

# Dental Lamina as Source of Odontogenic Stem Cells: Evolutionary Origins and Developmental Control of Tooth Generation in Gnathostomes

MOYA M. SMITH<sup>1\*</sup>, GARETH J. FRASER<sup>1,3</sup>, AND THIMIOS A. MITSIDIADIS<sup>2\*</sup>

<sup>1</sup>*KCL, Dental Institute, London, United Kingdom*

<sup>2</sup>*Institute of Oral Biology, ZZMK, University of Zurich, Zurich, Switzerland*

<sup>3</sup>*School of Biology, Georgia Institute of Technology, Atlanta, GA*

**ABSTRACT** This study considers stem cells for odontogenic capability in biological tooth renewal in the broad context of gnathostome dentitions and the derivation of them from oral epithelium. The location of the developmental site and cell dynamics of the dental lamina are parameters of a possible source for odontogenic epithelial stem cells, but the phylogenetic history is not known. Understanding the phylogenetic basis for stem cell origins throughout continuous tooth renewal in basal jawed vertebrates is the ultimate objective of this study. The key to understanding the origin and location of stem cells in the development of the dentition is sequestration of stem cells locally for programmed tooth renewal. We suggest not only the initial pattern differences in each dentate field but local control subsequently for tooth renewal within each family. The role of the specialized odontogenic epithelium (odontogenic band) is considered as that in which the stem cells reside and become partitioned. These regulate time, position and shape in sequential tooth production. New histological data for chondrichthyan fish show first a thickening of the oral epithelium (odontogenic band). After this, all primary and successive teeth are only generated deep to the oral epithelium from a dental lamina. In contrast, in osteichthyan fish the first teeth develop directly within the odontogenic band. In addition, successors are initiated at each tooth site in the predecessor tooth germ (without a dental lamina). We suggest that stem cells specified for each tooth family are set up and located in intermediate cells between the outer and inner dental epithelia. *J. Exp. Zool. (Mol. Dev. Evol.) 312B:260–280, 2009.* © 2009 Wiley-Liss, Inc.

---

**How to cite this article:** Smith MM, Fraser GJ, Mitsiadis TA. 2009. Dental lamina as source of odontogenic stem cells: evolutionary origins and developmental control of tooth generation in gnathostomes. *J. Exp. Zool. (Mol. Dev. Evol.) 312B:260–280.*

---

The evolution of a dental lamina, judged essential to produce teeth at the margins of the jaws, may have occurred independently in more than one gnathostome group (Smith and Johanson, 2003). If this character “teeth produced from a dental lamina” occurs more than once on separate branches of a gnathostome phylogeny then teeth would be separately evolved (a homoplastic character) and derive from nonhomologous developmental mechanisms. For example, teeth in extinct placoderm fish (those with jaws but armored) evolved separately from chondrichthyans (sharks and rays) and separately also from those in osteichthyans (fish and tetrapods). Consequently, the homology of the dental lamina in the two living clades of all gnathostomes, chondrichthyans and osteichthyans is also questionable.

The canonical view is that the formation of a subepithelial dental lamina as characterized for chondrichthyans (Reif, '82) defines an oral tooth, with its distinctive replacement patterns, as distinct from a skin tooth (placoid denticle) with replacement of each one on demand when space occurs. It has been assumed that the dental lamina of all osteichthyans (including mammals) forms

---

Grant sponsors: Leverhulme Emeritus Fellowship; European COST Action; Swiss National Foundation; University of Zurich.

\*Correspondence to: HRC DNB, School Biomedical and Health Science, KCL Guys Campus, London SE1 IUL. E-mail: moya.smith@kel.ac.uk or Thimios A. Mitsiadis, Institute of Oral Biology, ZZMK, University of Zurich, Plattenstrasse 11, CH-8032 Zurich, Switzerland. E-mail: thimios.mitsiadis@zzmk.uzh.ch

Received 19 November 2008; Accepted 20 November 2008  
Published online 20 January 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.b.21272

in this way. The genes required for each successive tooth induction may be conserved through evolution (Fraser et al., 2004) although the molecular network that regulates the spatio-temporal tooth pattern in mammals, assumed to be dependent on the dental lamina, could also have evolved more than once in the osteichthyan history. Alternatively, the developmental change could occur in the process of tooth regulation and involve heterochronic and heterotopic shifts in any stage of the odontogenetic gene cascade.

In mammals (e.g. rodents, humans), the dental lamina formation starts as an island of ectodermal epithelial thickening on each side of the primordium of the maxillary and mandibular processes. Additional epithelial thickenings are also created on each lateral border of the fronto-nasal processes. Thereafter, these epithelial thickenings fuse and form a continuous plate of epithelium for both upper and lower jaws in humans (Ooe, '56; Nery et al., '70; Hovorakova et al., 2005, 2007). All teeth will arise from this epithelium (the odontogenic band defined from molecular data) as it later forms a continuous dental lamina. We now have several gene expression studies using *sonic hedgehog (shh)* in fish (Fraser et al., 2004; Smith et al., 2008) and reptiles (Buchtova et al., 2008; Vonk et al., 2008) that show equivalent epithelial thickenings to relate to those of mammals with restriction of the dentate field as an odontogenic band. We have illustrated the odontogenic band in

mice with a molecular definition as the restricted expression of three genes *Pitx2*, *Notch1* and *Islet1* (Fig. 1) and show that this does almost simultaneously give rise to the first tooth bud.

However, different definitions of a dental lamina qualify the epithelial invagination as a permanent or temporary structure and both continuous and discontinuous as well as primary and then secondary. A consensus view of its development and function has been difficult to obtain, also its evolutionary origins have been assumed to have occurred in chondrichthyan fish and shared with osteichthyans (Schaeffer, '75). We now compare the new data with the proposals of Smith and Johanson (2003), who concluded that teeth had evolved at least twice, once in stem gnathostomes (placoderms) and again in crown group gnathostomes. These proposals accepted that the tooth module is homologous at a deep level in the vertebrate phylogeny (within agnathans), but not the mechanisms for patterning the tooth module in space and time, even varying on each dentate bone. Smith and Johanson (2003) and Johanson and Smith (2005) suggested that patterned teeth may have evolved independently in each of the other three clades, Osteichthyes, Acanthodes and Chondrichthyes.

Alongside the localized induction of tooth loci, it is hypothesized that a contra-active (counter-productive) event occurs and involves inhibitory signaling molecules. Reif ('78) and Reif ('84) proposed that the induction and repression of

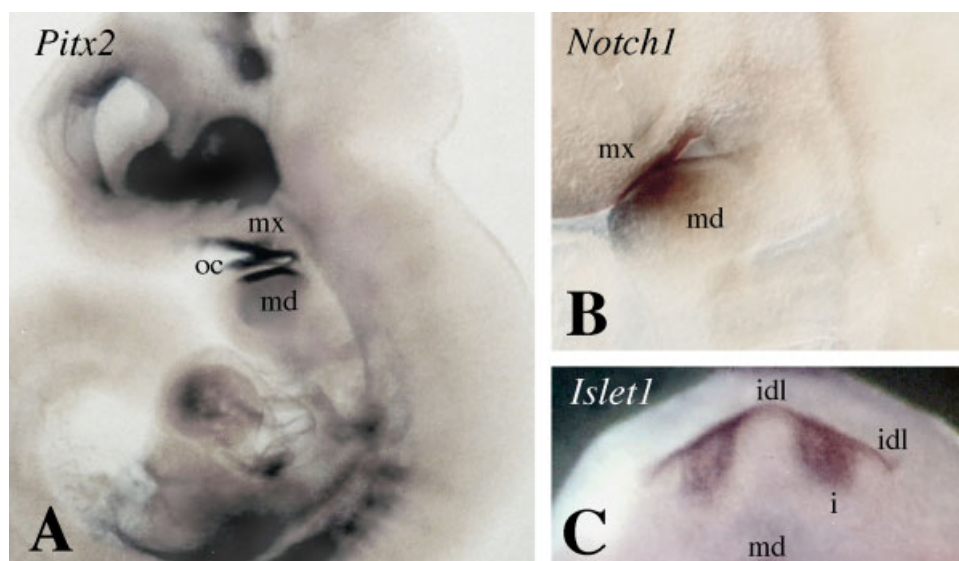


Fig. 1. Molecular definition of the dental lamina in mammals. (A) *Pitx2* expression in the oral epithelium of an E9.5 mouse embryo. (B) *Notch1* expression in the maxillary and mandibular processes of an E9.5 mouse embryo. (C) *Islet1* expression in the odontogenic band where incisors can grow and in the epithelial bud of the incisors of an E13.5 mouse embryo. i, incisor; idl, dental lamina where incisors grow; md, mandibular process; mx, maxillary process; oc, oral cavity.

teeth occurred in a reiterative fashion along the jaw in sharks. Smith (2003) further developed this model with putative genes for antagonism of tooth induction as applicable to all jawed vertebrates. This periodic patterning of iterative structures has recently been demonstrated in Lake Malawi cichlids through coordinated patterns of gene expression in early development, which prefigure the dentition (Fraser et al., 2008). Fraser and colleagues found that the combinatorial epithelial expression of *pitx2* and *shh* determines both initial tooth sites and also future tooth rows, and epithelial *wnt7b* with mesenchymal *eda* are expressed in the interspace regions. Their likely role would be to regulate the spacing of these *shh* positive loci, and illustrates the intrinsic patterning inherent in the odontogenic band together with the collaborative mesenchyme. It has been suggested that there is a fundamental mesio-distal periodicity of the dental lamina in the embryo mouse dentition (Peterkova et al., 2000, 2002) and that the spacing of tooth primordia (vestigial and actual) can be understood by a spatial variation in activator and inhibitor morphogens, fibroblast growth factors (Fgfs) and Shh for the activators and bone morphogenic proteins (Bmps) for the inhibitors. Previously, it had been shown in murine studies (Neubuser et al., '97) that one mechanism for positioning sites for teeth was the combined action of regulation of the mesenchymal gene *Pax9* by ectodermal signals to induce (Fgf8) alongside those to inhibit (Bmp2 and Bmp4), these acting in noncoincidental domains. Furthermore, variations in tooth number in humans are linked to mutations in both *PAX9* and *MSX1* genes, this proposed as a mechanism for the macroevolutionary change in tooth number in mammalian dentitions. This type of spacing mechanism has been experimentally determined in mammals for molar cusp pattern by ectodin, an ectodermal inhibitor of Bmp (Kassai et al., 2005); now spatial mechanisms in nonamniotes have also been investigated (Fraser et al., 2008).

Considering the molecular regulation of early mammalian tooth development (Fig. 1), the Notch signaling pathway has been implicated in cell differentiation (Mitsiadis et al., '95; Mitsiadis et al., '98a). *Notch1* in the rodent incisor is expressed in the cells of the cervical loop area and in the stratum intermedium, whereas *Notch2* is expressed in the cells of the stellate reticulum and the outer enamel epithelium (Mitsiadis et al., '98a). A recent study has demonstrated that activated canonical Wnt signaling induces contin-

uous tooth generation in mice, suggesting that Wnt signaling may be part of the molecular network regulating dental epithelial stem cells (Jarvinen et al., 2006). The general trend of the *Notch* expression is in accordance with the gradient of cytodifferentiation that exists from the cervical loop to the incisal end of the incisor. The proliferation of epithelial stem cells is governed by signals from the surrounding mesenchyme, such as Fgf molecules (especially Fgf3 and Fgf10) (Thesleff et al., 2007). Interactions between the Fgf and Notch signaling pathways would maintain stem cells of the cervical loop in an undifferentiated state. *Midkine* (*MK*), *HB-GAM*, *Bmps*, *Activin* and *Follistatin* are also expressed inside the stem cell niche and are known to regulate its maintenance and functionality through a complex integrative network (Thesleff et al., 2007; Wang et al., 2007; Mitsiadis et al., 2008). How these mechanisms relate to stem cells in the initiation of teeth has not been addressed despite considerable advances in identifying molecular regulation of the stem cell niche in the continuous growth teeth of rodents.

Against this broad comparative background, new data on the development of a dental lamina in chondrichthyans are compared with a synthesis of classic and new data on nonmodel osteichthyans. Although we have little idea of where a stem cell niche resides in the dental lamina, we do know that one exists in the mature but growing tooth germs of rodents. We propose that the stem cells reside in the odontogenic band and become set aside as progenitor cells with the role of regulation of time, position and shape for tooth production. This can be considered as a dental lamina primordium, one that may later develop into a deeper epithelial invagination, a mechanism for retaining a stem cell population for continuous tooth renewal deep to the oral surface.

