Origin of teeth in jawed vertebrates

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We explore how teeth emerged in living and fossil forms as one of the great transformations in vertebrate evolution; that of the evolution of biting jaws from jawless fishes.

Summary

Our research is concerned with the question of tooth origins and the relationship of teeth with the body scales, of both modern and extinct fishes. To unravel the evolutionary steps that led to tooth origins we focus our attention on both fossil and living fishes with potential ancestral dental characters. Our emphasis is on the organisation and development of teeth into functional toothed jaws in a range of cartilaginous fishes, including sharks and rays. We use non-destructive micro-computed tomography (xCT) to uncover morphological traits in these fishes (embryos and adults) as clues to the emergence of the early vertebrate dentition. Advanced histological techniques and segmented 3D models identify how tooth formation is achieved in several types of dentitions. Notably, we determine how replacement from successively developed teeth occurs through regenerative mechanisms that provide a continuous conveyor belt of many ready-made teeth for each jaw position in sharks.

We compare the arrangement and development of teeth with dermal scales – tooth homologues that cover the body of sharks and rays -- and how they might share evolutionary mechanisms, in a scenario of body scales evolving into organised dentitions when jaws emerged. Advanced histology highlights the specific expression of genes that suggest a shared genetic regulatory mechanism for body scales and teeth in sharks. Also, evidence from genes used to initiate teeth in chondricthyan fish shows a set of regulatory genes, a core signalling network that is common to all teeth.

Further, we discuss new information on rays and sharks that have extended rostra with saw-teeth along the lateral margins and outside the mouth, designed from modified placoid scales. One exceptional extinct, fossil sawfish has replacement saw-teeth in a unique arrangement strikingly similar to oral dentitions with a 'many for one' arrangement, but all nested beneath the divided roots, and distinct from that of oral teeth. Another unique and earlier jawless fish highlights the possible origins of tooth-like structures with skin-born (dermal) dentine fused to bone, which can repair damage and wear by continuously producing new dentine material, filling spaces in the bone from migratory odontoblasts.

Teeth organised into functional dentitions

Introduction

To understand how teeth first evolved we need to look back 420 million years to the origins of jawed vertebrates. We can gather information from fossil jawed vertebrates, and importantly we can also use developmental studies of living forms to provide clues to the origins of mechanisms that made teeth. In fact the major changes shaping our understanding of how teeth and dentitions evolved, were from the great transformation of jawless to jawed fishes, where teeth emerged together with jaws. This event resulted in jawed vertebrates becoming the dominant life forms on the planet. The earliest jawed vertebrate fossils can provide theories for the origins of teeth, including the classical theory where early tooth-like structures as dermal armour in the skin gave rise to teeth at the margins of the newly evolved jaws. This would have involved a shift of these dermal structures (placoid scales, or dentine tubercles) into the mouth by a form of developmental co-option and heterotopic transfer, where an existing structure performs a different function at a different site. Understanding developmental genetic mechanisms in living, jawed vertebrates can explain the process of how teeth may have evolved through small changes or 'tinkering' in key developmental genes over time, into precisely organised dentitions. The essential developmental difference between oral teeth and denticles in the skin is the evolution of a developmental structure (epithelial dental lamina) along the jaws that allows teeth to form in a separate morphogenetic field from that of placoid denticles.

Evolution and developmental origins

Classical and novel theories are actively debated today (Huysseune et al 2009; Fraser & Smith 2011; Witten *et al.*, 2014; Smith & Johanson, 2015), including gene expression data (Debiais-Thibaud *et al.*, 2011), as to whether teeth originate from external dermal armour, skin denticles or scales (placoid) that migrate into the mouth, or from similar denticles deep in the pharynx that have a serial order to them (Smith & Coates, 2000; Smith, 2003).

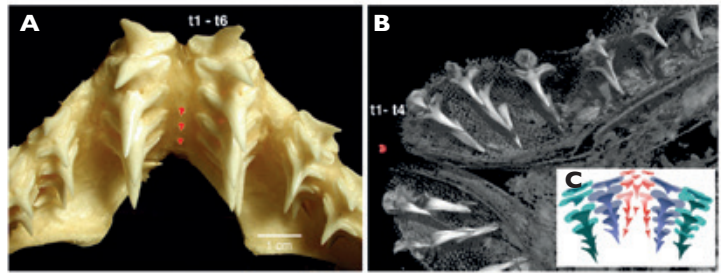


Figure 1 (A-C). *A, upper jaw of adult spotted woebegong shark (Orectolobus maculates) with sets of replacement teeth aligned as single files inside each functional tooth position along the jaw margins. Rudimentary teeth (red) are present at the midline symphysis, although in the lower jaw they form a set of full sized teeth. B, lower jaw of angel shark in late development (viviparous species) with sets of partly developed teeth aligned as single file replacements (four in each) with one fully formed tooth at the jaw margin (t1-4) and a single rudimentary symphyseal tooth (red). C, cartoon of lower jaw, adult Blacktip Reef shark (Carcharhinus melanopterus), shows single file replacement teeth but in alternate arrangement (purple - green) and three sympyseal sets (centre).*

