Integrative and Comparative Biology

Integrative and Comparative Biology, pp. 1–14
doi:10.1093/icb/icaa116

Society for Integrative and Comparative Biology

SYMPOSIUM

The Role of Core and Variable Gene Regulatory Network Modules in Tooth Development and Evolution

Alexa Sadier [1,*] Sharlene E. Santana [†] and Karen E. Sears [*]

*Department of Ecology and Evolutionary Biology, University of California at Los Angeles, Los Angeles, CA, USA; †Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, WA, USA

From the symposium "Biology at the Cusp: Teeth as a Model Phenotype for Integrating Developmental Genomics, Biomechanics, and Ecology" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2020 at Austin, Texas.

[1]E-mail: asadier@ucla.edu

Synopsis Among the developmental processes that have been proposed to influence the direction of evolution, the modular organization of developmental gene regulatory networks (GRNs) has shown particular promise. In theory, GRNs have core modules comprised of essential, conserved circuits of genes, and sub-modules of downstream, secondary circuits of genes that are more susceptible to variation. While this idea has received considerable interest as of late, the field of evo-devo lacks the experimental systems needed to rigorously evaluate this hypothesis. Here, we introduce an experimental system, the vertebrate tooth, that has great potential as a model for testing this hypothesis. Tooth development and its associated GRN have been well studied and modeled in both model and non-model organisms. We propose that the existence of modules within the tooth GRN explains both the conservation of developmental mechanisms and the extraordinary diversity of teeth among vertebrates. Based on experimental data, we hypothesize that there is a conserved core module of genes that is absolutely necessary to ensure tooth or cusp initiation and development. In regard to tooth shape variation between species, we suggest that more relaxed sub-modules activated at later steps of tooth development, for example, during the morphogenesis of the tooth and its cusps, control the different axes of tooth morphological variation.

Introduction

Why do some morphologies repeatedly evolve, while others, although theoretically possible, do not? In the 19th century, scientists proposed that this phenomenon could result from developmental processes biasing the evolution of phenotypes (Smith et al. 1985). The idea of developmental biases or constraints was revisited by Raup (1966), who proposed that biases imposed by the mechanisms of growth and development were responsible, at least in part, for his finding that only a small proportion of all possible snail shapes are realized in nature. This idea was also explored more recently by researchers in the field of genetics. For example, Schluter (1996) proposed that adaptive morphological differentiation tends to occur preferentially in certain directions along "lines of least resistance" (Schluter 1996). However, while the concept of developmental bias has existed for over a hundred years, the prevalence and significance of developmental bias in phenotypic evolution remain unresolved, in part due to a historical lack of relevant experimental data (reviewed in Uller et al. 2018; Brakefield 2006; Hendrikse et al. 2007).

In the past 20 years, advances in the study of developmental mechanisms and their ability to generate variation have begun to fill this critical knowledge gap. As one example of these recent, fine-scale studies, explorations of the mechanisms controlling limb development suggest that development constrains the phalangeal variation observed among vertebrates by limiting morphologies within

a continuum (Kavanagh et al. 2013). Additionally, developmental mechanisms have been shown to constrain organ positioning in the cephalic horns of scarab beetles (Busey et al. 2016). Moreover, study of development has generated insights into its role in the generation of phenotypic diversity in bird beaks; morphological variation in the bird beak has been linked to the structure of the gene network that controls beak development in Darwin's finches. Among Darwin's finches, species that eat hard seeds tend to have shorter, broader beaks, while finches that pick seeds out of cactus fruits tend to have longer, more pointed beaks. Researchers have found that development of the beak in Darwin's finches is decomposable into two modules: one that controls depth and width and is regulated by the BMP pathway through *BMP4*, and one that controls length and is regulated by the calmodulin pathway (Abzhanov et al. 2004, 2006; Mallarino et al. 2011). This finding suggests that there is a simple mechanism for independent evolutionary changes in beak length and width within Darwin's finches. Interestingly, while closely related finch genera display a morphological diversity similar to that of Darwin's finches, research suggests that the pathways generating that diversity are distinct from those observed in Darwin's finches (Mallarino et al. 2012). This appears to be true for more distantly related bird groups as well (Cheng et al. 2017). Taken together, these findings suggest that developmental processes might have the potential to bias the generation of form within groups, and they might themselves be highly evolvable.

