9 Evolution, Development and Regeneration of Fish Dentitions

Gareth J. Fraser and Alex P. Thiery

Abstract

The diversity of fishes provides a wealth of morphological variety to investigate at the developmental and genetic level. The diverse range of phenotypes offered by fish dentitions provides an excellent comparative context for studies of evolutionary developmental biology. This chapter discusses the evolution, development and regenerative capacity of chondrichthyan and osteichthyan dentitions. We provide an overview of the recent insights into how general fish dentitions are initiated and how they continue to redevelop over multiple tooth generations.

9.1 Introduction

The diversity of vertebrate dentitions, in both form and function, is staggering. Fishes reflect a huge proportion of this craniofacial diversity, with teleost fish species alone accounting for more than half of all extant vertebrates (upwards of 30,000 species). Cartilaginous fishes (Chondrichthyes) add even more diversity to the wider cohort of 'fishes' and another ~1,000 species, and so, collectively, these are the most dominant and diverse members of the vertebrate clade. Given this bias in nature, the emergence of more diverse fish models for research into evolution and developmental biology (Evo-Devo) is necessary in order to understand morphological diversity observed in these jawed vertebrates. Osteichthyan and chondrichthyan fishes show a comprehensive range of diverse phenotypes, not limited to but seen to an extreme in their dental characters that reflect the variety of trophic niches and habitats they exploit.

The iterative emergence of new tooth replacements within organized families (successional regeneration) associated with oral jaws, typifies the dentition of crown gnathostomes (Chondrichthyes, including acanthodians, plus Osteichthyes). Most fish, whether bony or cartilaginous, possess a polyphyodont dentition capable of developing multiple generations of teeth. In the case of these vertebrate groups, they routinely produce teeth throughout their entire lifetime (Berkovitz and Shellis, 2016).

Not only is polyphyodonty a plesiomorphic character within the chondrichthyan and osteichthyan clades, it is also thought to be the most ancestral condition for all vertebrate dentitions (Botella, 2006; Botella et al., 2009; Rücklin et al., 2012; Maisey et al., 2014). This suggests that the production of multiple generations of teeth was a character present in the earliest jawed vertebrates (Rücklin et al., 2012). Although dentine and enamel-type units (odontodes) were present in extinct jawless (agnathan) fishes, their capacity for multiple generations are unclear. However, evidence suggests that multiple generations of pharyngeal 'denticles' were produced in the oro-pharyngeal cavity of extinct jawless fishes (e.g., thelodont tooth whorls), suggesting that an early tooth replacement mechanism was in place before the evolution of jawed vertebrates (Smith and Coates, 1998; Rücklin et al., 2011, 2012). This, therefore, may provide evidence to suggest that polyphyodonty is not a gnathostome innovation but potentially a vertebrate novelty; however, it appears that pharyngeal tooth whorls in thelodonts could in fact represent a more derived condition in this group (Rücklin et al., 2011).

The origin of true teeth in early vertebrates is still a matter of contention, and it is still unclear whether teeth evolved first in the oro-pharyngeal cavity or the skin of jawless fishes (Smith, 2003; Fraser et al., 2009, 2010; Donoghue and Rücklin, 2016). However, it seems that at least a palaeontological consensus is emerging with recent support for a more external origin of odontodes before oro-pharyngeal teeth (Donoghue and Rücklin, 2016). Extant fishes still retain the capacity to make tooth-like units (odontodes) in both the mouth and in the skin, e.g., elasmobranchs (Fig. 9.1). Recent Evo-Devo studies have attempted to use the developmental genetic basis of these units to appreciate the potential relationship between these distinct but structurally similar tissues. What is now clear is that these two distinct internal and external classes of odontodes, once thought to be unified, are separate developmental modules, at least in extant gnathostomes (Fraser et al., 2009, 2010; Donoghue and Rücklin, 2016; Martin et al., 2016). But how this developmental independence will help resolve the origins of teeth requires further interdisciplinary investigation, incorporating both palaeontology and developmental biology to decipher whether

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

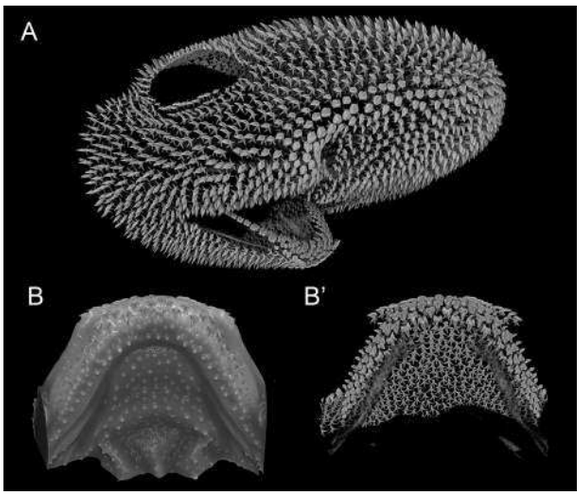

Fig. 9.1. Rendered micro-CT scans of a hatchling small-spotted catshark (*Scyliorhinus canicula*) **A)** Head showing coverage of skin denticles and teeth in the jaws. **B/B')** Dorsal view of the lower jaw with (**B**) soft tissue revealed through contrast staining showing the taste buds present in association with the teeth (coloured red/green) and (**B'**) hard tissues segmented highlighting the teeth and replacement teeth of the lower jaw (red/yellow) and the bases of the skin denticles on the outer surface (green). A black-and-white version of this figure appears in some formats. For the colour version, please refer to the plate section.

tooth-like structures first appeared inside (oropharyngeal endoderm) or outside (ectoderm) (Smith, 2003; Fraser et al., 2010). Whether these embryonic cell layers differ in their ability to produce potentially different odontodes (internal regenerative teeth versus non-regenerative external denticles [Martin et al., 2016]) is unclear. The endoderm and ectoderm could offer dissimilar induction events for these structures that converge on form, these based on the secondary instruction of mesenchymal cells derived from migratory neural crest cells (ectomesenchyme). Either cell type, or only mesenchyme can react to the initiatory signals of the epithelia (whether endodermal or ectodermal; Fraser et al., 2010). However, it is clear that the oral teeth of extant and extinct vertebrates have a unique regenerative capacity that is not shared by external odontodes (Martin et al., 2016). It has to be established whether or not this is related to the presence of other regenerative appendages, e.g., taste-like units (Martin et al., 2016) or to other factors that are specific to the endodermal oro-pharynx.