The idea that a pattern to the structural arrangement of teeth in the jaws of sharks and rays (Neoselachii; Chondrichthyes) was important to characterise teeth as part of a dentition and different from external denticles, or scales in the skin (tooth homologues), was discussed by Smith (2003). Inside the mouth neoselacians have very organised, multiple sets of developing teeth, able to regenerate throughout life in a conveyor belt-like process. The study of developing shark jaws shows how the teeth form precisely timed and ordered multiple replacement teeth for each functional tooth at the jaw margin (Figure 1A, B, C) Thus, sharks and rays provide a precise extant developmental model to examine how 'teeth in the skin' may have evolved

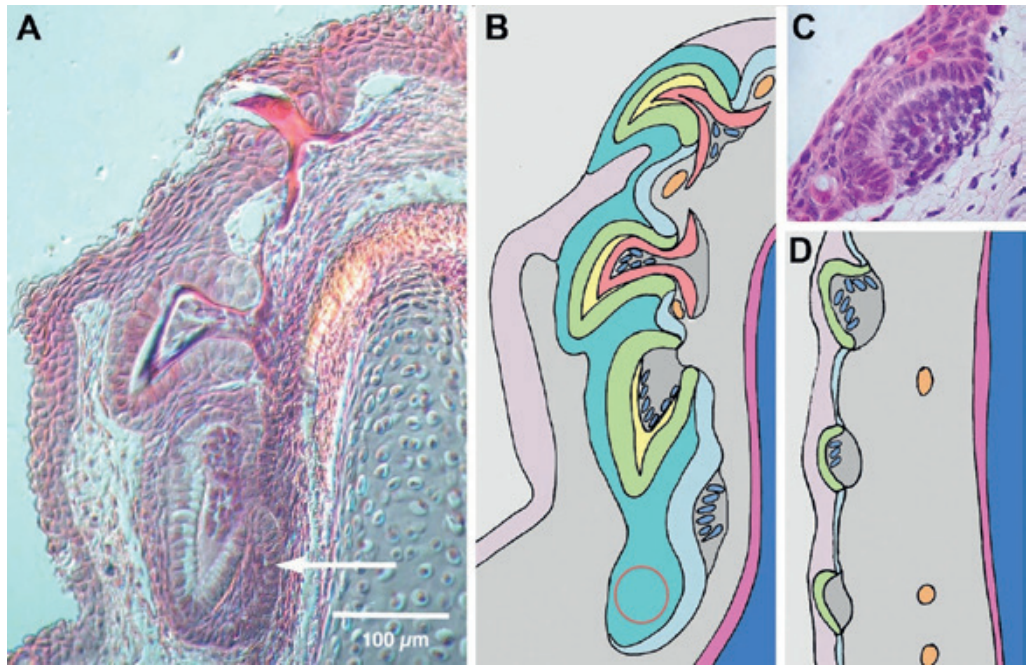


Figure 2 (A-D). *A, spotted catshark (Scyliorhinus canicula). (Haemtoxylin and Eosin stain, Nomarsky optics Zeiss x16) section is through the developing dentition on the jaw cartilage with three teeth at different stages, the first to form has not yet erupted through the oral epithelium, a new site for a tooth protogerm is on the inner side of the dental lamina (arrow), shown in B as a cluster of dental papilla cells. 100µm bar A-D. B, interpretive drawing of A with dental lamina cells in blue, proposed stem cell niche (red oval, oral epithelium pink, outer dental lamina epithelium blue, inner dental epithelium pale blue, ameloblasts green, enameloid yellow, dentine red, dental papilla blue cells). C, the first stage of development of skin denticles, at same posthatch stage as in D. D, diagram of three skin denticles as superficial development in the epithelium on the opposite side of the jaw to dentition in A, (differentiated skin epithelium green, papilla cells blue, basal epithelium pale blue) no dental lamina is formed here. (With permission from University of Chicago Press, Figure 1.4 in Great Transformations in Vertebrate Evolution 2015).*

into teeth organised within dentitions as proposed by the classical theory. However, positional ordering along the jaws and multiple replacement teeth stacked alongside each functional tooth are notable differences compared to dermal scales or denticles.