## CONCEPTUAL FRAMEWORK

The question of most importance here is what regulates the temporal and spatial sequences of the cascades of gene expression along the jaw at each tooth position? Is it the sole property of the epithelial cells to which the name odontogenic epithelial stem cell can be given? Alongside this we cannot ignore the related question of the timing of the involvement of the ectomesenchymal cells, is it before or after the commitment of epithelial odontogenic cells as they are set aside from a stem population with the potential to initiate a tooth?

Relative to these two cell populations is the timing and location of their interaction through differential gene activation to establish the location of an oral/aboral boundary and the possibility that it acts as an epithelial signaling center for tooth pattern. Smith (2003) proposed that a shifting ectodermal–endodermal boundary was the site of signaling to initiate pattern formation for the dentition in all crown group gnathostomes and that this would be the site of the odontogenic band (incipient dental lamina). In this model a reiterative occurrence of tooth initiation and tooth suppression was proposed to occur along the jaw axis. Harris et al. (2006) also proposed that the oral/aboral boundary was a signaling center in the mutant talpid chick, which controls the initiation and suppression of teeth, when in apposition with competent mesenchyme. We suggest that this might be the site of initial odontogenic stem cell segregation that could be tested. It would perhaps be fruitful to test for potential indicators such as those in hair or feather bud initiation (E-cadherin, Wnt, Noggin, Lef1,  $\beta$ -catenin, Bmp, Lhx2) (Barrandon, 2003; Jamora et al., 2003; Rhee et al., 2006; Mitsiadis et al., 2007). Indeed, Huysseune and Thesleff (2004) have suggested the involvement of epithelial stem cells in continuous tooth replacement but placed the site of this as the dental epithelium of either a functional or an erupting tooth and proposed the investigation of the regulatory mechanisms in such an epithelial stem cell niche.

Cobourne and Mitsiadis (2006) have reviewed the topic of primacy of epithelium or neural crest-derived mesenchyme (ectomesenchyme) and succinctly expressed the crucial relationship between oral epithelium and ectomesenchyme as a time-dependent influence of ectoderm (endoderm) on the short-lived plasticity of the ectomesenchyme. This restricted time window is the only opportunity to confer pattern information on the ectomesenchyme, after which specific domains of homeobox expression are fixed and this co-operative activity in space and time is an example of a developmental constraint on tooth production (Mitsiadis and Smith, 2006). An insight into the timing in evolution of this developmental interactive mechanism, gain or loss, is suggested by experimental tissue recombinations between mouse and the toothless bird embryos (Mitsiadis et al., 2003b, 2006; Mitsiadis and Smith, 2006). These experiments have shown that the embryonic chick epithelium could be activated by mouse ectomesenchyme to produce chimeric tooth structures at a

crucial time window between epithelial competence and ectomesenchymal migration time. However, in chick embryos the epithelial competence is not complete as *Pitx2* and *Fgf8* are expressed but *Bmp4* and *Shh* are not (Mitsiadis et al., 2006). Could the latter two molecules be activated by and depend on appropriate signals from mouse ectomesenchyme? It seems that they can and do, as in these chimeras focal expression of chick *Shh* and *Bmp4* is located in epithelial sites adjacent to mouse ectomesenchyme expressing *MK*, *Msx1* and *Pax9* (Mitsiadis et al., 2003b, 2006). From these results it was proposed that in the evolution chick ectomesenchyme has probably lost the signaling ability to induce odontogenesis (Mitsiadis et al., 2006). Another study describing the rudimentary teeth in the chick talpid mutant (Harris et al., 2006) has suggested that these simple conical shapes are of a reptilian type, and only form as the first generation, marked initially by the focal expression of *Shh* and *Pitx2*. The authors suggested that it is the displacement of the oral/aboral site (lateral boundary formation) in the epithelium that determines the novel expression of teeth, as it is now in contact with the competent ectomesenchyme. The model proposed from the mutant chick with atavistic teeth (Harris et al., 2006) was explained as a spatial change in the relative position of a lateral signaling center over competent mesenchyme, leading to loss of teeth in avians. Mitsiadis et al. (2006) also considered that only cranial neural crest (not trunk neural crest) retains odontogenic potential after migration and both these cells and the oral epithelium contribute equally to odontogenesis.

Only dynamic cell lineage studies combined with gene expression data will answer the question of where the regulation of differentiation of the odontogenic cells resides, epithelium or ectomesenchyme. Before that we can only pose the question of which genes may be essential and in what cell population by comparative observations to determine the site of this activity from the histological observations on cell differentiation combined with in situ expression data. A linear expression band of *shh* and *pitx2*, prior to focal expression at tooth sites, has been described in the trout (Fraser et al., 2004), in the talpid chick and alligator (Harris et al., 2006) and in the snake (Buchtova et al., 2008; Vonk et al., 2008) and mouse (Mucchielli et al., '97; Mitsiadis et al., '98b; Cobourne et al., 2004). This expression band is considered as the site for initiation of tooth developmental programs and has been equated with the incipient or true “dental lamina.”

One proposed shared developmental feature of all gnathostome dentitions (Smith, 2003) in the SAM (sequential addition model) model is the pioneer tooth on each of the dentate regions, observed in trout to coincide with first loci of upregulation of *Shh* and *Pitx2* (Fraser et al., 2004, 2006a). It is proposed that this pioneer site of gene expression initiates the cascade of genes regulating time and position of appearance of all subsequent sequentially added primary teeth under the control of this local signaling center. Replacement sets are then regulated from these first teeth at each site on the dentate bone by a system expected to involve sequestration of stem cells locally in each tooth family. These foci of gene expression follow the general expression of the odontogenic band appearing as separate regional areas for each premaxilla and maxilla as well as dentary and basihyal (Fraser et al., 2006a). This regionalized expression is an antecedent of the differential pattern of tooth replacement for each of the toothed regions. Each taxon has a unique signature for the spatial temporal pattern of initiation of the teeth and this may also vary within species for each part of the dentition.

## DEVELOPMENTAL ORIGINS IN CHONDRICHTHYANS

### Observations

Reif ('80a) has shown in embryos of *Scyliorhinus canicula* that the dentition and dermal skeleton (placoid scales) developed from separate and independent secondary fields. There is no gradation in the development of one into the other as has been suggested for their evolution. But even recently the view that posterior teeth resembled modified dermal scales was expressed in connection with the earliest fossil shark dentition (Miller et al., 2003) to support the origin of teeth from dermal denticles. Our own observations are that all the posterior jaw teeth of *S. canicula* do develop from a dental lamina and show that a clear difference in this respect between teeth and skin denticles is maintained. Hence this implies separate stem cell populations (Fig. 2A–C). We propose that by comparison of early development of dermal denticles (Fig. 2B) with that of the earliest teeth (Fig. 2A) an epithelial niche function for stem cells is represented by the dental lamina (Fig. 2C, \*) and may be the essential difference between them.

In an embryo of *S. canicula* of 38 mm length the beginnings of a dental lamina are observed as an epithelial thickening and inpushing of the basal

layer in both upper and lower jaws (Fig. 3A, pob arrows). This is against a condensation of mesenchymal cells (Fig. 3A–C) and located on the ventro-medial and dorso-medial aspects of the palatopterygoid and Meckel's cartilage, respectively. This aggregation of cells is separate from those for chondrogenesis and occurs before the development of any buds (placodes) for teeth or any for placoid scales in the skin or the oral epithelium. The thickening of the basal epithelium extends for about 200  $\mu\text{m}$  across the oral margins of the jaw cartilage with an inflection at its lateral margin. This margin determines the oral/aboral boundary and medially is the prospective odontogenic band. It is continuous along the proximo-distal axis of the jaws and polarized cell division can be seen in an oral to visceral axis (Fig. 3C).

At a later stage of development, just prehatch, when both placoid scales and the teeth are developing, only the latter develop within a deep epithelial dental lamina. There are two tooth germs in each jaw position, the first of which is a rudimentary shell of dentine (Fig. 4A,  $t_1$ ). The distinction is clear between the development of the scales superficially in the basal layer of the skin epithelium and that of the teeth deep to the oral epithelium from a dental lamina in the embayment of the upper/lower jaw cartilage (Fig. 2A, B). Teeth develop between the two layers of the dental epithelium where the cells differentiate into ameloblasts (inner dental epithelium (IDE), ide) and others remain undifferentiated as the outer dental epithelium (ODE) (Fig. 4A, B, ode). However, at the aboral end of the dental lamina the extension of the two layers is evident with many cells between (Fig. 4B, mde) and here a thickening (Fig. 4A, B,  $t_3$  arrow) suggests the site for the dental placode for the next tooth germ. Each thickening is formed on the innermost layer of the dental lamina in elasmobranchs (Fig. 4A,  $t_3$  arrows), close to the cells of the predecessor tooth as the ODE. However, the placodal type thickening of the dental lamina from the ODE adjacent to the cartilage has a differentiated region with mesenchymal cells aligned against it (Fig. 4A, B, arrow). By comparison, scale development is very superficial and involves only the basal layer of the dermal ectoderm (Fig. 2B) in the thickened placode with a large aggregation of mesenchyme cells as the scale papilla.

Posthatch 5-day fish have few erupted teeth (Fig. 4C,  $t_1$ – $t_3$ ), but fully differentiated tissue enameloid, dentine and bone of attachment in three tooth generations and the site for the next tooth placode (Fig. 4C,  $t_4$ ). The dental epithelium merges with but is distinct from the

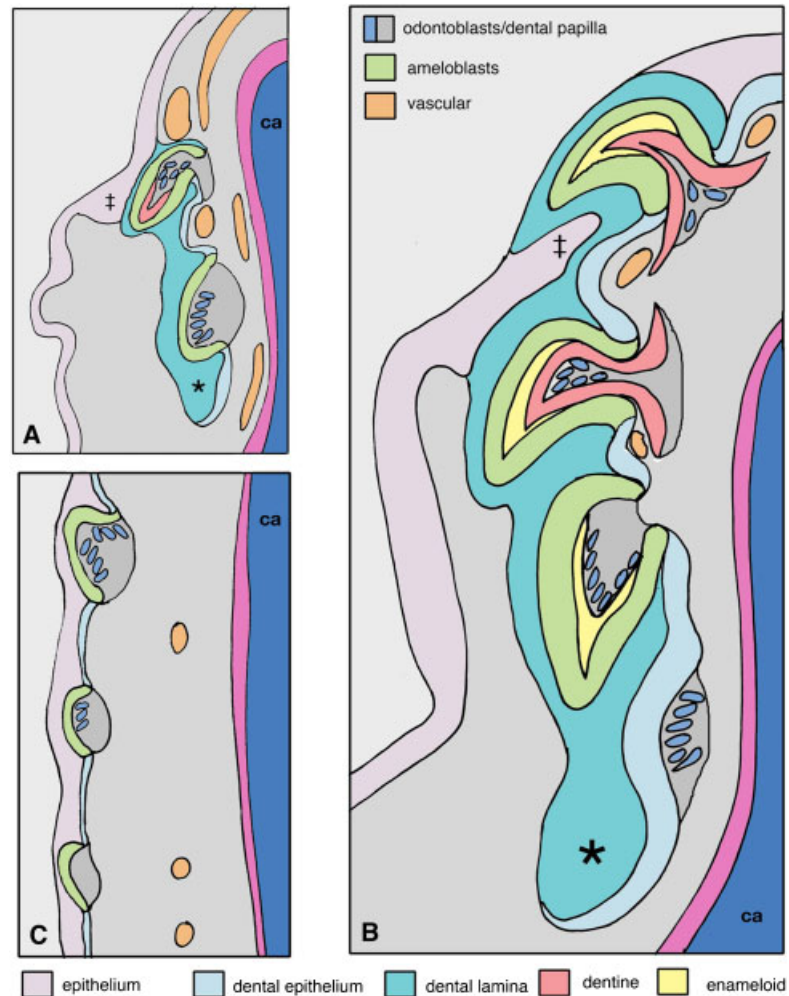


Fig. 2. Development of the dental lamina in chondrichthyans. Comparison of skin denticle (placoid scales) development with teeth in prehatch stages of *Scyliorhinus canicula* embryos. (A) Early tooth development with rudimentary tooth at first position and tooth cap stage at the second, both form within a dental lamina (stage 32). (B) Hatching stage of tooth development (11.5 cm BL) with three teeth in the family and cells of dental epithelium color coded as in the key; a new tooth bud develops from a region of the outer dental epithelium that lies close to the jaw cartilage and within an expanded region of the dental lamina with many intermediate cells proposed as equivalent to the stem cell niche. Regulation is proposed to occur in the dental lamina cells, either at the oral surface ( $\ddagger$  hypothesis 1) or at the growth extension away from the oral epithelium (\* hypothesis 2). ca, cartilage. (C) Dorsal surface of the jaw with early epithelial buds and papillae for dermal denticles but no hard tissue formed, stage 32 of development. BL, baseline.

oral epithelium as the tooth reaches the jaw margins (Figs. 2C, 4C,  $t_1$ ).