The process of tooth development and redevelopment (regeneration) has been well documented in several groups of fishes over recent years, and now a vast amount of data focused on these developmental mechanisms has emerged (Moriyama et al., 2010; Debiais-Thibaud et al., 2011, 2015; Fraser et al., 2012, 2013; Abduweli et al., 2014; Bloomquist et al., 2015; Ellis et al., 2015, 2016; Streelman et al., 2015; Martin et al., 2016; Rasch et al., 2016; Sahara et al., 2017; Thiery et al., 2017). This is a particularly exciting period for experimental evolutionary developmental biology (Evo-Devo), especially in light of recent genomic and transcriptomic advances expanding our collective knowledge of the genetic regulation of odontogenesis in many non-mammalian subjects. Fishes more generally, offer a set of emerging (non-classical) models for the study of evolutionary and developmental biology, and recently more derived teleost (actinopterygian) and chondrichthyan fish (elasmobranchs) have emerged as potentially new Evo-Devo models to expand the field into an exciting variety of morphological novelty and diversity.

9.2 Conservation of Tooth Development in Fishes

Regardless of the diversity observed in dental patterning, shape and the regenerative potential of the vertebrate dentition, there is an overwhelming conservation of the genetic mechanisms underlying tooth development and regeneration (multi-generational odontogenesis; [Debiais-Thibaud et al., 2015; Rasch et al., 2016]). It now appears clear that tooth development is a highly stable biological process and that irrespective of developmental tinkering over time, which can alter shape, rate of development, and number of tooth generations, the fundamental process of tooth development is strictly adhered to. This then suggests that a core set of conserved 'tooth' genes are known to regulate tooth development throughout the vast diversity of vertebrates from sharks to humans (Jernvall and Thesleff, 2012; Martin et al., 2016; Rasch et al., 2016; Thiery et al., 2017). Furthermore, the stability of this process across a large portion of evolutionary time (~400 million years), therefore, promotes the idea that at the earliest point in the evolution of jawed vertebrates, the genetic programme for tooth initiation and development emerged to provide the basis for tooth development that has remained relatively unchanged to the present day. This central criterion for the construction of a tooth is therefore also an important universal basis for translational advances in dental biology – essentially any tooth or dentition can provide the foundation for a deeper understanding of a variety of processes governing multi-generational odontogenesis (Martin et al., 2016; Thiery et al., 2017).

The literature includes many instances of defining dental characters on the basis of distinct morphologies, hard tissue mineralisation and fossil preservation; however, it is important to stress that in terms of development and regeneration there are a number of processes that are defined by subtle signals in otherwise homogeneous soft tissues that will not be detected or represented by these descriptors of later-stage development. Therefore, it is

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

important to think about these developmental mechanisms in terms of the whole process: initiation of the dental signalling cascade and maintenance of regenerative signalling are key 'characters' of dental anatomy without the clear signs of morphological distinction. This is especially important when considering fossils, where soft tissue and cellular level details cannot be determined. Therefore, the continued study of early genetic programmes of tooth development and regenerative signalling cascades across diverse groups of vertebrates will be essential to our interpretation of morphological traits in both extinct vertebrates without soft tissues or embryonic stages, and extant taxa with little or no access to embryonic specimens.

The current list of new Evo-Devo models for oral tooth development, and craniofacial diversity, more generally, include several teleosts: medaka (*Oryzias latipes*; Abduweli et al., 2014), stickleback (*Gasterosteus aculeatus*; Ellis et al., 2015, 2016), African cichlid fishes (Fraser et al., 2009; Streelman et al., 2003; Streelman and Albertson, 2006), Chondrostei: the paddlefish (*Polyodon spathula*; Smith et al., 2015), and chondrichthyan fishes, e.g., the catshark (*Scyliorhinus canicula*; Smith et al., 2009a; Martin et al., 2016; Rasch et al., 2016). This collection of 'fishes' covers a large phylogenetic portion of the vertebrate tree, offering new insights into the conserved development and genetic control of ancient and more basal characters of the craniofacial skeleton. Here, we will discuss some of the more recent information gathered on these species to provide a general understanding of the dental and regenerative mechanism underlying the diversity in these groups.

9.3 Making the First Tooth: Initiating the Development of a Dentition

Tooth development in most vertebrates begins with the expression of a number of initiatory signals prior to the onset of morphological shifts in the early oral epithelium (Thesleff and Sharpe, 1997; Cobourne and Sharpe, 2003; Tucker and Sharpe, 2004; Jernvall and Thesleff, 2012). It is now clear that these initiatory signals can be combined into a highly conserved 'core gene set', for all toothed vertebrates, which are involved in both initiation and tooth development more generally. This core gene set can be extrapolated toward a functional gene network for every tooth that has ever been made in nature, and importantly this core gene set (Fraser et al., 2009, 2010; Tucker and Fraser, 2014; Rasch et al., 2016) facilitates tooth development across a great range of evolutionary change from fishes (chondrichthyan and osteichthyan) to mammals (Jernvall and Thesleff, 2012). Regardless of the final dental form, the early beginnings of all vertebrate teeth share a classic process of initiation, and subsequent early morphogenesis.

