Evolutionary modification of development in mammalian teeth: Quantifying gene expression patterns and topography

Jukka Jernvall*, Soile V. E. Keränen, and Irma Thesleff

Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Post Office Box 56 Viikinkaariq, FIN-00014, Helsinki, Finland

Edited by David B. Wake, University of California, Berkeley, CA, and approved October 13, 2000 (received for review August 21, 2000)

The study of mammalian evolution often relies on detailed analysis of dental morphology. For molecular patterning to play a role in dental evolution, gene expression differences should be linkable to corresponding morphological differences. Because teeth, like many other structures, are complex and evolution of new shapes usually involves subtle changes, we have developed topographic methods by using Geographic Information Systems. We investigated how genetic markers for epithelial signaling centers known as enamel knots are associated with evolutionary divergence of molar teeth in two rodent species, mouse and vole. Our analysis of expression patterns of *Fgf4, Lef1, p21*, and *Shh* genes in relation to digital elevation models of developing tooth shapes shows that molecular prepatterns predict the lateral cusp topography more than a day in advance. A heterotopic shift in the molecular prepatterns can be implicated in the evolution of mouse molar, changing locations from which historically homologous cusps form. The subtle but measurable heterotopic shifts may play a large role in the evolution of tooth cusp topographies. However, evolutionary increase in the number of longitudinal cusps in vole molar has involved accelerated longitudinal growth and iterative addition of new cusps without changes in lateral cusp topography. The iterative addition of cusps after the establishment of lateral cusp topography may limit the independence of individual morphological features used in evolutionary studies. The diversity of mammalian molar patterns may largely result from the heterotopic and iterative processes.

One problem encountered in the attempt to link differences in developmental gene expression patterns with differences in morphology is that morphological patterns themselves are often complex. This complexity can be a challenge for interpreting gene expression data and inferring patterning mechanisms involved in morphological evolution (1, 2). Evolutionary inferences are complicated further by expression studies that use thousands of genes, which provide a multivariate view of developmental processes (1–5); that is, how can we connect expression data from an ever-increasing number of known genes to morphologies that themselves are complex multivariate entities? This question is especially crucial in evolutionary studies where one needs to detect *subtle* changes during development, because evolutionary patterns, as observed in the fossil record, usually involve little initial modification of morphology.

One system that offers promise for linking known evolutionary transformations with genetic control of development is mammalian dentition. Mammalian molar tooth morphology has played a central role in the analysis of mammalian paleoecology (6–9), and subtle changes, such as modification of cusp configuration, are usually the earliest available morphological evidence of major clades in the fossil record (10–16). In developmental biology, subtle changes in cusp patterns can be studied by analysis of several genetic markers for cusp development (17, 18). However, whereas the evolutionary aspects of molar morphology are well characterized, the exact ways that tooth morphogenesis has changed remain unclear (17–23). Species-specific morphologies of single teeth show complex interspecific patterns whose development and gene expression patterns are difficult to characterize quantitatively.

To be able to quantify evolutionary changes in developing morphology and link these changes to the activity of developmental regulatory genes, we offer methods with Geographic Information Systems (GIS). GIS is used for spatial analysis of geographical and large scale ecological data, but recently it has been shown to be suitable for analysis of fossil tooth shapes (24–26). In these analyses, tooth cusps are analogous to landscape features such as hills, and intercuspal areas are equivalent to valleys. Here we build on this method by linking the topography of developing tooth morphology with gene expression data from whole-mount *in situ* hybridizations. Rather than representing gene networks in linear pathway diagrams, topographic analysis allows linking of gene activity to complex changes in morphology. Another advantage of GIS methods is that they permit analyses of features before the appearance of morphological landmarks.

Comparisons between taxa are necessary to identify those developmental processes that change in evolution; thus we analyzed differences in cusp patterning in the first lower molar of two species of muroid rodents, a mouse (house mouse, *Mus musculus*, Murinae), and a vole (sibling vole, *Microtus rossiaemeridionalis*, Arvicolinae). Both were derived from a cricetid stock; the first members of the Murinae date back to the Middle Miocene, and the first arvicoline date back to the Early Pliocene (27–30). These species have contrasting molar morphologies that provide a suitable basis for inferring the modes of developmental change in molar evolution. Mouse lineages have commonly retained the ancestral number of main cusps, but the cusp pattern has evolved from having buccal and lingual cusps diagonal to each other to having them opposite or parallel (Fig. 1*A*). In contrast, voles have retained the diagonal cusp pattern, but have evolved several new cusps on their first lower molars (Fig. 1*A*). Thus, the vole molar is an example of extreme molarization. Additionally, voles have retained the longitudinal crest connecting anterior and posterior cusps, whereas this crest has been lost in mice. Because rodent molars function as an integrated unit, cusp positions of adjacent molars are similar, as also are the anterior and posterior portions of the same molar (31).