### Interpretation and discussion

At the early stages of development of the dentition in embryo sharks, Reif ('76, '80a, '84) recognized differences in the pattern formation from that of the other polyphyodont dentitions of osteichthyans, both lizards and trout. Significantly, rather than one initiator tooth as in osteichthyans in each toothed region, all teeth of the first generation form at the same time and the number

of tooth families is fixed then, suggesting an early spacing control and lack of a tooth-generating pool of stem cells to extend the tooth row. Only with the expansive growth of the jaws does the second row of alternate tooth germs form and these have more typical shapes than the rudimentary tooth germs of the first set (Reif, '78). The process of tooth generation is at a continuous rate and found not to be altered by damage or loss to the functional tooth (Reif, '80b). As discussed by Reif ('80a) regulatory aspects of the dental lamina still need to be explained as teeth only start to develop after the dental lamina forms and with increasing



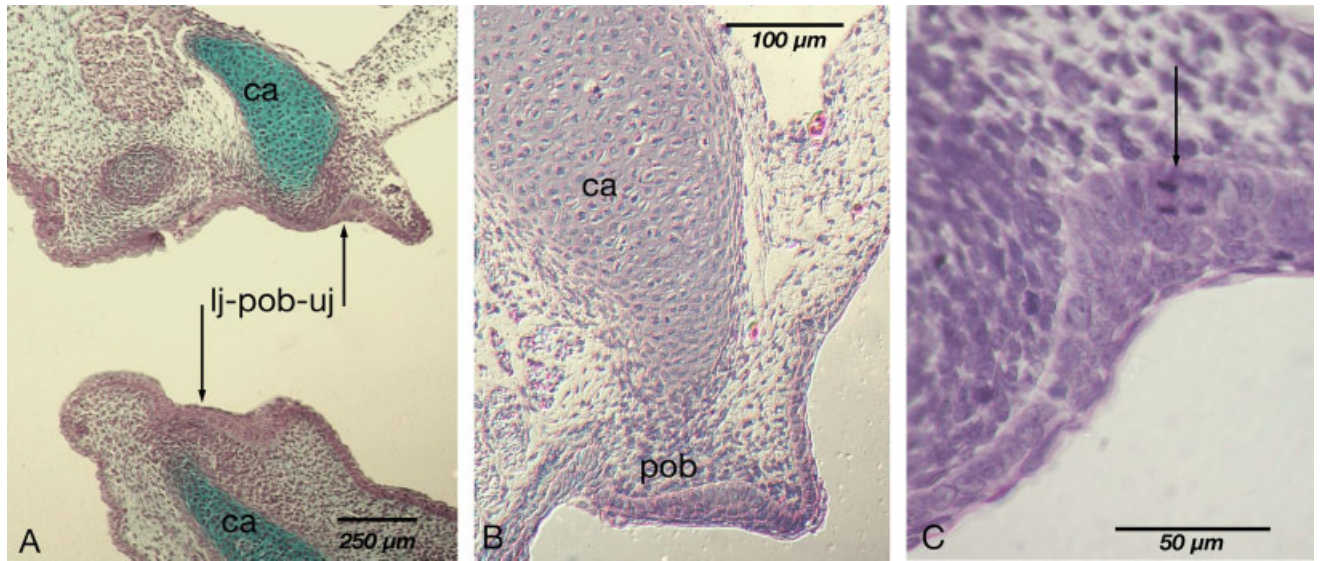


Fig. 3. Initiation of dental lamina in chondrichthyans. Coronal sections of stage 20, 38 mm BL *Scyliorhina canicula* lateral aspect left. (A) Early stage of dental lamina formation as the primary odontogenic band ( $1^\circ$ ,  $\downarrow\uparrow$ ) in both upper and lower jaws (ca, cartilage). (B) More posterior section of palatopterygoquadrate of upper jaw and  $1^\circ$  odontogenic band (pob). (C) Third field in higher power of upper jaw and  $1^\circ$  odontogenic band with polarized cell division in the basal oral epithelial cells ( $\downarrow$ ). BL, baseline.

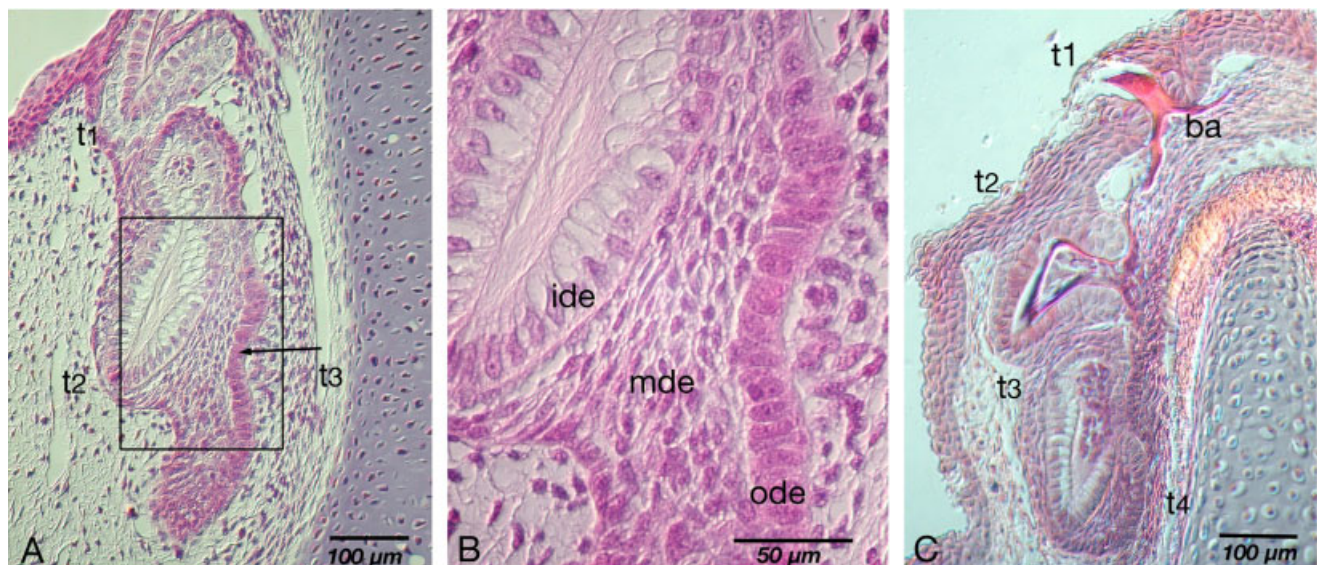


Fig. 4. Tooth family development in chondrichthyans. (A, B) Prehatch vertical section through lower jaw at two tooth stage *S. canicula*. (C) Five-day posthatch at start of tooth eruption. (A) Two teeth in tissue growth stage (ameloblasts, ide) and position of third ( $t_3$ ) as a thickening (dental placode, arrow) of the outer dental epithelium (ode) as a part of the extension of the dental lamina. (B) Field in (A) (rectangle) to show undifferentiated cells of middle dental epithelium (mde) and dental placode with grouped mesenchyme cells. (C) Three to four tooth family stage ( $t_1$ - $t_2$ ) with enameloid on tooth crown, pulp tissue and bone of attachment but  $t_3$  with enameloid matrix only and  $t_4$  as a placodal thickening.

morphological competence of the cells also they only form from one side of the dental lamina (see Fig. 4). We have proposed that the middle group of cells located at the distal end of the dental lamina has the role of regulation to induce a thickening of the ODE

at temporal and spatial focal points (Fig. 4B). For any functional roles we need essential information on cell activity states from gene expression data. New data on the pattern of sonic hedgehog expression in early embryos of the catshark show a

reiterative pattern of this gene coincident with tooth loci that occur first on the dental lamina, then in the location of the first cusp and the secondary cusps (Smith et al., 2009). Prior to this each jaw quadrant has a restricted but initially broad expression domain that corresponds with the early epithelial thickening, the odontogenic band (Smith et al., 2009). This becomes more restricted but extended posteriorly later in embryogenesis to define where the dental lamina will form and determine the positions of the teeth.

As Smith (2003) proposed in the SAM model two sequential sets of teeth provide the alternate tooth rows with intrinsic control for each so that disturbances to one site may result in only one affected tooth. The new study of gene expression for sonic hedgehog in the dental epithelium of each new tooth site shows the fine embryonic timing of these along the jaw and demonstrates that the alternate sites in the embryonic timescale are a later occurrence (Smith et al., 2009). New tooth families interpolated into the original set only arise if a single “protogerm” splits naturally or through injury (Reif, '80b), where these as active tooth induction sites alternate with non-active “intermediate cell clusters,” both recognized on the dental lamina. This site, the dental lamina in chondrichthyans, is well positioned to provide a stem cell niche with middle cells regulated by positional information and in contact with both active and nonactive dental epithelia. The epithelial cells in between the IDE and ODE are postulated to be the site of an odontogenic stem cell population or at least progenitor cells derived from stem cells in the early stage of dental lamina formation before tooth germs form here. These cells could regulate sites and times of odontogenic cell differentiation, both mesenchyme and dental epithelium. The functions or state of differentiation of the middle cells and the putative dental placode has not been characterized with in situ expression data. From histology alone they do seem to be a participant in the organization of cells that will be activated for tooth renewal.

## DEVELOPMENTAL ORIGINS IN OSTEICHTHYAN FISH

### Observations

Although differentiation of the tooth germs proceeds via similar sets of morphological stages as in all tooth developments, it is linked with gene expression data with subtle heterotopic

differences as summarized (Fig. 5). There is an initial thickening of the basal oral epithelium, the odontogenic band, where later at specific loci basal epithelial cells collaborate with condensing ectomesenchyme to form the tooth bud. The localized expression in the odontogenic band occurs prior to tooth formation in *Oncorhynchus mykiss* and is subsequently restricted to the tooth bud loci (Fig. 5; see also Fraser et al., 2006a).

The successional dentition appears from separate and transient thickenings of the ODE of the predecessor teeth (Berkovitz and Moore, '75; Fraser et al., 2006a). Each thickening is an

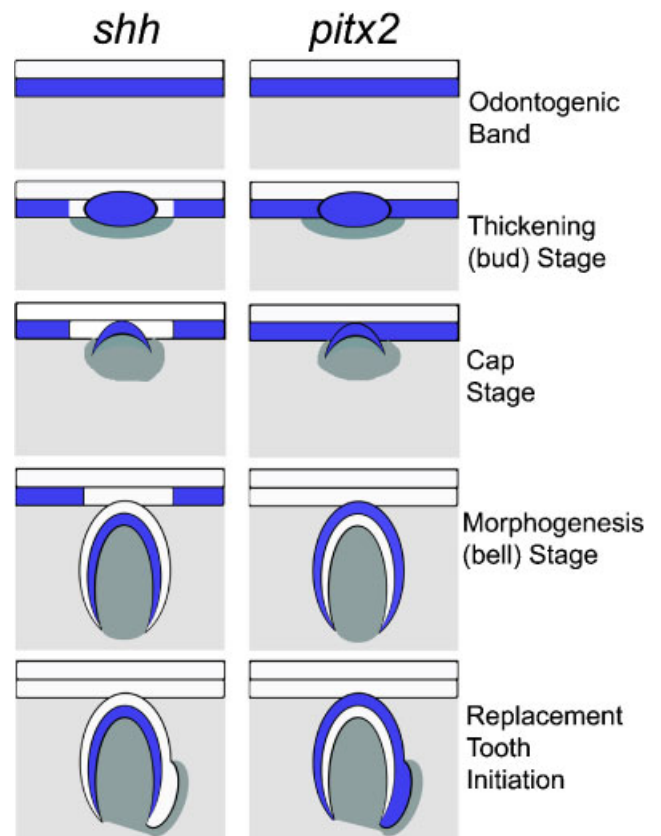


Fig. 5. Tooth renewal from ODE of tooth germ in Osteichthyes. Schematic diagrams from gene expression data of tooth development in the teleost fish *Oncorhynchus mykiss*. Two epithelial genes are represented, *shh* and *pitx2*. Both genes are expressed in the odontogenic band (basal oral epithelial layer). Through the bud stage to the cap stage, the two genes are similarly expressed in the dental epithelium. As morphogenesis progresses *shh* is confined to the inner dental epithelium, whereas *pitx2* is restricted to the outer dental epithelium. The initial step of replacement tooth development is observed as upregulation of *pitx2* concomitant with a thickening of the lingual outer dental epithelium of the predecessor tooth. Although expression of *shh* is localized to the primary tooth placode, it is not related to replacement tooth initiation. ODE, outer dental epithelium.