Among the developmental processes that have been proposed to bias the evolution of phenotypes, the modular organization of developmental gene regulatory networks (GRNs) is a good candidate (Davidson 2010; Hinman and Jarvela 2014). GRNs provide a map of the interactions between the transcription factors (TFs; see Box 1), enhancers, and target genes (Erwin and Davidson 2009) that control the development of organisms. As such, GRNs can be represented as networks or circuits with beginnings and ends and various possible topologies. Using theoretical and experimental approaches, researchers have started to investigate the inherent properties of GRNs and the impact of these properties on the evolution of form (Davidson and Levine 2008; Davidson 2010). From these studies, two properties of particular interest to the topic of developmental bias have emerged. First, GRNs are hierarchical, meaning that the portion of the GRN that controls the initial stage of development of an organism or an organ (generally its induction) precede other parts of the network, which in turn control more specific functions such as morphogenesis and cell differentiation (Erwin and Davidson 2009). Second, GRNs are modular, meaning that they can be decomposed into sub-circuits or modules, which are defined for this article as semi-autonomous units responsible for the development of a phase or a part of an organism or organ (Davidson and Erwin 2006; Davidson and Levine 2008; Erwin and Davidson 2009). Building on the hierarchical and modular architecture of GRNs, we can further predict that organ GRNs have two types of modules (Box 1): a core module, also called a kernel, comprised of a primary circuit of genes that are essential for correct initiation of the organ in question; and sub-modules of downstream and peripheral (i.e., at the periphery of the GRN, spatio-temporally) circuits of genes that control the phenotype of parts of the organ (e.g., morphogenesis or cell differentiation; Lipson et al. 2002; Erwin and Davidson 2009; Clune et al. 2013; Kouvaris et al. 2017; Uller et al. 2018).

Evidence in support of the hierarchical and modular nature of GRNs comes from studies in several systems. For example, research has shown that the GRN that controls the dorsal-ventral patterning of *Drosophila* embryos composed of several sub-circuits, each of which controls the specification of a single tissue type (e.g., mesoderm, ventral neurogenic ectoderm, and dorsal neurogenic ectoderm; reviewed in Levine and Davidson 2005; Ochoa-Espinosa et al. 2005). Similarly, studies suggest that the GRN that controls endoderm and mesoderm specification in the sea urchin is partitioned into modules that control distinct developmental processes, such as the specification of most larval cell types (Peter and Davidson 2010). However, while findings to date are consistent with GRN structure being inherently hierarchical and modular, research in a wider range of organisms and organs is needed to further test this hypothesis.

A hierarchical and modular GRN structure has been proposed to contribute to the non-uniform distribution of shapes across morphospaces (Espinosa-Soto and Wagner 2010; Andreas Wagner 2011; Uller et al. 2018). GRN modules have been described for different organs in different organisms (Raff 2007; Reno et al. 2008; Zeller et al. 2009; Lacquaniti et al. 2013) and the biasing impact of this modular GRN structure on evolutionary change has been theorized (reviewed in Uller et al. 2018). However, there remains little direct, experimentally-based evidence of how the modular structure of GRNs impacts evolutionary change and is modified in response to a fluctuating environment over evolutionary time.