We first constructed a developmental transformation series, as high resolution digital elevation models (DEMs), of developing mouse and vole first lower molars. We recorded the three-dimensional interface between epithelium and mesen-

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: DEMs, digital elevation models; GIS, Geographic Information Systems; E*n*, embryonic day *n*.

See commentary on page 14019.

*To whom reprint requests should be addressed: E-mail: jvakudaret@aol.com.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

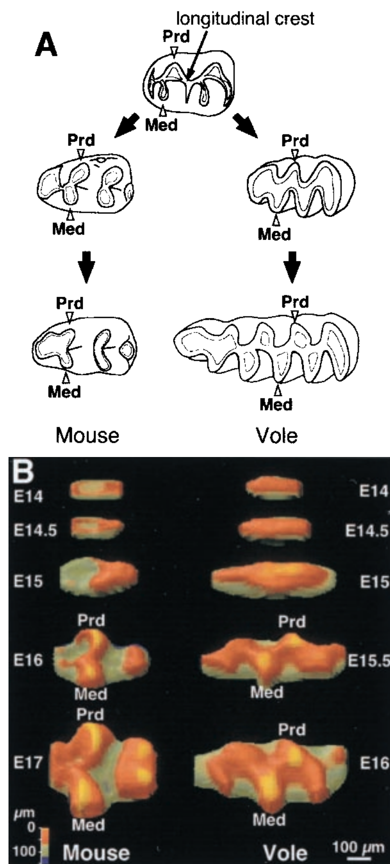

Fig. 1. Evolution and development of mouse and vole first lower molars with the two first forming cusps, the protoconid (Prd) and the metaconid (Med), labeled. (*A*) The basal diagonal cricetid cusp configuration with longitudinal crest (*Democricetodon*, top) has evolved in mouse lineages to lack the longitudinal crest and more parallel cusp configuration (*Antemus*) resulting in teeth with transverse lophs in extant *Mus*. The vole lineages have retained the longitudinal crest with diagonal cusp configuration (*Bjornkurtenia*), but the number of cusps is greatly increased (*Microtus*). In addition, voles have evolved high crowned molars by delaying the initiation of root formation. Examples, which illustrate a general morphological transformation series rather than strict ancestor-descendant relationships, are not to scale. (*B*) DEMs of molar shape development. The color ramp corresponds to height of the interface between epithelium and mesenchyme. Note how cusps emerge in their species-specific configuration around embryonic day 16 (E16) in mouse and E15.5 in vole, about 2 days after the initiation of tooth crown base (E14, the cap stage in tooth development). All of the main cusps have appeared in both species around E18 (not shown) at half the final length (final sizes are 1.4 mm in mouse and 2.5 mm in vole). Anterior side toward the left and buccal side toward the top.

