REVIEW ARTICLE

Evolution of Developmental Pattern for Vertebrate Dentitions: An Oro–Pharyngeal Specific Mechanism

GARETH J. FRASER[1]* AND MOYA MEREDITH SMITH[2]
[1]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, United Kingdom
[2]Biomedical and Health Sciences and Dental Institute, King's College London, London, United Kingdom

ABSTRACT
Classically the oral dentition with teeth regulated into a successional iterative order was thought to have evolved from the superficial skin denticles migrating into the mouth at the stage when jaws evolved. The canonical view is that the initiation of a pattern order for teeth at the mouth margin required development of a sub-epithelial, permanent dental lamina. This provided regulated tooth production in advance of functional need, as exemplified by the Chondrichthyes. It had been assumed that teeth in the Osteichthyes form in this way as in tetrapods. However, this has been shown not to be true for many osteichthyan fish where a dental lamina of this kind does not form, but teeth are regularly patterned and replaced. We question the evolutionary origin of pattern information for the dentition driven by new morphological data on spatial initiation of skin denticles in the catshark. We review recent gene expression data for spatio-temporal order of tooth initiation for *Scyliorhinus canicula*, selected teleosts in both oral and pharyngeal dentitions, and *Neoceratodus forsteri*. Although denticles in the chondrichthyan skin appear not to follow a strict pattern order in space and time, tooth replacement in a functional system occurs with precise timing and spatial order. We suggest that the patterning mechanism observed for the oral and pharyngeal dentition is unique to the vertebrate oro-pharynx and independent of the skin system. Therefore, co-option of a successional iterative pattern occurred in evolution not from the skin but from mechanisms existing in the oro-pharynx of now extinct agnathans. *J. Exp. Zool. (Mol. Dev. Evol.) 314B, 2010.* © 2010 Wiley-Liss, Inc.

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Currently there is active discussion and debate over the theories that might explain how the system for dentitions evolved from skin denticle systems, especially at the jaw margins. In order to assess the relationship between dental and skin denticle patterns, we present data on patterns of skin denticles in the catshark (*Scyliorhinus canicula*) and emphasize that, although almost nothing is known about molecular regulation of patterning in the skin (Johanson et al., 2007, 2008), at least from the structural pattern for spacing and replacement there is no obvious organization of these denticles that could be suitably co-opted and converted into a pattern that might have evolved into "tooth sets."

The dentition of jawed vertebrates is assumed to have evolved together with jaws with teeth classically considered as derived from the scattered skin denticles (Reif, '82). This theory was reconsidered from new fossil evidence in an agnathan

*Correspondence to: Gareth J. Fraser, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom.
E-mail: g.fraser@sheffield.ac.uk

group–Thelodonti (Smith and Coates, '98) when it was suggested instead that the dentition evolved from sets of denticles on the pharyngeal arches in existence before jaws had evolved. In fossil jawed vertebrates, occurrence of many separate tooth whorls in a dentition at the jaw margins was cited as developmental evidence for successive tooth formation, each whorl developed from a separate dental lamina (Reif, '82). Also, an organized pattern is seen in toothed pads on the 3rd–6th pharyngeal arches of the Carcharinidae as denticles "lined up in rows" (Nelson, 1970: Fig. 15). Following the same principal, the iterative sets of denticle whorls in the pharynx of agnathan fossils, (e.g. *Loganellia scotica*) homologous with those of sharks (Smith and Coates, 2001), were proposed to be pre-adapted for a tooth succession mechanism (Smith and Coates, '98, 2001) in a way that skin denticles were not. Hence, the developmental mechanism for pharyngeal denticle sets (sequential, joined odontode units) could be co-opted from "inside" the oro-pharynx to the margins of the oral jaws to pattern successive sets for teeth. This was proposed as a new theory to that of co-opted "outside" skin denticles forming dentitions on the inside, as skin denticles did not have similar time-and-space linked sets for replacement (Smith and Coates, 2001). This became known as the "inside-out theory" ("out" meaning only to the jaw margins), where sets from the pharynx are adapted to function at the margins of the jaws, as opposed to the classical "outside-in" theory where skin denticles came into the mouth to function as oral teeth, to distinguish the different concepts. More recently considering and evaluating both theories, Fraser et al. (2010) proposed an "inside and out" theory in which teeth and skin denticles are independent units that emerged separately (convergent) in endodermal (oropharyx: inside) and ectodermal (epidermis: outside) locations. In both locations elaboration of neural crest fates (ectomesenchyme) allowed union with the different epithelial tissues to form odontodes as separate and independent systems. Thus, in this view it is unlikely that skin denticles evolved into oro-pharyngeal teeth (Fraser et al., 2010). This theory predicts that gene expression groups and ultimately a gene network from the emerging and existing cell types (neural crest and any epithelium, respectively) came together in space and time to cause "collaborative innovation events" (merging networks for a new purpose). This gave rise to multiple odontodes (i.e. grouped skin denticles and teeth grouped as whorls) in numerous and varied locations in vertebrates (Fraser et al., 2010). Some of these theories have begun to be addressed by consideration of both "an ancient and a core gene network" for the evolution and development of the individual tooth (Fraser et al., 2009), but deeper still in phylogenetic time there is "a core gene network for skeletogenesis" (Hecht et al., 2008). However, much less is known about the genes that organize initiation of the unit teeth in space and time as a unified dentition.

Nearly all the information gathered on tooth development and associated genetic organization has emerged from studies on the mammalian dentition, specifically the mouse (*Mus musculus*). More recently, comparative gene expression data focused on the developing dentition has been collected from osteichthyan and chondrichthyan fish models including: the rainbow trout *Oncorhynchus mykiss* (Fraser et al., 2004, 2006a,b); the medaka, *Oryzias latipes* (Debiais-Thibaud et al., 2007); the Mexican tetra, *Astyanax mexicanus* (Jackman et al., 2004; Stock et al., 2006); zebrafish, *Danio rerio* (Jackman et al., 2004; Stock et al., 2006); a selected set of Lake Malawi cichlid species (Fraser et al., 2008, 2009) and also, studies limited to a single gene (*sonic hedgehog* (*shh*)) from the catshark, *S. canicula* (Smith et al., 2009a,b,c), and from the lungfish, *Neoceratodus forseri* (Smith et al., 2009a,b,c). These all highlight vast genetic conservatism in tooth development. From these collective studies, it is emerging that much of the genetic signature that defines a tooth across the vertebrate clades is well conserved. This general rule, however, does have some intriguing exceptions. The dentitions of osteichthyan fish (specifically teleost osteichthyans) exhibit specific genetic differences during development compared with that of mammals (Jackman et al., 2004; Laurenti et al., 2004; Borday-Birraux et al., 2006; Fraser et al., 2008, 2009). Even among teleost groups, divergence of gene expression patterns exist during tooth development to permit variation of type (Fraser et al., 2008). During the evolution of variety, diversity and adaptation of oro-pharyngeal teeth in teleost fish disparity has occurred in gene expression even between oral and pharyngeal tooth sites of the same individual (Fraser et al., 2009; Gibert et al., 2010).

An important issue to evaluate when discussing the evolution of the dentition is the genetic spatial and temporal parameters of those dental patterns that make each higher-level clade distinctively different (Smith, 2003). Little is known about the genetic regulators that orchestrate the intricate and precise patterns of vertebrate dentitions as they develop in time and space (Fraser et al., 2008). The initiation of teeth in one or multiple rows of primary teeth is often set in alternate odd and even unit positions and the successive teeth for each position of the dentition is set up for replacement (Fraser et al., 2004, 2006a,b; Jarvinen et al., 2009). Some advances have been made to our understanding of dental evolution in mammals, especially at the individual tooth level. Utilizing the mouse molars, Kavanagh et al. (2007) discovered the genetic mechanism behind the relative size and number of molar teeth. This study showed how levels of inhibitors and activators can affect tooth development and, therefore might produce adaptive change to the dentition as a whole. Alteration in gene regulation is thought to be a major mechanism for the production of great diversity in phenotypic morphology but little is known about conservation of these mechanisms regulating gene expression in nonmammalian vertebrates. Chan et al. (2009) presented that "many genes show conserved human/fish (non-tetrapod) expression". They conclude that strong evolutionary constraints exist in tissue-specific gene expression but caution that there are major challenges to

understand the precise mechanisms behind similar patterns of expression.

