Developmental genetics and early hominid craniodental evolution

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Summary

Although features of the dentition figure prominently in discussions of early hominid phylogeny, remarkably little is known of the developmental basis of the variations in occlusal morphology and dental proportions that are observed among taxa. Recent experiments on tooth development in mice have identified some of the genes involved in dental patterning and the control of tooth specification. These findings provide valuable new insight into dental evolution and underscore the strong developmental links that exist among the teeth and the jaws and cranium. The latter has important implications for cladistic studies that traditionally consider features of the skull independently from the dentition. *BioEssays* 23:481–493, 2001. © 2001 John Wiley & Sons, Inc.

Introduction

Between 3 and 2.5 mya novel behavioral and ecological exploitation by our ancestors led to the emergence of three distinct taxa: *Australopithecus aethiopicus* and *A. garhi* in East Africa[1,2] and *A. africanus* in South Africa.[3] Of these taxa, both *A. africanus* and *A. aethiopicus* increased the size of their postcanine dentitions relative to the ancestral condition displayed by *A. afarensis*, but they did so in very different ways. In *A. africanus* tooth enlargement relative to *A-atarensis* involved little alteration of molar crown morphology and was limited to the second and third molars (Fig. 1).[4,5] In *A. aethiopicus* (an example of "robust" *Australopithecus*) not only were all of the chewing teeth (premolars and molars) expanded (Fig. 1), but their distal segments (talonids) were differentially expanded as well (Fig. 2).[5] Quite clearly selection favored the development of large postcanine occlusal areas in Pliocene hominids. The variation in both tooth shape and dental proportions observed in these taxa indicate, however, that tooth enlargement could have been achieved through very different genic mechanisms. An understanding of the underlying basis of these dental variations is required if we are to appreciate fully the role played by these species in the evolution of subsequent taxa and the emergence of *Homo*. Here we review current evidence regarding the genic mechanisms involved in the patterning and morphogenesis of the murine dentition and apply this information to the early hominid fossil record.

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Dental patterning and the specification of tooth type

The development of the dentition, like that of all ectodermal organs, is directed by a series of reciprocal tissue interactions that occur between an epithelium and its underlying mesenchyme. In teeth, these interactions occur between the epithelium lining the future oral region and mesenchyme formed from cranial neural crest (CNC) cells that have migrated into the frontonasal, maxillary and mandibular processes from the midbrain, hindbrain and forebrain regions of the neural tube.[6,7] Although there is some evidence for the predetermination of CNC cells based on their axial origins,[7,8] it is clear that signals from the ectoderm and endoderm have a major influence on the fates of these migrating cells. At present, although the relative contributions of premigration and postmigration CNC determination remain to be firmly established, it is clear that however the migration pathway and destination are determined, both are strongly influenced by ectodermal/endodermal signals.

Teeth start to develop relatively early in embryogenesis, at around 6 weeks in humans and 10 days in mice. Thickening of the oral epithelium at the future sites of odontogenesis is the first indication that tooth morphogenesis is underway. During this transformation of oral epithelium into (primary) dental lamina, signals that pass from the epithelium to the mesenchyme initiate mesenchymal cell condensation. Reciprocal signals from the mesenchyme instruct the epithelium to invaginate into the mesenchyme and form the tooth bud. Although these events are the first morphological manifestations of the development process, they actually represent the final phases of a protracted initiation period in which signals originating from the epithelium establish both the type and placement of teeth within the developing jaws. Numerous signaling molecules are expressed by the epithelium of the jaw primordia at this time,[9] but those most important in the establishment of dental pattern appear to be FGF8 and BMP4.

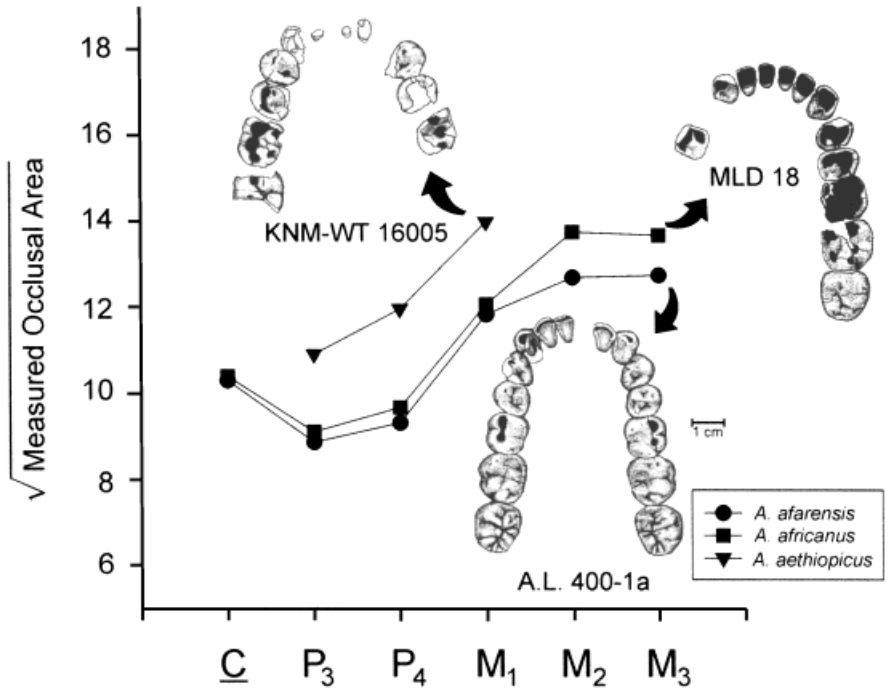

Figure 1. A comparison of the occlusal areas of the mandibular canine (C), premolars (P) and molars (M) of *A. afarensis*, *A. africanus* and *A. aethiopicus*. With the exception of canine area, which is represented as the buccolingual breadth of the canine, all measures represent the square root of the measured occlusal areas. All dental measures are in millimeters. For both *A. afarensis* and *A. africanus*, individual data points represent species means (see Fig. 6 for sample sizes). In comparison, occlusal areas of *A. aethiopicus* are represented by KNM-WT 16005 (shown), the only associated mandibular dentition currently known of this taxon. Scaled representatives of each of *A. afarensis* and *A. africanus* are also shown. Metric data provided by G. Suwa. Drawings © 2000 Luba Dmytryk Gudz.