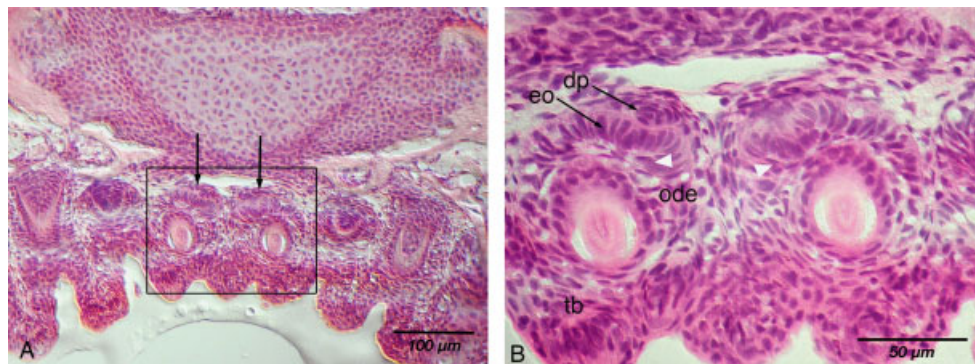


Fig. 6. Tooth germs for renewal in osteichthyans from primary tooth germ. Coronal sections of day 25 trout hatchling *Oncorhynchus mykiss*, premaxillae from the midline with three tooth positions each side and early replacement tooth buds ( $\Downarrow\Downarrow$ ) developed from the dental organ of the preceding tooth. (A) Field with several stages of developing teeth. (B) (From field of rectangle in (A)) two nonerupted teeth either side of the midline, each with a tooth bud, early dental organ and dental papilla (eo+dp) in continuity with the dental organ of the preceding tooth in the series (ode). Site of intermediate cells as potential stem cells (white arrowheads). tb, taste bud.

analogous event to that formed on the innermost layer of the dental lamina in elasmobranchs (Fig. 4, arrows), close to the cells of the predecessor tooth, and a participant in organization of cells activated for tooth renewal. The teleost successional tooth unites both cells of the predecessor tooth (now tooth germ progenitors) and cells of the surrounding mesenchyme peripheral to the dental papilla of the first-generation tooth (Fig. 6) and this ODE could be the site for local regulation of position and time for each replacement tooth. The onset of replacement tooth development coincides with strong upregulation of *pitx2* at this site, together with *bmp4* expression later in the papillary mesenchyme, and is equivalent to the same events as in the first-generation teeth (Fig. 5; see also Fraser et al., 2006a). Interestingly, another epithelial marker *shh* related to the initiation of the first-generation teeth of *O. mykiss* is restricted to the IDE during tooth morphogenesis and is never upregulated within the ODE where the replacement teeth originate (Fig. 5).

### Interpretation and discussion

It has previously been observed that all primary teeth in the trout (*O. mykiss*) develop superficially and separately from the basal layer of the oral epithelium (Berkovitz, '78), identified as the odontogenic band from the expression of both *shh* and *pitx2* in a restricted but diffuse area (Fraser et al., 2004). This precludes the necessity of a dental lamina as classically defined to initiate teeth. In a study showing that a primary dentition forms in the mutant talpid chick, Harris et al. (2006) similarly identify a restricted band of cells

expressing *Shh* and *Pitx2*. The authors suggest that this band represents the site of a putative dental lamina at the oral/aboral boundary. Significantly, Fraser et al. (2006a,b) have also shown from gene expression data that secondary teeth in the trout do not form from a stereotypic dental lamina but from the dental epithelium of the predecessor tooth. The site for the local regulation of position and time for each replacement tooth (Fig. 6A,  $\Downarrow\Downarrow$ ) is linked to that of the preceding tooth until it erupts, and the putative stem cells are shown intermediate between the ODE and IDE of the prior tooth germ (Fig. 6B,  $\nabla\nabla$ ).

These developmental stages are similar across osteichthyan fish although teeth can be located in more than one functional row, and tooth type and replacement mode vary considerably. Primary oral teeth of teleost fish develop superficially within the odontogenic band, as do pharyngeal teeth (Huysseune et al., '98; Jackman et al., 2004). This band is unclear using standard histology but is now recognized by dental epithelia-specific gene expression (Fraser et al., 2004). However, what is most apparent with gene expression is the lateral and medial restriction of this band along the anterior–posterior axis of the oral jaw quadrants, most notably in the rainbow trout (*O. mykiss*) (Fraser et al., 2009). This restricted band does not appear to correlate with the classic descriptions of a dental lamina, as described earlier for chondrichthyans, for which a shallow invagination of the oral epithelium also occurs from the earliest stages.

Recently, more extensive gene expression data have been obtained from several cichlid fish with different morphological patterns to their

dentitions, in which periodic patterning is assumed to be a fundamental process (Fraser et al., 2008). Here, it was found that gene expression patterns varied among the species and prefigured the phenotypic pattern in tooth shape, size and number in a coordinated pattern. They suggested that “developmental tinkering” of the conserved gene network had occurred to effect functional efficiency. The specific questions asked in the latest studies of the oral and pharyngeal dentitions (Fraser et al., 2009) examined modularity to see if they used common or independent gene regulatory pathways. They concluded that there were conserved patterns of tooth initiation in both oral and pharyngeal sites with control from a

common gene regulatory circuit. Interestingly, the pharyngeal gene regulatory pathway operates in a hox-positive environment, whereas in the oral jaws of mice and teleosts tooth development is independent of a hox-patterning program (James et al., 2002; Fraser et al., 2009).

## DEVELOPMENTAL ORIGINS IN URODELE AMPHIBIANS

### Observations

In posthatch larval stages each toothed bone has a set of teeth at many developmental stages (Fig. 7A), with a two-layered epithelial dental lamina linking them all to the erupted teeth and

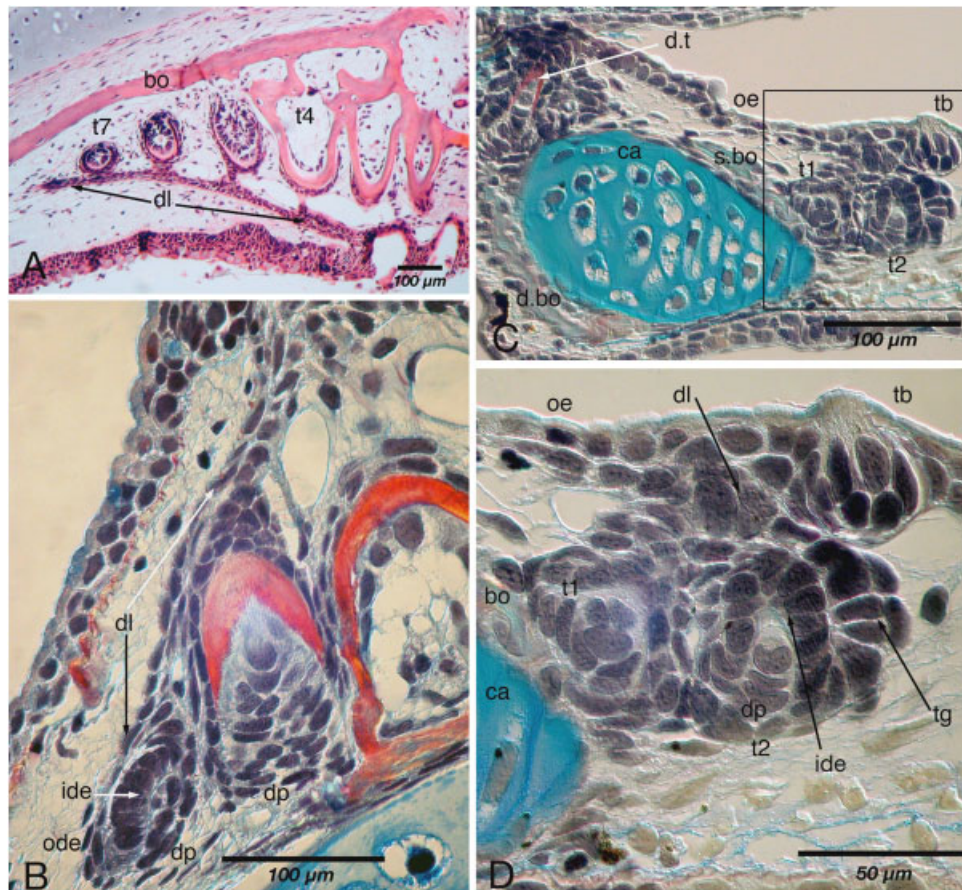


Fig. 7. Permanent dental lamina in amphibian tooth replacement. (A, B) Three-month juvenile stage of *Ambystoma mexicanum*, sections cut vertically along the tooth row, medial is left. (C, D) Twelve millimeter larval stage, vertical sections through lower jaw, medial is right. (A) Palatine tooth field with seven teeth ( $t_4$ – $t_7$ ) all connected to the dental lamina (dl), only three of them are attached to the bone (bo) with one erupted tooth, the distal end of the dental lamina is quiescent (left arrow). (B) Splenial tooth row of lower jaw through two tooth germs, one bud stage with columnar ide cells and new ode cells at the free end of the dental lamina (dl) with dental papilla cells (dp) and one attached tooth base. (C) Early tooth development prior to attachment to the dentary and splenial bones (d.bo, s.bo), Meckel's cartilage (ca), tooth germs ( $t_1$ ,  $t_2$ ) distinct from the oral epithelium (oe) and the taste bud (tb). (D) Dental lamina cells (dl) joining two tooth germs below the oral epithelium (oe) all separate from taste bud (tb), ameloblasts (ide) of new germ ( $t_2$ ), differentiated dental papilla (dp), with thickened outer epithelium as site of next tooth germ (tg).

the oral epithelium. The distal end of the lamina will be either quiescent or active when formed into a bud (Fig. 7B, ide and dp) deep to the oral epithelium, when IDE cells are thickened against a group of mesenchyme cells. The earliest teeth develop prior to perforation of the oral cavity into the gut, from a cluster of cells of the basal oral epithelium, and form an invagination distinct from the taste bud territory (Fig. 7C, D). We propose that this is the primary dental lamina, interpreted as competent cells for first-generation teeth as a set of stem cells from which the successional dental lamina will develop and produce sets of replacement teeth. Each dentate bone has a separate and permanent dental lamina for all regions of the upper and lower jaws (Fig. 7A, C, palatal bone: bo, dentary: d.bo, splenial: s.bo). In the young larval stage the third tooth in the series begins as a thickening of the ODE of the preceding tooth germ (Fig. 7D, tg) much as described for the trout secondary teeth, and the dental lamina is a short connection of few cells linked to the basal epithelial cells (Fig. 7D, dl). This becomes deeper in the connective tissues at the older juvenile stages of 40 mm length (Fig 7B, dl) and each toothed bone of the upper and lower jaws has a separate dental lamina as a double epithelial cell layer. When a new tooth bud forms it is at the free end of the dental lamina but this extension is continuous with the preceding tooth at the appositional growth stage and forms the new ODE (Fig. 7B, dl, ode).

### ***Interpretation and discussion***

The role of the dental lamina and the location of stem cells for tooth initiation may vary significantly between the three different amphibian groups (anurans, urodeles, apodans). Within urodele amphibians we propose that the transition from a tooth-based ODE site for replacement tooth initiation to that separate from this and on the dental lamina can be seen in development. Fraser et al. (2006b) suggested that tetrapod osteichthyans had evolved a persistent dental lamina as a budding process from the dental epithelium.

Anurans are examples with a toothless tadpole stage and activation of tooth initiation occurs only in the larval stage at metamorphosis (Shaw, '79). Urodeles initiate teeth before the bucopharyngeal membrane perforates in the prehatch stage (Chibon, '77), and apodans can be viviparous with fetal teeth, which function in utero (Wake, '76). These may illustrate the heterochronic develop-

mental control of initiation of the dentition and its continued replacement. Heterochrony in development is especially a factor for two other reasons, the change in the rate of replacement of tooth initiation during hibernation and emergence (Miller and Rowe, '73) and the regenerative ability to grow a new dental lamina. Teeth can regenerate from an amputated lower jaw (Graver, '73, '78) or after a section was taken from the premaxilla (Howes, '78). In fact, later Ghosh et al. ('94) and Ferretti ('96) commented that teeth regenerated in the adult lower jaw were the adult-shaped teeth, not a reversal to the larval monocuspid shape. Similarly, amputation of the larval lower jaw resulted in the regeneration of simple monocuspid teeth. Although they suggested that the maintenance of the correct shape was probably owing to hormonal influences, it would seem equally likely that a stem cell population of the dental lamina had been able only to regenerate the correct tooth shape for that series of teeth.