Having outlined why teeth and skin denticles are separate systems but made of similar units, with similar/shared genetic ("core" genes) and tissue characters, we address how these structures (skin denticles and the dentition) differ in their temporo-spatial organization and patterning mechanisms. Chondrichthyans have been used to formulate theories of tooth evolution (Reif, '82) due to the presence of both separate skin denticles and separate teeth in a dentition (none are attached to dermal bone). Although, it is unfortunate that currently little is known about gene expression and the interactions that lead to the formation of skin denticles. Inferring conservation of gene expression from teleost scale development, Fraser et al. (Fraser et al., 2010) discussed the separate evolution of skin-born odontodes vs. oral odontodes. However, we currently lack the information to conclusively determine the genetic relationships of all vertebrate odontodes. Here, we present new data comparing the temporo-spatial pattern of chondrichthyan skin denticles with that of the vertebrate dentition within a framework that considers essential concepts. These data provide further evidence for the separate evolution of skin vs. oro-pharyngeal odontodes during vertebrate diversification.

EVOLUTION OF DEVELOPMENTAL MECHANISMS BY CO–OPTION

Theoretical Concepts

The established but now well-challenged view of co-option from denticles in the skin is that they are turned into a more evolved system of teeth in a dentition. This is characterized by sequential iterative initiation along the jaw and successive initiation of tooth families for replacement of functional teeth. This should rely not only on evidence from morphology but the underlying molecular control of pattern regulation. At a deeper level of homology, a system of dentine-based modules (odontodes) is present in the skin and in the oropharynx of early, jawed vertebrates, which likely develop with a common core set of genes. This is an essential genetic concept to any discussion on how dentition patterns may have evolved in agnathan vertebrates, despite the fact that extant agnathans (lacking any odontodes) cannot offer any information. Morphological observations on pattern order for odontodes are mostly based on evidence from the fossil record, as living agnathan representatives (hagfish and lampreys; cyclostomes) are without any mineralized skeleton. At one level, co-option of the tooth module with the associated regulatory gene network, from a developmental unit that is present in some of the earliest vertebrate body plans, is perhaps deep in the phylogeny of vertebrates. Developmental modules are those that can undergo temporal transformation in development and can also undergo evolutionary transformation. The principles of how developmental modules may undergo evolutionary transformation is set out by Raff (Raff, '96). He discusses the evolution of feeding as a co-option event at both the morphological and genetic level. For the jaws and dentition this co-option may be of a serial homolog as are the gill arches, but there are also separate serial homologs in the ordered sets of denticles on each arch (Nelson, '69).

Genetic Experimental Studies

The genes that accompany this co-option of one system to function as another are modified in development, as is the "Hox" code for each pharyngeal segment (Hunt et al., '91; Miller et al., 2004). This idea has been taken up in experimental manipulation studies in the mouse (James et al., 2002) and also to interpret comparative expression data in cichlids (Fraser et al., 2009). In both, teeth expected to develop in a Hox-negative environment as postulated to come from the skin teeth, can form in a Hox-positive environment as in the endodermal pharyngeal sets. This would occur if the serial addition pattern mechanism (sequential addition model (SAM); Smith, 2003) had been co-opted from the pharyngeal denticle sets instead of from the skin. Part of this theory focuses on the developmental layer from which the pattern was generated, ectoderm, endoderm (Johanson and Smith, 2005; Huysseune et al., 2009), or from the union of the two tissue layers (Huysseune et al., 2009). As the emphasis changed from a skin-derived ectodermal system to one from the pharyngeal endoderm then more attention was paid to more suitable animal models than the elasmobranches, as for instance among the teleosts, and questions were directed at the importance, or not, of the embryonic germ layer to generate pattern information to a developing dentition (Soukup et al., 2008; Fraser et al., 2009, 2010).

It follows that as genetic information is mostly acquired from studies specific to the oral jaws, (as most amniotes house a dentition restricted to the oral jaws) they should be extended to include those with pharyngeal jaws as well (pharyngognaths) among the actinopterygians. Interestingly, the majority of the genetic information on the developing dentition comes from a collection of seemingly unfortunate dental model vertebrates. The mouse (*M. musculus*) has a single set of highly specialized teeth with no replacements (Thesleff and Sharpe, '97) and the zebrafish (*D. rerio*) has no oral dentition and teeth restricted to the lower pharyngeal jaws (Huysseune and Sire, '98; Huysseune et al., '98; Huysseune, 2006; Stock et al., 2006; Stock, 2007), although teeth are replaced frequently. More appropriate dental models have come to be recognized, like the cichlids, medaka and the Mexican tetra, offering a better understanding of general vertebrate dental systems, ones which include oral and pharyngeal dentitions and multiple rounds of tooth replacement. Evolutionary theories of co-option can be assessed from these studies, where more ancient systems of patterned structures, including their regulation, can be modified and changed in development to function and adapt to new uses.

SHARK SKIN DENTICLE PATTERNS AS A CO–OPTION MODEL

### Results From X-ray Microtomography

Because there is no certainty of whether, or not, a pattern can exist from the beginning of denticle initiation and continue with their addition in shark skin, we have investigated the skin denticle coverage in a standard area of the flank below the dorsal fin in six specimens of juvenile catsharks (*S. canicula*) (Fig. 1A and B). As demonstrated by volume rendering in microtomographs generated by X-ray scans at 8 μm intervals, there is no obvious regular geometric pattern to the skin denticle arrangement in juvenile shark. The region studied in the dorsal flank included a scatter of both mature and forming denticles (Fig. 1C and D). The average result of 1,000 random points covering the same area was used as a simulation model to compare with skin denticle pattern, where each was marked as a small spherical point, and a minimum distance of 0.25 mm was set to represent the size of the skin denticles.

Differences were detected in their initiation times by observing the stages of growth of the base from the visceral side, and the degrees of mineral density. Denticles that are early in development have less dense mineralization and also show a wide pulp cavity (Fig. 1D). As mineral density increases there is a gradual closure of this opening restricted to the blood vessels (Fig. 1D). It is apparent from these developmental observations that the denticles are not added sequentially in rows, nor is their timing of addition at regular intervals, nor is their size constant. Thus, the pattern mechanism for ordered rows in time seems not to exist (Fig. 1C). This randomness of size and spacing can be seen in a surface view of the skin denticles as well as the view from the bases (Fig. 1A–C). However, the shape and polarity of each skin denticle is constant and can be determined from the proportions and direction of the central tallest cusp, relative to the accessory cups (measurements not included here). The polarity of each unit is theoretically regulated by interactions between neighboring cells and tissues with positional co-ordinates as part of the field position.