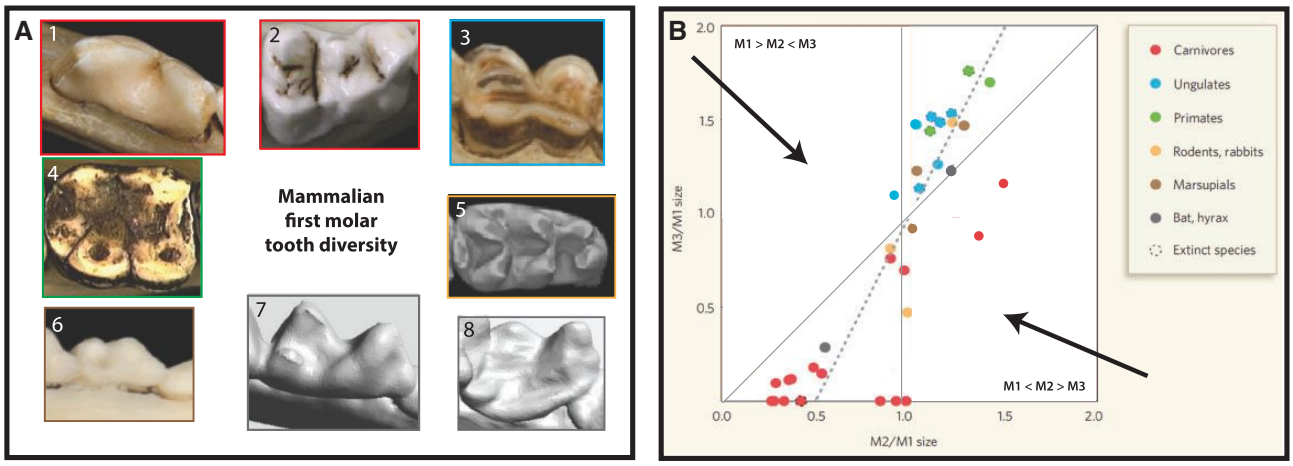


Fig. 1 Molar diversity in mammals and molar morphospace. (**A**) Mammalian molars exhibit an extraordinary diversity in terms of size, cusp number, and overall morphology. First molar of species from left to right, top to bottom: 1, cheetah (Phil Myers, ADW); 2, giant panda (Phil Myers, ADW); 3, big horn sheep (Phil Myers, ADW); 4, chimpanzee (Phil Myers, ADW); 5, mouse (Charles et al. 2009b); 6, water opossum (Phil Myers, ADW); 7, Waterhouse's leaf-nosed bat and; 8, tent making bat. ADW, Animal Diversity Web—Museum of Zoology of Michigan-Ann Arbor. (**B**) Molar inhibitory cascade morphospace. This schematic illustrates that not all possible molar proportions are realized in mammals: some regions of the morphospace (emphasized by black arrows) are not occupied. The uneven distribution of realized morphologies is thought to be due to developmental mechanisms (inhibitory cascades) controlling tooth proportions during molar development. Adapted from (Polly 2007). M1, first molar; M2, second molar; M3, third molar.

Vertebrate teeth: A model system for studying relationships between GRN structure and evolution

Teeth are serially homologous structures (i.e., repeating elements or organs within a single organism that share a large proportion of their genetic architecture and developmental pathways). Teeth are also functionally-important anatomical elements of the jaw that vary tremendously in number, shape, and location across mammals and vertebrates (Fig. 1A) (Stock 2001; Tucker and Sharpe 2004; Ungar 2010; Jernvall and Thesleff 2012). As teeth physically interface with food items during biting and mastication, their shape and number are thought to be under strong selection to match the demands imposed by the material properties of foods and the feeding behaviors used by vertebrates (Evans et al. 2007; Ungar 2010). Consistent with this, the evolution of teeth and their shape shows tight links with diet in many vertebrate groups (Evans et al. 2007; Ungar 2010, 2015). Beyond extrinsic factors, research in rodents suggests that teeth may also exhibit a differential evolvability along developmental lines of least resistance (Fig. 1B) (Renaud et al. 2006, 2011; Kavanagh et al. 2007). Furthermore, the genes that comprise the GRN that controls tooth development have been well described in model organisms, including mammals. Findings of this research suggest that the GRN controlling at least the initiation of tooth development is extremely conserved among mammals and potentially among all vertebrates (Jernvall and Thesleff 2012; Rasch et al. 2016). As a result, vertebrate teeth represent an ideal model system with which to study how the inherent architecture of GRNs can facilitate variation while ensuring that essential processes (e.g., organ formation) are preserved. In this article, we use mammalian teeth as a model system to illustrate how applying the GRN module framework to study of teeth can advance understanding of the ways in which development biases morphological evolution and facilitates the generation of variation. In addition, we discuss how the modular structure of GRNs might facilitate the reiteration of developmental units during development.

Teeth develop through successive signaling centers that act as key checkpoints for morphogenesis

During development, teeth initially form through interactions between two embryonic tissues: the dental epithelium and the underlying mesenchyme (Tucker and Sharpe 2004). These interactions cause a thickening of dental epithelium which then invaginates into the dental mesenchyme to form a tooth bud (Fig. 2A). After invagination, the epithelium wraps around the underlying mesenchyme and the tooth progresses into cap and bell stages of development that phenocopy adult tooth form (Fig. 2A). Like tooth initiation, tooth progression through the bud, cap, and bell stages is also regulated by cross-talk between the epithelium and mesenchyme. This cross-talk takes place in a series of signaling centers that express a set of genes in a pattern conserved

