How teeth are organised into functional dentitions

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This is an update on our article "Jawed vertebrate dentitions – when did teeth evolve" which appeared as a review for infocus 42, June 2016 but since then two important papers have just been published on the topic we choose to headline here.

1. Sharks cutting blade dentitions evolve from modified inherited order for tooth replacement by developmental tooth loss

This paper shows how changes in developmental order of individual blade shaped teeth can form a continuous, serrated blade along each jaw margin. To function effectively these teeth are replaced as a single unit along the lower and upper jaws as the blade erupts onto the functional margin.

2. Evolution of teeth from denticles in the skin by acquisition of tooth regeneration through a stem cell factor sox2

This links tooth development in sharks with the evolution of a replacement pattern by acquiring a new function of a gene that mediates regeneration of stem cells for making replaceable teeth.

These two new papers both use embryos of living species of shark to describe how the dentitions are patterned and regulated in development to achieve the adult dentition that is specific to the morphology that facilitates the shark's ability to survive at the top of the food chain, as apex predators. One huge advantage conferred on sharks and rays (neoselachians) is the rapid and impressive conveyor-belt method of tooth production. All teeth at the jaw margins having numerous replacement teeth located under the oral skin at each tooth position, made ahead of function. This replacement pattern emerges even from the earliest initiation of teeth at the jaw margins; with each site (tooth germ) generating an iterative set of successive teeth for future replacement,
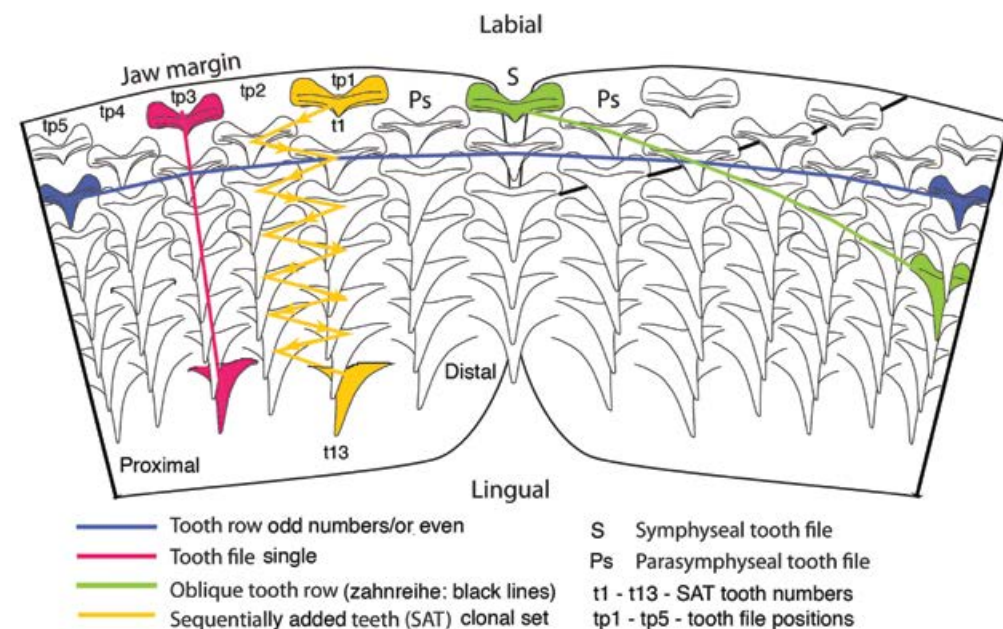

Figure 1. Diagram of sets of replacement teeth from a typical neoselachian jaw - closely packed teeth depicted in four different alignments to explain how iterative generation is ordered in development; red for a single file of clonally successive teeth; yellow for a linked clonal set that establishes the alternate pattern of tooth eruption; blue for the horizontal successive sets of either odd, or even numbered tooth positions, resulting from the developmental order each set may erupt synchronously, or asynchronously, along the jaw; green oblique row starting from a symphyseal tooth and representing teeth that normally erupt at different times as the files in which they were generated are in an iterative sequence from the jaw margin. From Underwood et al. 2016, Figure 3.