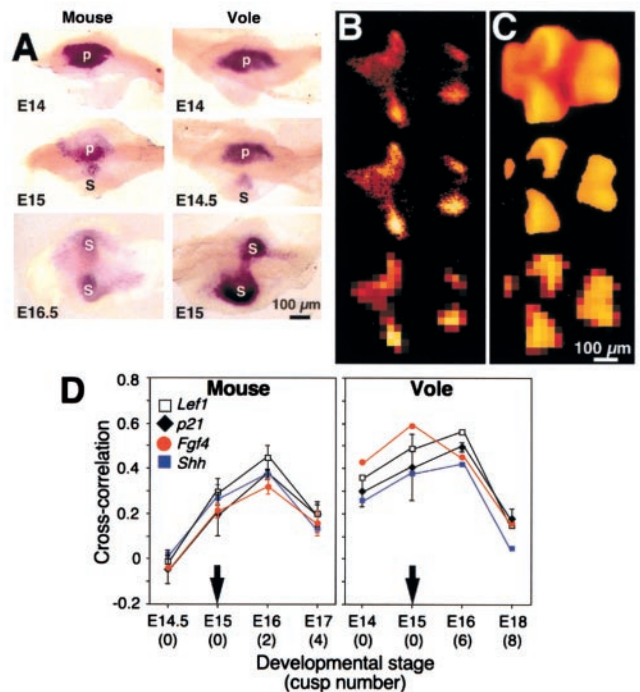

Fig. 2. Whole-mount *in situ* hybridization analysis of gene expression patterns and cusp topography. *Shh* expression in isolated tooth epithelia (*A*) showing the primary enamel knot (p) and the first secondary enamel knots (s). The gene expression data were digitized (*B*, top) and gene expression intensity of each explant was standardized (*B*, middle). For correlation analysis, average expression intensities in 30 μm × 30 μm squares were used (*B*, bottom). Cusps were delineated from DEMs (*C*, top and middle) and average heights in 30 μm X 30 μm squares were tabulated for correlation analysis (*C*, bottom). Anterior side toward the left and buccal side toward the top. (*D*) Cross-correlations between gene expression patterns of E15 teeth (large arrow) and shape topographies at different stages. Note how the E15 expression patterns have generally higher Spearman rank correlation coefficients (r_s) with E16 than with E15 shapes. The extremely fast emergence of cusps in the vole molar (Fig. 1*B*) result in smaller differences between E15 and E16 correlations. This shorter lag may also be partly due to the use of mouse and rat probes on vole tissues.

chyme, because the formation of cusps is a product of the folding and growth of this interface (17–19, 32–34). As the folding proceeds, the differentiating inner enamel epithelium facing the mesenchyme gives rise to enamel-forming ameloblasts, and the mesenchyme below gives rise to the dentine-forming odontoblasts (17–19, 32–34). Next, to link gene activity to molar crown topography, we used GIS methodologies to analyze changes in the DEMs and correlated these with molecular markers for cusp development. Tooth epithelium has localized molecular signaling activity in the primary enamel knot and the secondary enamel knots, areas forming the tooth crown base and individual cusp tips, respectively (18). We previously have shown that cells of the enamel knots are nonproliferative and express several signaling molecules common to other embryonic signaling centers, such as the apical ectodermal ridge and the zone of polarizing activity in limbs (18, 35). The cells of the enamel knots may control the folding of the inner enamel epithelium by ceasing to proliferate and by producing signaling molecules that stimulate adjacent cells to proliferate (18, 36). The spatial activity of four genes was investigated; each of these genes has been linked to a different signaling pathway: fibroblast growth factor-4 (*Fgf4*), sonic hedgehog (*Shh*), transcription factor *Lef1* (target of *Wnt* signaling), and cyclin-dependent kinase inhibitor *p21* (target of *Bmp* signaling). We chose this set of genes because in histological sections their expression seems to be associated with cusp development both in mouse and vole (37), and thus they might be useful markers for the process of tooth crown development. Also, FGF4 protein stimulates cell proliferation in isolated dental tissues (36); *p21* is a differentiation marker because it is expressed only in the cells that cease to proliferate (38, 39).

Our specific aim was to discern the nature of developmental processes responsible for evolutionary change in mouse and vole molar teeth.

Methods

DEMs were generated from horizontal sections of tooth germs by using the three-dimensional view version (public domain by Iain Huxley) of National Institutes of Health IMAGE software (http://www.physics.usyd.edu.au/physopt/3dview/; ref. 25).