As noted above, one evolutionary mechanism is to take a structure present in early groups of jawless fish and co-opt it, together with the genes and translational mechanisms, to form another structure in another position with selection through adaptation for different functional adaptations. To achieve a developmental change that transforms skin denticles into the functional, structural order of teeth in the dentition of the neoselachions depends on evolution of a mechanism for creation of a dental lamina. This is an invagination of epithelium from the oral mucosa as a double layer on the inner side of which (next to the jaw cartilage) epithelial placodes form to generate individual teeth (Figure 2A, arrow.) Also, this novel structure, absent in the skin, determines that teeth only form within the lamina that restricts by inhibition the morphogenetic field of odontogenic competence to make teeth, and in a timed order of development, rather than scattered around the mouth. Order is maintained by the continual renewal of new teeth as replacement sets (Figure 2A, B) (Smith *et al*., 2009a). The replacement teeth are in a particular labio-lingual order of new lingual teeth with cusps aligned, but these sets are either spaced out along the jaw in single file order (Figure 1A-C) or, an alternate arrangement (off-set crowns and bases overlapping; (Text Figure 1, Figure 3C). A rare articulated fossil, the earliest one for chondrichthyans (Lower Devonian, c. 397Ma, *Doliodus* (Maisey *et al.* 2013) with teeth in place in single file central cusp-aligned arrangement and labiolingual replacement, suggests that it is likely to be the primitive condition for *chondrichthyans*, or a *synapomorphy* with a 'shark-like" *acanthodian Ptomocanthus* (Brazeau, 2009). Although continuous regeneration is proposed to be basal for extant vertebrate dentitions (Rasch *et al*. 2016), this is not true for stem jawed vertebrates, as observed in placoderm fish, an entirely fossil group, (Johanson & Smith, 2005) where teeth are added, but not replaced, nor renewed.

The ordered dentition deep in the oral mucosa is in contrast to the superficial placoid scales present in the skin, developing without this order and without a dental lamina (Figure 2C, D). Consequently in the skin serially timed and spaced replacements are not formed (Smith & Fraser, 2010), new scales only forming when a gap occurs in the skin (Figure 2B, D). At the margins of the jaws button shaped scales are close packed with irregular size and spacing (blue, Text Figure1, Figure 2D).

All microcomputed tomography (xCT) images of the catshark (Figure 3A-E) and virtual sections through the jaw margin show the whorl of close packed, serially ordered teeth within the jaw cartilage furrow contrasting with the denticles in the skin. The adult Porbeagle shark illustrates this tooth-whorl arrangement so characteristically in a rendered image of one segment of the jaw (Figure 3C). A lingual view of a pre-hatch catshark embryo with three rows of teeth in development, none at the jaw margin yet, demonstrates the precise alternate arrangement of the first tooth crowns (Figure 3E), contrasting with the superficial, scattered arrangement of the early denticles. This difference is seen at the jaw margins in a juvenile catshark (Text Figure 1, Figure 3D) when ordered tooth loss at the margin of one set is also indicated.

Both sharks and rays provide fossil and extant forms that show ordered teeth along the jaw margins derived from a dental lamina, where alongside each functional one there are many developing tooth sets (successors, or 'many for one') that provide for their continuous replacement (Figure 1A, B). These tooth sets grade from a tooth germ deep within the jaw to those that are fully formed teeth, all are serially arranged, lingual to the functional tooth that is the first one ready to erupt (Figure 2A, B). Many of these tooth sets (large to small size, t1-t5, Figure 1B) may be preserved in articulated fossils.

Test of classical theory of teeth co-opted from skin denticles

Rostral saw-edge teeth from modified placoid scales

In order to discuss competing evolutionary theories, we can examine examples where enlarged skin denticles occur in some apparent order, but outside the mouth. Among neoselachian chondrichthyans, sawsharks and sawfish provide a model to test if skin denticles can be ordered in a similar way to teeth in the mouth. These allow comparison of pattern order that may further illuminate the evolution of developmental processes of oral tooth origins, in species where placoid scales can form 'saw-teeth" (Welten *et al*. 2015). Especially, we compared data from fossils to provide a range of adaptive diversity, and also extract developmental, morphological data where possible, to predict if a dental lamina had formed in their development, in order to test theories of the origins of teeth from skin denticles.

As potential examples of enlarged and ordered denticles, these elongate cartilaginous rostra with 'saw-teeth' along their edges have evolved within both major chondrichthyan crown groups. In elasmobranchs both rays and sharks (e.g., sawfish, sawsharks, extinct sclerorhynchid rays) have saw-teeth along the lateral margins of the extended rostra that are convergently derived and all described as modified placoid scales. Using the non-destructive technique of (xCT) we examined