Previous studies on the salamander (*Plethodon cinereus*) have indicated that initiation of teeth for replacement was controlled by local mechanisms rather than external stimuli generating a wave along the jaw of sequential tooth production (Lawson et al., '71; Shaw, '79). In the context of a dental lamina, the first-generation teeth developed in even-numbered positions but were all very superficial (Shaw, '79) and directly formed from the basal layer of the oral epithelium, without first forming a dental lamina. These were upper jaw teeth in relation to the ethmoid and were initiated in a posterior to anterior direction. In relation to the adult dentition of anurans in the frog *Rana pipiens*, Gillette ('55) described a deep epithelial invagination as the dental lamina, each replacement tooth formed from its dorsal border. He described this as remaining separate from the enlarging tooth, and as permanent but with six separate dental laminae one for each dentate bone. The sequence of development in the larval form, as in many examples, is from posterior to anterior, raising a question of the temporal control for this in the stem cell population.

### **DISCUSSION OF DEVELOPMENTAL ORIGINS**

Even as early as Gillette's study on the frog (Gillette, '55), the idea of local control based on each tooth family was proposed, through the continuous odontogenic potential of the dental lamina together with an inhibiting influence from

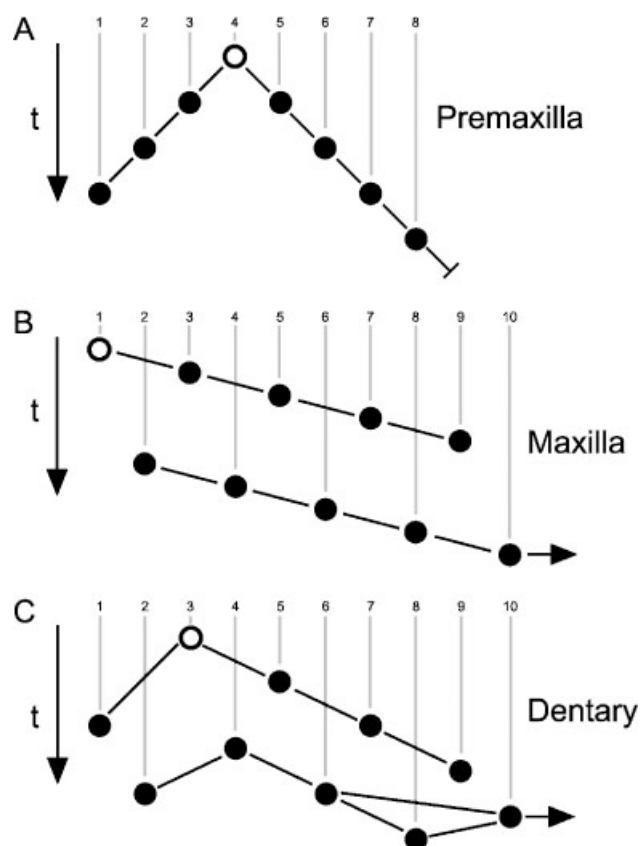


Fig. 8. Differential patterns of tooth initiation in an osteichthyan. Schematic diagrams of the spatio-temporal orders in tooth initiation on three dentate bones of the rainbow trout (*Oncorhynchus mykiss*; modified from Berkovitz, '77, '78). (A) Adjacent tooth initiation on the premaxilla, pioneer tooth (open circle) present in jaw position 4, followed in sequential order in both anterior and posterior directions, but stops at 8. (B) Alternate order of dental initiation related to the maxilla, with the pioneer tooth in position 1 and two sequences of development; the odd-numbered positions 1–9 develop first; even-numbered positions 2–10 follow in a posterior direction and continue till 12. (C) Alternate order of tooth initiation associated with the dentary: the pioneer tooth develops in position 3 followed by the odd-numbered positions 5, 1, 7, 9, and all continue in the posterior direction. t, developmental time.

existing germs. This idea was taken up later by Osborn ('71) for the reptilian dentition and proposed as specific for each tooth family in all gnathostome vertebrates (Smith, 2003). The more recent ideas of local control from a stem cell population in osteichthyans (Huysseune and Thesleff, 2004) consolidate these proposals.

If we assume that a dental lamina arises independently for each region of the jaws, then this assumes some regionalization of the stem cell population and questions both the spatial and temporal controls for this restriction. This is

shown by the different orders of tooth initiation in the rainbow trout for each of the dentate bones as established by Berkovitz ('77, '78) and figured here to show both alternate and adjacent forms of tooth initiation (Fig. 8) and different jaw positions for the pioneer tooth. Greven and Clemen ('85) describe separate dental laminae for each paired bone of the upper jaw and palate in hynobid urodeles. Also described is the regression of the dental lamina on the palatal bone as it becomes toothless at metamorphosis. Only one tooth row and dental lamina are left lateral to the tooth row on each vomerine bone, where the control of tooth positions obviously resides in the dental lamina and not elsewhere. A related problem is how regions form multiple rows of functional teeth (polystichous) rather than a single row (monostichous) and this can vary from region to region in salamanders (Ehmcke and Clemen, 2000) and with time as in cichlid marginal dentitions (Streelman and Albertson, 2006). New data on the genetic regulation of the different cichlid dentitions are now available (Fraser et al., 2008).

Although the site of the stem cell niche for the odontogenic epithelium is unknown, we can assume that the odontogenic band represents a restricted primary set of odontogenic stem cells to ensure continuous and regulated production of teeth related to jaw growth. Confirmation has to await dynamic studies of the genetic program associated with the location of transit cells and other uncertain parameters. A strong basis for these studies would be the data on differential location of initiation of tooth sites by spatial temporal gene expression. One can propose that these primary stem cells become restricted to a dental lamina for tooth renewal as evidenced from cell proliferation studies in mammals (Shigemura et al., '99; Guven et al., 2007). In a previous study, Kronmiller ('95) has shown that explanted mouse mandibles treated with exogenous epidermal growth factor (Egf) contained supernumerary buds in the diastema region. These results support the hypothesis that Egf interacts with the dental lamina in controlling, at least in part, the pattern of the dentition. The equivalent site for a stem cell niche in elasmobranchs and tetrapods would be within the dental lamina, but in the trout we could propose that the site would be within the dental epithelia of the tooth germs themselves.

As previously observed, all primary teeth in the trout (*O. mykiss*; Euteleostei: Salmoniformes) develop superficially and separately from the basal layer of the oral epithelium (Berkovitz, '78; Fraser



et al., 2004). This precludes the necessity of a dental lamina as classically defined to initiate osteichthyan teeth. Fraser et al. (2006a,b) have shown that secondary teeth in the trout do not form from a dental lamina but from the dental epithelium of the predecessor tooth. This has also been shown to occur for the replacement teeth of wild Atlantic salmon (Huysseune and Witten, 2006). It is evident that for these fish tooth placodes for successional teeth form separately in relation to each ODE of the developing predecessor and not from a permanent, continuous epithelial lamina. This sequence of developmental events is depicted as four stages from superficial initiation of the primary tooth, where the tooth germ forms within the oral epithelium (Fig. 9). At the nonerupted stage of the tooth germ, the ODE is the site for the local regulation of position and time for each replacement tooth germ (Fig. 9, i.t.g), where the tooth germ (t.g) remains linked to that of the preceding tooth until it erupts (Fig 9, r.t). At the stage prior to the differentiated tooth germ, the stem site would be where cells are intermediate between the IDE and ODE (Fig. 6, arrowheads). The hypothesized site for cells that can regulate tooth renewal is indicated in the developing tooth germ (Fig. 9, arrowheads), but allows for the participation of both epithelium and ectomesenchyme. It is currently uncertain which germ layer has the dominant role (Cobourne and Mitsiadis, 2006; Soukup et al., 2008).

One candidate gene for the regulation of tooth induction related to the initiation of both primary

and secondary dentitions is *Pitx2*. Along with its known importance during mammalian tooth initiation (Mucchielli et al., '97; Mitsiadis et al., '98b), these observations could indicate that it has an important function as regulator in odontogenesis (i.e. E8.5 in mice; Fig. 1A). *Pitx2* is expressed in ODE cells during morphogenesis, shown to be cells that form the lingual thickenings in trout, which initiate each replacement tooth (Fig. 5). In a study showing that a primary dentition forms in the mutant talpid chick, Harris et al. (2006) similarly identify a restricted band of cells expressing both *Shh* and *Pitx2* and suggest that this band represents the site of a putative dental lamina at the oral/aboral boundary. *Shh* is expressed later during the initiation of tooth formation (thickening of the dental epithelium) and its expression in the dental lamina is restricted to the areas where the future tooth germs will grow (Cobourne et al., 2004). It is noteworthy that the transcription factor *Islet1* is expressed as early as *Pitx2* in the dental lamina but its expression is limited to the part of the lamina where incisors (upper or lower) will grow (Fig. 1C). In later developmental stages, *Islet1* is expressed exclusively in the epithelium of incisors (Mitsiadis et al., 2003a).

Berkovitz and Shellis ('78) were the first to show that the first overt sign of successional tooth development was an outgrowth of the ODE, yet this was in the specialized dentition of piranhas (Euteleostei: Cypriniformes) with rows where teeth are joined as cutting blades. They further

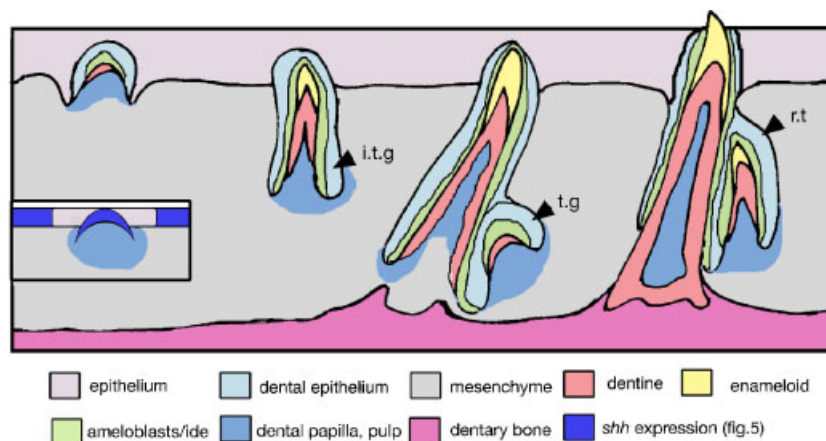


Fig. 9. Diagram of osteichthyan tooth renewal from the dental organ. This depicts the site of secondary tooth initiation ( $\Delta$ ) known from the gene expression in the outer dental epithelium of a prior tooth germ (see Fig. 5). Four stages of the primary tooth are shown from superficial initiation; inset is the stage with *shh* expression to eruption and attachment to the bone. The incipient site for tooth renewal (i.t.g) is in the thickened ODE of the previous tooth germ where the tooth germ (t.g) for the replacement tooth (r.t) develops. The putative site for stem cells ( $\nabla$ ) is within the dental epithelium of the preceding tooth germ. Color code for tissues is the same as in Figure 2 but note the absence of a dental lamina. ODE, outer dental epithelium.

suggested that control was local to each tooth position and regulated by the cycle of tooth development. They described almost synchronous replacement in each jaw quadrant but with a slight antero-posterior gradient. The linkage between each functional tooth and its successor was an epithelial cord. As in other cichlids the epithelial cord may simply physically link the tooth germ to its predecessor and monitor the general expansive growth, but Huysseune and Thesleff (2004) suggested a functional link with stem cells in the tooth crypt epithelium. In the piranha marginal teeth are analogous with a longitudinal row of the chondrichthyan dentition in a continuous series, but importantly this is a functionally convergent mechanism for successional tooth formation. It is fundamentally different in this teleost, nonpermanent and discontinuous, with the absence of a continuous, permanent dental lamina and only one developmental set formed at a time. One detailed study of tooth replacement in the blue fish (Perciformes), another voracious predatory feeder taking pieces cut from the prey, describes the origin of tooth germs from a discontinuous dental lamina (epithelial cords) and their migration through pores in the bone from the crypt (Bemis et al., 2005). The pattern of replacement is strictly alternate here (we assume with local control as in the piranha) and an example of intraosseous development in which the epithelial cord may only be important for tooth location to the functional tooth at the jaw margin and not for regulation.