Typically, the process of vertebrate tooth initiation begins with a set of initial signals, e.g., *shh*, *pitx2*, that help to direct the instigation of local changes to the seemingly uniform oral epithelium toward a dental fate. In fact, these signals may be present in a band of expression termed the 'odontogenic band' (at least in fishes; [Smith et al., 2009a; Fraser et al., 2004, 2008; Martin et al., 2016; Rasch et al., 2016]) prior to any morphological signs of cellular differentiation in the oral epithelium, therefore, molecular screens of this early initiatory cascade are truly the first indication of the dental character in vertebrates (Fraser et al., 2004, 2006, 2008; Debiais-Thibaud et al., 2015; Rasch et al., 2016). In fish, these signals act to trigger a morphological cascade in the tissue, leading to a thickening of the now dental epithelium toward the emergence of the first tooth placode – this thickened epithelial band is the emergence of dental lamina development (Smith et al., 2009b; Tucker and Fraser, 2014). However, in some fish, e.g., salmonids (rainbow trout, *Oncorhynchus mykiss* [Fraser et al., 2006] and the salmon, *Salmo salar* [Huysseune and Witten, 2008]), the odontogenic band does not develop into a typical dental lamina, and tooth development proceeds from this superficial thickening with subsequent tooth generations forming from the deep epithelial regions of the predecessor tooth rather than a deep or invaginated dental lamina (Berkovitz and Moore, 1975; Fraser et al., 2004, 2006; Huysseune and Witten, 2006, 2008).

Regardless of whether a species develops a true dental lamina or utilises a more superficial odontogenic band, the first tooth placode in fish is generally a superficial epithelial unit distinct from the surrounding oral epithelia. This is likely true among all fishes, regardless of dental form and diversity. It appears that the event of first tooth development is relatively well conserved among fish, at least from elasmobranchs to osteichthyan fishes (Smith et al., 2009a; Fraser et al., 2004, 2008, 2012; Martin et al., 2016; Rasch et al., 2016), where a thickened epithelial placode emerges from the neighbouring dental epithelium and begins the process of differentiation (Fig. 9.2). The tooth itself is a collaborative union between two tissue types: (i) the epithelium, where the signals first direct tooth placode initiation (Lumsden, 1988), and (ii) the underlying (ecto) mesenchyme, where local cells congregate to the site of the overlying thickened placode that envelops the mesenchyme, forming a cap-shaped unit (cap-stage) to begin the process of tooth morphogenesis (Thesleff and Sharpe, 1997; Tucker and Sharpe, 1999, 2004; Jernvall and Thesleff, 2000). These two cell layers then choreograph a three-dimensional interplay, transforming the thickened placode into the characteristic early tooth, where cell types begin the process of differentiation and the tooth takes shape.

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

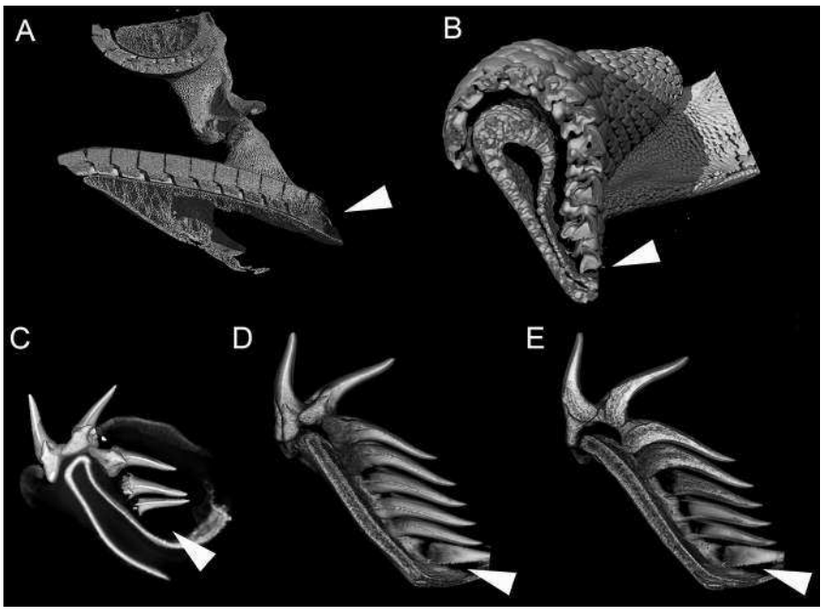

Fig. 9.2. The developing shark dentition. Serial sections of the lower (**A–D**) and upper (**E–H**) jaw dentition labelled for PCNA immunohistochemistry. Proliferating cells are observed throughout the initiating dentition from the early invagination of the odonto-gustatory band (OGB) to form the dental lamina (**A** and **E**), through to first tooth initiation (**B** and **F**) and morphogenesis (**C** and **G**), then through stages of second-generation tooth formation (regeneration) from the deep developing successional lamina (black arrowheads in **C–G**). Note the position of the superficial taste buds to the emergence of the dental lamina (arrows).

The formation of the first tooth, and the general initiatory sequence of events, is an important instigator for the collective development of the functional dentition. It appears, in fishes at least, that the first tooth and its initiation are essential for the normal development of the subsequent teeth, both within the first generation and subsequent generations (Fraser et al., 2008), this suggests a widespread phenomenon, where failure of first tooth development has a profound influence on the rest of the dental field. Therefore, the patterning and development of the entire dentition could rely heavily on the initiatory capabilities of the first tooth to begin the dental programme and may be essential for the correct patterning and development of the whole dentition. This is an intriguing scenario that suggests there might be no alternative initiation process; thus, if the first tooth does not initiate, then there may not be a 'back-up' mechanism for the neighbouring teeth to develop – even from a competent and existing odontogenic band. The initiatory trigger for wide-ranging developmental processes is an intriguing research topic in developmental biology and biomedical science that, across many systems, may utilise similar gene networks and cells types. The importance of the first tooth, therefore, has major implications for successional tooth sets and their morphology. Not only does the first tooth offer a starting point for the rest of the dentition to follow, but it also creates the key initiation point for a strict patterning mechanism (Fraser et al., 2006, 2008). In fishes, more generally, the developing first generation tooth set can materialise as an 'experimental' and rudimentary collection of tooth units with simple shape (unicuspid) compared to the subsequent generations (Smith et al., 2009a; Fraser et al., 2008; Rasch et al., 2016). In many cases the rudimentary, and often not functional, first-generation dentitions are followed by more complex tooth types that emerge as successional teeth.