Bioinformatic Analysis of Results

The intrinsic biological interest of pattern regulation is in the mechanism for information exchange. However, the mechanism for information exchange and pattern regulation is unknown.
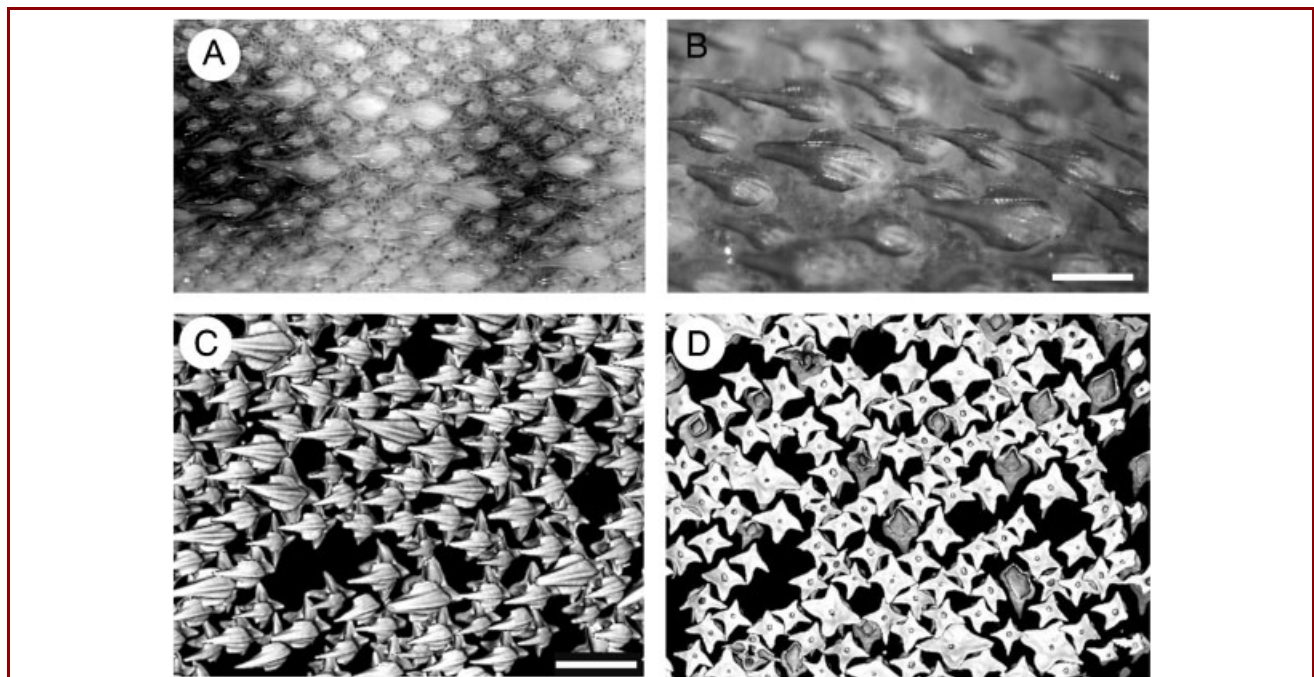
Figure 1. Shark skin denticle arrangement and superficial growth pattern. (**A–C** Rostral to the right, **D** rostral at top). (A) Photomicrograph of juvenile (BL–27.5 cm) spotted catshark (*Scyliorhinus canicula*) flank skin with denticles between two dark pigment spots: all show similar shape and polarity but are of irregular size and uneven spacing, (B) higher magnification field. (C) Micro-Xray tomogram of mineralized skin denticles, outer surface view; (D) visceral surface view. Size varies and spacing is irregular, also the stage of formation of each new denticle is different, as shown by forming, less mineralized ones (grey) with the open pulp cavity at various stages of base development, contrasting with the small pulp canal opening in the majority of denticles with star shaped fully formed bases. Scale bar in C = 500 μm (same for A and D). Scale bar in B = 200 μm.

Recognition of the pattern might go some way toward understanding this. As no obvious pattern was found in the microtomographs either from 3D rotation or snapshots in selected planes, the sample image was analyzed with a computer program to find patterns by comparison with a true but hypothetical random set of spheres. Using bioinformatic techniques (cluster analysis algorithm), we did produce scatter plots and nearest neighbor sub-graphs analyzed from 153 single points on the skin denticle bases. Lines joining the nearest two denticles to each denticle do not reveal a pattern by itself other than that each denticle is at least a minimum distance from one another and no single denticle is further away than a maximum distance (figures not given here). However, comparison with the random set did reveal a difference. The simulation model of a thousand spheres is significantly more spread out and there are many more isolated clusters of skin denticles where there are far fewer isolated points (denticles). We interpret this as regulation of the distance between the denticles by some biological patterning constraints. Possibly there is a requirement to be near other denticles to initiate denticle formation. This would depend on theories of inhibition zones around new denticles and concentration gradients within the epithelium to induce new denticle morphogenesis from competent subsets of mesenchyme cells. This theory is supported by research into wound healing in sharks (Reif, '78). Reif showed that in superficial wounds on the Nurse Shark (*Ginglymostoma cirratum*) and Leopard Shark (*Triakis semifasciata*) new skin denticles formed from the sides of the wound inwards. This creates the impression that it is essential to have denticles nearby to initiate new skin denticle growth, at least in wound healing. Established denticles probably provide the cells for new denticle development in the re-growth area.

Comparison of the morphology and spatial arrangement of skin denticles at a growth stage at the mouth margin with the alternate tooth sets of the dentition (Fig. 2A), formed by replacement sets from the inner, or lingual side reveals two apparently differently ordered systems. A dentition is ordered from early development both in time and space and the skin armour forms from scattered denticles that become packed into the space available, when free through growth (Fig. 2A, sk.d).

Current Hypotheses

One explanation for control of new sites of denticle formation is the "nearest neighbor hypothesis" (Reif, '85; Johanson et al., 2008), likely making use of local genetic and cellular components. Possibly there is a requirement to be near other denticles to initiate denticle formation from putative stem cell-like populations. This would depend on inhibition zones around new denticles and molecular concentration gradients within the epithelium to induce morphogenesis of new denticles from competent subsets of mesenchyme cells. The current observations have revealed that there is nothing in denticle arrangement to suggest an obvious pattern or order; individual denticles are

scattered unequally in space, their size on completion is variable across any area, and the timing of their initiation (as judged from the variation in closure time of the denticle base) forms no regular pattern. Importantly, this suggests that any denticle pattern that may later appear is only built up in later developmental time, as none was present at their initiation. It may be so, in earlier evolutionary time that no pattern was available to be co-opted from the skin to the mouth to function as tooth sets. It is noteworthy that earlier in embryology the tail pattern of denticles is very ordered but from caudal to rostral and it may be an older and alternative mechanism that is replaced in evolution by the secondary and later developing body pattern (Johanson et al., 2008).

ORDERED DENTITIONS AND A DENTAL LAMINA IN CHONDRICHTHYANS

As discussed from Reif's (Reif, '85) data on embryonic shark dentitions, pattern order for the dentition has been recognized in numerous different functional manifestations (as morphology can show in Fig. 2D and E), but all are generated from a dental lamina in the Chondrichthyes (Fig. 2B and C). Historically, time and space order of the tooth sets is described in different ways (Fig. 3A); Odontostichi, tooth rows along the jaw with the same morphology at five sequential times (same shade sets); tooth sets (†) as half of each tooth family (in the boxed area of five teeth); diagonal Zahnreihe (dashed line) as developmental tooth sets; each of these show increasing adult shape. Here the SAM of one tooth family (Smith, 2003), consists of the adjacent alternate tooth sets (indicated by joined arrows and within rectangle in Fig. 3A). The emphasis in the SAM is on the proposed biological entity for regulation, a double tooth set of two adjacent families as shown by arrows in upper jaw position S-1 and 2-3, and lower S-S_1 and 1-2, as data taken from Reif (Reif, '85). These families regulate the time of each successive tooth sequentially in the odd and even tooth positions to provide timed alternate replacement. Consideration of dentition pattern must take account of changing shape along the jaw (Figs. 2D and E, 3B), between jaws, and in developmental time in the tooth sets through successive tooth initiation (Fig. 3A). These show change in size, and differential tooth shape (Fig. 2E), as well as timed replacement and are all regulated to the specific pattern.