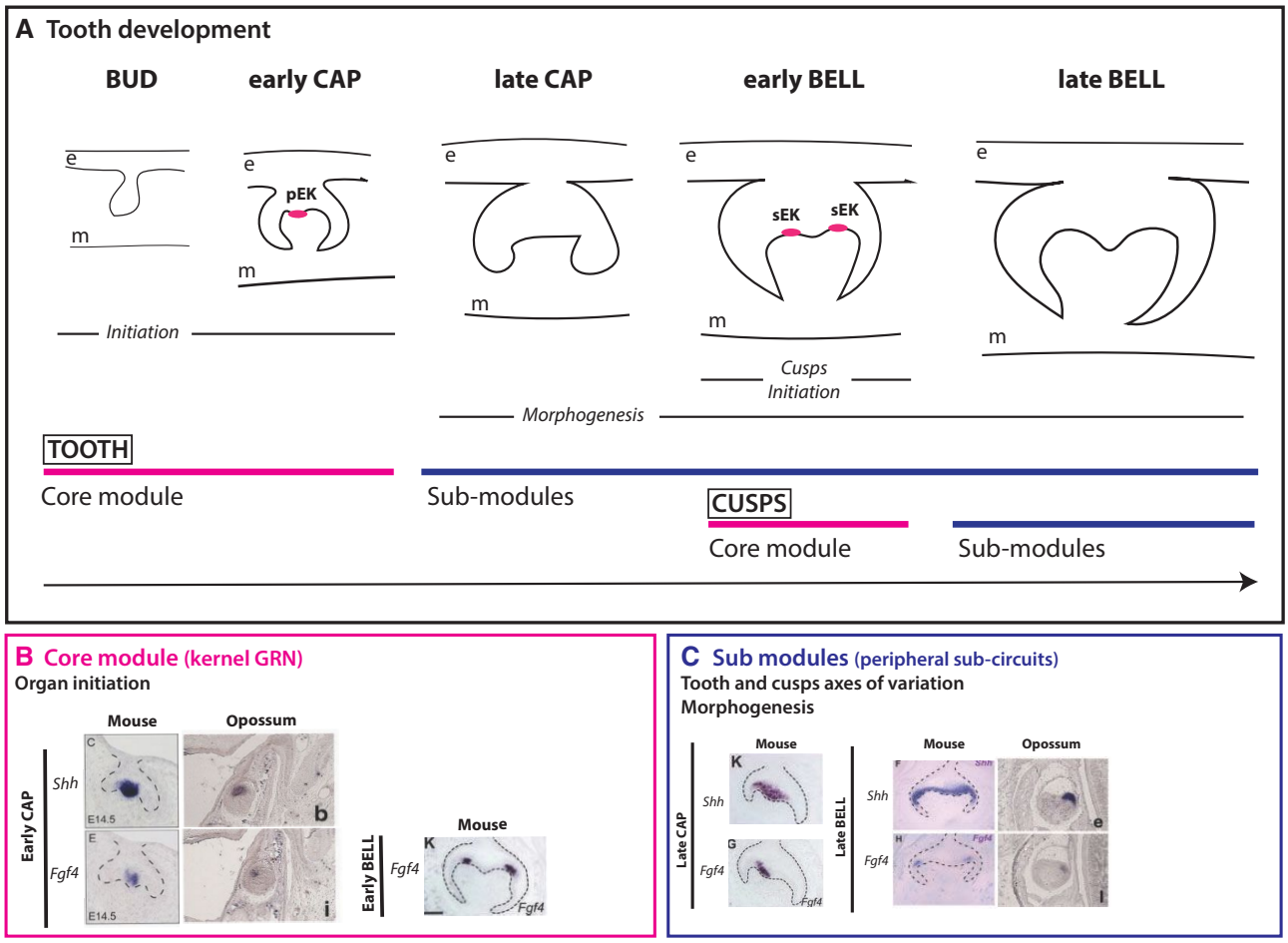
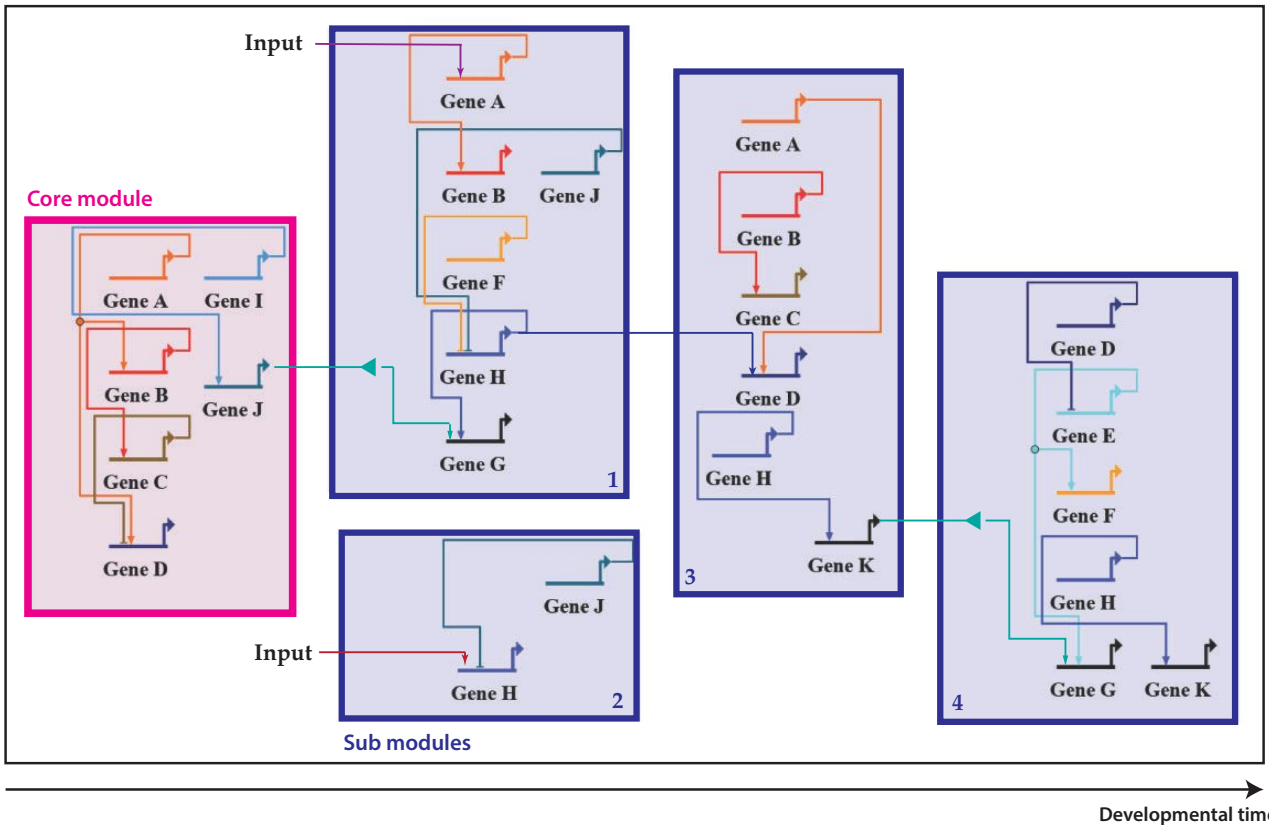