aligned below each functional tooth. This feature of vertebrate dentitions, the timed pattern order that generates these replacement tooth sets is the defining character, one that was emphasised in 'the origin of jawed vertebrate teeth' (Smith, et al 2016; Figures 1-3), a pattern 'fit-for-purpose' and inherited through 500 million years of evolution. We now know that a regenerative dentition requires the acquisition and a change of function of one stem cell factor, Sox2, distinct from that of non-replacing skin denticles (Martin et al 2016) that cover the external surface of sharks and rays. These denticles, at least in sharks, do not regenerate and only develop when space becomes available in the adjacent skin. This lack of regenerative potential among skin denticles is mirrored by the lack of certain key genetic factors in these 'skin teeth' (Martin et al. 2016). This new data offers an important addition to the canonical story of tooth origins that suggested teeth evolved from external skin to jaws.

A model for simultaneous replacement of cutting blade teeth

The squaliform sharks are a derived group, but the genus *Squalus* is commonly used as a 'model organism' for dissections. Most squaliforms have a modified dentition in the lower, or both, jaws wherein teeth are aligned on the jaw margin to form a continuous blade. The pattern of tooth replacement represents a highly modified version of the general alternating replacement seen in other clades (Underwood et al 2016; Figure 9). To function effectively, cutting blade teeth are replaced as a single unit along the jaws as the blade erupts onto the functional margin. In order to do this the inherited replacement pattern of alternately timed tooth eruption has evolved from one of each tooth erupting at alternate times, and asynchronously along the jaw (distal to proximal, Figure 1), to one which is modified in development so that teeth of altered, synchronised developmental times, erupt simultaneously at the jaw margin.
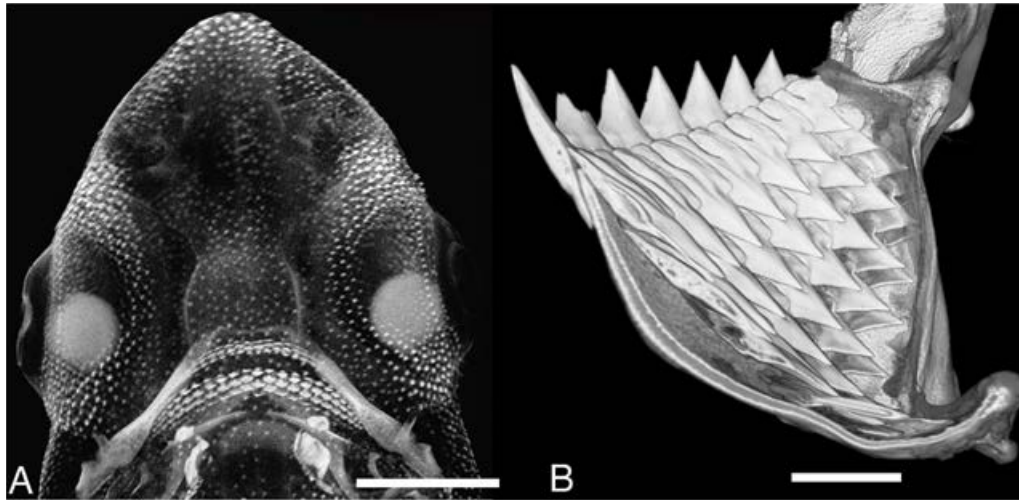
As conventionally assumed, the eruption time depends on the timing of initiation of replacement teeth, an example of perpetual biological regeneration that results in alternate tooth eruption. This model is the alternate, asynchronous set of replacement teeth, both the iterative set along the jaw (distal to proximal) and the iterative successional, clonal tooth sets with inbuilt alternate timing (yellow, Figure 1: see also Smith, MM, Johanson Z, Underwood C, & Diekwisch T. 2013). To create the cutting blade from teeth at the jaw margin either, the two horizontal rows of even and odd numbered teeth would have to change from asynchronous, to synchronous development, or the oblique developmental row would form those teeth that achieve simultaneous eruption at the jaw margin.