To date, virtually nothing is known regarding the genetic control that governs the production and patterning of the dentition in chondrichthyans. A recent study by Smith et al. (2009a) of the initial shark dental pattern showed the expression of a single gene, *shh*, (Fig. 4A and B) where they discussed its spatio-temporal congruence with the initiation of pattern for general vertebrate dentitions (Fraser et al., 2004, 2006b, 2008). The epithelium that forms the dental lamina in chondrichthyans folds inwards from a thickened epithelium, or odontogenic band (OB), and it is in this region that expression of *shh* is restricted in a manner comparable to that of all other studied vertebrates (Dassule et al., 2000; Fraser et al., 2004, 2008, 2009; Buchtova
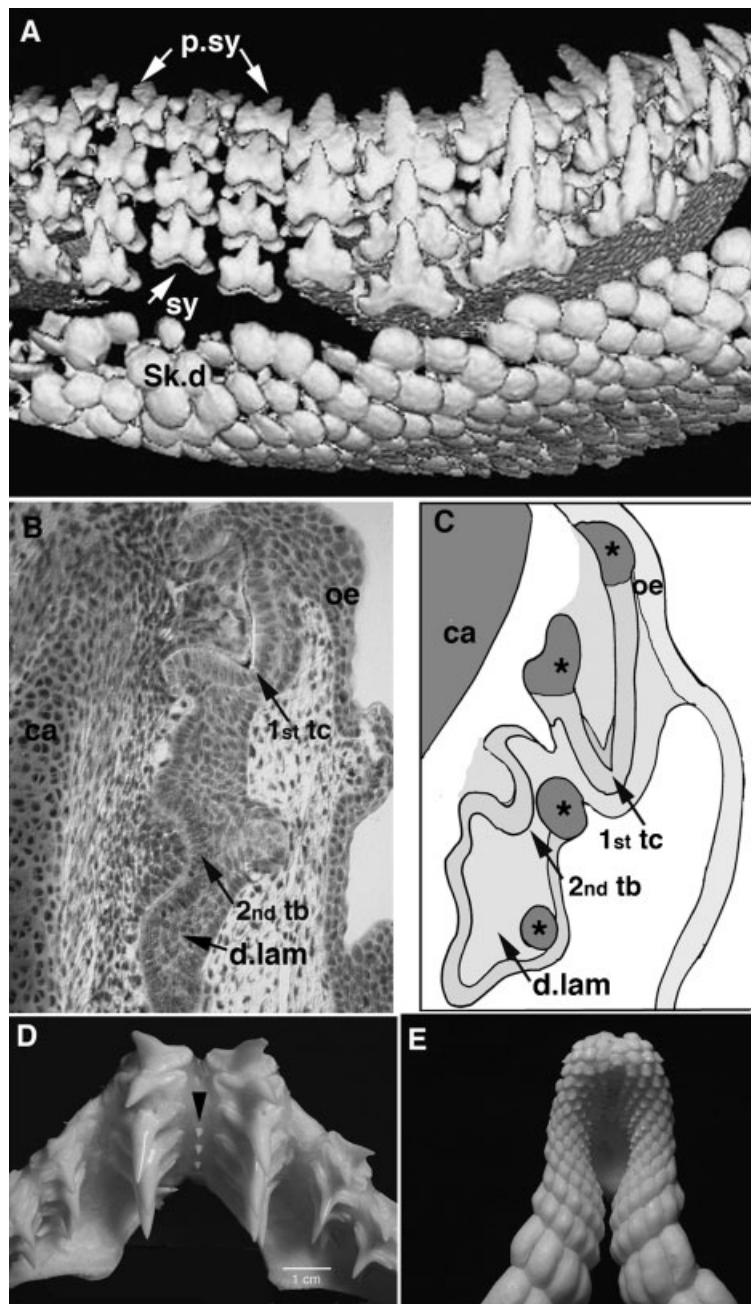
Figure 2. Shark dentition pattern as developed from a dental lamina. (**A**) X-ray tomogram of the juvenile (BL−27.5 cm) catshark mouth margin from labial view to compare the space-packed skin denticles (Sk.d) with regular, ordered tooth sets in alternate pattern starting from those of the symphyseal (sy) tooth row and left /right para-symphyseal (p.sy) sets. (**B**), (**C**) Photomicrograph and drawing of sagittal sections through the lingual dental lamina (d.lam), an epithelial fold from the oral epithelium (o.e) with the first developing teeth (arrows), first tooth cusp (tc) and successive tooth buds (tb) within a tooth family: (**C**) putative epithelial control centers (∗) for regulation of tooth development from the dental lamina (d.lam); Cartilage, ca. (**D**) *Carcharinus* sp. lower jaw, oral view of midline with all successive teeth in lingual sets for each functional tooth position, very small symphyseal row (arrowhead) flanked by larger parasymphyseal sets with left and right smaller teeth. (**E**) *Heterodontus* sp. symphyseal region with packed tooth sets and change in size and shape along the jaw, tooth families are here tightly packed with many functional teeth and the anterior and posterior tooth morphology is different. Scale bar in D = 1 cm; same scale for E.
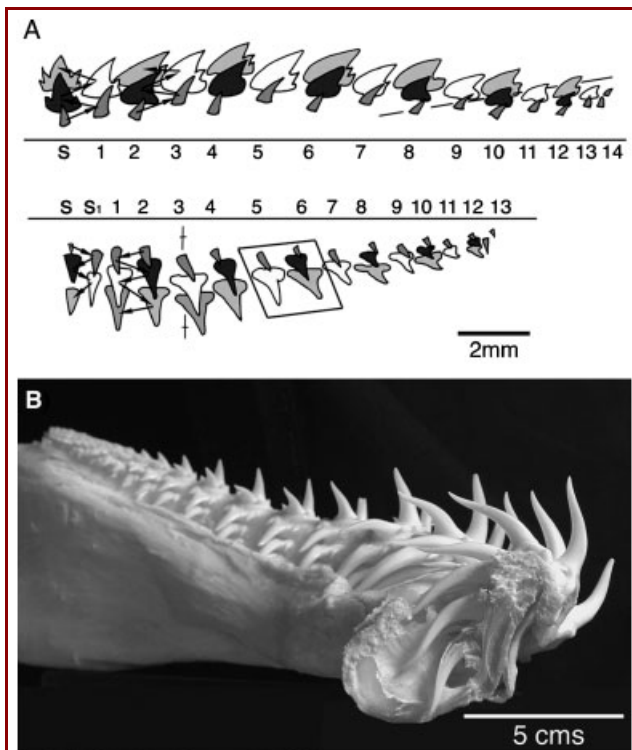
Figure 3. Embryonic and adult shark pattern of tooth sets. (A) Diagram of embryonic shark upper and lower half jaws from the symphyseal region (S/S$_1$) to last families (U13/14 and L12/13), oral margins are horizontal line, with numbered tooth positions, none have erupted to be functional teeth and first ones are rudimentary shapes. Vertical line (†) tooth set, diagonal line (- - -) zahnreihen, same shade teeth are the odontostichous rows with first ones as simple cones in even then odd alternate positions, boxed area is one regulated tooth family as also shown by linked arrows ($\rightarrow$ $\rightarrow$) at jaw positions S-1, 2-3 in the upper jaw, S-S1, 1-2 in the lower jaw. Note the gradual acquisition of adult shape within each family and difference in upper and lower jaws (*Carcharhinus brachyurus*; Reif, '84; Smith, 2003). (B) Adult left half jaw, *Carcharias taurus* (skeletal preparation) cut at the symphysis showing the functional teeth and their replacement series on the lingual aspect (total of seven teeth) normally covered by oral epithelium (Fig. 2B and C).

et al., 2008; Vonk et al., 2008; Smith et al., 2009a,b). The regulation of precise positioning for tooth competence involves a complex periodic pattern mechanism that first spaces the odd and then sequentially the even positions of each tooth set. This allows the chondrichthyan dental system to form separate replacement series linking adjacent odd and even teeth (as families) in a timed series along the jaw (Fig. 3A and C; from (Smith, 2003)). Further gene expression data is required to identify and to determine more precisely the putative control centers that regulate all parameters of the dentition (as shown in Fig. 2C). These include

an intermediate population of undifferentiated putative dental epithelial stem cells, proposed to be sequestered here and to control the time of new tooth replacements (Huysseune and Witten, 2008; Huysseune et al., 2009; Smith et al., 2009a).