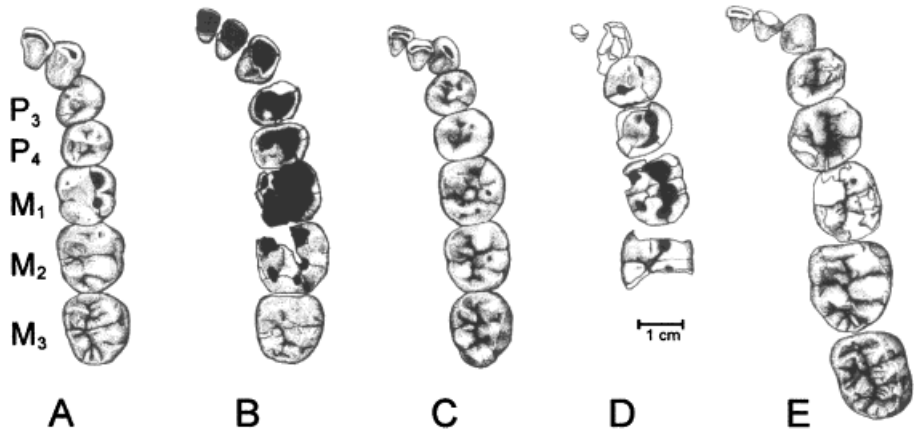

Figure 2. Occlusal variation in the early hominid mandibular dentition. **(A)** *A. afarensis* (A.L. 400-1a), **(B)** *A. africanus* (MLD 18), **(C)** *A. robustus* (SK 23), **(D)** *A. aethiopicus* (KNM-WT 16005 reversed) and **(E)** *A. boisei*. These scaled comparisons illustrate the variation in postcanine crown morphology discussed in the text. In particular, note the large size of the *A. aethiopicus* and *A. boisei* dentitions in comparison to those of *A. afarensis* and *A. africanus*, and *A. robustus* as well. Note also the expanded distal segments (talonids) of the *A. robustus* and *A. boisei* premolars and molars, and the unique character of the *A. boisei* P4. Drawings © 2000 Luba Dmytryk Gudz.

The fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling systems interact in the spatial patterning of a variety of developing tissues.[10–14] During early facial development, *Fgf8* and *Bmp4* are expressed in complementary domains in the epithelium of both the developing mandibular and maxillary primordia. Here FGF8 and BMP4 induce and/or maintain the mesenchymal expression of a variety of growth and transcription factors (Fig. 3A). For example, epithelial FGF8 induces and/or maintains the mesenchymal expression of the growth factor genes *activin* βA,[15] as well as the transcription factor genes *Dlx1* and *Dlx2*,[16] *Alx3*,[17] *Barx1*,[18] *Pax9*,[19] and *Msx1*.[20] Epithelial FGF8 also induces the mesenchymal expression of the LIM-domain transcription factors *Lhx6* and *Lhx7*.[21] Since expression of the latter define the oral–aboral axis of the mandible prior to the appearance of tooth primordia, FGF8 may well be the signal responsible for conferring odontogenic potential to the jaw mesenchyme.[21] BMP4 shares with FGF8 the ability to induce and/or maintain the expression of the homeobox-containing genes *Msx1*, and *Dlx2* in mesenchyme.[16,22,23] Whereas FGF8 induces the expression of the paired box gene *Pax9*, BMP4, however, antagonizes the FGF8 inductive signal and thereby restricts expression of this gene, which is required for tooth morphogenesis to proceed beyond the bud stage, to presumptive odontogenic mesenchyme.[19] In the presence of both *Pax9* and *Msx1*, BMP4 expressed in the epithelium induces its own gene expression in the underlying mesenchyme.[24] Mesenchymal *Bmp4*, along with FGF8-induced *activin* βA, are subsequently involved in the mesenchymal signaling that initiates epithelial budding at the sites of inidividual tooth formation (see below).[9]

That epithelial FGF8 and BMP4 contribute to the specification of tooth type is suggested by their ability to establish spatially restricted domains of homeobox-containing genes in the underlying mesenchyme of the murine jaw primordia (Fig. 4). Mice possess only two types of teeth: incisors and molars. During early initiation, BMP4 expressed in the distalmost oral