Initiation of successional teeth was also thought to be under local control by Huysseune and Witten (2006) from a study of three species of teleost and they linked this with the activation of putative stem cells (Huysseune and Thesleff, 2004). In addition, replacement teeth in the pharyngeal dentition of the zebra fish (Cypriniformes) are formed from a successional dental lamina shown to bud from the crypt epithelium of the functional tooth (Huysseune, 2006) and to be a separate downgrowth for each functional tooth, a nonpermanent and discontinuous dental lamina. The functional tooth was thought to be the mechanical link between it and the successor tooth that could trigger the putative stem cells to form a new tooth at the distal end of each epithelial strand and hence was under local control. Clearly, there is a difference between trout and zebra fish but a developmental similarity with piranha in the same family. Disruption of the epithelium through tooth eruption and formation of the separate dental

lamina are linked by Huysseune (2006) from data showing spatio-temporal coincidence between them; each could be an independent outcome of the same molecular event, such as downregulation of E-cadherin. It has been suggested that the activation of stem cells to make the new tooth is linked with eruption of the predecessor tooth (Huysseune and Thesleff, 2004). From the data on the trout of gene activation sites, this link is still local but more remote from eruption itself as the dental placode for the successional tooth forms from the ODE of the unerupted and unattached tooth germ, suggesting quite a difference between the two developmental types.

We should be able to relate newer concepts of stem cell regulation to the older descriptions of tooth development in nonamniote and amniote species to allow an evolutionary model of tooth development to be proposed. From descriptions of *Alligator mississippiensis* by Westergaard and Ferguson ('86), the first teeth are initiated from the oral epithelium and are both rudimentary and nonfunctional. However, in the lizard *Lacerta vivipara* (Osborn, '71), although the first teeth are rudimentary they develop from the free end of a short dental lamina and may be either aborted or resorbed before functioning. It is significant that Osborn ('71) commented that there is an increasing competence of the odontogenic epithelium, but it is implicit from his studies that only the dental lamina has the competence to initiate teeth in a temporal and regional dependent way. It is also apparent that the early pattern is less organized for tooth positions than had been assumed earlier, as they appear to be random at first. Although these teeth are developing, a new dental lamina forms at the lingual side from which a new tooth can be initiated and this is continuous with the ODE of the tooth germ (Osborn, '71), much like that of teleosts illustrated here. In another lizard *Chalcides sexlineatus* (Sire et al., 2002), the replacement dental lamina is shown extending from the lingual side free from the tooth germ but linked to the ODE, and the developing tooth remains joined by a dental lamina to the preceding tooth: an identical arrangement is shown for *Crocodylius niloticus* (Sire et al., 2002).

Since the many descriptions of genes specifying the odontogenic regions in fish, several authors have described the profile of *Shh* expression through developmental stages in a large range of snakes (Buchtova et al., 2008; Vonk et al., 2008). In a study of cell proliferation and apoptosis during craniofacial development of the python

(Buchtova et al., 2007), both first-generation and second-generation teeth are shown to develop from a deep dental lamina. This is noted as a striking difference from mammals. Here, the authors show that cell apoptosis is focused on the central cells of the dental lamina, where also there is a high proliferation rate, as there is in the outer enamel epithelium and the dental papilla. Although the authors ascribe this cell apoptosis to the simple conical morphology of snake teeth, it is perhaps more relevant to suggest that in the central cells of the dental lamina it does indeed relate to the stem cell niche for odontogenic cells proposed in the model discussed here. In two species of python and the corn snake, Buchtova et al. (2008) show that the expression of *Shh* first locates to the odontogenic band as the restricted oral expression, which determines the site of the dental lamina. In this study of initiation and patterning in the snake dentition, by blocking sonic hedgehog signaling with cyclopamine, they also show the dependence on this gene for both initiation and extension of the dental lamina. In a study to locate the developmental position of the poison fang in front-fanged and rear-fanged of numerous species (using 96 embryos), Vonk et al. (2008) used *Shh* expression profiling to visualize the odontogenic band and the origin of the dental lamina. They showed anterior and posterior dental laminae that are developmentally independent and suggested that the latter can become developmentally uncoupled to allow association with the poison gland. Because regulation of tooth type and position is sited first in the odontogenic band, the loss of this in the anterior region for front-fanged snakes can demonstrate that the anterior fangs develop from migration of the posterior-sited fang together with its close association with the poison gland. Vonk et al. (2008) suggest that this front position in the adult results from ontogenetic allometry and implies a posterior evolutionary origin for the front-fanged snakes. In the terminology of our discussion, this suggests a relocation of the specified stem cells for functional fangs.

There is a great deal to learn about replacement tooth production in the most basal living actinopterygian, *Polypterus*, the bichir, before anything more is understood about the origin on a phylogeny of a mechanism for ensuring that a stem cell niche is restricted to odontogenic regions. This primitive ray-finned fish is interpreted to show a derived condition for the dentition from a supposed generalized gnathostome fish, a scattering

of denticles without order according to Wacker et al. (2001). They do not give exact details of replacement tooth formation except to say that it derives from a process from the basal layer of the oral epithelium. It is not clear if this is similar to a nonpermanent dental lamina or if it forms from the side of the ODE of the tooth germ.

## DISCUSSION OF EVOLUTIONARY ORIGINS

Because ordered dentitions are an integral part of gnathostome dentitions, there is an imperative to understand their evolutionary origins. The developmental mechanisms behind this order can be elucidated in the cichlid fish (Fraser et al., 2008) and also in the medaka (Debiais-Thibaud et al., 2007) as they have both oral and pharyngeal jaws. Both these investigations explored the concept that the first teeth to be patterned in this way were in the pharyngeal region and hence this represented the origin of an ancient coordinated regulatory mechanism (Smith and Coates, '98; Smith, 2003; Johanson and Smith, 2005). The gene regulation for the pharyngeal dentition was proposed as the conserved ancient dental gene network, derived from embryonic endoderm but co-opted and subsequently modified for the oral jaw (Fraser et al., 2009). The current research on the embryonic origin of epithelial cells that form the earliest teeth in the Mexican axolotl shows that many oral teeth form from endoderm, whereas others are of mixed or entirely ectodermal origin (Soukup et al., 2008). They discuss these results in the context of the theory above that endoderm conveys the ancient pattern of tooth initiation order, originating from the pharyngeal teeth into the oral tooth germ (Smith and Coates, '98). However, they conclude that as these oral teeth are formed from either ectoderm or endoderm, the regulation of their induction comes from the neural crest in all instances and the epithelium has a secondary role (Soukup et al., 2008). Alongside this issue of an ancient gene network and its deployment in both sets of jaws is the question addressed here, where do the stem cells arise in evolution? Preliminary data have been obtained to show that many of these markers for stem cells are expressed in pharyngeal tooth replacement and, therefore, these may be ancient indicators of a stem cell niche at this evolutionary early tooth proficient site (Fraser et al., 2009). We have not addressed the issue of possible regulation from the neural crest-derived dental papilla, as the

remit of this study is to discuss the origin and development of the dental lamina.

The origin of the dental lamina (putative source of stem cells), whether conserved through evolution or independently acquired, is an important character in phylogenetic analyses of jawed vertebrates among crown gnathostomes. The relationship of placoderms (an entirely fossil group) with all other gnathostomes has reached a consensus view that these are the first stem group in the phylogeny and the sister group of all crown gnathostomes. This is based in part on the absence of renewable oral teeth, a consequence of the closer relationship of chondrichthyans and osteichthyans proposed by Schaeffer ('75), with the "presence of a dental lamina" as one of the five shared synapomorphies (Young, '86), views endorsed by Goujet (2001). With placoderms as the sister group of all other gnathostomes, their features are crucial to determine the shared (plesiomorphic) characters of gnathostomes (Goujet, 2001). There are two corollaries to this phylogenetic position: we need to be able to identify a developmental structure in an exclusively fossil group and distinguish shared characters of all gnathostomes from their distinctive autapomorphies. The feeding structures in placoderms are unique and bizarre and very varied, with their interpretation problematical, but in an inclusive review of all possible types of placoderm dentition the authors concluded that the origin of teeth in placoderms is late in their phylogeny (Johanson and Smith, 2005). In the more derived arthrodires, all three gnathal bones

provide evidence of tooth addition to polarized loci at the end of tooth rows, with gradual degrees of wear along them and a divergent arrangement of rows from the most worn and functional part of the jaws (Fig. 10). The position of new teeth was judged to provide evidence of development from an effective regulatory dental epithelium. This is especially convincing when compared with the new data in trout (Figs. 6 and 9) showing successional tooth buds added to the side of existing teeth without a dental lamina (Fraser et al., 2006a,b). Thus, these new teeth in arthrodires qualify as true teeth, but because of the consensus phylogeny teeth are secondarily acquired in the group (Smith and Johanson, 2003) and hence are divergent structures. However, the latest phylogenetic research reduces the Placodermi to a paraphyletic group (Brazeau, 2008) and patterned oral elements (teeth) occur in the one group arthrodires, but are shared by these and crown group gnathostomes. This would imply that patterned oral teeth originate at this node on the phylogeny before all other teeth in chondrichthyans and osteichthyans. The mechanism by which teeth are produced in placoderms as an ongoing activity, as in tooth replacement in chondrichthyans and osteichthyans, can never be demonstrated but only inferred by comparative observations on the position of new teeth in a growth series of fossil phenotypes. The same applies to acanthodians and a sequence was suggested for the addition of these teeth with a distinctive pattern for these fossil fish (Smith, 2003).

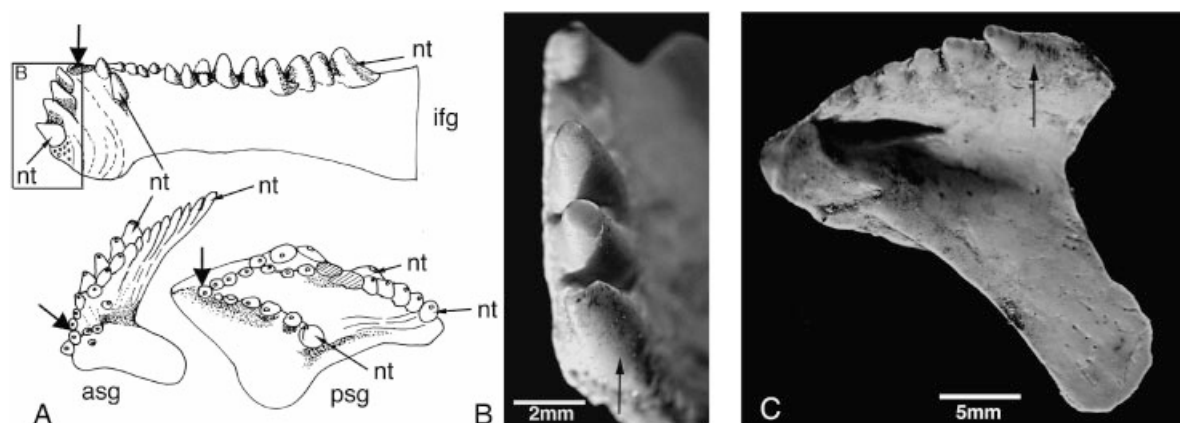


Fig. 10. Dentition in placoderms. Examples of tooth rows on the three gnathal bones (ifg, infragnathal; asg, anterior supragnathal; psg, posterior supragnathal) that make up the biting jaws in brachythoracid arthrodires from the Devonian period, a time when these fish of the jawed vertebrates were dominant. (A) Diagrams to show new teeth (nt) added to positions at the ends of all the previous teeth in the row, as these are retained until worn to a cutting edge; primordial tooth position (large arrow). (B) Field of rectangle in (A), lingual view of infragnathal symphyseal row, new tooth ( $\uparrow$ ). (C) Palatal view of anterior supragnathal with one row and new tooth ( $\uparrow$ ).



The pattern in which new teeth are generated has been proposed as unique for placoderms, as also for acanthodians by Smith (2003), and not the stereotypic pattern as produced from a dental lamina in chondrichthyans. Therefore, the pattern is under the genetic control of the type ensured by the restricted location of odontogenic epithelial cells, but not necessarily in a dental lamina. However, the position of the acquisition of patterned teeth on a placoderm phylogeny is crucial to our understanding of the evolutionary origin of a dental lamina. From the new phylogeny (Brazeau, 2008), it may be a synapomorphy shared with all other gnathostomes. Whether or not patterned teeth, as developed from a dental lamina, occur at the base of chondrichthyans still needs to be tested from a robust phylogeny comparing holocephalans with elasmobranchs as well as new fossil data. This may change the phylogenetic position of the origin of a dental lamina and show that this character is derived within this monophyletic group Chondrichthyes (for a phylogeny, see Stiassny et al., 2004) and, therefore, would not be homologous with other jawed vertebrates.