DENTAL GENE EXPRESSION PATTERNS IN ACTINOPTERYGIANS

Similar mechanisms for tooth patterning occur within actinopterygians (Fig. 4), as exemplified by a number of recently emerged model species for the development and evolution of the vertebrate dentition including the medaka, rainbow trout and Lake Malawi cichlids. It is very clear that the pattern by which the initial dentition emerges is extremely well conserved among teleosts, even among highly derived groups (Fig. 4), and in many this is without the classical dental lamina, like the deep epithelium formed in chondrichthyans. Always an OB demarcates the field of initiation, from which teeth of at least the first row develop. Some vertebrates, like the mammals, only possess a single row of teeth. The cichlids of Lake Malawi are exceptional evolutionary models for their diverse dental phenotypes (Streelman et al., 2003; Streelman and Albertson, 2006; Fraser et al., 2008) and for the genetic mechanisms regulating their extreme diversification (Albertson et al., 2003a,b, 2005; Sylvester et al., 2010). How teleosts organize and pattern multiple tooth rows through transfer of the OB lingually has recently been described in the cichlids (Fraser et al., 2008). Teleosts do not have a deep primary dental lamina; the epithelial OB is a restricted but superficial region that could be modified through evolution into a dental lamina as may have occurred in chondrichthyan and osteichthyan vertebrates including tetrapods. In fact some teleost fish do develop a successional lamina or secondary offshoot of the dental epithelium for each tooth that can extend deep into the mesenchyme for initiation of replacement teeth (Huysseune and Thesleff, 2004; Huysseune, 2006; Huysseune and Witten, 2006; Moriyama et al., 2010). The pattern mechanism for tooth initiation begins in teleost fish by extension of the OB (described above; Fig. 4D–I). This OB seems to be demarcated by the expression of a number of epithelial genes, common across vertebrates. Along with an OB specific to the cells of the oral epithelium, a similar band is observed in a corresponding field within the cells of oral mesenchyme (Fraser et al., 2006b). From this restricted, cooperative genetic and cellular field, the tooth buds emerge as a thickened population of epithelial cells that swell into the underlying mesenchyme that in turn responds to the growth of the epithelial bud. The budding of tooth placodes, in sequence along the mesio-distal axis of the jaw within the OB, plays out utilizing a complex orchestra of genetic expression. This cohort of genes are expressed during the organization of both the placode and the interplacodal regions in a temporal–spatial pattern that permits the development of tooth units with specific spacing mechanisms that govern adequate inter-tooth distance (Fraser et al., 2006b, 2008). Developmental data from Lake Malawi cichlids provides evidence of the conservation
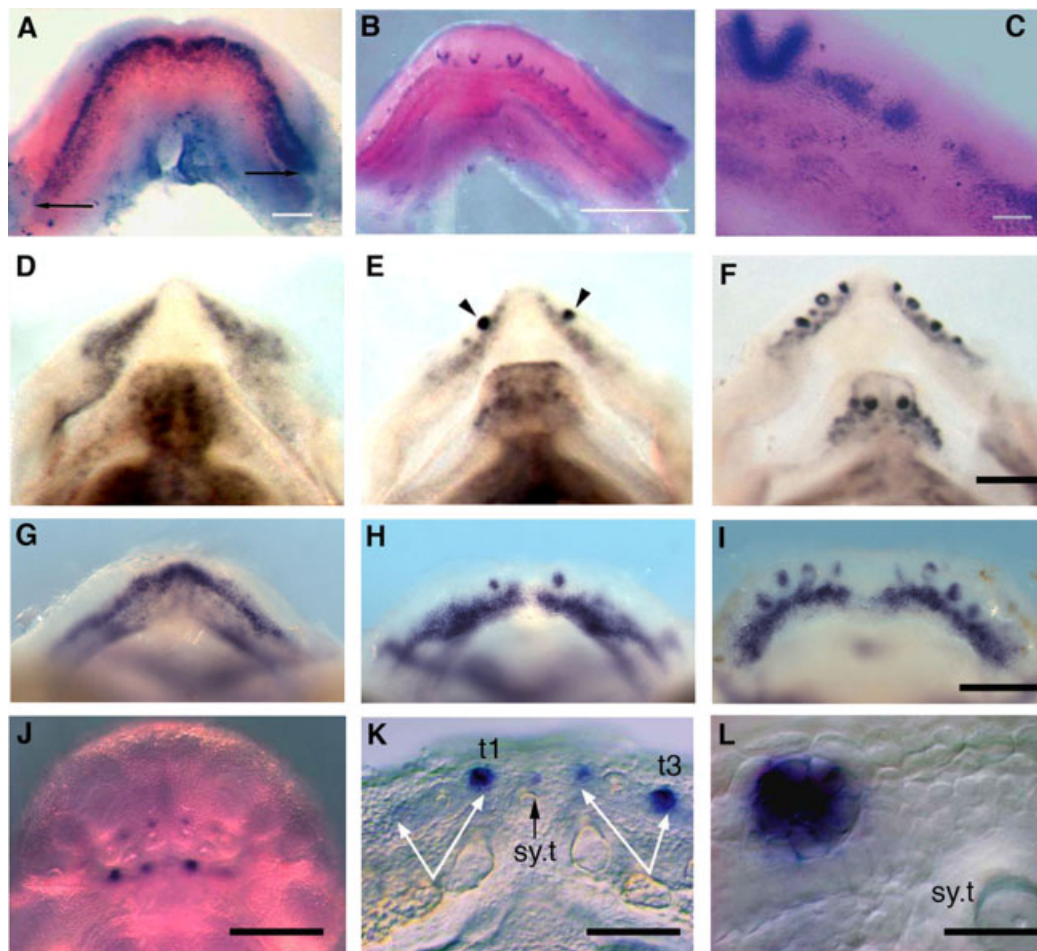
Figure 4. *shh* expression in comparative stages marking the initial pattern regulation of the primary dentition within four fish groups. (A–C) The catshark *Scyliorhinus canicula* (Smith et al., 2009a); (D–F) the Rainbow Trout *Oncorhynchus mykiss* (Fraser et al., 2004); (G–I) a Malawi cichlid *Labeotropheus fuelleborni* (Fraser et al., 2008); (J–L) the Australian lungfish *Neoceratodus forsteri* (Smith et al., 2009c). Note how similar the stages of tooth initiation are in all species. (A) Embryonic stage 30, lower jaw in dorsal oral view, *shh* is strongly expressed in the odontogenic band (OB), the lateral-distal extent of the OB is denoted by the arrows. (B, C) embryonic stage 32 lower jaw in dorsal oral view, when the OB invaginates deep within the jaw at the lingual margin and forms the dental lamina with restricted and weak *shh* expression (Fig. 2B and C), *shh* expression is strong when restricted to the primary tooth cusps of the most anterior teeth in the series (V-shaped expression located in the inner dental epithelium) and also in (C) expression lingers in the remaining OB for subsequent tooth sites of the initial tooth positions. (D) OB stage in *O. mykiss* with *shh* again restricted to the OB marking the epithelial competence of the dentary and tongue field before tooth development that initiates with the first epithelial tooth bud (black arrowheads in E), a ring of expression in the most developed tooth germ (F) for the same tooth position; (F) strong expression in additional tooth positions, including the first two on the tongue, and either side of the first tooth with earlier stage buds for alternate teeth in the series; lingering OB expression lingually for further tooth competence as successional teeth. (G) OB stage of lower jaw of the Malawi cichlid *L. fuelleborni*, (H) first tooth in the dentition initiated from the OB, *shh* expression restricted to the first dentary tooth and the OB, (I) multiple primary epithelial tooth buds as expression loci on the superficial OB as the dentition pattern develops and OB lingual expression is strong. (J–L) *shh* expression in the initial dentary tooth buds of *N. forsteri*. (J) frontal view of the whole head showing tooth buds with positive expression for *shh* in both the upper and lower jaws, with strongest signal in the lower tooth buds. (K), (L) expression is restricted to the epithelial buds of the dentary field in oral view (labial is top), where at this stage in development, because no other lingual teeth form here no expression appears in the OB (earlier stages of an OB before the thickening placode stages have not been recorded): dentary teeth 1–3 (white arrows) on either side of the symphyseal tooth (sy.t; black arrow) show differential expression timing between them from the strongest left (t1), close up in (L), and right (t3), to weak expression in right (t1), explained as due to exquisite timing differences (Smith et al., 2009a). Scale bars: (A) is 200 µm; (B) 1 mm; (C) 50 µm; (D–F) 200 µm; (G–I) 100 µm; (J) 300 µm; (K) 150 µm; (L) 50 µm.

of gene expression patterns, and thus suggest that these expression patterns permit the induction and control of space in adjacent tooth units across the vertebrates. The specific genes that occupy the inter-placode space or the zone of inhibition (ZOI), for example, *wnt7b* and *eda* have been reported both from the mouse and cichlid dentitions (Sarkar et al., 2000; Fraser et al., 2008). Not only are these genes implicated in the patterning of the cichlid dentition but also alterations in the expression of these genes across Lake Malawi species may be responsible for, at least, some of the phenotypic diversity observed through variation in tooth number and spacing (Fraser et al., 2008).