Fig. 2 Tooth development, spatiotemporal context of GRN modules activation, and gene expression patterns. (**A**) Tooth development involves two tissues: the epithelium and the neural-crest-derived mesenchyme. In mammals, the different stages of tooth development are called: bud, cap, and bell. Tooth development starts with the invagination of the epithelium into the underlying mesenchyme, followed by the induction of a first signaling center that is necessary to initiate tooth development. In multicuspid teeth, signaling centers are reactivated for the formation of each cusp. These signaling centers are transient structures that express signaling molecules and are thought to regulate tooth morphogenesis. In mammals, signaling centers are called pEK (bud/cap stages) and sEKs (bell stage). In the framework presented here, we propose that the GRN that controls tooth development is partitioned into modules that are active at different stages of tooth development: the core module is active at early stages of signaling center formation (pEK and sEKs in mammals) and the sub-modules are active at later stages of tooth or cusp development. (**B**) Core module gene expression pattern. The GRN of the core module is characterized by genes with a conserved expression pattern in signaling centers, with many of them exhibiting a restricted expression pattern to signaling centers. (**C**) Sub-modules gene expression pattern. Expression patterns of the genes expressed when the sub-modules are active vary between species and can be linked to the direction of variation. *In situ* hybridization: mouse early cap from Liu et al. (2014), opossum early cap from Moustakas et al. (2011), late cap and early bell in mouse from Nakatomi et al. (2013).

among vertebrates (Fig. 2B; Jernvall and Thesleff 2012). In teeth with one cusp (i.e., monocuspid), a single signaling center likely regulates tooth development through the bud, cap, and bell stages. In contrast, development of the multicuspid teeth of mammals is regulated by multiple signaling centers. In mammals, the first of these forms during the bud stage of development is called the primary Enamel Knot (pEK; Fig. 2). Additional signaling centers form later at the tips of future tooth cusps and are called secondary EKs (sEKs; Fig. 2; Jernvall and Thesleff 2000; Ungar 2015). Mammalian EKs are characterized by restricted expression patterns of key genes and distinct patterns of apoptosis (Lesot et al. 1996; Vaahtokari et al. 1996; Jernvall et al. 1998).

EKs are crucial to induction of the tooth as a whole and, in multicuspid teeth, induction of each tooth's individual cusps. Consistent with this, disruption of pEKs or sEKs leads to developmental arrest of whole teeth or individual cusps, respectively (Fig. 3B). In contrast, perturbation of tooth development after pEK or sEK induction (Fig. 3B) alters