After the development of the more superficial first teeth from a thickened epithelial odontogenic band, the epithelium continues to proliferate and invaginate into the underlying mesenchymal tissue (Fig. 9.2); this is the beginning of dental lamina formation. The dental lamina is a diverse structure that can form a single tooth or multiple sets of teeth, depending on the dentition. The dental lamina is a set of epithelial cells that connect the site of successional tooth initiation to the oral epithelium, and in many examples it connects teeth of the same family (Fig. 9.2). Recent studies among diverse vertebrates, including fishes, suggest that the preservation of the dental lamina is essential for subsequent generations of repeated tooth formation – this process of regeneration requires the maintenance of tooth competent cell progenitors, and the dental lamina adopts this housekeeping role (Juuri et al., 2013b; Martin et al., 2016).

9.4 The Dental Lamina – A Source for Diversity in Tooth Regeneration

The mechanism of tooth initiation – from a set of dental competent epithelial cells (odontogenic band) – marks not only the onset of first generation tooth formation but also the emergence of the dynamic epithelial dental lamina

(DL) (Smith et al., 2009b). The dental lamina is a structure defined by a competent dental epithelium from which new teeth can emerge. The lamina, in simple terms, is a connecting sheet of cells that links the oral epithelium at the surface (oral cavity) with the region where new tooth generations will form (successional lamina) – and this is usually an invaginated structure deep within the jaw. The dental lamina essentially links each 'related' tooth with a given family, whether this is a continuous and permanent unit as in the case of elasmobranchs (Fig. 9.2) or less permanent and sometimes discontinuous in teleost fishes, where the dental lamina is specific to each tooth and family of related teeth, for a given position (including predecessor and successor teeth of the same position along the jaw). Typically in fishes, the DL emerges during the formation of the first teeth (Smith et al., 2009a, 2009b; Fraser et al., 2008, 2009; Rasch et al., 2016) and continues to develop into a continuous sheet of cells via epithelial cell recruitment from more superficially located cells contained within the lamina proper (Fig. 9.2; Martin et al., 2016; Rasch et al., 2016). A dental lamina is necessary for multiple generations of teeth (Smith et al., 2009b; Martin et al., 2016; Rasch et al., 2016). The lamina houses important epithelial progenitor cells for the continuation of next-generation tooth production (Martin et al., 2016; Thiery et al., 2017). Recently, populations of dental progenitor cells (and potentially stem cells) housed within the epithelial dental lamina have been identified in a number of fish species (Fraser et al., 2013; Abduweli et al., 2014; Bloomquist et al., 2015; Martin et al., 2016; Rasch et al., 2016; Thiery et al., 2017). Taken together, these data confirm the fundamental nature of the dental lamina as a precondition for tooth regenerative capacity, especially continuous tooth regeneration. Our understanding of the regenerative capacity contained within the dental lamina in a large collection of fishes, and vertebrates, has emerged in the past few years, and now the dental lamina is becoming a very exiting structure for the more general appreciation of epithelial regenerative biology.

9.4.1 Developing the Elasmobranch Dentition

The elasmobranchs include sharks, skates and rays, and, as a whole, have a particularly diverse set of dental phenotypes. However, together they have a conserved dental character that defines the 'elasmobranch' clade – a many-for-one, continuous production of teeth. The elasmobranch dentition is characterised by a permanent and continuous dental lamina (Fig. 9.2) that is capable of producing multiple teeth per set/family ahead of the single functional position (Rasch et al., 2016). The lamina houses a complete series of developing teeth with functional teeth emerging at the oral surface anteriorly (labially) and new teeth are initiated from within the deep furrow of the cartilaginous jaw at the posterior (lingual) extent of the lamina, called the successional lamina – the region named from where successional teeth are born (Figs. 9.2, 9.3; Smith et al., 2009b; Martin et al., 2016; Rasch et al., 2016).

Whether flattened crushing teeth of the skates and rays (Batoidea) (Underwood et al., 2015) or pointed blade-like teeth of some sharks (Selachii) (Rasch et al., 2016), the teeth of elasmobranchs are highly conserved in their development, despite these morphological differences. All elasmobranch teeth develop within an epithelial dental lamina, where many teeth develop in a synchronised timed series, ahead of function at the oral surface (Figs. 9.2, 9.3). The development of teeth, through a series of cellular (epithelial and mesenchymal) and genetic interactions, is incredibly similar across these elasmobranch groups. In fact, the development of all vertebrate teeth is highly conserved in terms of the genes involved in the morphogenesis of the unit tooth. A core set of genes is vital for normal tooth development in all toothed vertebrates, and the teeth of sharks and rays do not deviate from this evolutionarily stable developmental process.

9.4.2 Developing the Osteichthyan (Teleost) Dentition

With an estimated 30,000 species, teleosts are unquestionably the most diverse vertebrate clade. They have colonised almost every aquatic habitat on earth and have adapted to their specific trophic niches. This adaptation has led to an incredible amount of morphological diversification, even between closely related species (i.e., cichlids). Teloestean diversity is immediately apparent through observation of the dentition.

As a central organ for food processing, the dentition has become highly specialised to diet. Within teleosts, teeth can be found on almost every surface of the oral cavity, including the pharynx. Extreme modification of the pharyngeal teeth has given rise to elaborate crushing pads, which act as primary food processors in multiple teleost genera (Fraser et al., 2009). In many teleost species, it is not uncommon for dental morphology to differ between the juvenile and adult (Fraser et al., 2008). For example, pufferfish exhibit a dramatic shift in dental morphology from a simplistic first-generation dentition to an elaborate beaked dentition in the adult (Fraser et al., 2012; Thiery et al., 2017). These unusual dental forms provide an ideal opportunity to broadly study the developmental basis of morphological change, as well as investigate more specific questions on the molecular regulation of regeneration (Thiery et al., 2017).