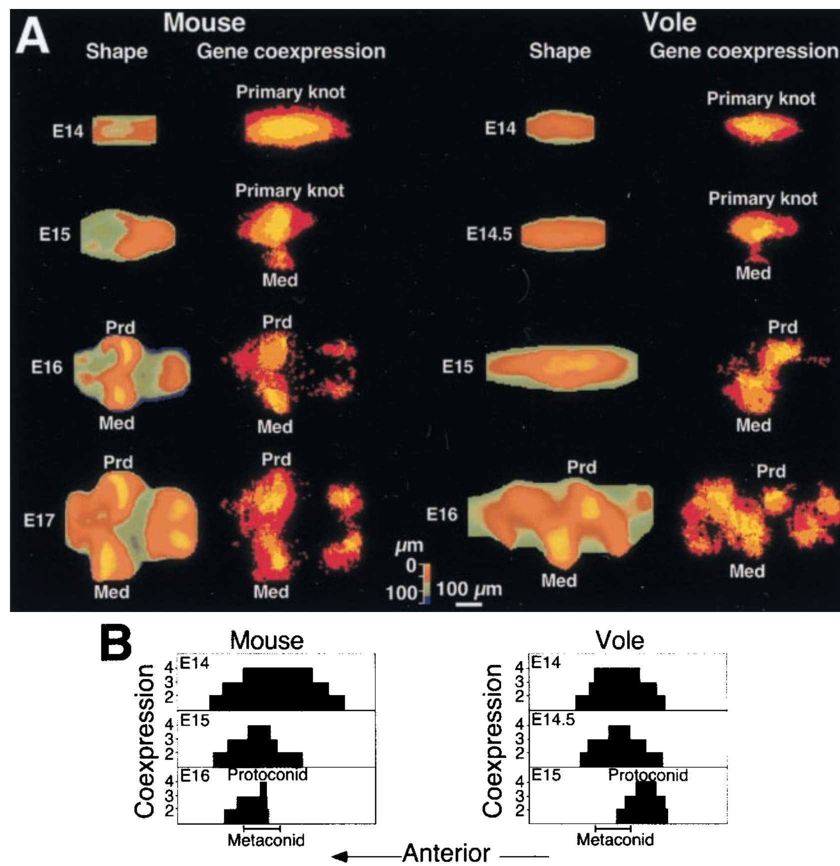

Fig. 3. GIS analysis of gene activity and topography with the two first forming cusps, the protoconid (Prd) and the metaconid (Med), labeled. (*A*) DEMs showing the formation of cusps and corresponding epithelial coexpression patterns of *Fgf4*, *Shh*, *Lef1*, and *p21*. The coexpression of all of the four genes (in yellow) marks the core of the enamel knots, surrounded by areas lacking *Fgf4* (in orange) and *Fgf4* + *Lef1* expressions (in red). The cells expressing *p21* (approximately the area in red) are nonmitotic, and down growth of the tooth epithelium happens outside the *p21* gene expression domains. Note how the lingual secondary enamel knot for the metaconid (Med) cusp forms next to the primary enamel knot. Anterior side toward the left and buccal side toward the top. (*B*) A bar graph showing the nested coexpression domains of the genes in a longitudinal section through the primary enamel knot. Note how, within the primary enamel knot, the secondary enamel knot of the protoconid forms in the anterior end in mouse and in the posterior end in vole. These shifts in gene expression patterns result in a parallel cusp configuration of the protoconid and the metaconid in mouse and in a diagonal cusp configuration in vole.

Initially, the interface between tooth mesenchyme and epithelium was marked with a light line in stacks of digitized frontal serial sections (7- and 10-μm sections), and then the digital stacks were resliced horizontally to generate the occlusal DEMs (Fig. 1*B*). Orientations of DEMs were corrected in AMORPHIUM (www.play.com, www.electricimage.com). Also, gene activity was recorded from dark-field images of serial sections. Whole-mount *in situ* hybridization was done on embryonic tooth epithelia that were first separated from the mesenchyme after pancreatin-trypsin digestion (ref. 40; Fig. 2*A*). *In situ* preparations (whole-mount and serial) of the same genes showed similar expression patterns. Epithelia were digitized from above and sorted into length groups corresponding roughly to E14, E14.5, E15, E16, and E17 tooth germs (cap, late-cap, early-bell, bell, and late-bell stages). Because mouse and vole lineages have separated recently, with respect to the origins of the genes studied, we used mouse (*Fgf4*, *Lef1*, and *p21*) and rat (*Shh*) probes for both species. For details in materials, probes, and tissue specificity, see ref. 37.

For time-series analysis, we grouped teeth into four stages and generated cross-correlations between precusp-stage gene expression patterns and shapes of the four stages. Superimposition of epithelia was done by using the anterior and posterior margins of the epithelial perimeter as landmarks, as well as cusps in older specimens. Then, tissue and background were filtered (Adobe PHOTOSHOP), and gene expression intensity of each explant was standardized to range from 0 to 255 with five rank values (Fig. 2*A* and *B*), generating a robust measure of gene activity patterns. Morphological data were filtered to delineate cusps as isolated convex areas, and mean heights in 30 μm × 30 μm squares were tabulated (Fig. 2*C*). The topography of early tooth germs, where no cusps were yet visible, was mapped similarly. Finally, mean gene expression intensities in every 30 μm × 30 μm square were cross-correlated with corresponding height values in the topographic shape maps. Calculations were done by using a three dimensional view version of National Institutes of Health IMAGE and the MFWORKS GIS package (www.thinkspace.com).