How this could change was a big evolutionary question and one that had to occur through developmental plasticity, but how this was achieved was previously unknown. From a study of embryos in this group it was clear that the bladed dentition involved a change in the inherited, alternate developmental pattern of relative timing, where teeth are discarded *in utero* before their use so that all remaining teeth of the different initiation time, aligned along oblique rows, (green, Figure 1) can erupt at the same time at the jaw margin.

Micro-CT scans of embryos of squaliforms such as *Squalus* revealed the inherited, neoselachian alternate tooth pattern with addition of all new teeth in alternate positions, also with asynchronous timing of tooth production from distal to proximal along the jaw margin. In this pattern (from the growth series) successive tooth rows parallel to the jaw margin (blue, Figure 1) contained additional teeth added proximally. However, in the bladed forms tooth loss prior to birth brings the timing of proximal teeth (none lost before birth) gradually into register with distal ones where teeth had been lost, prior to use. In this type the oblique rows (green, Figure 1) come to erupt at the same time.

Figure 2. Morphogenetic fields comparing skin denticles with replacement teeth - A, density difference render from micro-CT data of a 15cm embryo of the long-nose spurdog (Squalus blainville) in ventral view, reveals a regular, alternate replacement tooth pattern for developing tooth sets in upper and lower jaws, that is also quite distinct from the scattered pattern for the enhanced, morphogenetic fields of skin denticles, around the eyes and rostrum. B, Rendered micro-CT of lower jaw segment of the kite-fin shark (Dalatias licha), virtual dissected view of exposed lingual replacement teeth in sets of five for each file, aligned 180° to erect functional teeth. Scale bar = 1cm. From Underwood et al. 2016; Figures 5b, 4d.

This data from embryos revealed that the squaliform dentition pattern in the adult represents a highly modified version of tooth replacement to that seen in other clades. The developmental process involves loss of unused distal teeth until the tooth germs initiated proximally at later times, can erupt with those distally generated at the same time and simultaneously come to the jaw margin. Comparing the embryonic *Squalus* dentition with the adult shows that the common neoselachian alternate pattern is present in the embryo (Figure 2) and is converted into an adult pattern where the earlier oblique rows are now parallel to the jaw margins,

facilitated by a presumed shift in timing of tooth initiation to achieve synchroneity. All these derived dentitions of squaliform sharks have become what is a single file type, but is a transformation of the inherited alternate, iterative double clonal set, pattern of tooth addition.

The interlocked teeth along the cutting blade (Figure 3) form effective serrations and all teeth are replaced together and can function together as an adaptation to a specialised feeding mode, a set of teeth that is functional along the whole jaw. This is likely to have been one of the key innovations that allowed the squaliforms to become a diverse
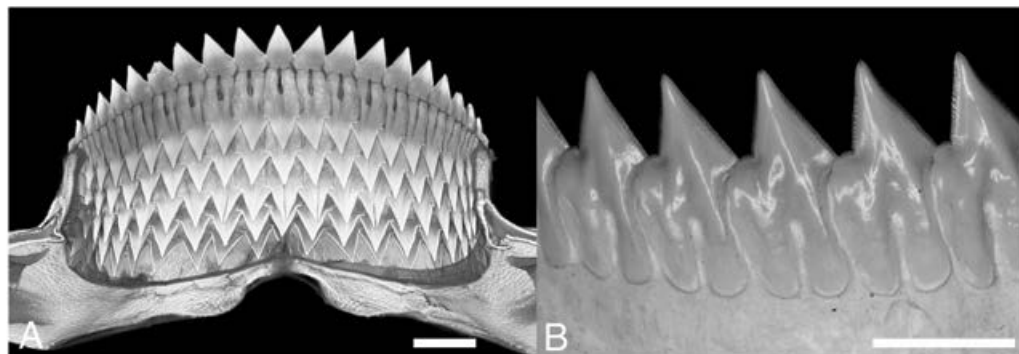