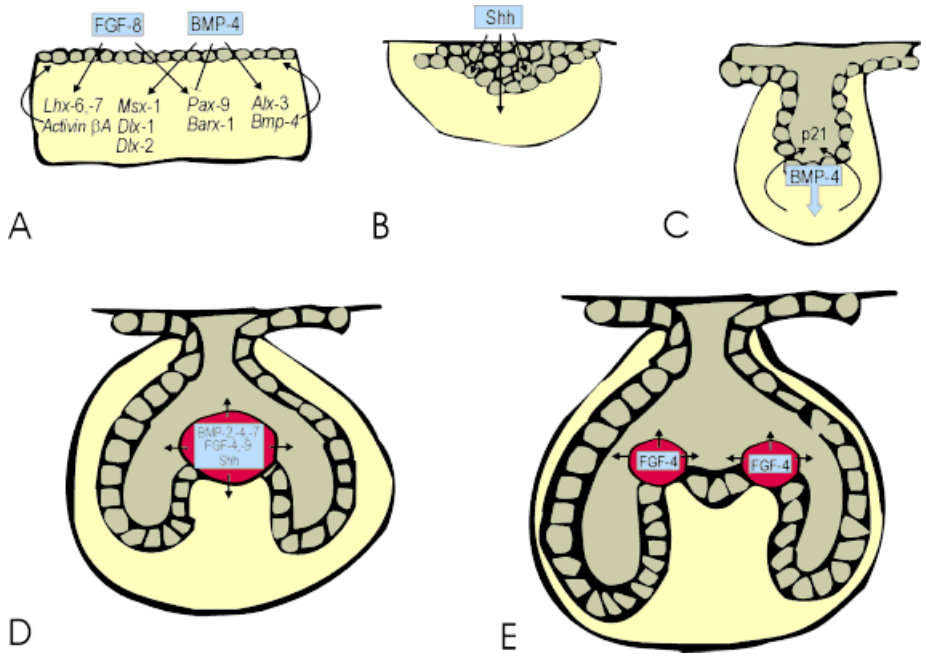

Figure 3. Molecular signals involved in dental patterning. During early initiation **(A)** epithelial FGF8 and BMP4 induce and/or inhibit mesenchymal expression of a number of transcription factors which confer odontogenetic potential to the mesenchyme and specify tooth type. These epithethelial signals also induce mesenchymal expression of *activin* βA and *Bmp4* which signal back to the epithelium to initiate epithelial bud formation. Subsequent Shh signaling by the dental lamina **(B)** further directs epithelial cell proliferation to produce a tooth bud (Bud stage). Upon formation of the tooth bud and establishment of an early epithelial signaling center **(C)**, both instructive potential and *Bmp4* expression shift to the mesenchyme. In response to BMP4 signals, epithelial cells at the tip of the tooth bud stop proliferating and form the (primary) enamel knot of the cap stage tooth **(D)**. Signaling by the enamel knot organizes the development of crown shape by regulating both epithelial proliferation and the activation of secondary enamel knots **(E)** at the precise locations that cusps will form (Bell stage). Continued growth and folding of the epithelium results in the acquisition of tooth shape. Terminal differentiation of the dentin-forming odontoblasts and enamel-forming ameloblasts accompanies the latter process. Green, tooth epithelium; red, enamel knots; yellow, tooth mesenchyme.

epithelium (presumptive incisor region; in tooth development studies distal implies anterior) induces the mesenchymal expression of *Msx1* and *Alx3* in this region.[17,18] At the same time, BMP4 actively inhibits FGF8-induced expression of *Barx1* and thereby restricts expression of this gene to presumptive molar mesenchyme.[18] In a manner analogous to the specification of vertebral type by overlapping domains of Hox gene expression, it is thought that the expression patterns of these homeobox-containing genes in the presumptive dental mesenchyme (e.g., *Barx1* and *Dlx2* proximally, in presumptive molar field; *Msx1*, *Msx2* and *Alx3* in presumptive incisor field) provide the spatial information necessary to determine tooth type (Fig. 4).[25–27] Functional analyses of murine homeobox genes support the existence of an "odontogenic homeobox code". Specifically, double mutations in *Dlx-1* and *Dlx-2*, genes co-expressed in the proximal (but not distal) mesenchyme of the mandible and maxilla, result in the absence of maxillary molar tooth development.[28,29] In these studies, the resulting hypodontia is a primary defect, which correlates directly with the expression domains of *Dlx* genes (the normal development of mandibular molars in *Dlx1/Dlx2* mutants implies functional redundancy with other Dlx family members that are expressed in proximal mandibular arch mesenchyme).[29] Also consistent with an odontogenic homeobox code is the transformation of tooth type that occurs when homeobox genes are misexpressed in oral mesenchyme. In mice, the inhibition of epithelial BMP4 signaling by Noggin results in both the loss of mesenchymal expression of exclusively distal genes and the ectopic expression of *Barx1* distally.[18] As a consequence of this combined loss of distal genes and gain of a proximal one, multicuspid teeth develop in place of incisors. It should be emphasized that the code model predicts a change in tooth type only when a loss of gene function is accompanied by an appropriate gain of function of some other gene involved in tooth specification. Thus in the *Dlx1/2* double mutants, the loss of *Dlx1/Dlx2* gene function does not result in molar to incisor transformation because it is not accompanied by the gain of function of an "incisor-specifying" gene. The ectopic expression of *Barx1* that results from inhibition of BMP signaling does, however, result in an incisor to molar transformation because it is accompanied by the loss of "incisor" gene (*Msx1*, etc.) expression.

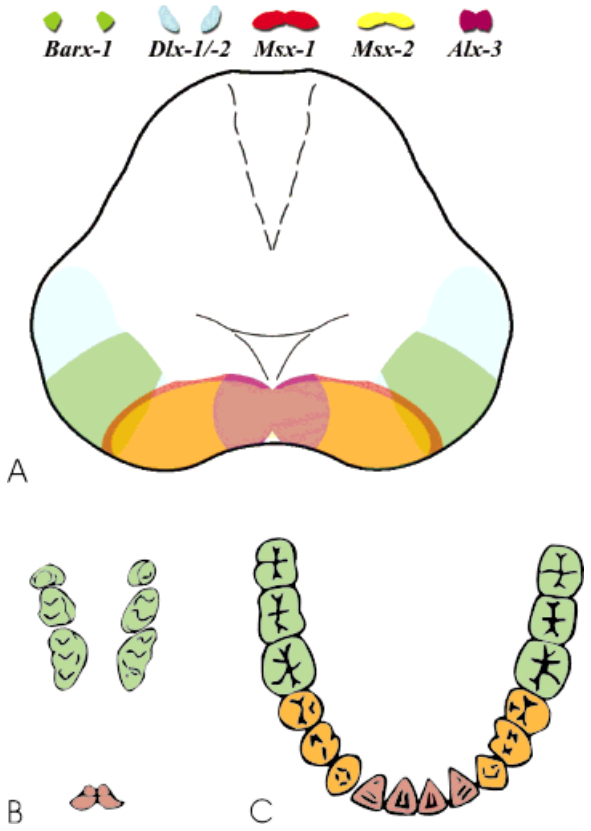

Figure 4. The odontogenic homeobox code. Epithelial FGF8 and BMP4 expressed during early initiation induce the mesenchymal expression of a number of homeobox-containing genes in the underlying mesenchyme. In a manner similar to the specification of vertebral type by overlapping domains of *Hox* gene expression in the developing neuraxis, it is thought that the overlapping domains of homeobox-containing genes expressed in dental mesenchyme provide the spatial information necessary to determine tooth type. In the mandibular primordium of the mouse **(A)** domains of *Barx1* (green) and *Dlx1/2* (blue) expression overlap in the mesenchyme of the presumptive molar region, while domains of *Msx1* (red), *Msx2* (yellow) and *Alx3* (purple) overlap in presumptive incisor mesenchyme **(B)**. Expression domains of *Dlx1/2*, *Msx1* and *Msx2* overlap to varying degrees in the non-tooth forming diastema of the murine jaw [compare **A** and **B**]. These combinations of homeobox-containing genes may code for the canine and premolars of other mammalian groups [compare **(B)** and **(C)**].