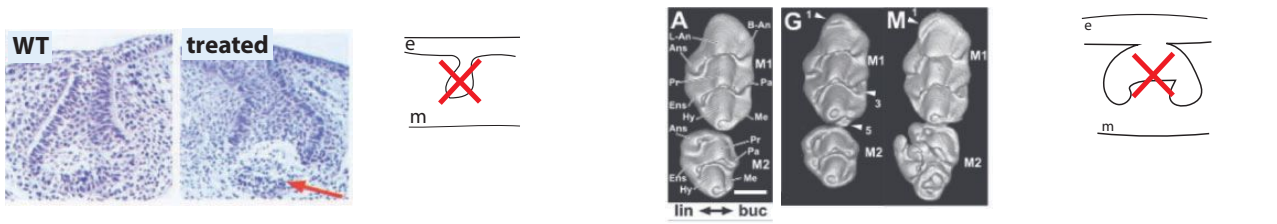


Fig. 3 GRN, core, and submodules. (**A**) Representation of a theoretical GRN. This GRN is both hierarchical and modular, and can be decomposed into circuits or modules: a core module, also called kernel, is comprised of an essential, primary circuit of genes that ensure the correct initiation the organ (here, the tooth and its cusps); and sub-modules of downstream, secondary peripheral circuits of genes are responsible for more peripheral functions during morphogenesis (here, the shape of the tooth and its cusps). In this theoretical example, the GRN is composed by 10 genes involved in different modules (one core and four sub modules). The core module is responsible for organ development initiation and each sub-module (1–4) is responsible for a given biological function or controls the development of a given trait (e.g., sub-module 1 could control tooth length). As many pathways and genes are pleiotropic and active during the development of a given organ (here, the tooth), many are shared between modules. However, each module circuit is unique as the regulatory loops inside these modules are specific to it. For example, Gene D is involved in the core and sub-module 3 and 4 but involved in different regulatory loops. As a result, disrupting gene D function in the core and sub-modules 3 and 4 will have different consequences (see below). (**B**) Example of a gene shared by different modules and functional consequences of disruptions. *Shh* is known to be involved both in tooth initiation and morphogenesis. Because of this, we hypothesize that Shh is involved in at least two different regulatory loops responsible for two different functions. As a disruption of *Shh* at an early stage leads to developmental arrest (Cobourne et al. 2001), we predict that *Shh* is part of the core module. Because late *Shh* inhibition leads to cusp number modification and tooth size change (Kim et al. 2019), we propose that *Shh* is also involved in sub-modules controlling these tooth traits through a different regulatory loop.

the morphology of teeth or cusps, respectively, but does not impact their presence (reviewed in Catón and Tucker 2009; Jernvall and Thesleff 2012). We, therefore, hypothesize that there are phases of tooth development that are critical (i.e., when EKs are formed; pre- and early cap for pEK and early bell stages for sEKs) and phase that are peripheral (i.e., after EK formation; late cap for pEKs, and late bell for sEKs). We further speculate that the critical, early phases are indispensable for tooth and cusp formation and are highly conserved across species, while the peripheral later phases are more sensitive to evolutionary change and thus more variable across species. In this framework, these phases could represent modules of a tooth GRN, with the critical phase representing a core module of tooth development that controls presence/absence of structures and peripheral phases representing sub-modules that control distinct aspects of those structure's shapes.

A core gene network controls tooth initiation and development and is conserved across vertebrates

The genes that underlie the early phases of tooth development have been intensively studied in model and, over the last 10 years, non-model organisms (Fraser et al. 2009, 2020; Moustakas et al. 2011; Jernvall and Thesleff 2012; Rasch et al. 2016; Landova Sulcova et al. 2020; Sadier et al. 2020). As a result, researchers have identified many of the developmental pathways that are required to make a mouse molar and its cusps: (TGFβ [BMP], Fgf, HH, Wnt, Eda, and Notch; Thesleff and Jernvall 1997; Tucker and Sharpe 1999, 2004; Jernvall et al. 2000; Tucker et al. 2000, 2004; Pispa et al. 2003; Catón and Tucker 2009; Jernvall and Thesleff 2012; Jussila and Thesleff 2012; O'Connell et al. 2012). When these pathways are genetically disrupted in mouse at early stages, this generally results in arrest of tooth development at early stages, or significant changes in tooth shape. For example, manipulation of *Shh* (Dassule et al. 2000) or *Bmp4* (Jia et al. 2013) signaling at early stages of tooth development leads to a total arrest of tooth development before the cap stage, and manipulation of *Eda* or *Edar* signaling at early stages disrupts the correct establishment of the EKs (Fig. 4) (Tucker et al. 2000).

Comparative studies of transcriptomes have provided additional insights into the core developmental program controlling tooth development within a species. In particular, comparisons of the transcriptomes of the upper and lower molars (Pantalacci et al. 2017) and upper and lower molars and incisors (Laugel-Haushalter et al. 2013) in mice have identified some key regulatory interactions with similar roles in the development of different tooth types. Other studies suggest that the core module could contain a Wnt-BMP feedback loop, and the peripheral pEK module could contain a Wnt-Shh-Sostdc1 negative feedback loop (Cho et al. 2011; and see O'Connell et al. 2012). However, while some signaling pathways have been implicated in tooth developmental modules, the TFs and their controlling enhancers that regulate tooth development remain more elusive.