The development of teeth is dependent upon the presence of both competent epithelial and mesenchymal progenitors, and the spatio-temporal expression of a suite of initiatory signals. Small shifts in the expression of these markers can lead to dramatic changes in the patterning of

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

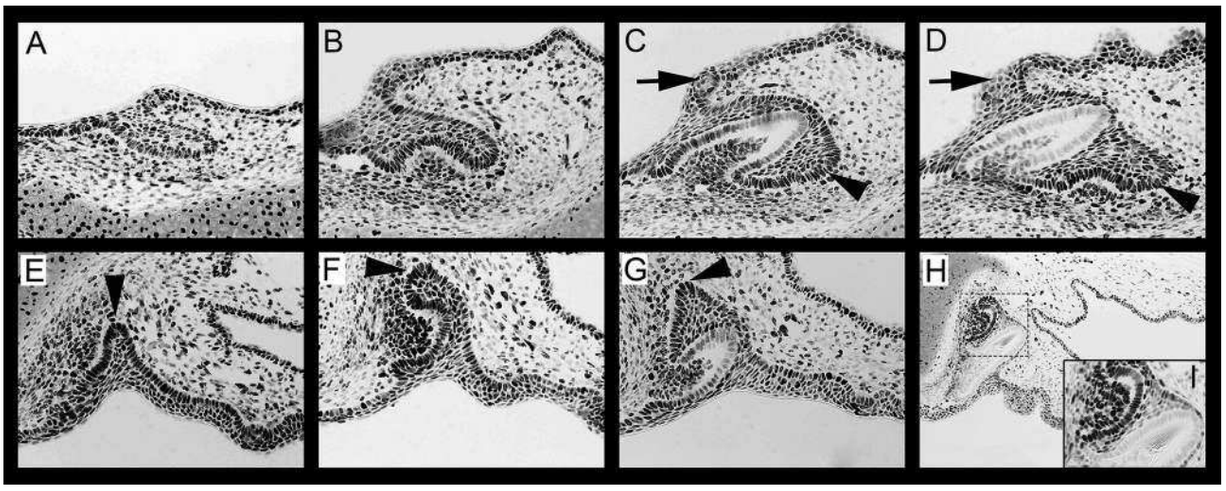

Fig. 9.3. Micro-CT scans of elasmobranch tooth replacement diversity. **A)** Eagle ray, *Myliobatis* sp. Virtual sections (upper and lower jaw in sagittal plane). **B)** Thornback ray, *Raja clavata*, tooth replacement section with surface of tooth pavement (lower jaw in sagittal virtual section). **C)** The porbeagle shark, *Lamna nasus* (lower jaw in sagittal virtual section). **D, E)** The Great White Shark, *Carcharodon carcharias* (lower jaws in sagittal virtual section). Images show the functional teeth and replacement teeth made in advance of function within the same tooth family (sagittal). Replacement tooth number varies in elasmobranchs, **A**, *Myliobatis*=11; **B**, *Raja*=12; **C,** *Lamna*=5; **D, E**, *Carcharodon*=8. Partial mineralisation of the newest tooth in the *Myliobatis* lower jaw (**A**, arrowhead) highlights the mechanism of tooth linkage that forms the basis of the pavement-like crushing dentition. Mineralisation occurs close to where the successional lamina will reside (arrowheads). *L. nasus* (**C**) scans captures the time point before mineralisation of the newest tooth, and likely during early initiation, as an open cavity is visible. 90° tooth rotation in *C. carcharias* (**D/E**) allows more space to develop new tooth at the level of the successional lamina (arrowhead), which could suggest a slower rate of tooth regeneration where most teeth in the family are mineralised and close to functionality.

the dentition. Unlike other polyphyodonts, most teleosts do not possess a deeply invaginated, permanent dental lamina from which new teeth develop (Fig. 9.5). Dental progenitor cells reside close to the oral surface, with a transient elongation of this epithelium arising during dental regeneration (Thiery et al., 2017). It is not inconceivable that the association of the teeth with dental progenitors on the oral surface in teleosts has facilitated diversification of dental pattern. Teleost fishes typically develop their teeth relatively superficially in comparison to tetrapods; however, given the sheer diversity observed within the group, almost all forms of dental development are observable. Between species, teeth can develop either labially or lingually, one for one or many for one, and unicuspid or multicuspid. This diversity renders teleosts an ideal Evo-Devo model for tooth development.

9.5 Rate, Age and the Lifelong Production of Teeth

The wonderful arrays of teeth within the elasmobranch jaw develop and progress through the dental lamina toward functionality in a conveyor belt-type mechanism (Figs. 9.2–9.4). The rate of transition from one generation to the next can vary in sharks (Motta, 2012), from a rapid turnover of 8–10 days in the lemon shark (*Negaprion brevirostris*; Moss, 1967) to a few weeks in some species, e.g., the nurse shark, *Ginglymostoma cirratum* (Luer et al., 1990), and leopard shark, *Trikias semifasciata* (Reif et al., 1978), to potentially several months in the largest sharks, e.g., the great white (*Carcharodon carcharias*).

The rate and production of teeth can vary quite considerably in sharks. The number of teeth in a family series can shift from few (*Lamna* and *Prionace*) to more in *Carcharodon* (Fig. 9.3; Moyer et al., 2015; Schnetz et al., 2016) to 10 or more tooth rows in batoids (Underwood et al., 2015). The rate of tooth replacement and, therefore, the speed of mineralisation in shark teeth should be directly related to diet type. However, this must also be related to frequency of feeding events and speed of digestion. Those species that eat large amounts with long periods between feeding events should, in theory, not need to replace their teeth so rapidly. Whereas, those species that exhibit a continuous feeding strategy are likely to replace their teeth more frequently.