In the murine dentition, there is a large toothless gap between the anterior (incisor) and posterior (three molars) teeth, the diastema. In mice, the expression domains of proximal and distal genes overlap in the diastema (Fig. 4). Such overlaps, for example between Msx and Dlx genes, may code for the canine and premolars of other mammals. In this respect it is significant that, in the BMP4 inhibition studies, teeth with an incisor–molar hybrid shape, similar in form to premolars, were occasionally produced.[18] Also of interest is the finding that many rodent species, including mice, possess rudimentary tooth germs in their maxillary diastema regions.[30–32] These rudimentary tooth germs are believed to be remnants of the two incisors, one canine and four or five premolars that were lost during the evolution of the murine dentition from the primitive Eutherian condition.[32] Studies of the genic mechanisms involved in the initiation and subsequent developmental arrest of "diastema teeth" may shed

additional light on the molecular mechanisms involved in the specification of tooth type. At present it is known that, unlike molars, but as in incisors, the diastema tooth buds of both mice and voles do indeed express *Msx1*.[32,33]

Although data are still limited, FGF8 and BMP4 appear to be involved in the patterning of facial structures as well.[34,35] In the maxillary primordia of the chick, mesenchymal expression of *Barx-1* is complementary to that of *Msx-1*, and both correlate with the overlying epithelial expression of *Fgf8* and *Bmp4*, respectively.[35] In mice, *Barx1* is expressed in the molar-coding region of the oral mesenchyme and developing molars, and is further expressed in a variety of neural-crest-derived tissues of the first and second branchial arches prior to their overt differentiation.[36] In addition in mice, the loss-of-function gene mutations that result in tooth patterning phenotypes additionally produce severe craniofacial skeletal abnormalities.[28,34,37] In these cases, the skeletal phenotypes include both primary (a direct result of gene loss) and "secondary" downstream defects. No genes have yet been identified that affect early tooth patterning, but fail to affect jaw skeletal development. There are several genes, however, including *goosecoid*, that have very severe effects on jaw bone development but have no effect on tooth development.[38–40] Therefore, whereas mutation-induced skeletal changes have been shown to occur in the absence of changes in tooth pattern, changes in tooth pattern have thus far always been accompanied by changes in skeletal development.

Tooth morphogenesis

With both the location and identity of individual teeth specified, the oral epithelium thickens and begins its transformation into the tooth bud (Fig. 3B). This outgrowth of the dental lamina is initiated following mesenchymal expression of both *Bmp4* and *activin* βA.[9] As noted above, mesenchymal expression of both of these signals is dependent on the epithelial expression of FGF8 and BMP4 that occurs during early initiation. Sonic hedgehog (Shh) is also active during epithelial thickening and bud formation. Like FGF8 and BMP4, Shh has been implicated in the patterning of a variety of vertebrate tissues and organs.[41,42] In the developing jaw primordia, *Shh* expression is restricted to the budding tooth germs. Interestingly, the expression pattern of the homeobox gene *Pitx-2* (*Otlx-2*; RIEG) is similar to that of *Shh* in oral and dental epithelium.[32] In avian embryos, *Pitx-2* interacts with *Shh* to establish left–right asymmetry.[43,44] Although a relationship between *Pitx-2* and *Shh* in tooth bud formation has yet to be established, mice homologous for mutations in *Pitx-2* show an early-bud-stage arrest of tooth development and humans heterozygous for mutations in PITX-2 display Reiger syndrome, a congenital malformation that includes the absence of teeth.[45–48]

According to the current model of Shh signal transduction, the Shh ligand binds to the Patched (Ptc) receptor and, in response to this binding, Ptc, which normally represses the action of Smoothened (Smo), releases this inhibition, thus allowing Smo to activate the transcription of downstream targets via the Gli1, Gli2 and Gli3 transcription factors.[49–51] In the developing dentition, *Shh* expression is restricted to the epithelial compartment, but components of its signaling pathway (the Ptc receptor and Gli transcription factors) are expressed in both epithelium and mesenchyme.[52] This implies that Shh is involved in both epithelial–epithelial and epithelial–mesenchymal signaling.[52–54] During late initiation *Shh* expression is restricted to the future sites of cell proliferation and bud formation.[52] This, along with findings that early intact tooth germs exposed to exogenous Shh form abnormal epithelial invaginations, and that ectopic epithelial invaginations are produced when recombinant Shh is placed in oral epithelium prior to its thickening, indicate that Shh activity affects epithelial cell proliferation.[52] It may be that the function of Shh activity during early tooth development is to guide epithelial cell proliferation to produce a tooth bud.[52]

The formation of the epithelial tooth bud coincides with the intense expression of a number of signaling molecules in a small subset of budding cells.[9] Included among the signaling molecules expressed by this "early" epithelial signaling center are FGF8, BMP4, Shh and members of the Wnt family of signaling molecules, as well as *p21*, *Msx2* and *Lef1*.[9] The precise role of many of these signaling molecules during early budding is currently unknown. It is known, however, that although the HMG-box gene *Lef1* is expressed in both epithelium and mesenchyme throughout the remainder of tooth development, it is required only in the epithelium.[45–57] In mice lacking a functional Lef1 protein, tooth development is arrested at the bud stage. This suggests that Lef1 is in some way involved in the regulation of an epithelial signal that acts on the bud-stage dental mesenchyme.

Upon establishment of the early epithelial signaling center the potential to further guide tooth development shifts from the epithelium to the mesenchyme (Fig 3C).[58,59] Once this occurs, the undersurface of the epithelial bud invaginates and begins to fold around the condensing mesenchymal cells at its tip (Fig. 3D). Folding of the epithelial bud always begins in the mesial (anterior) end of the tooth bud and proceeds distally (when describing teeth distal implies posterior). Once established, rapid downward growth of the folds results in the formation of cervical loops that later surround the mesenchymal dental papilla. During this transition from bud to cap stage, and apparently in response to BMP4 signaling,[60] the epithelial cells at the tip of the tooth bud express p21 (an inhibitor of cell proliferation) and stop dividing. The resulting, tightly packed epithelial cells, the (primary) enamel knot, soon begin to express many of the same signals as the early epithelial signaling center.[60–63] Specifically, shortly after its formation, cells of the enamel knot begin to express FGF4, BMP2, -4, and -7.[61–63] In addition, expression domains of FGF9 and Shh, which previously encompassed the entire

dental lamina, become restricted to the enamel knot.[52] As is indicated by the expression of FGF receptors in the non-enamel knot epithelium at this time (few FGF receptors are found within the enamel knot),[64] the FGFs expressed by the enamel knot are key signals regulating the growth and folding of the epithelial sheet that occurs during early cap stage.[65] However, FGF receptors are also expressed within the mesenchyme of the dental papilla.[64] This, along with the findings that FGF4 and FGF9 are capable of stimulating cell proliferation in both epithelium and mesenchyme,[64,65] suggest that enamel knot proliferative signaling targets the underlying mesenchyme as well.