For superimposition of gene activity, mean expression domains for each gene in each stage were calculated in National Institutes of Health IMAGE. Means of gene expression patterns were superimposed by using MFWORKS combine operation, which allows separation of all of the possible spatial combinations of gene expressions. The number of *in situ* specimens ranged from one (late stages) to six (early stages) for each developmental stage.

Estimation of epithelial growth in relation to the mesenchyme was done by measuring DEM's three-dimensional surface area (MFWORKS incremental area-operation) and dividing it by DEM's two-dimensional area (in occlusal view). Thus, when epithelial growth is equal to mesenchymal growth, the resulting value is one.

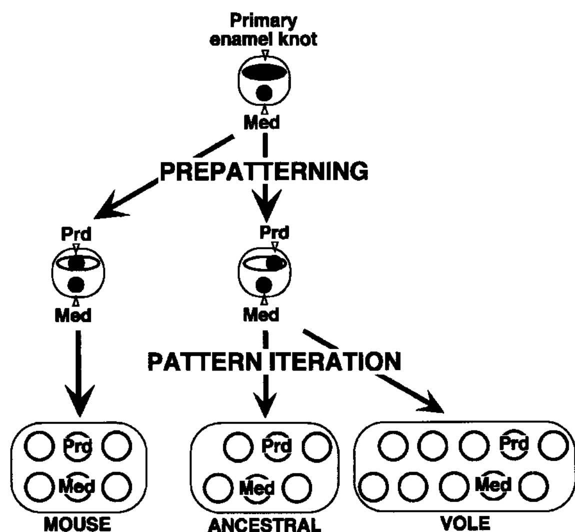

Fig. 4. The principal changes in development leading to the evolution of mouse and vole molar cusp patterns. In the mouse lineages (left), inferred development of the ancestral morphology (Fig. 1*A*) is altered early in development (enamel knots in black) by heterotopic shifts in the molecular prepatterns that change the location within primary enamel knot giving rise to the protoconid (Prd) next to the metaconid enamel knot (Med). In the vole lineages (right), no large changes in cusp topography are needed, but the number of times that the diagonal cusp pattern is iterated is increased.

Results and Discussion

First, we reconstructed the developing tooth shapes in three dimensions by recording the interface between epithelium and mesenchyme. The DEM data show that in both species the first cusps to appear are one buccal and one lingual cusp. Furthermore, these cusps appear at their species-specific positions, parallel to each other in mouse, diagonal in vole (Fig. 1*B*). Therefore, the derived parallel cusp configuration of the mouse molar (Fig. 1*A*) is not generated by local (e.g., allometric) changes in growth patterns *after* the initiation of cusp development. This observation suggests that cusp configuration may be changed before the initiation of cusp development by changes in molecular regulation of the domains from which cusps develop. This mode of evolutionary modification of development is often called heterotopy (for discussion on heterotopy, heterochrony, and allometry, see refs. 41–44). Furthermore, the data show that longitudinal growth is faster in vole than in mouse tooth germ (3.6 μm/h in mouse, 8.5 μm/h in vole), resulting in a more extensive iteration of the vole cusp pattern longitudinally (Fig. 1*B*). These results suggest that distinct developmental processes may regulate lateral cusp patterns (bucco-lingual) and longitudinal cusp patterns (anterior-posterior).

Because the DEMs show that there is a gap of up to 2 days between the initiation of tooth crown base and the onset of visible cusp formation (Fig. 1*B*), we next focused our analysis on this early period by using molecular markers for cusp development. Specifically, we examined how the patterns of gene activity, as detected by using whole-mount *in situ* hybridization, correlate with the emerging cusp patterns in DEMs (Fig. 2). First, we tested with time-series analysis whether the expression patterns of the enamel-knot genes predict the actual cusp topography. We obtained cross-correlations (Spearman rank) between precusp gene expression patterns and DEMs of different stages. The cross-correlations measure how closely the precusp gene expression patterns resembled tooth shapes when shifted forwards or backwards in developmental time.

The data show that precusp-stage expression patterns of the genes examined have generally a higher correlation with the future cusp topography than with their current topography (Fig. 2*D*). Thus, the configuration of enamel knots precedes the morphological appearance of future cusps. Furthermore, because *Fgf4*, *Shh*, *Lef1*, and *p21* show a similar pattern of correlations (Fig. 2*D*), they are all likely to be part of the mechanisms involved in cusp patterning, although the exact nature of molecular-level integration of the corresponding signaling pathways remains unclear (45–49). This temporal lag between gene expression and morphology further corroborates the role of enamel knots in tooth-shape development. The cross-correlation analysis linking gene activity to morphology provides a valuable tool in elucidating developmental processes that are currently not understood at the gene level. For example, mutant mice lacking a functional *p21* gene develop normally, without any reported dental anomalies (50). Our analysis may provide evidence that in addition to *p21*, which may be redundant in teeth, other members of the same gene family (51) may participate in cell-cycle control during tooth development.