Certainly, the age of an organism will also play a role in the rate and capacity of the continuous production of teeth in fishes. It could be presumed that in order to organise and set up the regenerative cycles of the dentition, the early (embryonic and juvenile) production of teeth would be

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

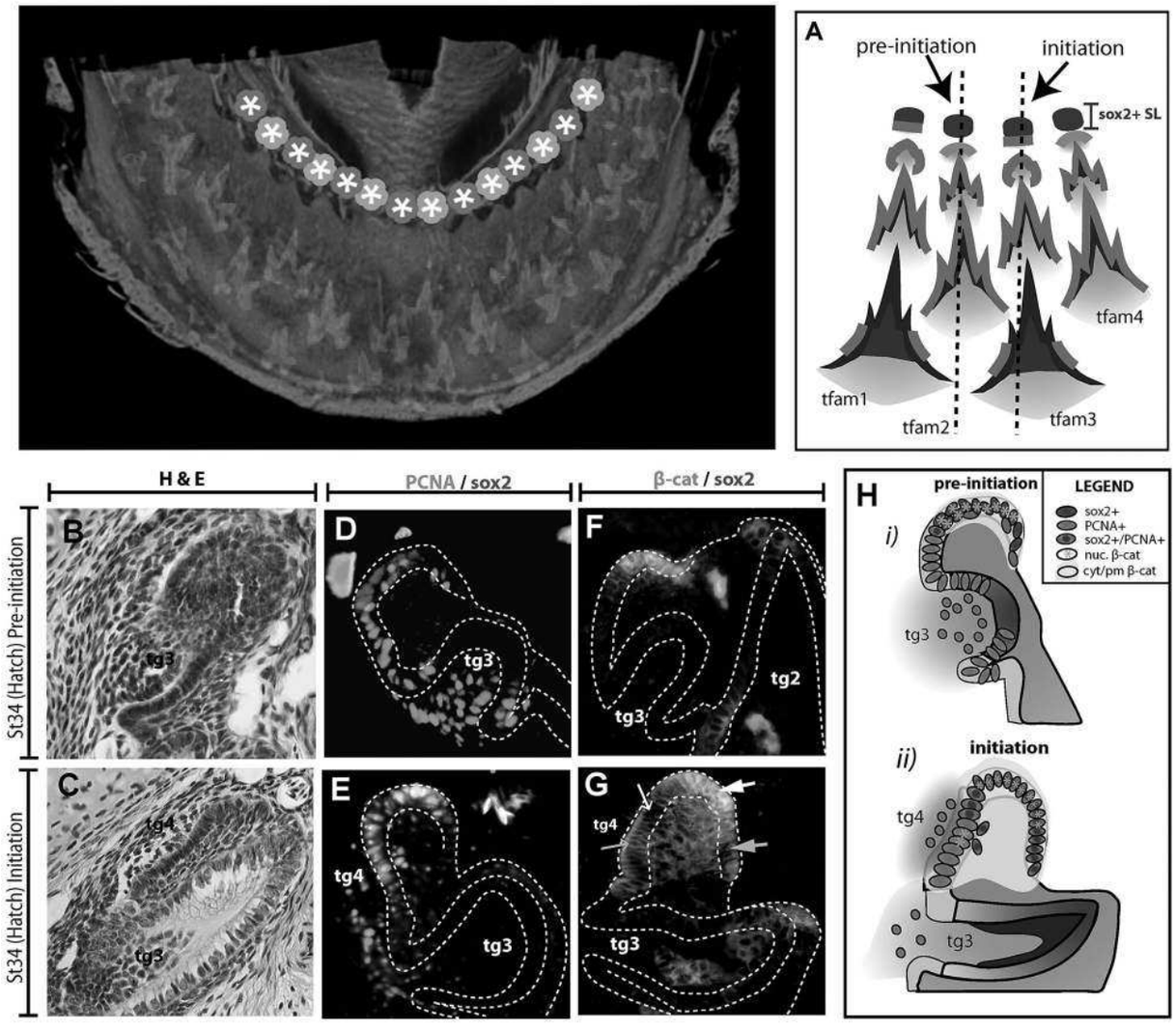

Fig. 9.4. Alternate and cyclical tooth regeneration mechanism in the small-spotted catshark (*Scyliorhinus canicula*). **A)** Alternate initiation (green asterisk) and pre-initiation (magenta asterisk) of teeth in the lower jaw of the catshark, with *sox2* expression (purple) associated with the successional lamina (SL). Dotted lines (**A**, tfam2/tfam3) indicates the position of pre-initiation (tfam2; **B, D, F**) and initiation (tfam3; **C, E, G**) sections for histology (**B, C**), *sox2* and PCNA (proliferating cell nuclear antigen; **D, E**) immunofluorescence, and (**F, G**) *β-catenin* and *sox2* immunofluorescence. **H)** Schematic diagram of the (***i***) pre-initiation phase of the successional lamina with immunofluorescence represented in **D** and **F**, and (***ii***) the initiation phase of the successional lamina with immunofluorescence seen in **E** and **G**. Numbered tooth families; tfam1–4 (Martin et al., 2016). A black-and-white version of this figure appears in some formats. For the colour version, please refer to the plate section.

relatively rapid; then the process may slow down to account for the energetic and steady production of large mineralised units later in development. However, in contrast it could also be true that tooth production becomes more rapid as the organism matures in order to deal with the mechanics of active feeding. Currently, data on tooth production rates and aging is lacking for any fish species. But one thing is more certain, later in development, during the process of senescence, we might expect the production of teeth, in continuously regenerating species, to slow. Even some groups that rapidly produce multiple generations of teeth, e.g., sharks and rays, could show a slow-down or complete cessation of tooth development during the latter periods of a long life. Therefore, to label sharks and other continuously regenerating groups ‘lifelong’ or ‘endless’ producers of teeth without evidence is incorrect.

Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

It is intriguing to think of the continuous nature of teeth, especially in long-lived species, i.e., the larger sharks. The recent news that some shark species can live to at least 400 years old, e.g., the Greenland Shark, *Somniosus microcephalus* (sleeper sharks; Family Somniosidae) (Nielsen et al., 2016) would certainly suggest the need for the production of an incredible number of teeth over this length of lifetime. Whether slow metabolism and cold Artic temperatures, coupled with the potentially infrequent need for prey in *S. microcephalus*, it is conceivable that this species in particular may have an extremely slow rate of tooth production and loss. Therefore, producing new teeth at an old age, in this case, does not seem implausible. In early chondrichthyans, the tooth replacement rate is estimated to be slow (Botella et al., 2009) compared to more derived, extant chondrichthyans. It seems that the rate of tooth replacement is directly related to function (Moyer et al., 2015).