Unique among osteichthyan and chondrichthyan fishes is the propensity to produce numerous rows of teeth, whether reserved for a later use, as in chondrichthyans or multi-rowed functional teeth, as in teleostean osteichthyans, e.g. cichlids. Interestingly, the genetic mechanism that sets up the initial dental field and first generation teeth is reutilized to organize each new row, one after the next until a termination mechanism ends the cycle (Fraser et al., 2008; Mikkola, 2009; Smith et al., 2009a,b; Zhang et al., 2009; Cobourne and Sharpe, 2010). In effect, the OB in teleost fish that kick-starts the initial dental pattern can migrate lingually to produce row after row of functional teeth. It appears that the co-expression of two key genes, *pitx2* and *shh*, is necessary within the epithelial cells of the OB to maintain the OBs initiatory competence (Fraser et al., 2008). It has been proposed that when one or both of these genetic regulators are lost from the OB then the mechanism for tooth row addition breaks down (Fraser et al., 2008; Mikkola, 2009). The genes expressed in the inter-tooth spaces or zones of inhibition, which surround each new tooth placode during early establishment, are reutilized to take on the putative role of inter-tooth row spacers, namely *eda* and *wnt7b*, at least in the cichlid models (Fraser et al., 2008). It would be surprising if the tooth row mechanism were not co-opted from the already established ZOI mechanism that restricts individual tooth sites. Thus, it is conceivable that ZOI genetic restriction of individual units along the jaw was co-opted for separate and distinct tooth rows during developmental evolution. This genetic mechanism likely continues to be used in developmental time to restrict sites for replacement of the individual tooth units, forming the entire "tooth family" (Fig. 4).

TOOTH PATTERN ORDER IN SARCOPTERYGIANS

The most precise timing of individual tooth germs was obtained by use of in situ gene expression for *shh* in *N. forsteri* where it was shown to reveal a staggered sequence of timing across left and right sides of the tooth row at the margins of the lower jaw (Fig. 4J–L) (Smith et al., 2009c). Here we have illustrated the data from cleared skeletal preparations to see, at selected larval and hatchling stages, the tooth addition order (Fig. 5C, F, G). This shows the stereotypic osteichthyan order for the dentary bone as mentioned earlier in this study (Fig. 5C). Drawings show the whole lower jaw of *N. fosteri* (Fig. 5A) with the marginal dentition starting with the symphyseal tooth after formation of three teeth of the prearticular, then shown on the right half of the jaw (Fig. 5B), two dentary teeth at positions 2 and a younger one at 3. When three dentary teeth are present (Fig. 5C), the youngest one of the three is at position 1, inserted between the symphyseal tooth (with bone of attachment) and tooth 3. Regarding the dental lamina, it can be seen from early tooth development (Fig. 5D and E) that, like in *O. mykiss*, tooth initiation is very superficial in the dental epithelium of the adjacent tooth germ (arrow Fig. 5E) so again, none form from a dental lamina as was previously thought (Smith, '85).

DISCUSSION

It appears that a regular pattern was not present in the early stages of denticle formation in the shark skin but that close spacing only developed with time, as Reif ('82) recorded that the first generation of skin denticles (placoid scales) is very widely spaced and with variable positions. In contrast to this lack of order in the skin, it is known that much earlier than the development of any body denticles, a set do form in a sequential, regular time and space order on the embryonic tail in at least some sharks, even though they are lost by shedding soon after hatching (Johanson et al., 2007, 2008). Denticles are rarely shed from the body skin, so that the cover arises slowly with growth as more and more individual denticles are added.

Our observations are in complete agreement with a statement by Reif ('80) that "the dentition and dermal skeleton belong to two independent secondary developmental fields that differ both developmentally and structurally." In the catshark (*S. canicula*) we can compare two skin patterns in the same species, the early regular, iterative caudal tail rows added from caudal to rostral (Johanson et al., 2007, 2008) and the irregular general skin denticles, which might also be initially set from two axial dorsal rows (Reif, '80; Ballard et al., '93). Completely different pattern mechanisms from either of these must regulate the dentition, as new teeth are added sequentially along the jaw and then within the family as generations of teeth are timed to succeed the functional teeth, but with consistent regular size increase and shape change as next discussed.

We can question from which system the dentition pattern was co-opted to evolve into the diverse gnathostome types living today, as we know little about skin denticle patterns in early sharks. The classic theories arose from developmental studies in the extant shark, where it was proposed that shark-type skin denticles had migrated into the mouth from the skin, evolving into oral tooth sets when forming a dental lamina (Reif, '82). Extant sharks were considered basal within the gnathostome phylogeny; however, recent phylogenies (Brazeau, 2009) place the fossil group Placodermi below the Chondrichthyes as the earliest jawed member of the total group Gnathostomata. Importantly, placoderms were considered originally as without teeth as tooth whorls were not present, "evidence of teeth as
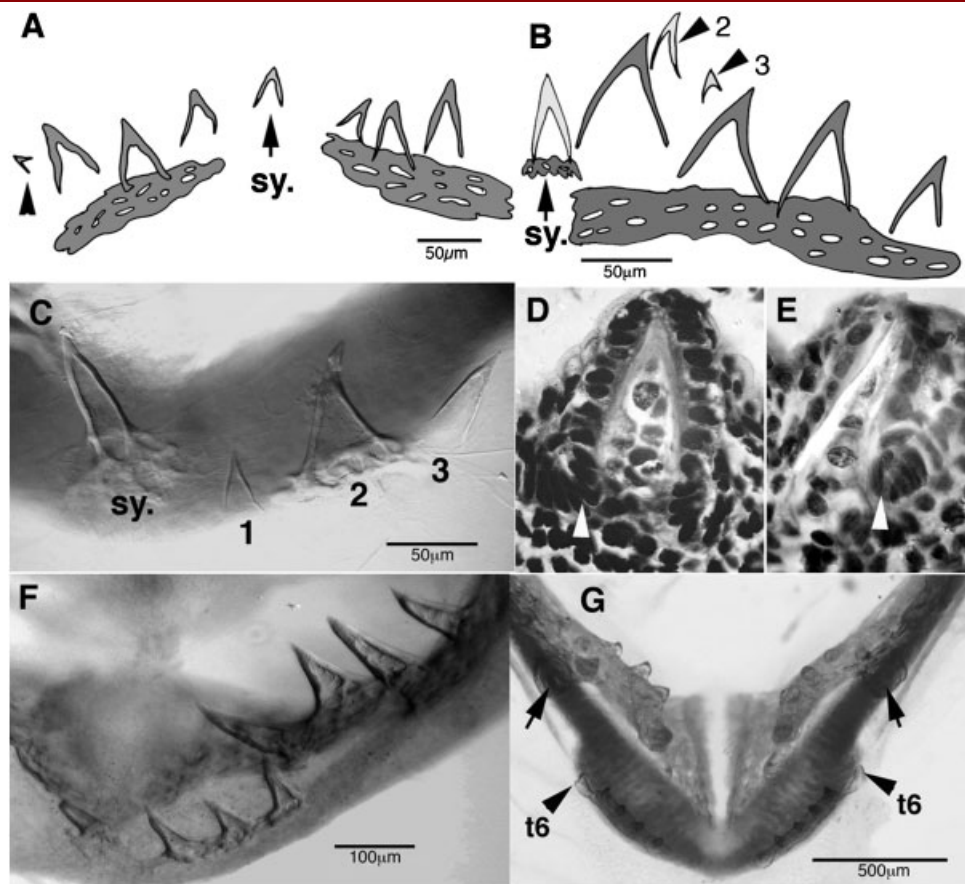
Figure 5. *Neoceratodus forsteri* larval to hatchling, stages of tooth development order in the lower jaw. **A, B** drawings, **C, F, G** microphotographs from alcian blue stained and cleared skeletal whole mounts. (A) Youngest lower jaw at stage 39, on both sides all teeth are simple dentine cones without their bone of attachment, first three–four prearticular teeth with the newest fourth on the left (arrowhead), one symphyseal (sy.), but no dentary teeth. (B) Later stage 41 of right side only, with four large prearticular teeth, one older symphyseal tooth (sy.) with its bone of attachment, and the two new dentary teeth in jaw positions 2 (largest), and 3 (smallest; arrowheads), drawn as dentine cones (see C 1and 3). (C) Stage 44 lower jaw of only right dentary teeth, bone of attachment on symphyseal tooth (sy.) and oldest dentary tooth at position 2, then a large tooth cone at 3 with the newest small tooth cone at 1, this size and stage difference reveals the pattern order of the osteichthyan dentary field. (D, E) histological sections of developing teeth in the lower jaw, superficial dentine cones with next tooth bud (white arrowheads) formed within the dental epithelium on the lingual side alongside the older tooth. (F) Larval stage 47 with all three dentary teeth now attached together by their own bone as none forms for the dentary homologue, their size is small relative to the 4 prearticular teeth shown. (G) Stage 52 of whole lower jaw with two prearticular tooth plates now formed but new teeth added antero-laterally (arrows), the sixth tooth is added to the marginal dentary row on the outside and above Meckel's cartilage (t6; arrowheads).