Our discovery that gene expression patterns predict future cusp patterns suggests that evolutionary changes in lateral-cusp configuration affect very early stages in development. We examined the nature of these changes in molecular prepatterns by superimposing expression patterns of the four genes (means-of-expression domains in each stage, total number of *in situ* specimens, $n = 76$) to locate the places of origins of the two cusps that form first. The patterns show that both vole and mouse molars initiate the second cusp (the metaconid) midway on the side of the primary enamel knot (Fig. 3*A*). However, this common pattern of gene expression in mouse and vole is followed by a shift to expression patterns that corresponds to the future species-specific cusp topographies. In vole, the first cusp, the protoconid, forms from the posterior part of the primary knot, whereas in the mouse, the protoconid forms more anteriorly, next to the metaconid (Fig. 3 *A* and *B*). Therefore, the evolutionary shift of the protoconid and the metaconid cusp configuration (Fig. 1*A*) is achieved during development by changing the location within the primary enamel knot that gives rise to the protoconid (Fig. 3*B*). Furthermore, the evolutionary direction of the change appears as an anterior shift of the first cusp. This shift in molecular prepatterns implies that cells forming the tip of the protoconid are not the same in mouse and vole. Therefore, specific cusps cannot be traced to any particular populations of cells in early embryo before the tooth germ formation; rather, historically homologous cusps stem from the cusp prepatterning process itself. Because first buccal and lingual cusps become apparent in DEMs more than a day later than the emergence of the bucco-lingual prepattern of gene expression in the crown base, heterotopic shifts can be inferred. Accordingly, heterotopy may be more common than generally believed (43, 52, 53).

The GIS analysis suggests, however, that the classic distinctions (e.g., heterotopy, heterochrony) may also depend on the level of analysis. The earliest stages of mouse and vole molars are morphologically distinct (Figs. 1*B* and 3*A*), and the heterotopic shift can be hypothesized to be underlain by differential growth of the tooth germs. Indeed, the analysis of growth patterns shows that, in relation to the underlying mesenchyme, the epithelium grows consistently 20% more in vole than in mouse molar (mouse and vole values ranged 1.2–1.4, and 1.4–1.7 respectively). This process is a different one than the faster elongation of the vole tooth germ, which requires equal acceleration of growth in both the epithelium and the mesenchyme, resulting in a simple iteration of the cusp topography. Later during development, future cusp tips form a string of connected gene-activity centers along the future longitudinal crest in the vole, whereas in the

mouse the anterior and posterior cusps have no connection at the level of gene activity (Fig. 3*A*).

In conclusion, we suggest that evolutionary divergence in molars has involved at least two separate hierarchical developmental processes in mouse and vole. First, the anterior shift of buccal cusps in mouse lineages is achieved by changing the spatial regulation of genes operating in signaling networks before the formation of first cusps (Fig. 4). This early shift in lateral topography changes the locations of the enamel knots and consequently the future tips of the homologous cusps. Additionally, in mouse lineages, the heterotopic shift alone may have removed the longitudinal crest connecting anterior and posterior cusps. Second, increased cusp number in vole lineages is caused by a greater number of iterations of the established lateral topography (Fig. 4). This latter process relates to the degree of molarization of teeth and may thus be linked with the determination of tooth identity (18, 54), as well as with the evolvability of new cusps without changes in lateral cusp topography (55). Whereas the developmental regulation of cusp number may resemble the formation of, for example, new phalanges in limbs (56), the prevalence of early heterotopic shifts, refining species-specific morphologies, remains to be determined. These distinct developmental processes underlying interrelationships between lateral topography and longitudinal iteration of topography may limit (at least in teeth) the independence of individual morphological features used in evolutionary studies. Limited construction rules of dental morphology could also greatly increase the likelihood of homoplasy in dental evolution, as has been suggested for tetrapod limb evolution (57). Considering broader comparisons among organs, GIS also can be used to explore how morphology and gene expression patterns are connected to organ specificity, because molecular signaling pathways, including the ones studied here, are largely shared among organs and organisms.

