feather patterning and cusp patterning have many similarities. Feathers and cusps form as iterations of similar sets of signaling molecules. For example, as a model to regulate patterning, FGF4 functions as an activator promoting cusp/feather bud initiation and growth. Inhibitors such as BMP4, which have a more diffuse expression domain, control the minimal distance between adjacent secondary enamel knots/feathers. The total number of cusps on a tooth would be limited by the size of the tooth crown base.

inhibit the production of the activator that is needed for the production of the inhibitor itself, production and spreading of the inhibitor are eventually terminated (Fig. 5). The result is a spatial pattern of differentiated domains that can be altered by any changes in activator-inhibitor production, decay, diffusion, or interaction. As Weiss et al. (1998) pointed out, reaction-diffusion models describe self-organized systems (or Bateson-Turing processes), which are systems that do not need external information to execute a developmental program once it is initiated. It is noteworthy that many processes, and models, can produce similar effects, and even cell density is known to affect feather patterning (Jung et al., 1999a). Furthermore, in reaction-diffusion models, the inhibitors would have to diffuse faster than the activators in order to create spacing among buds. Interestingly, Hammer (1998) showed that analytically, the effects of signaling can also be realized by cell-cell-mediated propagation of the signal and not by long-distance diffusion. Thus, while reaction-diffusion models are compatible with current data, several molecular-level processes may be involved in the production of patterns.

The reaction-diffusion model might not be limited to feathers and teeth. Localized expression of *Fgf*s, *Bmp*s, and *Shh* can be found in other parts of the skin, such as in the developing mouse tongue papilla (Jung et al., 1999b), and even the branching morphogenesis of mammal lungs and insect trachea shows similarities with avian feathers (Hogan, 1999; Metzger and Krasnow, 1999). Indeed, secondary enamel knots do not strike us as markedly different from feather primordia. In teeth, the control of both the timing and location of secondary enamel knots within the crown base has to be accurate (Fig. 5). As the same signaling molecules are used in teeth, it could be that the process of patterning is quite similar to that of feather primodia. However, some differences do exist. The exact locations of gene expression are slightly different. For example, *Bmp4* is expressed in a diffusive, inhibition-field manner in the dental mesenchyme, and not in the epithelium. Of course, other *Bmp*s (at least *Bmp9*) are known to be expressed in the dental epithelium (Åberg et al., 1997). Indeed, one of the main differences among epithelial organs appears to be in the details of domains that express the shared molecular signals. For example, enamel knots express *Fgf4* in the epithelia, but lung buds express *Fgf10* in the mesenchyme next to the tips. However, the common property for signaling centers (partly by definition) is their superimposed pattern of gene expression.

One aspect that seems to set teeth apart from feathers is their robustness in defying external perturbations. Experimentally, teeth cultured in vitro are remarkably resistant to perturbation by added proteins. It is known that, as in feathers and limbs, FGF proteins do, while BMP proteins do not, stimulate growth in separated tooth epithelium and mesenchyme. But only in rare cases will the addition of FGF protein increase the number of cusps, as in the rescue of mutant *Tabby* teeth (Pispa et al., 1999). Indeed, teeth appear to be exceedingly self-organized units. A single tissue containing primodia for a mouse first molar can give rise to all the molars in vitro. We suspect

that the robustness of tooth development is related to the fact that teeth can change shape after eruption only by wear, not by remodeling. Thus, evolutionarily speaking, there has been perhaps a good reason to make tooth development as robust as possible. Interestingly, Coin et al. (2000) showed experimentally that dividing cap-staged mouse molar germs to anterior and posterior halves caused disorganization of the primary enamel knot that subsequently became restored in the halves. When cultured, the distal halves produced almost normal sets of cusps, suggesting that portions of early tooth germs have a high self-organizing capability.