The developmental genetic pathways associated with controlling the rate of tooth replacement in any vertebrate are currently unknown. However, we do have some indication of signalling pathways that could affect the rate of dental lamina regeneration and, therefore, tooth production. In teleosts, the Wnt/ß-catenin and Notch pathway could both have a role in the timing and cyclical mechanism of tooth replacement (Martin et al., 2016). The cyclical nature of continuous tooth regeneration, in fishes, suggests that there must be an underlying mechanism to pulse the production of teeth, with a species-specific rate. Cichlid fishes have been well studied, and the rate of tooth regeneration depends greatly on the species (Fraser et al., 2008); however, the assumed estimate in African cichlids is 30–100 days for a new generation of teeth to become functional (Tuisku and Hildebrand, 1994). Indeed, one understudied element of tooth regeneration is the cyclicity of the tooth programme – that is, the regulation of a timed series of regeneration events. Sharks would appear to be an ideal model in which to study this phenomenon, with a continuous process of initiation and paused initiation ('stop-start' regeneration) with the successional lamina, at the free end of the dental lamina (Martin et al., 2016; Fig. 9.4). The catshark (*Scyliorhinus canicula*) jaw contains numerous tightly spaced tooth families so that neighbouring families exhibit an alternate file tooth replacement (Smith et al., 2013; Underwood et al., 2016), where odd tooth positions are timed to become functional lingual to the even-numbered positions. This is an exciting scenario when considering the study of regenerative cyclicity, for example, when one family is undergoing an initiation phase (at the level of the successional lamina), the adjacent families are paused (Fig. 9.4). One theory suggests that the paired families of tooth sets that show the stop-start initiation of new teeth are developmentally linked as clonal derivatives (SAM: sequential addition model; Smith, 2003; Smith et al., 2013). However, our interpretation of discrete, autonomous tooth families, timed to initiate new teeth in alternate neighbouring phases will be the subject of future research. The mechanism and the underlying genetic control of this 'stop-start' epithelial regeneration should be considered further, with some data inferring a link between the regulation of repeated tooth initiation and certain signalling pathways (i.e., Wnt/ß-catenin pathway; Fig. 9.4; Martin et al., 2016). The alternate nature of tooth initiation in the catshark allows each adjacent tooth family to have a slightly offset initiation within the successional lamina (Fig. 9.4A). Therefore, one family will be undergoing initiation of a new replacement tooth (initiation), while the adjacent tooth family will be timed to pause (pre-initiation), thus creating an alternative and cyclical tooth regeneration programme. The developmental basis of this initiation/pause mechanism within the successional lamina is highlighted by the expression of *sox2* and *ß-catenin*; wherein these two markers are restricted and co-expressed in a small population of progenitor cells (Fig. 9.4F, H*i*) during the pre-initiation phase of the regeneration cycle. During the initiation phase (Fig. 9.4G, H*i*) of the cycle (regeneration) the territory of *ß-catenin* expression expands throughout the entire successional lamina (localised to the active tooth family) to begin tooth regeneration. This pause/initiate mechanism appears to be, in part, directed by Wnt/*ß*-catenin signalling. In contrast, *sox2*-positive cells within the pause phase (pre-initiation) of the cyclical mechanism of initiation are separate from the actively proliferating cells (PCNA; Fig. 9.4D), and then during the initiation phase of the process, the separation of these cell populations is lost as the region of proliferation expands (Fig. 9.4E; Martin et al., 2016). This type of information will be invaluable for the translational developments of dental research to aid our understanding of why certain vertebrates, e.g., mammals, have lost the ability to develop additional tooth generations past the typical diphyodont system.

9.6 Developmental Basis for Tooth Regeneration

The essential property of the dental lamina, especially in the sharks and rays, is the regenerative capacity of this structure. This double layer of dental epithelial cells regulates the production of new teeth in a consistent, specifically timed, repeating mechanism where teeth develop in developmental sequence before eruption and functionality at the jaw margin. Recent developmental studies on the vertebrate polyphyodont system have begun to elucidate the genetic and cellular mechanism through which teeth are regenerated (Martin et al., 2016; Rasch et al., 2016). Early

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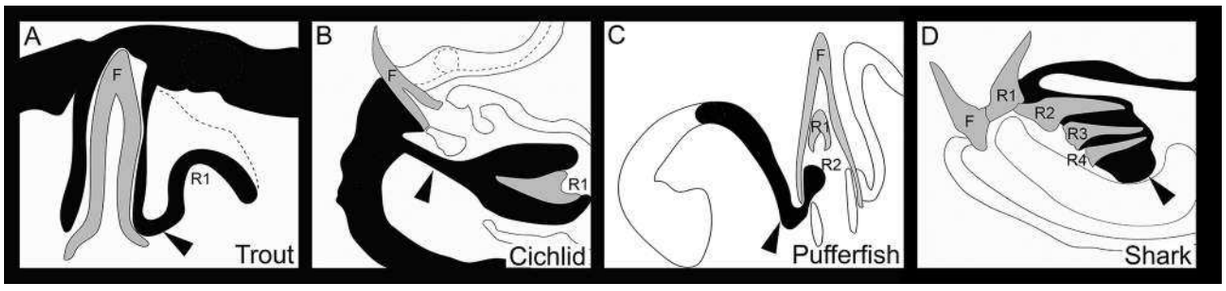

Fig. 9.5. Diversity of the dental lamina in fishes. Continuous tooth regeneration in fishes is governed by the activity of the dental lamina (black). In some teleosts, e.g., the rainbow trout (**A**, *Oncorhynchus mykiss*) the dental lamina is less obvious, and new tooth replacements form from the outer dental epithelial cells associated with the predecessor tooth (arrow; Fraser et al., 2006). There is great diversity of the dental lamina in teleosts, with some groups, e.g., the cichlids (**B**), developing new replacement teeth from a labial dental lamina that invaginates from taste-linked epithelia (arrow) into bony crypts (intraosseous replacement) directly beneath the functional tooth (Fraser et al., 2012). Highly derived teleosts such as the pufferfish (Tetraodontidae, **C**) also develop a dental lamina from taste-linked epithelia that invaginate from a labial position, where new tooth replacements appear to bud off (arrow) within the beak cavity (Thiery et al., 2017), adding new tooth 'bands' that reinforce and replace the beaked dentition. In elasmobranchs (e.g., the shark, **D**), a lingual and permanent dental lamina develops from an odonto-gustatory band that forms both the dental lamina and the taste bud–rich oral epithelia. New replacement teeth (R1–4) are made within the deeply invaginated successional lamina (arrow) at the recess of the jaw cartilage.