The core module for tooth development may be conserved across vertebrates. Studies have revealed remarkable conservation of the genes involved, and their expression patterns, at early stages of tooth development (e.g., bud to cap stages, Fig. 2) across vertebrate groups: (1) in mice and other rodents (e.g., vole; Keränen et al. 1999; Laffont et al. 2009), the pEK has been shown to express a large set of genes (e.g., *Shh*, *Fgf4*, *Edar*, *Edaradd*, *Spry2*, *Wnt10a*, *p21*, *Pitx2*, and *Lef1*) in a spatially-restricted fashion during tooth induction (see http://bite-it.helsinki.fi/); (2) in opossum (Moustakas et al. 2011), the pEK has been shown to express *Fgf3*, *Fgf10*, *Fgf4*, *Shh*, *Spry2*, *Spry4*, (3) while no true EKs were traditionally thought to be formed in lizards and snakes, tooth signaling centers in these groups express a set of genes similar to those expressed in mammalian EKs (*Wnt6*, *Wnt7a*, *Bmp4*, *Shh*, and *Edar*; Buchtová et al. 2008; Handrigan and Richman 2010, 2011; Richman and Handrigan 2011; Landova Sulcova et al. 2020), and a recent study revealed "EK-like" structures complete with apoptosis in the veiled chameleon as well as in crocodiles and geckos (Landova Sulcova et al. 2020); (4) a similar set of genes is conserved in bony fishes (zebrafish), medaka (Fraser et al. 2004, 2006, 2009), catshark (Debiais-Thibaud et al. 2015; Rasch et al. 2016), and cichlids (Hulsey et al. 2016). Using existing and new transcriptomic data, this last study by Hulsey et al. (2016) found strong support for the conservation of tooth gene expression across vertebrates.

Taken together, these and other studies support the existence of a core module of genes within the tooth GRN at early stages of tooth development (bud and early cap stage) that is absolutely necessary for correct tooth development (i.e., disruption leads to developmental arrest or severely impacts shape), which has been conserved over 450 million years of vertebrate dental evolution. The genes comprising this core module include, but are likely not limited to, *B-catenin*, *Bmp4*, *Fgf3*, *Fgf10*, *Lef1*, *Pitx1*, *Pitx2*, *Shh*, and *Sox2* (Rasch et al. 2016; see Box 1). Using computer science terminology, this core module