### Evolvability of tooth cusps

Secondary enamel knots are helpful in explaining cusp development, but to use them to examine molar evolution requires us to interpret them in their micro- and macroevolutionary contexts. Evolution of molar shapes is manifested by the convergent acquisition of new cusps. Most notably, the distolingual cusp in the upper molars, the hypocone, has evolved at least 20 times in mammals (Hunter and Jernvall, 1995). Even among primates, the hypocone has evolved multiple times (Fleagle, 1998). In many cases, the details (e.g., which part of the crown gives rise to the hypocone) differ, and subsequently different names are often used. However, the derived morphologies can often be such that it is difficult (Sánchez-Villagra and Kay, 1996) or impossible to infer the mode of hypocone evolution without a good fossil record. The evolution of the hypocone has been suggested to manifest the capacity of tooth development to promote the evolution of new morphologies (Gerhart and Kirschner, 1997). The capacity to evolve, or evolvability, has been traditionally inferred in population genetics studies (in response to selection), but evolvability is also considered to be a property of biological systems that can be studied at various levels, e.g., by studying development (Caporale, 1999; Gerhart and Kirschner, 1997; Kirschner and Gerhart, 1998; Wagner and Altenberg, 1996; West-Eberhard, 1998).

Primate molars, while lacking some more extreme morphologies (Fig. 2), still exhibit moderate evolutionary diversity in the number of cusps and conules which may have properties of evolvability. Indeed, the iterative activation of secondary enamel knots may in itself be a mechanism of evolvability, as the enamel knots can be understood as developmental "modules." Modularity of development has been suggested to be one of the mechanisms of evolvability (Gerhart and Kirschner, 1997) because it facilitates local, independent changes in morphology. That is, pleiotropic effects of genes may be limited if development uses modules that have only partly overlapping pleiotropic effects (Minelli, 1998; Wagner and Altenberg, 1996). For example, while gene knockout experiments with dental defects usually have effects on a wide range of organs, in some cases there is redundancy in gene function, and several genes have to be deactivated in order to have a specific effect. One such case is with *Dlx* homeobox genes, where both *Dlx1* and *Dlx2* have to be deactivated in order to have a dental effect, which is the lack of upper molars (Thomas et al., 1997). The other mouse teeth are unaffected, most likely due to the expression of other *Dlx* genes in their regions (Zhao et al., 2000b). However, no cusp-specific differences in homeobox gene expression have been reported (Zhao et al., 2000b), and the secondary enamel knot "cusp-making modules" appear to be alike among all the cusps at the level of molecular signaling. This would mean that pleiotropy among secondary enamel knots, and cusps in general, should be high. Obviously, *cis*-regulatory elements can be assumed to be involved in the activation of individual secondary enamel knots. Indeed, regulatory regions of genes evolve faster than actual coding regions (Huynene and Bork, 1998). Could this rate difference, for example, implicate that new regulatory elements evolve with every cusp? This scenario would be a case of extreme genetic determinism that does not necessarily make it much easier to evolve new cusps. But how then has molar tooth diversity evolved and, moreover, can teeth really be evolvable?

A possible solution is a patterning cascade mode of cusp spacing that may promote the evolution of new cusps (Jernvall,

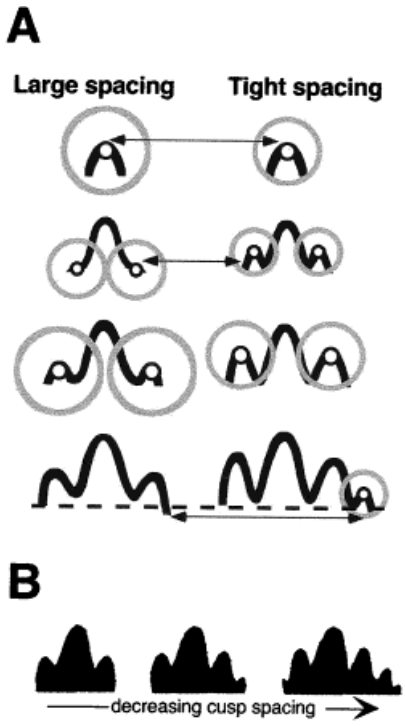



Fig. 6. Patterning cascade mode of cusp development. **A:** Small initial differences in cusp spacing have cumulative effects on later developing cusps. **B:** Only small changes in the spacing of cusps can increase or decrease cusp number and the size of small cusps. The crown height affects the realized cusp numbers globally, while the patterning cascade affects the potential cusp pattern. More complex teeth are likely to have partly independent patterning cascades at different parts of the crown (e.g., paracone-protocone-Carabelli cascade; paracone-metacone cascade).