Text Figure 1. *arrangement of four tooth files as replacements in the dentition at the jaw margin of a young catshark; alternate green and red files with a space where one tooth has been shed from the most distal parasymphyseal tooth file: contrasting blue scattered button-like denticles in the marginal skin. See figure 3 D for the same regions.*

extant (adults and embryos) and fossil species to see if these rostral saw-teeth showed order as in oral teeth, with multiple successor teeth aligned for replacement alongside functional ones (Smith *et al*. 2015;Welten *et al*. 2015). Fossil and extant, sawsharks and sawfish show ordered saw-teeth along the jaw margins, where each functional saw-tooth is associated with developing ones (successors below the skin) that provide for their replacement (Figure 4). However, much as these demonstrate a pattern order of initiation and replacement for the rostral saw-teeth, it is different from that in oral dentitions, each species also has an ordered dentition of close packed, alternate arrangement teeth, with many

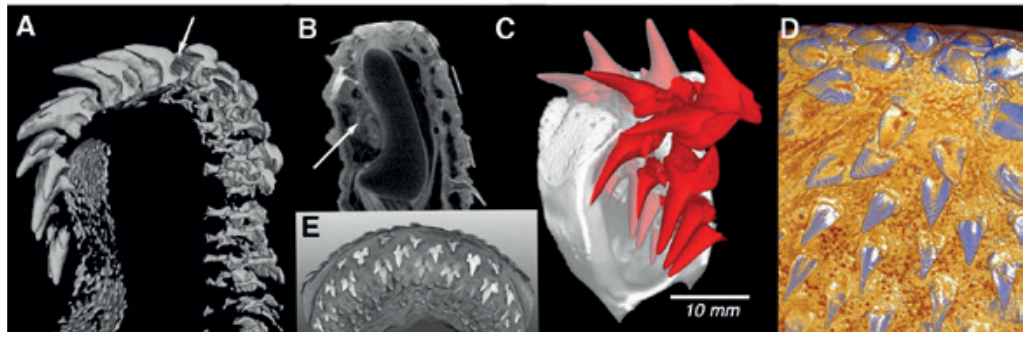


Figure 3 (A-E). *xCT 3D renders of tooth 'whorl' arrangement of timed replacement tooth sets compared with skin denticles at jaw margin; (Scyliorhinus canicula) catshark lower jaw A, B, juvenile, D, E prehatch. A, segment of tooth whorl (replacement teeth) up to jaw margin (white arrow), denticle clusters right. B, virtual section teeth on left, (tooth germ arrow) skin denticles right. C, Porbeagle shark, Lamna nasus lower jaw functional teeth and tooth whorls in cartilage furrow, segmented teeth red in Avizo 9. D, labial view of marginal button shaped denticles, scatterd skin denticles. E, density rendered in Drishti of lingual, view with three rows of unerupted, developing teeth in alternate arrangement.*