Figure 3. Lower jaw teeth of the adult Kite-fin shark (Dalatias licha) – (A) lower jaw as a virtual dissection of all replacement teeth exposed as developmental sets, five in each file below the functional tooth position. Each horizontal row is parallel to the jaw margin and teeth can erupt together linked by overlapping bases. (B) close up demonstrates the interlocking (imbrication) of functional teeth so that all are joined below the cutting edge by the expanded bases to make a serrated blade. Scale bar = 1cm. From Underwood et al. 2016; Figures 4c, 2e.
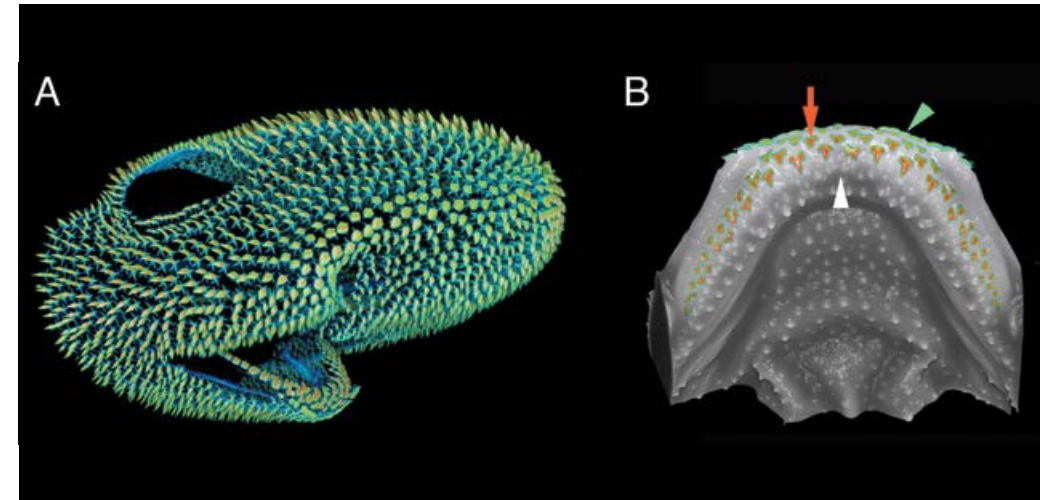

Figure 4. Teeth, skin denticles and taste buds - Micro-CT rendered images of (A) the head of a hatchling catshark (Scyliorhinus canicula) covered in skin denticles (green) structures that are distinct from the oral teeth (red). Note two rows of teeth are highly organised, denticles in the skin are not. (B) micro-CT rendered dorsal view of the catshark lower jaw showing the external skin denticles (green, green arrow), oral teeth (red, red arrow) and the taste buds that line the oropharynx, that are in a regular patterned rows (white arrow) are immediately adjacent to the erupted rows of teeth.

and successful clade of sharks, dominating many deep and cool seas, their success arising from key modifications before birth that allowed a cutting blade to form at the jaw margins. All result from the dental lamina controlled pattern information in chondrichthyans that mediates development of extremely diverse dentitions, as explained below (Martin et al., 2016). This previously unrecognised, extreme developmental plasticity is enabled from one developmental mechanism, shared amongst chondrichthyans.

Stem cell control for ordered continuous tooth regeneration

This new paper develops a model to formulate the developmental genetic basis of successional regeneration of teeth that differs from non-successional odontode addition of skin denticles as an irregular, replacement process.

A defining character of true teeth is the expression of the stem cell factor *Sox2*, known to maintain stem cell potential in oral epithelial cells to ensure teeth regenerate continuously (Juuri et al. 2013; Martin et al. 2016). In contrast, this tooth-specific character is not active within skin denticles, although they are

structurally equivalent to teeth, but do not undergo continuous, controlled regeneration. This genetic study aimed to answer the question of how key differences between skin denticles (placoid scales; Figure 4) and functional teeth, emerged in evolution of teeth at the jaw margins, as both are based on the same unitary developmental module.