2000). As in the models explaining feather bud patterning, the position of a new secondary enamel knot may depend on the previous enamel knot, and the evolution of new cusps would not require the evolution of any new developmental information. Rather, a parameter change in the enamel knot program affecting the reaction-diffusion process would alter cusp patterns. This also implies that no external positional information is needed for any particular cusp, but that the whole cusp pattern is a product of the dynamic program itself (Jernvall, 2000; Zhao et al., 2000a). For example, a broader inhibition field around an enamel knot will displace the formation of new secondary knots further away. Furthermore, as crown shape results from the folding and downgrowth of the inner enamel epithelium, delay in cusp initiation results in a shorter cusp (Fig. 6). And as the secondary enamel knot program is repeated for every cusp, any small difference in cusp spacing will have a cumulative effect on later-developing cusps (Jernvall, 2000) (Fig. 6). The patterning cascade mode of cusp development predicts that variation in small cusps should be cumulative and that random variation should not necessarily increase in short, later-developing cusps. This seems to be the case at least in a population sample of seal dentition and where the height of the three tallest cusps can be effectively used to predict the height and number of short cusps in a tooth (Jernvall, 2000). However, seal dentition is essentially two-dimensional, and it remains to be seen whether the patterning cascade explains cusp size variation in more complex teeth, such as in primate molars. Interestingly, Polly (1998) studied variation in cusp position, apart from height, in viverravid carnivores and found that the degree of growth between cusps may contribute additional variation to cusp positions. However, it is not known if this is also the case with cusp height.

A possibly useful character in human molars that could be linked to the patterning cascade development is Carabelli's cusp, an extra mesiolingual cusp emerging from the protocone (Hillson, 1996). If a simple seal-like patterning cascade is applicable to human molars, then a larger Carabelli's cusp would be expected to be present when the height of the protocone, relative to the paracone, is large. However, as the differences are cumulative, only a very small increase in size of the protocone would be needed to have a large difference in the size of Carabelli's cusp (Fig. 6). While it remains to be documented, this kind of variation could explain why the number and size of small cusps and cingula are variable in populations, as natural selection limiting variation in large cusps would not be as effective on small cusps. Inversely, selection to increase the size of small cusps would have relatively small effects on large cusps. Thus, the evolution of new cusps may be a good example of evolvability. Obviously variation in the parameters affecting the patterning cascade

mode of cusp formation has to be inherited in order to respond to natural selection (thus, to be evolvable in terms of population genetics). Indirect evidence can be found in the case of seals where a hybrid cusp between two species (*Halchoerus grypus* and *Phoca hispida*) had an intermediate dental morphology, including the configuration and number of cusps (Lönnberg, 1929). Thus the patterning cascade parameters are inheritable, but to what extent remains to be determined. It is worthwhile to point out that the patterning cascade mode of cusp development is quite compatible with the "morphogenetic triangle" that Keene (1991) used to approach heterochrony in tooth development. For example, the iterations that the patterning cascade is allowed to go through appear to be controlled independently by crown height (Jernvall, 2000). Therefore, the potential cusp number and the actually developmentally realized cusp number result from the patterning cascade and crown height, respectively.

The repeated activation of molecularly similar secondary enamel knots as a basis for cusp evolvability may have influenced the likelihood of cusp evolution in general. Not only may the evolution of new cusps be a relatively small change developmentally, but the loss of cusps may also be equally simple. Nevertheless, loss of cusps seems to be rare in mammalian radiations (e.g., mainly in seals and whales). Among primates, the loss of the hypocone in marmosets and tamarins (Callitrichinae) is perhaps the only documented case (Kay, 1994: Fleagle, 1998).