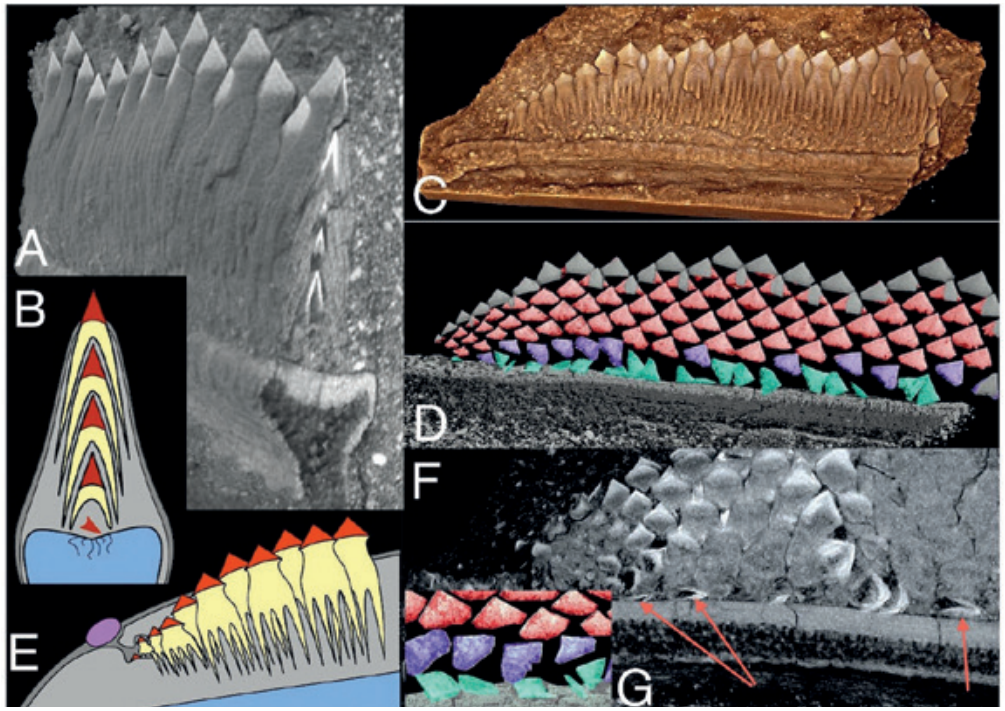


Figure 4 (A-G). *xCT 3D renders of partial rostrum of young Scihizorhiza stromeri (Cretaceous, Morocco), right side of rostrum, saw-teeth in situ (A, C) with growth of new small teeth at the tip (D-G), exposed lateral surface shows arrow shaped crowns in close packed, alternate arrangement (A, C). A, oblique view part of saw-tooth edge, density rendered crowns (in VG studio max), virtual section revels stack of replacement saw-teeth enclosed within the long roots attached to mineralised cartilage. B, cartoon of stacked replacement saw-teeth with new developing crowns, located in the cartilage furrow. C, whole specimen, volume rendered (Drishti software) from xCT. D, lower density roots virtually segmented and removed, revealing stacked crowns (higher density) of replacement saw-teeth extending laterally, occupying all space above the cartilage support surface, youngest crowns lie flat (green), then half rotated (purple), fully rotated to come below the roots (red), exposed at the surface (grey); inset G, higher magnification of first crowns to develop (green). F, growth-tip in virtual horizontal section, new saw-tooth crowns first lying flat against the cartilage, (red arrows). E, cartoon of the growth tip depicts, soft tissue reconstructed, how gene regulation at the surface (pink) might start the new saw-teeth as they differentiate from placoid scales and grow from the intucked epithelium.*

replacements lingual to the functional ones as standard for chondrichthyans.

Sawfish saw-teeth in sclerorhynchid fossils

One exceptional example of a species of the extinct ray *Schizorhiza*, Late Cretaceous, has sets of replacing teeth that look to model those of an oral dentition so we produced xCT scans of this fossil to analyse what was beneath the exposed surface of the rostrum comprising a near continuous sharp edge of close packed saw-teeth (Smith *et al*, 2015). These saw-teeth are arranged on the lateral margins only of the extended rostral cartilage, producing the functional saw-edge (Figure 4 A, C). Beneath the long roots of each tooth replacement teeth are stacked (Figure 4A, B), the youngest developing teeth in the cartilage furrow are lying flat (Figure 4D, F, G). We benefited from this particular and special fossil that had a growing tip to the rostrum and had preserved saw-teeth in life position up to the growing rostral tip (Figure 4C-G). Remarkably this specimen showed from the xCT with density rendered models that within the fully formed saw-edge all developmental stages of the nested set of replacement teeth had been retained (Figure 4D, F, G). These replacement teeth sit below the functional saw-teeth at the edge in nested sets like Russian Dolls (Figure 4A, B). The extended rostrum possesses saw-teeth with each functional one in an alternate pattern that have the replacement teeth beneath them (Figure 4A, B).

This fossil ray models complex 'tooth' replacement outside the mouth, as an example of diversification of skin denticles but the process of differentiation from regular placoid scales is decoupled from the process of internalisation of denticles into the mouth.

How oral teeth are organised in vertebrates

Development and evolution

Although modern sharks represent derived members of the Neoselachii, one shark species, the catshark *Scyliorhinus canicula* has become the model of choice as a laboratory animal for cartilaginous fish. Data on tooth development and patterning from a diverse range of animals, such as the shark, is key to our overall understanding of the core developmental processes that lead to modifications of the vertebrate dentition, including human teeth. Currently, we know a great deal about the genes and their association with patterning and development of teeth in mammals, primarily from studies on the mouse (the mammalian standard model for development). However, more details have recently emerged highlighting the diversity of dentitions and that the mouse dentition, like many 'standard' models of development, is highly restricted with such a low number of teeth in each jaw and no replacements (Jernvall & Thesleff, 2012). Therefore there has been a recent drive to develop more unusual models of tooth development that are replaced many times, like fishes and reptiles (Debiais-Thibaud et al. 2011; Tucker and Fraser, 2014). It appears that tooth development and the initial patterning process setting up the dentition is highly conserved among these vertebrate clades, showing a very tightly regulated developmental mechanism with a core set of genes. These regulate processes in all toothed vertebrates, with the core signaling networks likely to be present very early in vertebrate evolution, when teeth first appeared.