in the development of the dental lamina (first as the odontogenic band), epithelial dental progenitors are born within this specialised dental epithelium of the oral surface (Fig. 9.5) and become sequestered in the now expanding dental lamina. This scenario is true of all toothed vertebrates, whether polyphyodont, monophyodont (a single generation of teeth), or diphyodont (two generations of teeth; Jernvall and Thesleff, 2012; Juuri et al., 2013a). However, the major difference between those vertebrates with few and those capable of multiple generations of teeth is the persistent nature of the dental lamina and, importantly, the connection, or not, to a population of epithelial dental progenitors.

A number of recent studies have illustrated the role of one transcription factor in particular, *Sox2*, that appears to be important for the production and maintenance of epithelial progenitor/stem cells in renewable or regenerative dentitions (Smith et al., 2009b; Juuri et al., 2012, 2013a, 2013b; Martin et al., 2016; Rasch et al., 2016; Sun et al., 2016; Thiery et al., 2017). This key marker of dental progenitor cells has paved the way for new investigations into tooth development and redevelopment, by identifying active stem cell compartments within the dental lamina and lamina-derived cell populations. In fishes, *sox2* is an early marker of the epithelial odontogenic band and the emerging dental lamina (Fraser et al., 2013; Martin et al., 2016; Rasch et al., 2016). This epithelial expression of *sox2* appears alongside the expression of *shh* and *pitx1*/2, defining tooth initiatory competence (Bloomquist et al., 2015; Martin et al., 2016; Rasch et al., 2016); these 'tooth' genes are likely essential for the normal development of the entire dentition. Comparative analysis of the molecular control of dental regeneration in polyphyodont vertebrates has begun to reveal an incredible conservation of progenitor cell regulation and tooth initiation, suggesting that this ancient mechanism of dental regeneration has always relied upon the same key molecules for the replenishment of the dentition. Importantly, the region of the oral epithelium that becomes competent, active dentally to begin the process of tooth initiation in fishes, is also competent to develop another vital, spatially linked epithelial unit, key for the survival of vertebrates – the taste buds (Martin et al., 2016).

The gustatory system is a more ancient vertebrate innovation compared to the later appearance of odontodes (Kirino et al., 2013; Atkinson et al., 2016). Jawless and toothless ancestors of toothed gnathostomes likely swam the oceans with oral cavities filled with taste bud–like structures. Thus, when oral odontodes first appeared in the mouth (ecto-/endoderm) of ancient jawless fishes, they would have done so in taste-rich regions. We can speculate that the epithelium, from which both teeth and taste buds first emerged, also expressed *sox2*, similar to their extant vertebrate descendants, e.g., modern sharks. Evidence for the close developmental link between teeth and taste buds has recently demonstrated, with data showing that odontogenic epithelium is also bi-functional, that it develops both taste buds and teeth in close proximity (Martin et al., 2016). Thus, at least in sharks (and now we can certainly consider this true of some teleosts, including cichlids; Bloomquist et al., 2015) this tooth/taste competent region of the oral epithelium can now been termed the 'odonto-gustatory band' (Martin et al., 2016). When these epithelial junction cells between the tooth and taste territories are

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labelled with a lypophilic dye (DiI) in the shark (*Scyliorhinus canicula*) the cells either move into the invaginated dental lamina, and are incorporated into new teeth, or remain close to the oral surface and contribute to the supporting cells of adjacent taste buds (Martin et al., 2016). This therefore suggests an important developmental link between teeth and taste buds in the shark, highlighting a potential dual fate for these epithelial cells, from which tooth progenitors can emerge.

9.7 Future Implications for Comparative Dental Evo-Devo

Comparative Evo-Devo remains a relatively understudied field, with the focus of developmental research primarily on biomedical science and evolutionary research on the genome. A recent surge in new developmental techniques is facilitating the selection of almost any organism as a developmental model. Whole transcriptome sequencing can now be carried out on a cellular level, whilst CRISPR-CAS9 is revolutionising the approach in which we take towards experimental embryonic modification.

Intriguingly, the zebrafish is the 'go-to' biomedical model of choice. Given various evolutionary events that have taken place within the teleost lineage (notably an independent whole genome duplication), a comprehensive understanding of the evolution of teleosts is fundamental for the comparative study of development between zebrafish and humans. Within the field of tooth development and regeneration, classic developmental models are less than suitable because of the lack of teeth or the restriction to the pharynx. Given the availability of new developmental techniques, there is the exciting and unique opportunity to develop new Evo-Devo models which can be effective for understanding the developmental basis for regeneration as well as the evolution of the vertebrate dentition.

The catshark is emerging as an exciting model for tooth regeneration. Its large size and ease of accessibility make it ideal for physical manipulation and electroporation, and the expected development of both genome and transcriptome assemblies will further add to resources available for studying this model. In the study of lifelong dental regeneration, one major question, which remains to be answered, is how cyclicity (timed repetition) is developmentally regulated? This is important, as it provides clues as to how regenerative potential is maintained throughout adulthood. In the catshark, adjacent tooth families are staggered in their development. When one tooth is initiated, there is a pause before its neighbour is initiated (Smith et al., 2009a, 2013; Martin et al., 2016). However, all teeth develop across an extensive, continuous, jaw-length dental lamina, with genetic control of site specificity localised within this structure. Through the use of comparative transcriptomics, this developmental unit could provide an ideal opportunity to study the developmental timing of dental regeneration. As the availability of lab models increases and cost of developmental techniques decrease, the study of fish Evo-Devo could provide key information required to understand both the evolution of the vertebrate dentition and regulation of regeneration.

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Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA

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Gareth J. Fraser, Department of Biology, University of Florida, Gainesville, USA