Marshall et al. (1994) suggested that reactivation of genes can be quite common after up to 6 million years of evolution. This reactivation can greatly increase the likelihood of repeated disappearance-appearance, or "flickering," of character states, as they might be lost and gained repeatedly after divergence of lineages. Whatever the actual time limits really are, the likelihood for flickering can be predicted to be substantially greater in a situation such as the repeated activation of secondary enamel knots. The increase and decrease in cusp number do not need to depend on complete activation of single gene but in a mutation affecting, for example, the binding property of a *cis*-regulatory element. Flickering can, however, be even more likely if we consider the reaction-diffusion models for signaling center activation discussed above. For example, the evolution, or loss, of the hypocone does not need to involve the same gene or molecular-level change in every case. A similar developmental effect (e.g., delay in secondary knot activation) can be produced in some evolutionary lineages by altering production/diffusion of activators/inhibitors, and in other lineages by altering the growth rate or differentiation rate. This situation, while currently hypothetical, would be an extreme case of phenogenetic drift (Weiss and Fullerton, 2000), as the same change in phenotype could be produced by different changes in genotype. It is worthwhile to bear in mind that while several mouse knockout experiments, including the inactivation of homeobox genes, have produced morphologies that superficially resemble evolutionary reversals, these changes fail in closer scrutiny (Smith and Schneider, 1998). Thus the lack of "true" evolutionary reversals in knockout experiments suggests that at least the simplest genotype to phenotype map is unwarranted.

Considering cusps as products of the repeated activation of the same molecular machinery raises the question, how much individual variation is really allowed for a single cusp? Evolutionary versatility (Vermeij, 1973) is likely to play a role in the development of cusps in different parts of the crown. Thus, trigonid and talonid portions of the crown may have partly independent patterning cascades (Jernvall, 2000). Moreover, Schwartz (2000) demonstrated a highly diverse patterning of enamel thickness on hominoid cusps. This diversity shows that at least enamel formation can be controlled in a highly specific manner. Indeed, structural enamel genes may be a case of "structural" genetic determinism. Mathur and Polly (2000) showed that the diversity of amelogenin splicing variants correlates positively with the diversity of enamel microstructure among mammalian lineages. Interestingly, the formation of enamel seems to be decoupled with, or at least more sensitive than, crown growth, as repeated

enamel hypoplasia has been found not to be correlated with crown height (Guatelli-Steinberg and Skinner, 2000).

### Is there a homeobox code for teeth?

Visible tooth crown development commences with the formation of the primary enamel knot. The repeated use of signaling centers in cusp development is compatible with dynamic models of development (such as the reaction-diffusion models). The similarities in molecular signaling in cusp, feather, and other periodic patternings suggest that the principles for adjusting cusp spacing may be quite "commonly" used during development. Another aspect of development inferred to play a role in morphological evolution is a combinatorial code of homeobox transcription factors that select different differentiation fates. In particular, *Hox* genes are sequentially arranged in the genome, and they are expressed in a cumulative sequence along the body axis. Different combinations of *Hox* genes are required for the correct identity of body segments, and altering this code can cause homeotic changes, i.e., changes in the morphology of one part into that of another (Krumlauf, 1994). The homeobox code is thus an example of genetic determinism where structure's identity can be derived from its individual pattern of gene expression. However, the identity of structures (e.g., thoracic or cervical vertebrae; Burke et al., 1995) does not equate with the morphology of individual structures. By analogy, a dental homeobox code could potentially be involved in the developmental separation of incisor, canine, premolar, and molar tooth families rather than in the morphology of an individual molar per se.

Of the various families of homeobox genes, *Hox* genes cannot be used to explain tooth patterning because *Hox* gene expression does not extend to the first branchial arch, which gives rise to jaws and teeth (Hunt and Krumlauf, 1992; Krumlauf, 1994). Thus, other homeobox genes would have to be involved in tooth development (Weiss, 1993; Weiss et al., 1998). At least *Dlx*, *Msx*, and *Barx* homeobox-containing genes have been recognized as having potential for tooth identity-coding (Sharpe, 2000; Zhao et al., 2000a). For example, *Barx1* is found to be expressed only in the future molar region, while *Msx1* and *Msx2* are expressed in the preincisor region. Six known *Dlx* genes have a complicated combinatorial code-like expression pattern where the region of the upper incisors lacks *Dlx* activity altogether, the region for the upper molars expresses two (*Dlx1* and *Dlx2*), the lower molars express four (*Dlx1*, *-2*, *-5*, and *-6*), and the lower incisors express three (*Dlx2*, *-5*, and *-6*) genes (Zhao et al., 2000b). The only informative *Dlx* knockout experiment involves the deactivation of *Dlx1* and *Dlx2*. These mice lack upper molars (Qiu et al., 1997; Thomas et al., 1997). Therefore, *Dlx* genes appear not to be an indication by themselves of a dental homeobox code, as the *Dlx1* and *Dlx2* knockout upper molars should transform into upper incisors (Weiss et al., 1998). Weiss et al. (1998) suggested that the *Dlx5* and *Dlx6* double knockout would be an effective test for the *Dlx* code, as these mutants can be hypothesized to transform their lower molars into upper molars.