As noted, living species of Neoselachii can offer an opportunity to study potential 'ancient' developmental mechanisms, not available in clades without living representatives, such as placoderms and acanthodians. By using probes designed to reveal gene expression in specific subsets of dental cells, we can show how different genes coordinate the developmental timing and spacing of each set of new teeth (Figure 5) and compare them with those expressed in the skin denticles. Expression data for one key tooth initiatory gene, sonic hedgehog (*shh*), an epithelial signalling molecule (Smith *et al.*, 2009b) shows that intense *shh* expression is restricted to loci coincident with a temporal series of teeth in iterative jaw positions. This gene is implemented in the earliest signals for tooth positional information that sets off a cascade of signaling that directs tooth development and morphogenesis. As each tooth position forms, the process of tooth replacement can also be initiated, as the dental lamina emerges from the placodes at each locus for tooth development (Rasch et al. 2016), thus each tooth position is capable of making timed sets of replacement teeth that are regulated by site specific gene expression (Figure 5). This shift from a unitary locus to many in the same jaw position with continuation of lamina development shows that tooth regeneration may be an essential difference between the structural arrangement of teeth and that of skin denticles in the evolution of these integumentary mineralised units (Text Figure 1, Figure 3). As more information on tooth development and replacement has become available we can start to infer the evolutionary trajectory of tooth development in vertebrates. As noted, it has become clear that even though vertebrates possess great variation and complexity in their dentitions, they all share a common set of core genes that govern the process (Figure 5), including members of the Bmp, Fgf, Hh and Wnt/β-catenin signalling pathways (Fraser *et al.*, 2013; Rasch *et al.*, 2016).

Renewal and regeneration

One important characteristic of chondrichthyan dentitions is to provide a continuous supply of new teeth, as in sharks and rays (Figure 1A-B), where each new tooth is formed within the dental lamina by the initiation from epithelial stem cells (Figures 3A, B; 5D, F). The sharks are important to discuss when comparing vertebrate dentitions, as they have a very characteristic set of teeth, all seen as a whorl-like arrangement (Figure 3A, C), quite different from those of osteichthyan fish (Smith *et al.* 2009a) which develop without a continuous dental lamina along the