Roughly 24 hours after its formation, the enamel knot is apoptotically removed (also in response to BMP4 signaling) from the cap-stage tooth.[66] Shortly thereafter, a number of secondary enamel knots appear at the precise locations where cusps will form (Fig. 3E).[67] Unlike primary enamel knots, which express signaling molecules expressed in varying combinations in several, well-known organizing centers throughout the embryo (e.g., the notochord, zone of polarizing activity, apical ectodermal ridge), secondary enamel knots have thus far only been associated with *Fgf4* expression.[67] The expression domains of all other signaling molecules at this time are far more diffuse. It has recently been suggested that the nested expression of signals such as *Shh* and *BMP4*, which accompany the more localized expression of *Fgf4* by secondary enamel knots may regulate their spacing by actively inhibiting the *Fgf4* signal.[9]

Once cusp pattern and tooth shape are established, crown morphogenesis proceeds with the terminal differentiation of dentin-forming odontoblasts and the enamel-forming ameloblasts at the epithelial–mesenchymal interface of the bell-stage tooth. Unfortunately, relatively little is known of the molecular controls involved in this final phase of tooth development. The expression of *Fgf9* in the inner dental epithelium prior to the differentiation of odontoblasts suggests it may act as an epithelial signal regulating differentiation of the underlying mesenchymal cells into odontoblasts.[64] Alternatively, FGF9 may regulate ameloblast differentiation via an autocrine mechanism.[64] A role for FGFs in the differentiation and/or secretory functions of odontoblasts and ameloblasts is further suggested by the intense expression of FGF receptors in these cells (e.g., FGF1 in odontoblasts and FGF1 and FGF2 IIIb in ameloblasts).[64] BMP2, BMP4 and BMP7 are also expressed in the inner enamel epithelium during early bell stage in a pattern that is consistent with these factors acting as epithelial signals regulating the differentiation of mesenchymal cells into odontoblasts.[23] That BMP2 stimulates the differentiation of odontoblasts in cultured dental papillae supports this conclusion.[68] Whether mesenchymal BMPs are involved in the signaling leading to the differentiation of the inner enamel epithelium is currently unknown. They are present at the right time and right place for such a role, but, thus far, the ability of BMPs to induce ameloblast differentiation has not been demonstrated.[23]

Recently it has been demonstrated that expression of *Fgf4* and *Fgf9* in the tip of the epithelial tooth bud coincides with the start of *Cbfa1* expression in the subjacent underlying mesenchyme.[69] The transcription factor Cbfa1 is a critical regulator of osteoblast differentiation.[70] In the developing dentition, expression of *Cbfa1* in dental papilla mesenchyme follows the latter's acquisition of odontogenic potential, and is downregulated coincident with the disappearance of the enamel knot. In addition, Cbfa1-deficient mice display poorly differentiated odontoblasts and a highly disorganized dentin matrix.[69] In light of these findings, it is thought that Cbfa1 regulates the expression of mesenchymal signals (e.g., BMPs) that influence both the morphogenesis and histodifferentiation of the epithelial enamel organ.[69] It may be that inductive signals are initiated in dental epithelium through a Cbfa1-dependent pathway and then act back upon mesenchyme to stimulate odontoblast differentiation. Alternatively, Cbfa1 may regulate the expression of other inductive mesenchymal signals that directly influence odontoblast differentiation.[69]

Genic models of early hominid dental divergence

Given the highly derived nature of the rodent dentition, it is somewhat unfortunate that the mouse has become the predominant model system for experimental studies of mammalian tooth development. In most mammals, including hominids, a large portion of the permanent dentition of adults, essentially all antemolar teeth (incisors, canine and premolars of each jaw quadrant), develop from successional laminae that differentiate from the outer enamel epithelium (remnant of primary dental lamina) of their bell-stage (deciduous) precursors. The remainder of the permanent dentition (molars) develops directly from extensions of the primary dental lamina that secondarily form distal (posterior) to the terminal deciduous (pre)molar. Mice do not possess a deciduous dentition. Consequently, the murine permanent dentition develops directly from the primary dental lamina. Of the numerous specializations of the murine dentition (e.g., loss of specific teeth, absence of a deciduous dentition, possession of a continuously erupting incisor that lacks ameloblasts on its lingual surface), the failure to develop successional teeth may be the most significant obstacle to overcome in extrapolating details of rodent dental development to more generalized mammalian dental patterns. Although it is generally assumed that the same regulatory networks and signaling cascades are involved in the development of both the primary and successional dentitions, it is important to recognize that the permanent dentition of most mammals is of mixed developmental origin.

With respect to the development of the early hominid dentition, much of the dental variation observed in early

hominid taxa relates to differences in tooth size and crown morphology. It should be emphasized that this variation is most evident in the mandibular dentition.[4,71] That the maxillary and mandibular dentitions differ in their ability to distinguish early hominid taxa implies that morphogenesis of the upper and lower teeth is under the control of two different genic programs. The absence of maxillary molar development in mice lacking functioning *Dlx1* and *Dlx2* genes,[29] as well as the phenotype of *activin*βA mutant mice where maxillary molars are the only teeth to develop,[15] confirm that this is indeed the case.

Developmentally, larger teeth ultimately result from an up-regulation of epithelial cell proliferation either during initial bud formation or in association with cap-stage enamel knot signaling. Distinguishing between these two (Shh-mediated?) proliferation events is critical. The cap stage tooth is a relatively autonomous structure and as such its morphogenesis could be selectively modified in isolation from the remainder of the dentition. In comparison, initiation-phase epithelial proliferation occurs in an oral epithelium molecularly partitioned into discrete regions (i.e., fields or modules) that will give rise to buds (both primary and successional) of a similar tooth type. At this level of the developmental hierarchy, selection might alter tooth size individually, perhaps by operating on variations in the individual Shh domains associated with each tooth, or universally, by operating throughout a more inclusive tooth field. Although considerable comparative data attest to the relative ease with which all of the members of a single functional tooth class can be universally modified (despite their differing developmental origins, mammalian premolars and molars often evolve as a single unit Ref. 72), the genic/molecular controls involved in such comprehensive change are presently not fully understood.