A significant distinction between teeth and skin denticles is that succession of teeth is by an organised iterative process, biological regeneration, deep to the dentition at the jaw margins; denticles do not have this potential. As reviewed (Martin et al. 2016) the deep epithelial fold from the oral surface (dental lamina; Rasch et al. 2016) allows a compartment of stem cells to form from a common epithelium that develops both teeth and taste papillae in relation to a dental/taste junction (Martin et al. 2016). The growth of the dental lamina deep into the jaw produces new tooth replacements from the 'free end' of the lamina fed from the surface by progenitor cells specifically destined for odontogenesis (Rasch et al. 2016). The relationship between the taste regions of the oral jaws and the developmental emergence of the dental lamina and tooth regenerative capacity is linked to the presence in the region of the gene, *sox2* (Figure 5).
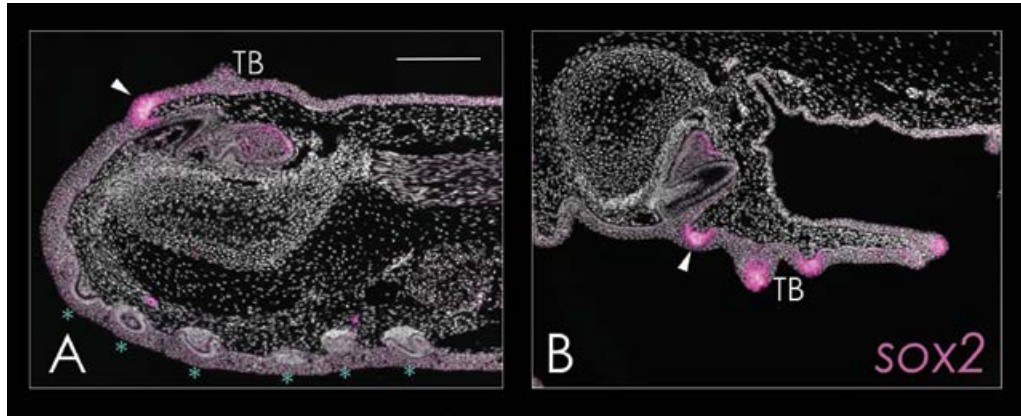
Figure 5. Stem cell marker sox2 related to continuous tooth regeneration in sharks in the catshark (S. canicula). Thin sections of sox2 gene expression (magenta) at the taste/tooth junction (arrows) of the lower (A) and upper (B) jaws, where Sox2 is expressed in association with teeth and taste buds but not skin denticles, a key distinction between skin denticles (lower surface in A) and teeth. Scale = 200µm.

Skin denticles do not have regenerative potential akin to teeth of the oral jaws, and do not express this key stem factor (*sox2*), we can therefore propose a new scenario for 'true' (regenerative) tooth origins that involves the cooption of oral taste-linked signals in association with tooth competence of the dental epithelium (Figure 5). Therefore, we could infer that teeth may have acquired their distinct regenerative capacity from taste-linked stem cells that provided key properties that transformed tooth-like structures, similar to skin denticles, into an organised and repetitively programmed dentition. This evolutionary event characterises crown gnathostomes, but humans as do most mammals, only reiterate this regenerative process once for each of the 20 first teeth, with the three permanent molars forming once to be retained.

Methods

Specimens were scanned using the Metris X-Tek HMX ST 225 XCT scanner at the Imaging and Analysis Centre, Natural History Museum, London. Three-dimensional volume rendering and analyses were performed using Avizo Standard software (v. 8.0.1) (https://www.fei.com/software/amira-avizo/), VGStudio MAX v. 2.0 (http://www.volumegraphics.com/en/products/vgstudio-max.html)

The catshark (*Scyliorhinus canicula*) head was scanned using microCT (ZEISS Xradia 520 Versa,

at 60kV with pixel size of 20µm) and 3D volumes were reconstructed using the free software 'Drishti' (github.com/AjayLimaye/drishti) using one transfer function to reveal the mineralised tissue (Red = most dense; Blue = least dense).

Acknowledgements

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