Interestingly, Tucker et al. (1998) showed how inhibition of *Bmp4* signaling in the preincisor region of the jaw can alter incisors to become molars. As cusp number is increased and root development seems apparent in the transformed incisors, this effect can indeed be interpreted as an identity change, and not just addition of a new cusp. Moreover, *Barx1*, a homeobox gene expressed in the premolar region, is expressed in the preincisor region as a result of *Bmp4* inhibition, suggesting that homeobox genes are involved in the identity change of incisor to molar. It is noteworthy that the tooth family-specific expression of genes, including the *Dlx* genes, is limited to early branchial arch development. In later developmental stages, the genes are reactivated in all the teeth. The signaling molecule *Bmp4* appears to be used repeatedly in various stages of development, including perhaps even in the induction of the primary enamel knot as a mesenchymally expressed signal (Jernvall et al., 1998).

Evolutionarily, an apparent role for a homeobox tooth code could be a role in the molarization of the premolars. Currently

there is no comparative evidence to test this hypothesis, as all studied mammals have only incisors and molars. There are even fewer data to infer how a homeobox code could direct the morphology of individual teeth (e.g., evolution of the hypocone, evolution of carnivore carnassials) or tooth size. Presumably this would require a highly accurate "cocktail" of homeobox genes regulating all aspects of tooth shape (see below) at stages which would precede the formation of the primary enamel knot by 2 days in the mouse. In practice, the homeobox code might influence the initial parameters in the enamel knot program, which in turn would influence the patterning cascade mode of cusp development. In the end, a dental homeobox code is a question of the degree and nature of genetic determinism in the control of morphology.

## DEFINING DENTAL CHARACTERS

### Grouping characters based on development

The concern that morphologic characters may be unreliable in phylogenetic studies stems partly from inferred frequency of convergent evolution (e.g., Collard and Wood, 2000; Hunter and Jernvall, 1995). Indeed, detailed studies of morphological characters, stimulated by molecular hypotheses, have illustrated the subtle differences and similarities in dental character evolution (e.g., Fleagle and McGraw, 1999). The evolvability of tooth cusps may add to this concern, as from the developmental point of view, there is very little to prevent convergent evolution of new cusps. Some morphologies may even be rare due to the patterning cascade mode of cusp development (Jernvall, 2000) increasing the likelihood for convergent evolution. Another concern of characters in phylogenetic studies is even more fundamental, i.e., what is a good, or real, character anyway? Ideally, each character provides *reliable* information about ancestry and descent (discussed in Lieberman, 1999; Wiesmüller et al., 2000). Taking a developmental biology point of view, a reliable dental character should be discretely identifiable so that its developmental basis can be understood. Only by knowing the developmental processes generating a character can we make reliable inferences on inheritance and character independence. One example is character redundancy. The correlated variation of cusp heights as a function of patterning cascade may cause phenotypic correlations that reduce character independence. However, simply ignoring short cusps in a phylogenetic study is perhaps too a simplistic "cure," as short cusps are inherently more variable in a population, making them useful for lower taxonomic distinctions. Studies mapping the extent of morphological variation in small cusps can be especially valuable in estimating the variational properties of primate teeth (Cuozzo and Sauther, 1999).