In herbivorous mammals tooth enlargement is often limited to the terminal elements of the molar row.[73] Tooth enlargement in *A. africanus* follows this same pattern (Fig. 1). As noted above, the permanent molars of mammals develop directly from secondary extensions of the primary (deciduous) dental lamina that occur distal to the terminal deciduous (pre)molar. In general, the initial elongation of the primary dental lamina that leads to formation of the first molar tooth bud occurs at the same approximate time that successional tooth buds begin to form.[74] Once the first molar has reached late bell stage, the primary dental lamina elongates a second time and the bud for the second molar forms. Development of the third molar follows the same sequence. Little is known of the genic patterning of the late-developing (from secondary extensions of the primary dental lamina) molars of non-rodent species (although recent molecular evidence has implicated mutations in human PAX9 as being responsible for some forms of oligodontia in which the permanent molars are preferentially affected Ref. 75). Nevertheless, the spatial and temporal isolation of second and third molar development, which is characteristic of the generalized mammalian dentition, provides ample opportunity for selection to modify terminal molar size individually, by acting either on the first phase of epithelial proliferation, during bud formation, or later, during proliferation of the cap-stage epithelium.

Unlike *A. africanus*, in which tooth enlargement relative to the ancestral condition is limited to the terminal members of the molar row, *A. aethiopicus* displays enlargement of its entire postcanine dentition (Fig. 1).[5] Although this pattern of dental expansion could be the result of independent selection on each tooth (either at budding or during cap stage), it is far more likely that such comprehensive change is the result of selection operating at a higher, more inclusive level of dental development. But at what level? One strong possibility is that selection operated on genetic variations expressed within the restricted boundaries of an established (homeobox-coded?) premolar–molar tooth field. The *A. aethiopicus* dentition is certainly consistent with this explanation. A second possibility is suggested not by the evidence provided by *A. aethiopicus*, but by that of its immediate descendant, *A. boisei* (see below). In *A. boisei* large molars and premolars are combined with a canine that is small in comparison to those of non-robust taxa[76] and is exceptionally small when considered relative to the size of its postcanine teeth (Fig. 5).[5] Although no canines are currently attributable to *A. aethiopicus*, the dentition of *A. boisei* suggests that expansion of the postcanine dentition may have been developmentally correlated with reduction of the canine. Such an effect might result if enlargement of the postcanine dentition was achieved by reproportioning the domains of the homeobox-containing genes expressed in the early initiation-stage oral mesenchyme. As these genes appear to partition what is essentially a spatially-restricted block of mesenchyme into smaller subunits[25–27] (in a manner analogous to the partitioning of the vertebral region by *Hox* genes), it is conceivable that increasing the size of any one subunit may occur at the expense of others. In this respect, a simple "distalization" (anterior relocation) of the boundary between posterior and anterior tooth fields might result in the correlated development of large postcanine teeth and small canines and incisors. As such a simple change involves neither the gain nor loss of homeobox gene function at any tooth location, a transformation of tooth type (such as occurred in the BMP4 inhibition studies) would not be expected.

In addition to their large size, the molars and premolars of *A. aethiopicus* display both expanded distal segments (talonids) and rearranged cusp/fissure patterns (Fig. 2).[5] These changes in postcanine crown morphology reflect an alteration in the spatial and/or temporal activation of secondary enamel knots.[61] That the modifications in crown morphology observed at each permanent tooth are similar in character suggests their acquisition through a single, "global" control mechanism. (Such global control is also indicated by the similar character of the *A. boisei* deciduous (pre)molars Ref. 77). Metric studies indicate, however that the magnitude of the

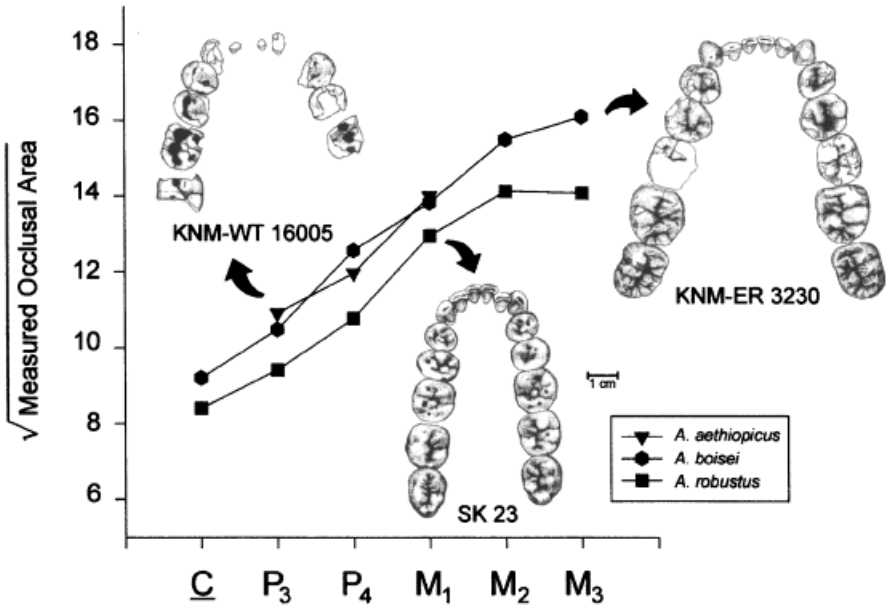

Figure 5. A comparison of the occlusal areas of the mandibular canine (C), premolars (P) and molars (M) of *A. aethiopicus, A. boisei* and *A. robustus*. As in Fig. 1, measures of the premolars and molars represent the square root of measured occlusal area. Canine size is represented by buccolingual breadth. All dental measures are in millimeters. Individual data points for both *A. boisei* and *A. robustus* represent species means (see Fig. 6 for sample sizes). Occlusal areas of *A. aethiopicus* are represented by KNM-WT 16005 only. Scaled representatives of each of these taxa are also shown. Metric data provided by G. Suwa. Drawings © 2000 Luba Dmytryk Gudz.

taxonomic differences in early hominid postcanine occlusal morphology vary from tooth to tooth along the dental row and are most notable in the mandibular third premolar (P3).[5] In non-robust taxa, this tooth is either unicuspid (a remnant of its sectorial history) or bicuspid with only little elaboration of its distal segment. In the *A. aethiopicus* P3, talonid expansion appears to be extreme. These differences in P3 crown shape suggest that some additional, independent control factor may have been involved in the morphogenesis of this tooth in early hominid taxa.