produced from a dental lamina." A comprehensive examination of all available placoderm dentitions and their pharyngeal denticles by Johanson and Smith concluded that rows of ordered teeth were present on the jaw bones (gnathalia) of the more derived placoderms (arthrodires) (Smith and Johanson, 2003). They also showed that ordered and patterned denticles did occur in the pharynx of all placoderms, even the least derived ones (Johanson and Smith, 2003, 2005). That is, ordered development did occur despite the absence of tooth whorls in this fossil group,

the criterion provided by Reif ('82) to extrapolate developmental information. It was argued that these dentine-based teeth in placoderms with a timed order of addition were true teeth. Also, in the latest discussion of evolutionary origins of ordered dentitions and the link with a dental lamina, Smith et al. (2009b) considered that the stereotypic chondrichthyan pattern of teeth produced from a dental lamina was not true for osteichthyan fish, nor for placoderms. They showed that the developmental model for producing teeth in the rainbow trout

(*O. mykiss*) from each previous tooth germ, rather than from a dental lamina, could equally apply to placoderm tooth production. From this it can be concluded that a dental lamina (typified by the shark model) is not essential for pattern information and that independent evolution of patterned dentitions occurred perhaps several times on the phylogeny of jawed vertebrates (Smith and Johanson, 2003).

In order to examine timing and spatial pattern of tooth initiation, gene expression data for *shh* in three species of fish, *S. canicula*, *O. mykiss* and *N. forsteri* were compared (Fraser et al., 2004, 2006a,b; Smith et al., 2009a,c). This gene, associated with the initiation of each tooth, may be conserved through evolution and is expressed reiteratively during continuous tooth morphogenesis (Fraser et al., 2006a; Smith et al., 2009a,c).

Because the spatial temporal expression patterns show the order of sequential timing of each tooth in the dentition we have been able to compare tooth order across taxa. In the rainbow trout (*O. mykiss*) *shh* marks the sites for initiation of the first row of teeth in two phases for alternate even-odd positions, then when these are established, *shh* is also expressed during morphogenesis of the replacement teeth (Fraser et al., 2006a). In the catshark, *shh* marks the order of teeth along the jaw and their first cusp positions, then the sites of the alternate series of teeth in a reiterative way (Smith et al., 2009a) followed by the second cusp positions, as also documented for mammalian molars (Jernvall and Thesleff, 2000). In all jawed vertebrates the dentition pattern in the most general sense has a common feature of sequential tooth addition along the tooth rows and then along the tooth families (or the replacement series), proposed as the SAM (Smith, 2003). The timing of the pattern in space and how this occurs is a topic of debate at present, as for example this pattern is different on each dentate bone for the rainbow trout, *O. mykiss* (Berkovitz and Moore, '74, '75). In the Australian lungfish (*N. forsteri*), only a single row of dentary teeth occurs and this is not added to by replacement teeth, but gene expression data has revealed that, in this divergent dentition, teeth form initially in the same sequence as in most osteichthyan fishes (Smith et al., 2009c). This temporal sequence for the dentary tooth field suggests a conserved pattern in tooth initiation order and is the proposed plesiomorphic state for osteichthyan dentitions. This time and space order in the Australian lungfish provides the phylogenetic link between these derived and specialized dentitions and those of other osteichthyans.

An important question is how was a stereotypic osteichthyan pattern transformed early in the evolution of the lungfish dentition to become the highly specialized paired sets of crushing tooth plates characteristic of the group. Although the lungfish (Dipnoi) belong within the Osteichthyes, their adult dentitions are radically different from other osteichthyans. Lungfish dentitions also show uniquely high structural disparity during the early evolution of the group (Ahlberg et al., 2006). The structure and pattern of construction of the paired palatal tooth plates has been conserved for 400 Myr since the early Devonian (Reisz and Smith, 2001), and as the closest living relatives of Tetrapoda this raises some fundamental questions (Ahlberg et al., 2006). Assuming that co-option of the dental pattern was initially from osteichthyan developmental regulatory genes used during the Devonian period, then how can extant forms provide any information on mechanisms that might guide evolutionary change? Some ideas on how this was achieved have been proposed after a study of the timed order of tooth initiation in *N. forsteri* (Smith et al., 2009c), the only extant form with a marginal set of teeth, although functional in only the youngest stages.

CONCLUSIONS AND NEW PERSPECTIVES

We have only just begun to understand the localization of gene expression groups associated with tooth development across vertebrates with an aim to decipher the functional genetic regulatory network that (i) instigates tooth development (ii) patterns these dental units within a functional dentition (iii) regulates the formation of multiple rows, (iv) controls the regulation of cyclical tooth replacement over many generations and (v) decouples tooth resorption from tooth production as in the lungfish and others, where all teeth are retained, neither lost nor replaced.

One area that requires attention in the future is the pattern order of chondrichthyan and osteichthyan denticles when present; specifically what genes are expressed during the early initiation and later spacing in development of the skin denticle coverage. Little is known about the developmental basis of even osteichthyan scales (Kondo et al., 2001; Sharpe, 2001; Sire and Akimenko, 2004; Fraser et al., 2010). We suggest a focus of future research toward a direct comparison of the genes and their interactions involved in both chondrichthyan tooth and skin denticle formation; can this inform us of the intrinsic patterning mechanisms that may decouple these two systems? A decoupling might reflect the obvious disparity between the tissue origin of skin denticles (strictly ectodermal) and teeth (likely endodermal or a mixed population).

The conserved, multi-purpose dental co-expression group includes members that are associated with each of the main developmental pathways: Notch, Fibroblast Growth Factors, Wnt/ß-catenin, Hedgehog, and Bone Morphogenetic Protein. These dental co-expression group associations provide unique pattern information to the oro-pharynx that must differ from the pattern mechanism that organizes the epidermal structures like the skin denticles of sharks and their relatives. We suggest that this unique oro-pharyngeal patterning mechanism has produced and organized dentitions from the first vertebrate dentition some 500 million years ago in jawless fishes to our own teeth.

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LITERATURE CITED

Ahlberg PE, Smith MM, Johanson Z. 2006. Developmental plasticity and disparity in early dipnoan (lungfish) dentitions. Evol Dev 8:331–349.

Albertson RC, Streelman JT, Kocher TD. 2003a. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. Proc Natl Acad Sci USA 100:5252–5257.

Albertson RC, Streelman JT, Kocher TD. 2003b. Genetic basis of adaptive shape differences in the cichlid head. J Hered 94:291–301.

Albertson RC, Streelman JT, Kocher TD, Yelick PC. 2005. Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. Proc Natl Acad Sci USA 102:16287–16292.

Ballard WW, Mellinger J, Lechenault H. 1993. A series of normal stages for development of *Scyliorhinus canicula*, the lesser spotted dogfish. J Exp Zool (Mol Dev Evol) 267:318–336.

Berkovitz BK, Moore MH. 1974. A longitudinal study of replacement patterns of teeth on the lower jaw and tongue in the rainbow trout Salmo gairdneri. Arch Oral Biol 19:1111–1119.

Berkovitz BK, Moore MH. 1975. Tooth replacement in the upper jaw of the rainbow trout (*Salmo gairdneri*). J Exp Zool (Mol Dev Evol) 193:221–234.

Borday-Birraux V, Van der Heyden C, Debiais-Thibaud M, Verreijdt L, Stock DW, Huysseune A, Sire JY. 2006. Expression of Dlx genes during the development of the zebrafish pharyngeal dentition: evolutionary implications. Evol Dev 8:130–141.

Brazeau MD. 2009. The braincase and jaws of a Devonian "acanthodian" and modern gnathostome origins. Nature 457:305–308.

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.

Chan ET, Quon GT, Chua G, Babak T, Trochesset M, Zirngibl RA, Aubin J, Ratcliffe MJ, Wilde A, Brudno M, Morris QD, Hughes TR. 2009. Conservation of core gene expression in vertebrate tissues. J Biol 8:33.