Below we present groupings of some commonly used dental characters. We stress that the groupings of characters are based on inferred mode of tooth development only, allowing us to make evolutionary predictions when the variational properties of traits are known. This also implies that our discussion on the likelihood of homoplasy is strictly meant as the *developmental potential* for character state reversals. Character grouping schemes incorporating additional knowledge of fitness, functional biology, and natural selection in connection to traits have also been suggested (Lovejoy et al., 1999). While this discerning use of information is proposed to allow "formally state the presumed morphogenetic basis" of traits, it remains to be seen how the role of development can be discerned from the other factors involved in character grouping. Another general problem with groupings of character is that our knowledge of variational properties of characters in connection to *development* is quite limited. Defining tooth characters based on molecular-level models directly (e.g., enamel knots, homeobox code) is of limited use without a population-level model of development. For example, inferring cusps to be independently variable because they have their own signaling centers (secondary enamel knots) or inferring cusps to be highly redundant characters because their secondary enamel knots express the same genes are both exceedingly simplistic conclusions. This is particularly apparent when the population-

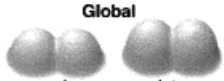
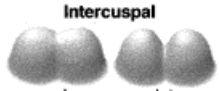
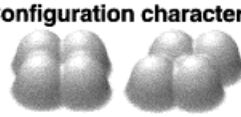
| | Developmental process | Character properties | Example characters |
|---|---|---|---|
| **Initiation characters**<br>  <br>early late | Patterning cascade mode of cusp development, likely to relate to the repeated activation of the secondary enamel knots at the onset of cusp development (Jernvall, 2000). Affects individual cusp size and cusp number. | Short cusps variable, homoplasy common. Unequal likelihood of character states. | Hypocone size, Nanopithex-fold size, Paraconule size, Talonid height, Cristid obliqua size (length). |
| **Termination characters** | | | |
| **Global**<br>  <br>early late | Global termination of crown formation. Timing mechanism is unknown. Affects crown height and indirectly cusp number (Jernvall, 2000). | Global character (e.g., tooth size). May affect the presence of short cusps. | Presence of small Hypocone, presence of conules, crown width, crown length. |
| **Intercuspal**<br>  <br>early late | Local termination of intercuspal growth. Timing mechanism is unknown, may be related to shape characters. Affects the presence of lophs and may also affect distances among cusps (Polly, 1998). | ? | Cusp relief, lophs. |
| **Cusp shape characters**<br>  <br>weak folding strong folding constant folding | Relative growth of the epithelium and mesenchyme affects the degree of folding (blunt or pointy cusps). The constancy in the epithelium/mesenchyme growth affects how straight the cusp slopes are. | Likely to be a global variable? Not very homoplastic? | Protocone lingual inflation, crown cusp relief, cusp inflation. |
| **Configuration characters**<br>  | Lateral cusp topography likely to be established prior to cusp formation. Affects initially the first lateral cusp pairs (e.g., Paracone-Protocone) and the pattern is then propagated longitudinally. Delayed cusp initiation may affect position of terminal cusps (e.g., Paraconid)? | Phenotypic correlation of anterior and posterior cusp configurations? | Hypocone position, Paraconid position, Cristid obliqua orientation |

Fig. 7. Basic tooth crown characters grouped into four developmental categories. Note that degree of homoplasy is here meant as developmental potential for homoplasy, not actual realized homoplasy.

level mode of variation is known (Jernvall, 2000) (Fig. 5). Furthermore, taking the opposite strategy by measuring character correlations without any consideration of development may miss developmentally relevant correlations altogether (Jernvall, 2000). Thus, classification of characters without any mechanistic models of development in microevolution could be little more than geometrical exercises on character correlations. These reservations in mind, we grouped dental characters below into four categories: initiation, termination, cusp shape, and configuration characters.

***Initiation characters.*** In this paper we have mostly addressed initiation characters (Fig. 7) because their variational properties are somewhat known (Fig. 6). These characters are likely to stem from the patterning cascade mode of development that makes short cusps evolvable by increasing their cumulative variation. The initiation characters may not always be independent from each other, but this would only be a problem when, for example, a large hypocone is found to have phenotypic correlation with a large metacone. However, the patterning cascade mode of development may increase the likelihood of homoplasy and flickering (repeated character state reversals). This is particularly the case with short cusps and dental features. In general, it will be interesting to test whether evolvability of morphology increases the actually realized homoplasy.