Like *A. africanus* and *A. aethiopicus*, the postcanine teeth of the contemporaneous *A. garhi* are large.[2] However, current knowledge of the *A. garhi* dentition is limited to the evidence provided by a single maxilla. It is therefore difficult to determine whether the large teeth of this specimen reflect a specialized adaptation of the species or are instead an artifact of large individual body size. It is known, however, that anterior/posterior tooth proportions in the *A. garhi* specimen are most similar to those of *A. afarensis* and unlike those of *A. aethiopicus*.[2]

Implications for early hominid phylogeny

Subsequent to 2.5 mya, the fossil record of early hominid evolution becomes significantly more complex with the appearance of *A. boisei, A. robustus* and *Homo* (Fig. 6). In East Africa the relatively intact fossil sequence of the Shungura Formation of Ethiopia preserves the transition from an *A. aethiopicus* to an *A. boisei* dental morphology.[5] In comparison to *A. aethiopicus, A. boisei* possesses larger postcanine teeth, a larger fourth premolar (P4) with a distinctive morphology, and distinct molar cusp proportions (Figs. 2,5). The Shungura sequence demonstrates that these derived features of the *A. boisei* dentition were acquired in a mosaic fashion—the increase in size and morphological specialization of the P4 preceded the reproportioning of molar cusps.[5] It is of note that the *A. boisei* mandibular P4 is the only early hominid premolar to possess accessory cusps on its distal margin that approach the primary cusps in size.[5,78] This "molarized" aspect of the *A. boisei* premolar (Fig. 2), together with the fact that it was acquired independently of other crown modifications, suggests that it may have arisen through an alteration of the homeobox code at this tooth position.

Although the fossil record yields considerable evidence concerning the emergence of *A. boisei* from *A. aethiopicus* approximately 2.3 mya, the origins of *A. robustus* and *Homo* from earlier forms is less clear (Fig. 6). In the absence of time-successive hominid samples many researchers have turned to quantitative cladistic methods as a means of resolving the phylogenetic origins of these taxa. A number of different phylogenetic scenarios have been proposed.[78–84] All are derived, however, from the analysis of large numbers of

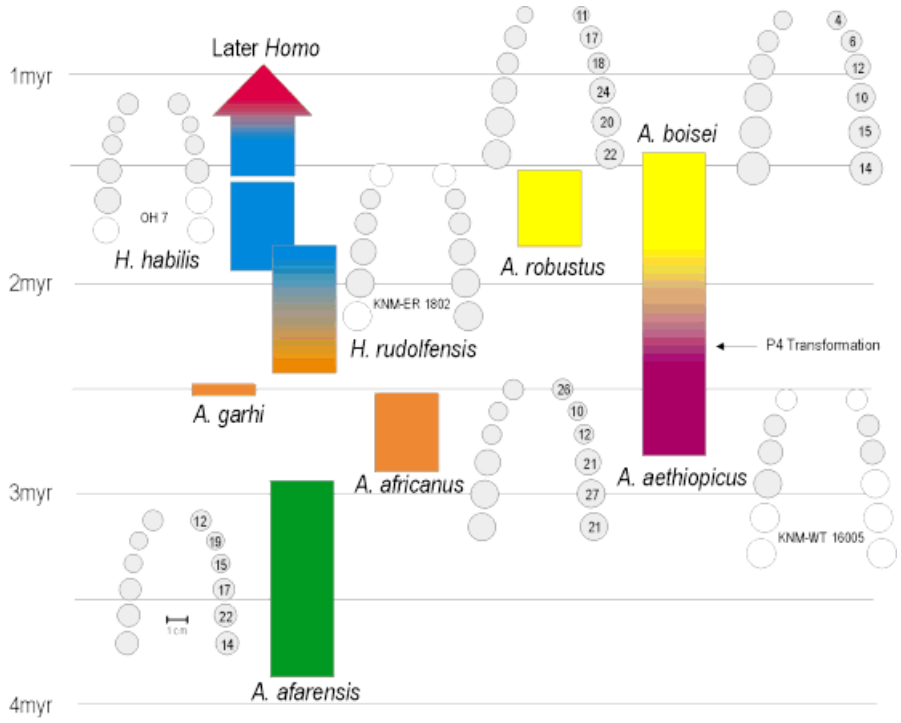

Figure 6. The chronological relationships of early hominid taxa. Note that the transition from *A. aethiopicus* to *A. boisei* occurs at ca. 2.3 myr.[5] Accompanying diagrams illustrate the variation in tooth size and dental proportions discussed in the text. The diameter of the circles representing the mandibular premolars and molars equals the relative square roots of their measured occlusal areas. The diameter of the circle representing the mandibular canine equals relative buccolingual breadth of this tooth. For *A. afarensis*, *A. africanus*, *A. robustus* and *A. boisei*, species means are represented and numbers within each circle represent the number of specimens from which mean occlusal areas were derived. The mandibular dentitions of *A. aethiopicus*, *H. rudolfensis* and *H. habilis* are represented by the preserved teeth (shaded circles) of fossil specimens KNM-WT 16005, KNM-ER 1802 and OH 7 respectively. No mandibular specimens are currently attributed to *A. garhi*.

craniodental characters, all of which are necessarily assumed by cladistic methodology to have arisen independently from one another. In reality, many of the cranial features regularly incorporated into these studies are redundant with respect to dental dimensions. For example, enlargement of postcanine occlusal areas, regardless of underlying genic mechanism, would have necessarily lowered the magnitude of occlusal pressure generated during chewing unless the cross-sectional area of the masticatory musculature was correspondingly increased. It is therefore not surprising to find that the "megadont" early hominid taxa extant at 2.5 mya (*A. aethiopicus* and to a lesser extent *A. africanus*), and later megadont taxa as well, all display anteriorly positioned maxillary zygomatic roots, well-developed extracranial crests and other features indicative of their enlarged masticatory musculature. Nevertheless, in cladistic studies, these features are often considered independent not only of dental size, but of each other as well.