Cobourne MT, Sharpe PT. 2010. Making up the numbers: the molecular control of mammalian dental formula. Semin Cell Dev Biol 21:314–324.

Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. 2000. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 127:4775–4785.

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 Zoolog B Mol Dev Evol 308:693–708.

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 Zoolog B Mol Dev Evol 306:183–203.

Fraser GJ, Bloomquist RF, Streelman JT. 2008. A periodic pattern generator for dental diversity. BMC Biol 6:32.

Fraser GJ, Hulsey CD, Bloomquist RF, Uyesugi K, Manley NR, Streelman JT. 2009. An ancient gene network is co-opted for teeth on old and new jaws. PLoS Biol 7:e31.

Fraser GJ, Cerny R, Soukup V, Bronner-Fraser M, Streelman JT. 2010. The odontode explosion: the origin of tooth-like structures in vertebrates. BioEssays 32:808–817.

Gibert Y, Bernard L, Debiais-Thibaud M, Bourrat F, Joly JS, Pottin K, Meyer A, Retaux S, Stock DW, Jackman WR, Seritrakul P, Begemann G, Laudet V. 2010. Formation of oral and pharyngeal dentition in teleosts depends on differential recruitment of retinoic acid signaling. FASEB J.

Hecht J, Stricker S, Wiecha U, Stiege A, Panopoulou G, Podsiadlowski L, Poustka AJ, Dieterich C, Ehrich S, Suvorova J, Mundlos S, Seitz V. 2008. Evolution of a core gene network for skeletogenesis in chordates. PLoS Genet 4:e1000025.

Hunt P, Gulisano M, Cook M, Sham MH, Faiella A, Wilkinson D, Boncinelli E, Krumlauf R. 1991. A distinct Hox code for the branchial region of the vertebrate head. Nature 353:861–864.

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, Sire JY. 1998. Evolution of patterns and processes in teeth and tooth-related tissues in non-mammalian vertebrates. Eur J Oral Sci 106:437–481.

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 Zoolog 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.

Huysseune A, Sire J-Y, Witten EP. 2009. Evolutionary and developmental origins of the vertebrate dentition. J Anat 214:465–476.

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.

Jarvinen E, Tummers M, Thesleff I. 2009. The role of the dental lamina in mammalian tooth replacement. J Exp Zoolog B Mol Dev Evol 312B:281–291.

Jernvall J, Thesleff I. 2000. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 92:19–29.

Johanson Z, Smith MM. 2003. Placoderm fishes, pharyngeal denticles, and the vertebrate dentition. J Morphol 257:289–307.

Johanson Z, Smith MM. 2005. Origin and evolution of gnathostome dentitions: a question of teeth and pharyngeal denticles in placoderms. Biol Rev 80:303–345.

Johanson Z, Smith MM, Joss JMP. 2007. Early scale development in Heterodontus (Heterodontiformes; Chondrichthyes): a novel chondrichthyan scale pattern. Acta Zool 88:249–256.

Johanson Z, Tanaka M, Chaplin N, Smith M. 2008. Early Palaeozoic dentine and patterned scales in the embryonic catshark tail. Biol Lett 4:87–90.

Kavanagh KD, Evans AR, Jernvall J. 2007. Predicting evolutionary patterns of mammalian teeth from development. Nature 449:427–432.

Kondo S, Kuwahara Y, Kondo M, Naruse K, Mitani H, Wakamatsu Y, Ozato K, Asakawa S, Shimizu N, Shima A. 2001. The medaka rs-3 locus required for scale development encodes ectodysplasin-A receptor. Curr Biol 11:1202–1206.

Laurenti P, Thaeron C, Allizard F, Huysseune A, Sire JY. 2004. Cellular expression of eve1 suggests its requirement for the differentiation of the ameloblasts and for the initiation and morphogenesis of the first tooth in the zebrafish (*Danio rerio*). Dev Dyn 230:727–733.

Mikkola ML. 2009. Controlling the number of tooth rows. Sci Signal 2:pe53.

Miller CT, Maves L, Kimmel CB. 2004. moz regulates Hox expression and pharyngeal segmental identity in zebrafish. Development 131:2443–2461.

Moriyama K, Watanabe S, Iida M, Sahara N. 2010. Plate-like permanent dental laminae of upper jaw dentition in adult gobiid fish, *Sicyopterus japonicus*. Cell Tissue Res 340:189–200.

Nelson GJ. 1969. Gill arches and the phylogeny of fishes, with notes on the classification of vertebrates. Bull Am Mus Nat Hist 141:479–552.

Raff RA. 1996. The shape of life: genes, development, and the evolution of animal form. Chicago: The University of Chicago Press.

Reif W-E. 1978. Wound healing in Sharks: form and arrangement of repair scales. Zoomorphologie 90:101–111.

Reif WE. 1980. Development of dentition and dermal skeleton in embryonic *Scyliorhinus canicula*. J Morphol 166:275–288.

Reif W-E. 1982. Evolution of dermal skeleton and dentition in vertebrates: the odontode-regulation theory. Evol Biol 15:287–368.

Reif W-E. 1984. Pattern regulation in shark dentitions. In: Malacinski GM, Bryant SV, editors. Pattern formation a primer in developmental biology. New York: Macmillan. p 603–621.

Reif W-E. 1985. Squamation and ecology of sharks. Courier Forschungsinstitut Senckenberg 78:1–255.

Reisz RR, Smith MM. 2001. Developmental biology. Lungfish dental pattern conserved for 360 Myr. Nature 411:548.

Sarkar L, Cobourne M, Naylor S, Smalley M, Dale T, Sharpe PT. 2000. Wnt/Shh interactions regulate ectodermal boundary formation during mammalian tooth development. Proc Natl Acad Sci USA 97:4520–4524.

Sharpe PT. 2001. Fish scale development: hair today, teeth and scales yesterday? Curr Biol 11:R751–R752.

Sire JY, Akimenko MA. 2004. Scale development in fish: a review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). Int J Dev Biol 48:233–247.

Smith MM. 1985. The pattern of histogenesis and growth of tooth plates in larval stages of extant lungfish. J Anat 140:627–643.

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, Coates MI. 2001. The evolution of vertebrate dentitions: phylogenetic pattern and developmental models (palaeontology, phylogeny, genetics and development). In: Ahlberg PE, editor. Major events in early vertebrate evolution. London and New York: Taylor and Francis. p 223–240.

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. 2009a. Reiterative pattern of sonic hedgehog expression in the catshark dentition reveals a phylogenetic template for jawed vertebrates. Proc Biol Sci 276:1225–1233.

Smith MM, Fraser GJ, Mitsiadis TA. 2009b. Dental lamina as source of odontogenic stem cells: evolutionary origins and developmental control of tooth generation in gnathostomes. J Exp Zoolog B Mol Dev Evol 312B:260–280.

Smith MM, Okabe M, Joss J. 2009c. Spatial and temporal pattern for the dentition in the Australian lungfish revealed with sonic hedgehog expression profile. Proc Biol Sci 276:623–631.

Soukup V, Epperlein HH, Horacek I, Cerny R. 2008. Dual epithelial origin of vertebrate oral teeth. Nature 455:795–798.

Stock DW. 2007. Zebrafish dentition in comparative context. J Exp Zoolog B Mol Dev Evol 308:523–549.

Stock DW, Jackman WR, Trapani J. 2006. Developmental genetic mechanisms of evolutionary tooth loss in cypriniform fishes. Development 133:3127–3137.

Streelman JT, Albertson RC. 2006. Evolution of novelty in the cichlid dentition. J Exp Zoolog B Mol Dev Evol 306: 216–226.

Streelman JT, Webb JF, Albertson RC, Kocher TD. 2003. The cusp of evolution and development: a model of cichlid tooth shape diversity. Evol Dev 5:600–608.

Sylvester JB, Rich CA, Loh YH, van Staaden MJ, Fraser GJ, Streelman JT. 2010. Brain diversity evolves via differences in patterning. Proc Natl Acad Sci USA 107:9718–9723.

Thesleff I, Sharpe P. 1997. Signalling networks regulating dental development. Mech Dev 67:111–123.

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.

Zhang Z, Lan Y, Chai Y, Jiang R. 2009. Antagonistic actions of Msx1 and Osr2 pattern mammalian teeth into a single row. Science 323:1232–1234.