We thank K. Kettunen, M. Mäkinen, P. Pekkarinen, and R. Santalahti for their expert technical help and C. Basilico, T. Edlund, R. Grosschedl, and B. Vogelstein for probe constructs. J. van Dam, M. Fortelius, J. Hanken, J. P. Hunter, E. Stone, and P. C. Wright provided comments on earlier versions of the manuscript. This paper was greatly improved by comments from D. B. Wake and three anonymous reviewers. This work was supported by the Academy of Finland and University of Helsinki Graduate School for Biotechnology.

1. Gilbert, S. F. & Sarkar, S. (2000) *Dev. Dyn.* **219,** 1–9.
2. Weiss, K. M. & Fullerton, S. M. (2000) *Theor. Popul. Biol.* **57,** 187–195.
3. Tamayo, P., Slonim, D., Mesirov, J., Zhu, Q., Kitareewan, S., Dmitrovsky, E., Lander, E. S. & Golub, T. R. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2907–2912.
4. White, K. P., Rifkin, S. A., Hurban, P. & Hogness, D. S. (1999) *Science* **286,** 2179–2184.
5. Tanaka, T. S., Jaradat, S. A., Lion, M. K., Kargul, G. J., Wang, X., Grahovac, M. J., Pantano, S., Sano, Y., Piao, Y., Nagaraja, R., *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97,** 9127–9132.
6. Kay, R. F. (1984) in *Adaptations for Foraging in Nonhuman Primates*, eds. Rodman, P. S. & Cant, J. G. H. (Columbia Univ. Press, New York), pp. 21–53.
7. Janis, C. M. & Fortelius, M. (1988) *Biol. Rev. Camb. Philos. Soc.* **63,** 197–230.
8. Jernvall, J., Hunter, J. P. & Fortelius, M. (1996) *Science* **274,** 1489–1492.
9. Janis, C. M., Damuth, J. & Theodor, J. M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 7899–7904.
10. McKenna, M. C. (1982) *Geobios* **6,** 213–223.
11. Gingerich, P. D. (1989) *Univ. Mich. Mus. Paleontol. Pap. Paleontol.* **28,** 1–97.
12. Fox, R. C. & Youzwyshyn, G. P. (1994) *J. Vertebr. Paleontol.* **14,** 382–404.
13. Meng, J., Wyss, A. R., Dawson, M. R. & Zhai, R. (1994) *Nature (London)* **370,** 134–136.
14. Butler, P. M. (1995) *Mammal Rev.* **25,** 3–14.
15. Gheerbrant, E., Sudre, J. & Cappetta, H. (1996) *Nature (London)* **383,** 68–70.
16. O'Leary, M. A. & Uhen, M. D. (1999) *Paleobiology* **25,** 534–556.
17. Weiss, K., Stock, D. & Zhao, Z. (1998) *Crit. Rev. Oral Biol. Med.* **9,** 369–398.
18. Jernvall, J. & Thesleff, I. (2000) *Mech. Dev.* **92,** 19–29.
19. Butler, P. M. (1956) *Biol. Rev. Camb. Philos. Soc.* **31,** 30–70.
20. Van Valen, L. (1970) *Dev. Biol.* **23,** 456–477.
21. Osborn, J. W. (1978) in *Development, Function and Evolution of Teeth*, eds. Butler, P. M. & Joysey, K. A. (Academic, London), pp. 171–201.
22. Butler, P. M. (1995) *Int. J. Dev. Biol.* **32,** 25–34.
23. Polly, P. D. (1998) *Paleobiology* **24,** 409–429.
24. Zuccotti, L. F., Williamson, M. D., Limp, W. F. & Ungar, P. S. (1998) *Am. J. Phys. Anthropol.* **107,** 137–142.
25. Jernvall, J. & Selänne, L. (1999) *Palaeontol. Electron.* **2,** http://www-odp.tamu.edu/paleo/1999_1/confocal/issue1_99.htm.
26. P. Ungar, P. & Williamson, M. (2000) *Palaeontol. Electron.* **3**, http://www-odp.tamu.edu/paleo/2000_1/gorilla/issue1_00.htm.
27. Jacobs, L. L. & Downs, W. R. (1994) in *Rodent and Lagomorph Families of Asian Origins and Diversification*, eds. Tomida, Y., Li, C. K. & Setoguchi, T. (National Science Museum Monographs 8, Tokyo), pp. 149–156.