***Termination characters.*** These characters fall into two groups: global and local (Fig. 7). Global termination of tooth crown formation will affect tooth crown size and height and indirectly the presence of short cusps. Thus, variation in tooth crown height is an additional factor increasing the likeli-

hood of homoplasy and flickering in short cusps. It is worthwhile to bear in mind that tooth size characters may be indirect results of global termination of tooth crown growth, and thus size measures may actually correlate with the presence of, for example, a small hypocone. Also, the global termination of crown development may actually have local components (e.g., lingual/buccal). The local termination of growth affecting intercuspal areas may influence presence of lophs. The varitional properties of the termination of intercuspal growth remain to be determined.

***Cusp shape characters.*** The shape of cusps, apart from enamel thickness, is largely due to the relative growth of the inner enamel epithelium and the underlying mesenchyme (Fig. 7). Cusp shape characters are likely to be global (affecting the whole crown), but this remains to be properly tested. As these characters do not result from simple initiation or termination events, but instead from changes in growth rate, it will be interesting to see if cusp shapes may be less prone to homoplasy and flickering than the initiation and termination characters.

***Configuration characters.*** The lateral cusp topography is one of the main aspects of tooth shapes as they ensure, in addition to cusp heights, proper occlusion of opposing teeth. There is preliminary evidence that changes in lateral topography result from coordinated changes in molecular prepatterns. This prepatterning happens within the primary enamel knot preceding the appearance of individual cusps (unpublished results). The early prepatterning of lateral topography may promote the likelihood of homoplasy, because the positions of cusps can be altered once during development. This could be developmentally a simpler and a more robust mechanism than altering cusp positions by allometric growth during the whole period of crown formation. The degree of character independence within a crown remains to be determined. As is the case with initiation characters, these characters are likely to be strongly linked between occluding teeth and thus, for example, an increase in the transverse orientation of lophs in upper and lower molars may be developmentally the same process. Furthermore, the configuration of cusps is likely to limit configuration and number of roots (Butler, 1956).

## CONCLUSIONS: SHOULD WE WORRY ABOUT DEVELOPMENT?

Perhaps the greatest value of teeth in evolutionary biology is their usefulness in inferring diets. The extensive convergence in tooth morphology allows reliable inferences of dietary correlates and morphology (e.g., Fleagle and McGraw, 1999; Jablonski, 1994). The evolvability of tooth cusps may even promote this usefulness, as detailed dental morphologies (e.g., Kay, 1984; Yamashita, 1998) may be hypothesized to evolve quickly to reflect dietary demands. However, most evolutionary studies rely on phylogenetic hypotheses that often use dental characters. And while the evolvability of new cusps may be an exciting phenomenon from the evolutionary biology point of view, it may also create complications when dental characters are used in constructing phylogenies.

A developmental basis for characters may be particularly important to know in cases where tooth shape (and biological shapes in general) turns out to be coded by dynamic processes that cannot be reduced into a simple genetic code. Already the repeated activation of the developmental modules (i.e., the enamel knots) during tooth development suggests that homologous cusps and crests are not coded as such into the genome, but that the whole cusp pattern is a product of a dynamic program (Jernvall, 2000; Zhao et al., 2000a). Also, the patterning cascade mode of cusp development and its morphological manifestation, the initiation characters, point toward dynamic processes in the control of tooth morphogenesis. It is important to note that cusps can still be historically homologous. Homology is not just a static genetic code readable deep inside the genome, but rather, it is a readout of the information stored in the dynamic cusp-making program. Comparisons to other developmental systems, e.g., to the patterning of avian feather primodia,

should aid us in discerning the molecular-lever components of dynamic processes (e.g., reaction-diffusion systems). Another aspect where comparative work is needed is to understand the role of homeobox codes in dental patterning. Particularly within primates, comparative work has great potential to help to quantify variational properties of developmental systems. Indeed, in contrast to the initiation characters, the variational properties of other character types are less understood from the developmental biology point of view. We predict that examining dental variation by incorporating developmental information in primate populations will offer new ways to test models of development. In this respect, biological anthropology may prove to be a test bench (and hopefully not only the source) for molecular-level "just-so stories" about development and evolution. And, almost as a side product, we may finally be able to discern reliable dental characters for reconstructing phylogenies. Maybe then we would not have to worry about development but instead use it as a tool in evolutionary studies.

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