Similarly, the same will apply to osteichthyans, the putative shared character for all living gnathostomes “teeth from a dental lamina” seems not to be true for teleosts. In actinopterygians, data are not yet available from polypterids and the sturgeon; hence, we do not know if it is present in stem groups at the base of the phylogeny. In addition, among sarcopterygians (tetrapods, etc.), data are needed from the coelacanth and lungfish. The current research on *Neoceratodus forsteri* shows that initial teeth form from the previous tooth germ (Smith et al., 2009) and replacement teeth never form. The occurrence of stem cells in a dental lamina is one step removed from this evidence and has yet to be demonstrated. As an ontogenetic transformation, one proposal is that stem cells, as progenitors of odontogenesis, become restricted to the subepithelial compartment and function as a dental lamina in the classic sense. Alternative developmental strategies will have evolved, which maintain that each taxon-specific pattern, as proposed here, is the value of this comparative approach inclusive of fossil data.

#### DISCUSSION OF A DENTAL LAMINA AND THE HUMAN DENTITION

In most osteichthyan tetrapods, a continuous and permanent dental lamina forms deep to the

oral surface, the free end of which is the site for regulated successive tooth induction. The same is true for mammals except that the primary dental lamina extends deeply backwards in the arc of the jaw and may delay the timing of the extended primary tooth series. An example is that of the delayed initiation of the human third molar until 7 years old, where although not known regulation is assumed to be within this posterior dental lamina. The production of secondary teeth may be eliminated or confined to the anterior tooth set where a successive separate dental lamina forms for each tooth. Consequently, it is an open question where any stem cell niche might be located for successive tooth production in mammals.

A dental lamina is variously associated with the creation of the mammalian primary dentition (Peterkova et al., 2006) and its extended development in the jaw axis for the permanent and nonreplaced molars as in humans. A concept that the phylogenetic memory is retained in the continuous dental lamina and evidenced by the vestigial structures that form in regions such as the postincisor diastema was proposed by Peterkova et al. (2006). This was from a survey of evidence in mammals where dental placodes may occur as focal histological change in the epithelial cells whose position and timing are related to the developing primary dentition. Although we do not know if this occurs in early human tooth development, six thickened epithelial zones are described at 35 days as the beginning of dental lamina formation (Nery et al., '70). These are two on each of the facial processes (lateral fronto-nasal, maxillary and mandibular) and it is not certain which teeth these represent (but see later, Hovorakova et al., 2007). Their term “dental lamina” referred to the “arch-shaped continuous plate of odontogenic epithelium” in upper and lower jaws once the fronto-nasal and maxillary zones had fused at 37 days. It is generally accepted that all upper and lower teeth develop exclusively from the arch-shaped dental lamina. The dental lamina is markedly elevated from the level of the oral epithelium and does not run in a smooth curve. Corresponding to the deciduous tooth germs, eight nodes are projecting from the dental lamina so that the free margin of the lamina shows a wavy contour (Ooe, '56; Nery et al., '70). Three-dimensional reconstruction showed that in hemi-mandibles of 40-day embryos, two thickened epithelial zones are formed: the first corresponds to the region of the deciduous central and lateral incisors and the second to the region of the

deciduous canine and first molar (Hovorakova et al., 2007). The close position of the canine to the lateral incisor, together with the deep notch between them, is characteristic of the lower jaw. The interdental spaces between the developing incisors are shorter in comparison with the interdental spaces between canine and first molar. The most significant interspace is located between the canine and first molar (Hovorakova et al., 2007). This could be explained by the reduction in tooth number during human evolution (basic formula comprises three incisors, one canine, four premolars and three molars). The interspaces gradually diminish and are lost finally because the germs are enlarged more rapidly as the jaws grow. Lack of space is responsible for the zigzag arrangement of the tooth germs (Ooe, '56). In older embryos, a "permanent" dental lamina can be observed, which is projected in a tongue shape on the lingual side of the deciduous set of teeth (Ooe, '56). The development of the lower dentition precedes that of the upper dentition. In the maxilla, the thickening of the oral epithelium starts during the sixth embryonic week. In the upper jaw quadrant, the dental lamina is constituted from two parts separated by a deep gap at the site of the earlier fusion of the medial nasal and maxillary facial outgrowth at 40 days (Ooe, '56; Hovorakova et al., 2005). At 44 days the dental lamina represents a continuous common area where it is possible to determine distinct epithelial swellings corresponding to the primordial germs of the deciduous incisors, canine and first molar. In older embryos, the "permanent" dental lamina is isolated, protruding in a waveform on the palatine side of the deciduous germs (Ooe, '56).

### CONCLUSIONS

The dental lamina as a structure essential for the formation of secondary teeth in all gnathostomes with replacing dentitions can be questioned from the evidence in some osteichthyans. The arrangement of the dental lamina is far from stereotypic as perhaps once thought and hence the location of stem cells could be quite diverse, related to the dental lamina itself, or to a cognate region of the dental epithelium such as intermediate cells in the preceding tooth germ. We can, however, conclude that perhaps there is a cascading competence in the development of the odontogenic stem cells for replacement tooth initiation. This occurs as they become increasingly restricted, either to the dental epithelium of the separate

tooth germs or to the free end of the dental lamina. This competence starts from superficial sites in the oral epithelium, and next resides in the dental epithelium of a specific time stage tooth germ. Later a budding process forms the dental lamina, either separately for each tooth or as a continuous lingual epithelial extension. It is clear that one fundamental character of the dental lamina is to provide epithelial continuity through developmental time. From this epithelium the tooth can be initiated at each cognate site, using local controls to restrict teeth in their positions. It is still uncertain if local control is universally from epithelial cells of the tooth germ or lamina or from the ectomesenchymal population. We propose that the dental lamina, or its equivalent the dental epithelium, provides a protected environment for the odontogenic stem cells created in the initiation stages to become subepithelial and set aside as a reservoir of regulatory cells. We have referred to these cells as intermediate cells in the shark dental lamina and similarly in the trout, but as part of the epithelial tooth germ. Huysseune and Witten (2008) have considered that the middle dental epithelium in the salmon is a source of stem cells and propose that functionally it substitutes for a dental lamina. This has previously been proposed for hair follicle stem cells (Rhee et al., 2006) and to occur similarly in continuous tooth replacement (Huysseune and Thesleff, 2004).

The role of the specialized odontogenic epithelium (odontogenic band) is considered as that in which the stem cells reside and become partitioned; this occurs in all species in which molecular data are available. Positions of the cells for tooth renewal within the dental epithelium may vary between the major clades of jawed vertebrates and be either in a classic dental lamina or part of the dental epithelium of the preceding tooth germ. We have proposed that there is a difference between chondrichthyans with an extended and continuous dental lamina (Smith et al., 2009) and euteleosts where the ODE is a transient dental lamina (Fraser et al., 2006b). Both ensure intrinsic control for time and site of replacement tooth initiation, probably through an intermediate epithelial cell population.

In amphibians, a vertebrate group at the transition to land living, there could be an example of a change in developmental topography (heterotopic mechanism) for the site of gene activation from tooth germ based to dental lamina based, one able to translate into evolutionary change for timing and position of replacement tooth initiation. In

addition, because heterochronic change can occur naturally and in regeneration, this raises fundamental questions about both location and control of stem cell activation.

### ACKNOWLEDGMENTS

M. M. S. was supported by Leverhulme Emeritus Fellowship and European COST Action. The authors thank the members of WG1 for a vigorous discussion on the definitions of a “dental lamina” chaired by Ann Huysseune and Ralph Radlansky. T. A. M. was supported by Swiss National Foundation grant and grants of the University of Zurich.

### LITERATURE CITED

- Barrandon Y. 2003. Developmental biology: a hairy situation. *Nature* 422:272–273.
- Bemis WE, Giuliano A, McGuire B. 2005. Structure, attachment, replacement and growth of teeth in bluefish, *Pomatomus saltatrix* (Linnaeus, 1776), a teleost with deeply socketed teeth. *Zoology (Jena)* 108:317–327.
- Berkovitz BK. 1978. Tooth ontogeny in the upper jaw and tongue of the rainbow trout (*Salmo gairdneri*). *J Biol Buccale* 6:205–215.
- Berkovitz BKB. 1977. The order of tooth development and eruption in the rainbow trout (*Salmo gairdneri*). *J Exp Zool* 201:221–226.
- Berkovitz BKB, Moore MH. 1975. Tooth replacement in the upper jaw of the rainbow trout (*Salmo gairdneri*). *J Exp Zool* 193:221–234.
- Berkovitz BKB, Shellis RP. 1978. A longitudinal study of tooth succession in piranhas (Pisces: Characidae), with an analysis of the tooth replacement cycle. *J Zool Lond* 184:545–561.
- Brazeau MD. 2008. The braincase and jaws of a Devonian “acanthodian” and modern gnathostome origins. *Nature*. DOI: 10.1038/nature07436.
- Buchtova M, Boughner JC, Fu K, Diewert VM, Richman JM. 2007. Embryonic development of *Python sebae*—II: craniofacial microscopic anatomy, cell proliferation and apoptosis. *Zoology (Jena)* 110:231–251.
- Buchtova M, Handrigan GR, Tucker AS, Lozanoff S, Town L, Fu K, Diewert VM, Wicking C, Richman JM. 2008. Initiation and patterning of the snake dentition are dependent on sonic hedgehog signaling. *Dev Biol* 319:132–145.
- Chibon P. 1977. Vitesse de croissance et renouvellement des dents chez les amphibiens. *J Embryol Exp Morphol* 42:43–46.
- Cobourne MT, Mitsiadis T. 2006. Neural crest cells and patterning of the mammalian dentition. *J Exp Zool B Mol Dev Evol* 306:251–260.
- Cobourne MT, Miletich I, Sharpe PT. 2004. Restriction of sonic hedgehog signalling during early tooth development. *Development* 131:2875–2885.
- Debiais-Thibaud M, Borday-Birraux V, Germon I, Bourrat F, Metcalfe CJ, Casane D, Laurenti P. 2007. Development of oral and pharyngeal teeth in the medaka (*Oryzias latipes*): comparison of morphology and expression of *eve1* gene. *J Exp Zool B Mol Dev Evol* 308:693–708.
- Ehmcke J, Clemen G. 2000. Development of the pattern of dentition and dental laminae of Costa Rican plethodontid salamanders (Amphibia: Urodela). *Ann Anat* 182:327–338.
- Ferretti P. 1996. Re-examining jaw regeneration in urodeles: what have we learnt? *Int J Dev Biol* 40:807–811.
- Fraser GJ, Graham A, Smith MM. 2004. Conserved deployment of genes during odontogenesis across osteichthyans. *Proc R Soc Lond B Biol Sci* 271:2311–2317.
- Fraser GJ, Berkovitz BK, Graham A, Smith MM. 2006a. Gene deployment for tooth replacement in the rainbow trout (*Oncorhynchus mykiss*): a developmental model for evolution of the osteichthyan dentition. *Evol Dev* 8:446–457.
- Fraser GJ, Graham A, Smith MM. 2006b. Developmental and evolutionary origins of the vertebrate dentition: molecular controls for spatio-temporal organisation of tooth sites in osteichthyans. *J Exp Zool (Mol Dev Evol)* 306B:183–203.
- Fraser GJ, Bloomquist RF, Streelmann JT. 2008. A periodic pattern generator for dental diversity. *BMC Biol* 14:6–32.
- Fraser GJ, Hulsey CD, Bloomquist RF, Uyesugi K, Manley NR, Streelmann JT. 2009. An ancient gene network is co-opted for teeth on old and new jaws. *PLoS Biol* 7(2):e31.
- Ghosh S, Thorogood P, Ferretti P. 1994. Regenerative capability of upper and lower jaws in the newt. *Int J Dev Biol* 38:479–490.
- Gillette R. 1955. The dynamics of continuous succession of teeth in the frog (*Rana pipiens*). *Am J Anat* 96:1–36.
- Goujet DF. 2001. Placoderms and basal gnathostome apomorphies. In: Ahlberg PE, editor. Major events in early vertebrate evolution: palaeontology, phylogeny and development. London: Systematics Association. p 209–222.
- Graver HT. 1973. The polarity of the dental lamina in the regenerating salamander jaw. *J Embryol Exp Morphol* 30:635–646.
- Graver HT. 1978. Re-regeneration of lower jaws and the dental lamina in adult urodeles. *J Morphol* 157:269–279.
- Greven H, Clemen G. 1985. Morphological studies on the mouth cavity of Urodela VIII. The teeth of the upper jaw and palate in two hynobius-species (Hynobiidae: Amphibia). *Z Zool Syst Evolforsch* 23:136–147.
- Güven G, Gunhan O, Akbulut E, Cehreli ZC. 2007. Investigation of proliferative activity in the developing human tooth using Ki-67 immunostaining. *Med Princ Pract* 16:454–459.
- Harris MP, Hasso SM, Ferguson MW, Fallon JF. 2006. The development of archosaurian first-generation teeth in a chicken mutant. *Curr Biol* 16:371–377.
- Hovorakova M, Lesot H, Peterka M, Peterkova R. 2005. The developmental relationship between the deciduous dentition and the oral vestibule in human embryos. *Anat Embryol (Berl)* 209:303–313.
- Hovorakova M, Lesot H, Vonesch JL, Peterka M, Peterkova R. 2007. Early development of the lower deciduous dentition and oral vestibule in human embryos. *Eur J Oral Sci* 115:280–287.
- Howes RI. 1978. Regeneration of ankylosed teeth in the adult frog premaxilla. *Acta Anat (Basel)* 101:179–186.
- Huysseune A. 2006. Formation of a successional dental lamina in the zebrafish (*Danio rerio*): support for a local control of replacement tooth initiation. *Int J Dev Biol* 50:637–643.
- Huysseune A, Thesleff I. 2004. Continuous tooth replacement: the possible involvement of epithelial stem cells. *Bioessays* 26:665–671.
- Huysseune A, Witten PE. 2006. Developmental mechanisms underlying tooth patterning in continuously replacing