28. Fejfar, O. (1999) in *The Miocene Land Mammals of Europe*, eds. Rössner, G. E. & Heissig, K. (Verlag Dr. Friedrich Pfeil, München, Germany), pp. 365–372.
29. Kälin, D. (1999) in *The Miocene Land Mammals of Europe*, eds. Rössner, G. E. & Heissig, K. (Verlag Dr. Friedrich Pfeil, München), pp. 373–387.
30. Freudenthal, M. & Suárez, E. M. (1999) in *The Miocene Land Mammals of Europe*, eds. Rössner, G. E. & Heissig, K. (Verlag Dr. Friedrich Pfeil, München, Germany), pp. 401–409.
31. Butler, P. M. (1985) in *Evolutionary Relationships Among the Rodents: A Multidisciplinary Approach*, eds. Luckett, W. P. & Hartenberger, J.-L. (Plenum, New York), pp. 381–401.
32. Swindler, D. R. & McCoy, H. A. (1964) *Science* **144,** 1243–1249.
33. Marshall, P. M. & Butler, P. M. (1966) *Arch. Oral Biol.* **11,** 949–965.
34. Popowics, T. E. (1998) *J. Morphol.* **237,** 69–90.
35. Vaahtokari, A., Åberg, T., Jernvall, J., Keränen, S. & Thesleff, I. (1996) *Mech. Dev.* **54,** 39–43.
36. Jernvall, J., Kettunen, P., Karavanova, I., Martin, L. B. & Thesleff, I. (1994) *Int. J. Dev. Biol.* **38,** 463–469.
37. Keränen, S. V. E., Åberg, T., Kettunen, P., Thesleff, I. & Jernvall, J. (1998) *Dev. Genes Evol.* **208,** 477–486.
38. Bloch-Zupan, A., Leveillard, T., Gorry, P., Fausser, J. L. & Ruch, J. V. (1998) *Eur. J. Oral Sci.* **1,** 104–111.
39. Jernvall, J., Åberg, T., Kettunen, P., Keränen, S. & Thesleff, I. (1998) *Development (Cambridge, U.K.)* **125,** 161–169.
40. Vainio, S., Karavanova, I., Jowett, A. & Thesleff, I. (1993) *Cell* **75,** 45–58.
41. Gould, S. J. (1977) *Ontogeny and Phylogeny* (Belknap, Cambridge, MA).
42. Raff, R. A. (1996) *The Shape of Life, Genes, Development, and the Evolution of Animal Form* (Chicago Univ. Press, Chicago).
43. Zelditch, M. L. & Fink, W. L. (1996) *Paleobiology* **22,** 241–254.
44. Hall, B. K. (1998) *Evolutionary Developmental Biology* (Kluwer, Dordrecht, The Netherlands).
45. Zúñiga, A., Haramis, A.-P. G., McMahon, A. P. & Zeller, R. (1999) *Nature (London)* **401,** 598–602.
46. Fraidenraich, D., Iwahori, A., Rudnicki, M. & Basilico, C. (2000) *Dev. Biol.* **225,** 392–406.
47. Moon, A. M., Boulet, A. M. & Capecchi, M. R. (2000) *Development (Cambridge, U.K.)* **127,** 989–996.
48. Sarkar, L., Cobourne, M., Naylor, S., Smalley, M., Dale, T. & Sharpe, P. T. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 4520–4524.
49. Zhang, Y., Zhang, Z., Zhao, X., Yu, X. & Hu, Y. (2000) *Development (Cambridge, U.K.)* **127,** 1431–1443.
50. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J. & Leder, P. J. (1995) *Cell* **82,** 675–684.
51. Zhang, P., Wong, C., Liu, D., Finegold, M., Harper, J. W. & Elledge, S. J. (1999) *Genes Dev.* **13,** 213–224.
52. Wray, G. A. & McClay, D. R. (1989) *Evolution* **43,** 803–813.
53. Guralnick, R. P. & Lindberg, D. R. (1999) *Evolution* **53,** 447–459.
54. Tucker, A. S., Matthews, K. L. & Sharpe, P. T. (1998) *Science* **282,** 1136–1138.
55. Jernvall, J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 2641–2645. (First Published March 7, 2000; 10.1073/pnas.050586297)
56. Dahn R. D. & Fallon, J. F. (2000) *Science* **289,** 438–441.
57. Wake, D. B. (1991) *Am. Nat.* **138,** 543–567.