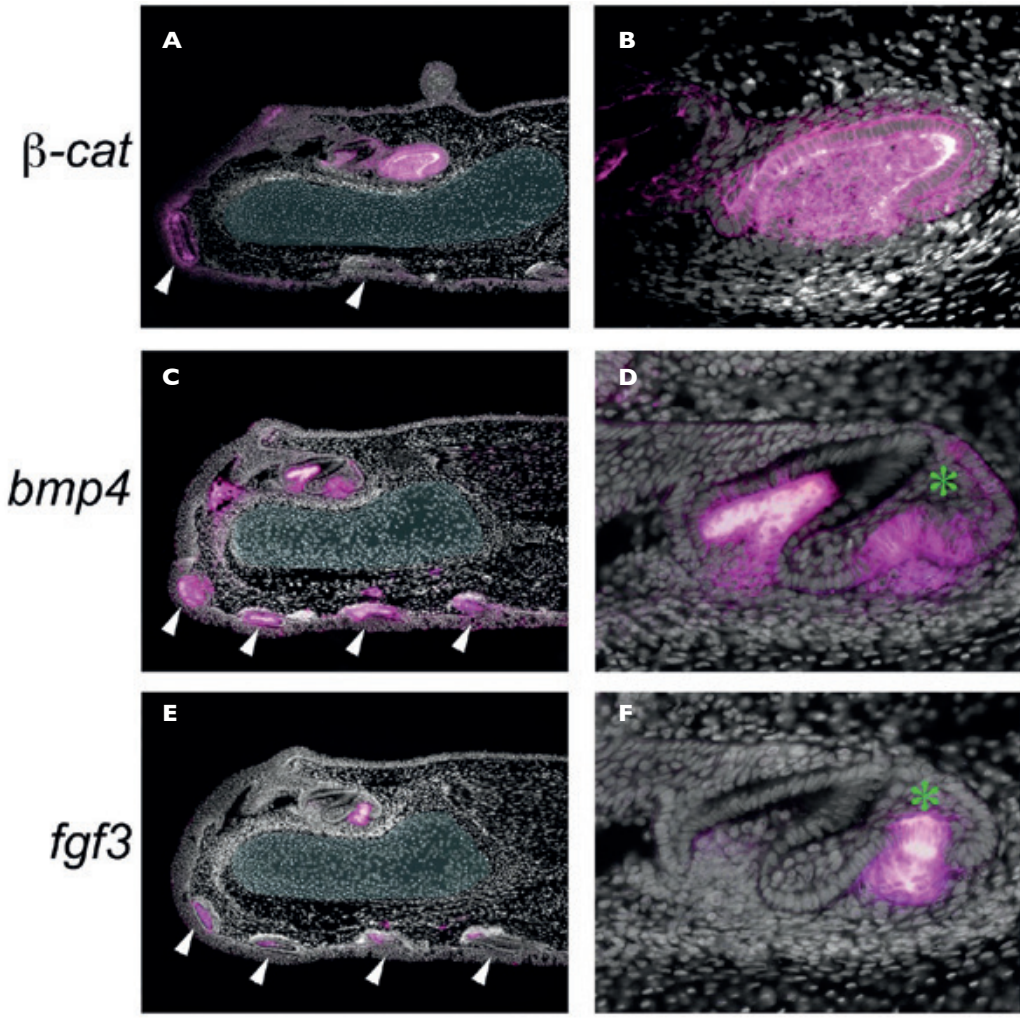


Figure 5 (A-F). *Gene expression (in situ hybridization) associated with tooth development in the lower jaw of the small spotted catshark (Scyliorhinus canicula). Functional and replacement teeth are present in developmental succession within the dental lamina, below the oral epithelium as a deep infolding of epithelial cells, in the furrow of Meckels cartilage. Developing teeth express a set of gene markers, including b-catenin (A, B), bmp4 (C, D), and fgf3 (E, F). All genes show expression (pink colour) in both the mesenchymal and epithelial cells of developing teeth and the skin denticles on the outside of the jaw (arrow heads). Teeth and skin denticles are examples of serial homology for these reasons although they are unrelated in developmental time and space. White cells are those labeled with DAPI (DNA) fluorescence. Asterix marks the likely position of stem cells in the dental lamina for the replacement tooth bud.*

jaws. In sharks and rays individual teeth attach from their base to a band of fibres that rolls around the cartilage, similar to a conveyor-belt mechanism for new tooth replacements. In this way they are able to regenerate their dentition within the lingual furrow with teeth formed in rapid succession because they are without a bony attachment to a jaw.

We, and others (Huysseune & Thesleff, 2004; Smith *et al*., 2009a; Tucker & Fraser, 2014; Rasch *et al*. 2016 believe that the dental epithelium contains stem cells (Figure 5 green asterix) that are capable of initiating new tooth formation in multiple sites throughout the life of the animals with continuous tooth regeneration, for example, in sharks, bony fishes and some reptiles. However, during mammalian evolution, indefinite tooth replacement was lost due to an inability to maintain the dental lamina necessary for succession of teeth. This ability is reduced in mammals, including humans, to only one replacement set with the coincident loss of the replacement dental lamina (Buchtova *et al*., *2012*). In these studies, investigating tooth replacement in the pig has shown that loss of essential epithelial cells (putative stem cells) was the main mechanism for loss of tooth replacement in these and other animals.

Teeth co-opted from dentine tubercles in dermal armour

The origins of teeth can also be informed by the diversity of structures in fossil jawless vertebrates, as early in vertebrate evolution, the skeleton was dominated by dermal bone with arrays of dentine tubercles as superficial ornament, these are related to teeth, as also are placoid denticles. Although isolated placoid denticles as dermal armour in which bone did not exist, are considered to give rise in evolution to oral teeth in chondrichthyans, other separate origins of teeth have been considered for fish with bone in their skeletons (Smith & Johanson 2003).

Evolution of teeth from ancient forms with bony armour

Regular tubular dentine is present in the tubercles of the ornament in dermal armour of a jawless fish that lived 380 million years ago (Figure 6A, B). This dentine has fine tubules of the same size as in human dentine, with growth from within the pulp cavity, and is also known to spread from the vascular pulp cavities to fill open spaces of spongy bone (Ørvig 1976). The ingrowing, space-filling dentine is deposited in response to regular wear and is recruited to repair damage to the bony armour (Figure 6C-G: Johanson *et al.* 2013).

In the agnathan fish (*Psammolepis*) although only existing as fossil forms, the secretary path of living odontoblastic cells is preserved as empty tubules created by the cell processes of the cells as they formed dentine. The migratory pathway of cell bodies as they secrete dentine, can be traced by these