- osteichthyan dentitions. *J Exp Zool B Mol Dev Evol* 306:204–215.
- Huysseune A, Witten PE. 2008. An evolutionary view on tooth development and replacement in wild Atlantic salmon (*Salmo salar* L.). *Evol Dev* 10:6–14.
- Huysseune A, Van der Heyden C, Sire JY. 1998. Early development of the zebrafish (*Danio rerio*) pharyngeal dentition (Teleostei, Cyprinidae). *Anat Embryol (Berl)* 198:289–305.
- Jackman WR, Draper BW, Stock DW. 2004. Fgf signaling is required for zebrafish tooth development. *Dev Biol* 274:139–157.
- James CT, Ohazama A, Tucker AS, Sharpe PT. 2002. Tooth development is independent of a Hox patterning programme. *Dev Dyn* 225:332–335.
- Jamora C, DasGupta R, Kocieniewski P, Fuchs E. 2003. Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature* 422:317–322.
- Jarvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J, Thesleff I. 2006. Continuous tooth generation in mouse is induced by activated epithelial Wnt/beta-catenin signaling. *Proc Natl Acad Sci USA* 103:18627–18632.
- Johanson J, Smith MM. 2005. Origin and evolution of gnathostome dentitions: a question of teeth and pharyngeal denticles in placoderms. *Biol Rev Camb Philos Soc* 80:303–345.
- Kassai Y, Munne P, Hotta Y, Penttila E, Kavanagh K, Ohbayashi N, Takada S, Thesleff I, Jernvall J, Itoh N. 2005. Regulation of mammalian tooth cusp patterning by ectodin. *Science* 309:2067–2070.
- Kronmiller JE. 1995. Spatial distribution of epidermal growth-factor transcripts and effects of exogenous epidermal growth factor on the pattern of the mouse dental lamina. *Arch Oral Biol* 40:137–143.
- Lawson R, Wake DB, Beck NT. 1971. Tooth replacement in the red-backed salamander, *Plethodon cinereus*. *J Morphol* 134:259–269.
- Miller WA, Rowe DJ. 1973. Preliminary investigation of variations in tooth replacement in adult *Necturus maculosus*. *J Morphol* 140:63–76.
- Miller RF, Cloutier R, Turner S. 2003. The oldest articulated chondrichthyan from the Early Devonian period. *Nature* 425:501–504.
- Mitsiadis TA, Smith MM. 2006. How do genes make teeth to order through development? *J Exp Zool (Mol Dev Evol)* 306B:177–182.
- Mitsiadis TA, Lardelli M, Lendahl U, Thesleff I. 1995. Expression of Notch 1, 2 and 3 is regulated by epithelial-mesenchymal interactions and retinoic acid in the developing mouse tooth and associated with determination of ameloblast cell fate. *J Cell Biol* 130:407–418.
- Mitsiadis TA, Hirsinger E, Lendahl U, Goridis C. 1998a. Delta-notch signaling in odontogenesis: correlation with cytodifferentiation and evidence for feedback regulation. *Dev Biol* 204:420–431.
- Mitsiadis TA, Mucchielli ML, Raffo S, Proust JP, Koopman P, Goridis C. 1998b. Expression of the transcription factors *Otlx2*, *Barx1* and *Sox9* during mouse odontogenesis. *Eur J Oral Sci* 106:112–116.
- Mitsiadis TA, Angeli I, James C, Lendahl U, Sharpe PT. 2003a. Role of *Islet1* in the patterning of murine dentition. *Development* 130:4451–4460.
- Mitsiadis TA, Cheraud Y, Sharpe P, Fontaine-Perus J. 2003b. Development of teeth in chick embryos after mouse neural crest transplantations. *Proc Natl Acad Sci USA* 100:6541–6545.
- Mitsiadis TA, Caton J, Cobourne M. 2006. Waking-up the sleeping beauty: recovery of the ancestral bird odontogenic program. *J Exp Zool (Mol Dev Evol)* 306B:227–233.
- Mitsiadis TA, Barrandon O, Rochat A, Barrandon Y, De Bari C. 2007. Stem cell niches in mammals. *Exp Cell Res* 313:3377–3385.
- Mitsiadis TA, Caton J, De Bari C, Bluteau G. 2008. The large functional spectrum of the heparin-binding cytokines MK and HB-GAM in continuously growing organs: the rodent incisor as a model. *Dev Biol* 320:256–266.
- Mucchielli ML, Mitsiadis TA, Raffo S, Brunet JF, Proust JP, Goridis C. 1997. Mouse *Otlx2*/RIEG expression in the odontogenic epithelium precedes tooth initiation and requires mesenchyme-derived signals for its maintenance. *Dev Biol* 189:275–284.
- Nery EB, Kraus BS, Croup M. 1970. Timing and topography of early human tooth development. *Arch Oral Biol* 15:1315–1326.
- Neubuser A, Peters H, Balling R, Martin GR. 1997. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. *Cell* 90:247–255.
- Ooe T. 1956. On the development of position of the tooth germs in the human deciduous front teeth. *Okajimas Folia Anat Jpn* 28:317–340.
- Osborn JW. 1971. The ontogeny of tooth succession in *Lacerta vivipara* Jacquin (1787). *Proc R Soc Lond B Biol Sci* 179:261–289.
- Peterkova R, Peterka M, Viriot L, Lesot H. 2000. Dentition development and budding morphogenesis. *J Craniofac Genet Dev Biol* 20:158–172.
- Peterkova R, Peterka M, Viriot L, Lesot H. 2002. Development of the vestigial tooth primordia as part of mouse odontogenesis. *Connect Tissue Res* 43:120–128.
- Peterkova R, Lesot H, Peterka M. 2006. Phylogenetic memory of developing mammalian dentition. *J Exp Zool B Mol Dev Evol* 306:234–250.
- Reif WE. 1976. Pattern formation and function of the dentition of *Heterodontus* (Selachii). *Zoomorphology* 83:1–47.
- Reif W-E. 1978. Shark dentitions: morphogenetic processes and evolution. *N Jb Geol Palaeontol* 157:107–115.
- Reif WE. 1980a. Development of dentition and dermal skeleton in embryonic *Scyliorhinus canicula*. *J Morphol* 166:275–288.
- Reif WE. 1980b. A mechanism for tooth pattern reversal in sharks: the polarity switch model. *Will Rou Arch* 188:115–122.
- Reif W-E. 1982. Evolution of dermal skeleton and dentition in vertebrates. The odontode regulation theory. In: Hecht MK, Wallace B, Prance GT, editors. *Evolutionary biology*. New York: Plenum Press. p 287–368.
- Reif W-E. 1984. Pattern regulation in shark dentitions. In: Malacinski GM, editor. *Pattern formation. A primer in development biology*. New York: Macmillan. p 603–621.
- Rhee H, Polak L, Fuchs E. 2006. *Lhx2* maintains stem cell character in hair follicles. *Science* 312:1946–1949.
- Schaeffer B. 1975. Comments on the origin and basic radiation of the gnathostome fishes with particular reference to the feeding mechanism. In: Lehman JP, editor. *Problèmes actuels de Paléontologie: Evolution des Vertébrés*. Paris: Colloques Internationaux de Centre National de la Recherche Scientifique. p 101–109.



- Shaw JP. 1979. The time scale of tooth development and replacement in *Xenopus laevis* (Daudin). *J Anat* 129:323–342.
- Shigemura N, Kiyoshima T, Kobayashi I, Matsuo K, Yamaza H, Akamine A, Sakai H. 1999. The distribution of BrdU- and TUNEL-positive cells during odontogenesis in mouse lower first molars. *Histochem J* 31:367–377.
- Sire JY, Davit-Beal T, Delgado S, Van Der Heyden C, Huysseune A. 2002. First-generation teeth in nonmammalian lineages: evidence for a conserved ancestral character? *Microsc Res Tech* 59:408–434.
- Smith MM. 2003. Vertebrate dentitions at the origin of jaws: when and how pattern evolved. *Evol Dev* 5:394–413.
- Smith MM, Coates MI. 1998. Evolutionary origins of the vertebrate dentition: phylogenetic patterns and developmental evolution. *Eur J Oral Sci* 106:482–500.
- Smith MM, Johanson Z. 2003. Separate evolutionary origins of teeth from evidence in fossil jawed vertebrates. *Science* 299:1235–1236.
- Smith MM, Fraser, GJ, Chaplin N, Hobbs C, Graham A. 2009. Reiterative pattern of sonic hedgehog expression in the catshark dentition reveals a phylogenetic template for jawed vertebrates. *Proc R Soc Lond B Biol Sci* 276(1660):1225–1233.
- Smith MM, Okabe M, Joss J. 2009. Spatial and temporal pattern for the dentition in the Australian lungfish revealed with sonic hedgehog expression profile. *Proc R Soc Lond B Biol Sci*. *Proc R Soc Lond B Biol Sci* 276(1657):623–631.
- Soukup V, Epperlein HH, Horacek I, Cerny R. 2008. Dual epithelial origin of vertebrate oral teeth. *Nature* 454:14.
- Stiassny MLJ, Wiley EO, Johnson GD, de Carvalho MR. 2004. Gnathostome fishes. In: Cracraft J, Donoghue MJ, editors. *Assembling the tree of life*. London: Oxford University Press. p 410–429.
- Streelman JT, Albertson RC. 2006. Evolution of novelty in the cichlid dentition. *J Exp Zool (Mol Dev Evol)* 306B:216–226.
- Thesleff I, Wang XP, Suomalainen M. 2007. Regulation of epithelial stem cells in tooth regeneration. *C R Biol* 330:561–564.
- Vonk FJ, Admiraal JF, Jackson K, Reshef R, de Bakker MA, Vanderschoot K, van den Berge I, van Atten M, Burgerhout E, Beck A, Mirtschin PJ, Kochva E, Witte F, Fry BG, Woods AE, Richardson MK. 2008. Evolutionary origin and development of snake fangs. *Nature* 454:630–633.
- Wacker K, Bartsch P, Clemen G. 2001. The development of the tooth pattern and dentigerous bones in *Polypterus senegalus* (Cladistia, Actinopterygii). *Ann Anat* 183:37–52.
- Wake MH. 1976. The development and replacement of teeth in viviparous caecilians. *J Morphol* 148:33–63.
- Wang XP, Suomalainen M, Felszeghy S, Zelarayan LC, Alonso MT, Plikus MV, Maas RL, Chuong CM, Schimmang T, Thesleff I. 2007. An integrated gene regulatory network controls stem cell proliferation in teeth. *PLoS Biol* 5:e159.
- Westergaard B, Ferguson MWJ. 1986. Development of the dentition in *Alligator mississippiensis*. Later development in the lower jaws of embryos, hatchlings and young juveniles. *J Zool Lond* 212:191–222.
- Young GC. 1986. The relationships of placoderm fishes. *Zool J Linn Soc* 88:1–57.