That some of the features commonly incorporated into cladistic studies may be functionally integrated with the dentition, and therefore not truly independent, has been recognized for some time. However, it is becoming increasingly more evident that selective modification of the dentition can generate dramatic, seemingly unrelated changes in skull form. For example, the facial skeleton of the *A. aethiopicus/A. boisei* lineage (and of *A. robustus* as well) is characterized by such features as a greatly thickened hard palate, a vertically tall infraorbital region in which the infraorbital foramina are set relatively low, and a relatively high hafting of the face onto the neurocranium. Although these features are usually considered genetically and developmentally independent features of the face of robust *Australopithecus*, it has recently been argued that a more likely explanation is that they are all the developmental byproducts of a pattern of facial growth in which the orbitonasal and oral skeletons were displaced in opposite directions from the nasal cavity floor during ontogeny.[85] Instrumental in the establishment of this particular pattern of facial growth are (1) an extreme amount of upward maxillary rotation produced by extensive vertical growth of the mandibular condyle, and (2) a derived subnasal morphology in

which the anterior tip of the bony nasal septum, the vomer, inserts above the premaxilla, on the facial aspect of the nasal cavity floor (a projection of the vomer below the premaxilla and into the incisive canal is the primitive condition). The first of these features, vertical elongation of the mandibular ramus, is a feature known to be functionally integrated with postcanine occlusal area[86] and is therefore not unique to robust taxa. The most likely explanation for the second feature, the unusual vomeral morphology that characterizes all robust taxa, is that it represents either a primary or secondary effect of changes in the genetic patterning of anterior/posterior dental proportions. In this respect, it is of some interest that *Barx1,* the homeobox gene expressed specifically in presumptive molar dental mesenchyme, is also expressed throughout the neural-crest-derived ectomesenchyme of the presumptive primary and secondary palates.[36] If the dental pattern of *A. aethiopicus/A. boisei* does indeed reflect a reproportioning of anterior and posterior tooth fields during early initiation, then a more distal (anterior) expression of proximal genes, such as *Barx1,* may have repatterned other aspects of upper jaw morphology as well, including the vomeral insertion.

There is little question that phylogenetic studies of early hominid species would benefit from a greater understanding of the developmental basis of the variation in craniodental form observed among taxa. There is also little question that, in the absence of time-successive samples, intimate study of variations in hard-tissue anatomy is the only means of establishing cogent hypotheses of early hominid phylogeny. It is therefore surprising that some researchers interested in this topic have come to conclude, based upon observed discrepancies between the phylogenies obtained from independent cladistic analyses of molecular and craniodental data, that little confidence can be placed in phylogenies generated solely from craniodental evidence.[87] Such a statement is both unfortunate and unwarranted. The craniodental data that have been used in these studies to date consist of ''standard'' measurements of the skull and dentition. Such measurements (examples of which include cranial length, facial height and lower facial prognathism, as well as the lengths and breadths of individual teeth) are advantageous because they can be readily defined across a variety of taxa and are relatively easy to code. They need not and often do not, however, reflect any specific developmental phenomenon directly accessible to the evolutionary process. Instead, standard craniometrics either incompletely characterize a single underlying developmental mechanism (e.g., interorbital breadth, occipital sagittal chord), or, more often, sample more than one (e.g., facial height, midfacial prognathism). In fact, craniometric data such as these are inherently poor morphological surrogates of an organism's underlying developmental program. It is therefore of little surprise that the phylogenies obtained from their cladistic analysis differ from those generated from molecular data.

In considering the phylogenetic origins of *A. robustus* and *Homo* from the perspective of the developmentally significant dental features discussed here, South African *A. robustus* shares with *A. aethiopicus/A. boisei* large premolars and molars in which the distal segments are differentially expanded.[4,5] *A. robustus*, however, lacks all of the derived features of the *A. boisei* dentition (large, distinctive mandibular P4, distinct molar cusps) and, although large, the postcanine teeth of *A. robustus* are smaller than those of the current *A. aethiopicus* sample (Figs. 2,5).[5] This variation in postcanine tooth size implies either that a reduction in tooth size occurred during the evolution of *A. robustus* from an *A. aethiopicus* ancestor, or that the East and South African robust taxa evolved independently. If the latter is true then large, distally expanded premolars and molars evolved in parallel in the two lineages. That this is certainly feasible is indicated by the convergent evolution of a similar suite of postcanine dental features in African suids[88] and perhaps early *Homo*[76,89] and also by the fact that the individual cusps involved in talonid expansion in *A. robustus* and *A. boisei* are not always the same.[4] *A. robustus*, however, also shares with *A. boisei* an absolutely small canine (Figs. 2,5).[76] Therefore an independent-origins model requires that canine reduction occurred in parallel as well. Reduction of the canine to the extent observed in the robust taxa has no obvious functional advantage, but one interesting possibility is that both the canines and incisors in robust *Australopithecus* were reduced to prevent dental crowding and malocclusions.[90] Also relevant to the issue of robust hominid phylogeny is the morphology of the mandibular P3 crown. If the morphology of this tooth in the robust taxa does indeed reflect an independent adaptation beyond the more general restructuring of all postcanine tooth crowns then, in an independent-origins model, it too must have evolved in parallel. Clearly, resolution of the origins of South African *A. robustus* requires a better understanding of the developmental basis of both of the latter dental features.

With respect to the origins and early evolution of the genus *Homo*, the earliest dental evidence of this genus dates to approximately 2.5 mya and is limited to a handful of isolated teeth and fragmentary mandibles.[5] Some of the teeth in this sample fall comfortably in the size range of *A. afarensis*. Interestingly, others are as large as or are larger than those of the more ''megadont'' robust taxa.[5] The somewhat larger sample of pre-*erectus Homo* that dates to between 2 and 1.5 mya is equally variable. The exact meaning of this variation is a matter of some debate. It may be that the large- and small-toothed individuals within this sample represent two distinct species of early *Homo* (*H. habilis/H. rudolfensis*).[76] Alternatively, it may be that the variation present in the pre-*erectus* sample reflects that of a single species (*H. habilis*) characterized by a high degree of body size variation. It is also very possible that the large- and small-toothed forms reflect the individual and inter-demic variation present in a

single species undergoing a rapid reduction in dental dimensions.[5,76] Unfortunately, in light of the small and highly fragmentary nature of the early *Homo* sample currently available, developmental data can as yet contribute little to the resolution of these important issues.

Conclusion

The information provided from studies of dental development in mice is beginning to shed light on the underlying genetic basis of mammalian craniodental evolution. Although many questions remain unanswered, it is nevertheless now possible to consider the divergence of taxa as a consequence of selection acting on specific attributes of the dental development process. Here we have applied principles of mammalian dental development to the early hominid fossil record. In doing so we have identified lucrative new pathways of palaeoanthropological research. Developmental genetics is revealing those genetic pathways that interact to control development of craniodental characteristics. The more that we learn about these pathways the more will genetic information impact the analysis and interpretation of hominid evolution.

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