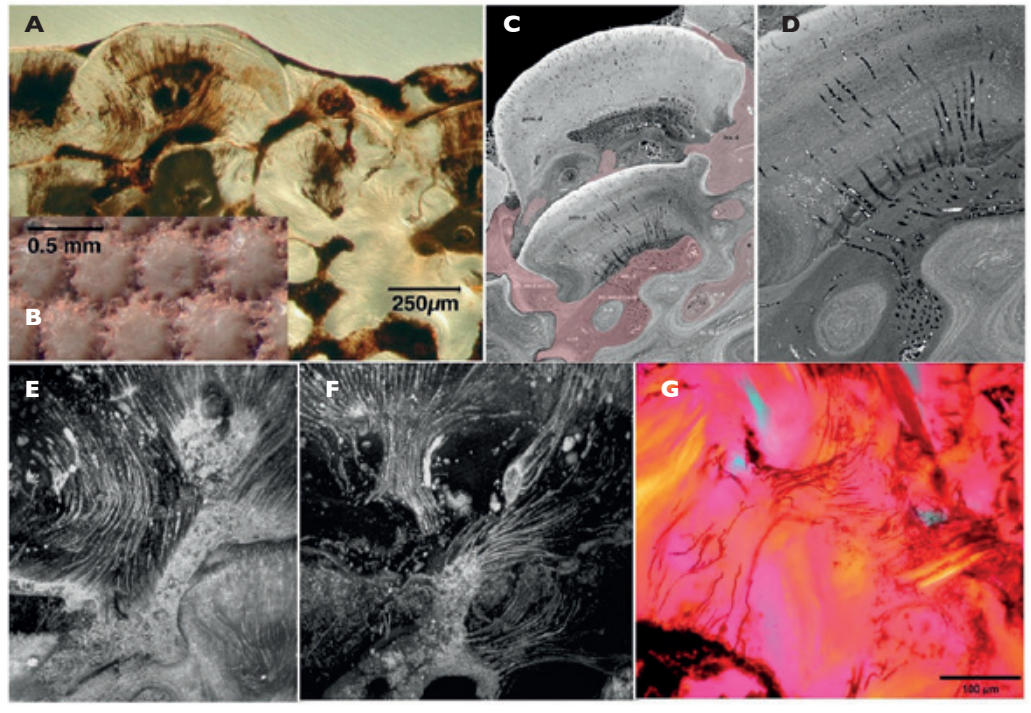


Figure 6 (A-G). *Sections through the dermal armour of Psammolepis venikovi, using different types of microscopy; A, phase contrast with inset surface macrophoto, tubercles fused to bone, B; C, D, backscattered electron scanning of section surface, pink translucent layer superimposed for secondary, infilling dentine; E, F, confocal, reflective scanning; G, polarisd light with a gypsum plate for sign of birefringence (blue/ yellow). Because the dentine tubules fill differentially with fossil deposits post-mortem they can be detected as brown in phase contrast (A), as irregularly deposited crystals by back-scattered electron microscopy (C, D) and by optical scanning microscopy as reflective, fluorescent, non-biological mineral (E, F). The distribution of invasive, secondary dentine within the fragments of bone that has been repaired by this dentine, seen as brown filled tubules (A, G); also fibre bundle orientation of the bone fragments are blue, or yellow, in groups aligned in opposite directions with a gypsum plate between the polars. Clusters of the tubules emerge from small vascular spaces in the bone (E, F) where they terminate as sites of (living) cell bodies of the odontoblasts at the hard tissue surfaces.*

tubules as the cells relocate into the vascular spaces in the bone (Figure 6E-F), hence its growth is shown where they had remained active at the surfaces of the new dentine. These various types of microscopy identify the tubule spaces in dentine and show that by invasion into the vascular spaces the bone has been filled with reparative dentine. We show that secondary dentine has filled in both the pore spaces between the individual tubercles and is continuous below them within the bone (false pink in Figure 6C). This results in a compact tissue, combined from dentine and bone tissue (Figure 6G) from one that was open as spongy bone with trabeculae around soft tissue spaces.

Dermal armour of jawless forms and co-option of regeneration

This invasive ability, a prophylactic action to resist damage used in wear and in response to a wound at the surface (Johanson *et al*., 2013), prompted the idea that properties of these tubercles were suitable for co-option to jaws gaining teeth; i.e. odontogenic potential from migratory cells is an essential component in the evolution of dentitions and their constant regeneration (Smith & Sansom 2000). Cell migration and continuous deposition of dentine from the tubercles within the bone as a regenerative process are properties of odontoblasts that are retained in teeth (Smith 2000) and are essential in growth and repair of all dentine (Couve *et al*. 2013).

Similar dentine tubercles co-opted as jaws had evolved could have transferred together with developmental genetic regulation to the inside of the mouth. Many new lines of research in progress will provide new data from fossils that address some of these points. For example, Long *et al*. (2010) proposed a phylogenetic origin of jaws where the first arch cartilages (jaws) of the pharynx were enhanced by bone to support teeth, with co-option of the developmental mechanism that can make these bones, but in particular sclerotic bone around the eye. Experimentally, scleral epithelium can induce bone formation in the chick when transferred to the embryonic jaw (Hall, 1981) so that a heterotopic process could occur and these regulatory mechanisms co-opted to the inside of the jaws.

Conclusions

These observations (Johanson *et al*. 2013) in *Psammolepis* showed that the superficial pores and associated canals were filled with invasive dentine, from migrating odontoblasts, supporting the idea that odontogenic stem cells were located within this network of blood vessels. Renewal of new teeth, and their regeneration that may involve stem cells is an important property that defines dentitions and allows their adaptation and evolution. Thus the properties of teeth in the form of dentine tubercles, were first evolving in jawless vertebrates over 380 million years ago, and persist in bony fishes until now when involved in tooth repair in living animals, including humans.

The topic of the origins of teeth and their historical evolution is currently an active research topic in the fields of palaeontology, developmental biology and molecular skeletogenesis. Some research endorses our conclusions, as expressed by Rasch et al. (2016) "at the dawn of the vertebrate lineage teeth were most likely continuously regenerative structures and utilised a core set of genes" but we are still uncertain of which structure existed that gave rise to teeth inside the mouth co-incident with the origin of jaws.

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