Linking sub-modules and phenotypic variation

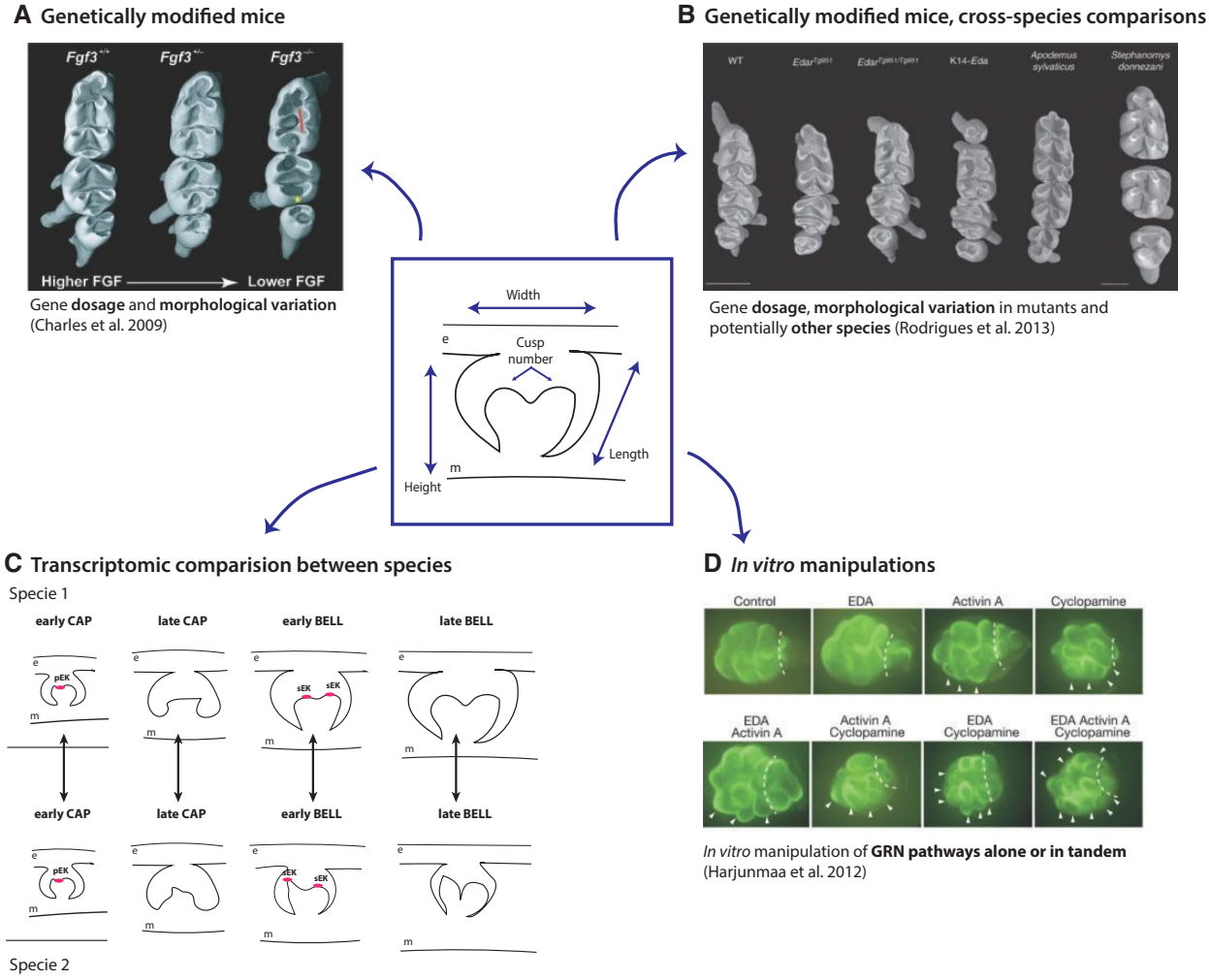


Fig. 4 Identifying the regulatory loops of modules. Identifying regulatory loops of each module require integrative approaches using morphometrics, transcriptomics, *in vitro* and genetic manipulation, species comparison, and modeling. (**A**) Genetically modified mice—genetically modified mice are useful tools to identify the role of a gene in the initiation and morphogenesis of an organ, and thus help resolve the genotype–phenotype map. In our framework, we hypothesize that dosage variation of genes implicated in the sub-modules could lead to variation in tooth morphology (e.g., width, length, or cusp number). For example, variation in FGF dosage in the *fgf3* mutant leads to variation in tooth size and cusp number (Charles et al. 2009a). (**B**) Genetically modified mice and cross-species comparison. Cross-species comparison between species and with genetically modified mice can help identifying the role of particular pathways in the development of particular morphologies seen in nature. For example, variation in Eda signaling through mutation (*Edar* mutants) or overexpression (K14-*Eda*) leads to variation in tooth and cusp number and size, leading to a stephanodont-like tooth morphology (Rodrigues et al. 2013). (**C**) Transcriptomic comparison between species. Transcriptomic is crucial to identify changes between the regulatory states of two or more different species. Comparison of transcriptomes of similar developmental stages between species exhibiting different tooth morphology will help identifying the regulatory loops of each tooth developmental modules. (**D**) *In vitro* manipulations—teeth can be cultured *in vitro* fairly late in their developmental processes. Because of this, it is possible to manipulate the pathways that control their development and test hypothesis regarding their implication in core and/or sub-modules. For example, the modulation of some pathways dosage *in vitro*, alone or in tandem, has been shown to increase the number of cusps and overall dental complexity (Harjunmaa et al. 2012). This suggests a circuit between these genes controls cusp number (see also Kim et al. 2019).

represents a "kernel" for the tooth development program (Fig. 3a, box 1). Additional investigation across diverse species is needed to generate a comprehensive understanding of the genes involved in this putative core module and their interactions.

Peripheral sub-modules of the tooth GRN shape tooth variation along distinct axes

The existence of a conserved, core developmental module across vertebrates poses a conundrum: If molar shape is controlled by such a robust, conserved

developmental network, then how did the great diversity in vertebrate tooth shape arise? Answering this question requires, in part, thorough comparative and quantitative analyses of tooth morphology across clades to identify: the major axes of morphological variation, and how homologous features of teeth (e.g., cusps) changed over evolutionary time and with changes in the tooth GRN. However, homologous traits are not always easily identifiable across the widely divergent tooth morphologies seen across vertebrates, rendering some current approaches (e.g., geometric morphometrics) very difficult to apply.

As a potential solution, researchers have begun to investigate this question using mouse assays, genetic manipulations, and computational *in silico* approaches. Results of these studies suggest that modifications in the expression levels of genes from the primary molar developmental signaling pathways are sufficient to modify molar shape along some axes of variation, without compromising the initial formation of the tooth and its cusps. Pathways whose perturbation modifies shape along single axes of variation, without disturbing other aspects of shape, could represent peripheral sub-modules of the tooth GRN. One such pathway is Eda. Disruption of *Eda* or *Edar* levels in mice modifies tooth size and crown shape in a dose-dependent manner (Fig. 4). *Eda* and *Edar* loss of function mice exhibit a trend toward a reduction of tooth size and cusp number, as well as a disorganization of tooth patterning (Tucker et al. 2000; Charles et al. 2009b). Genetically modified mice that overexpress *Edar* have misshapen molars, with phenotypes ranging from fewer cusps to more cusps, and sizes ranging from shorter/narrower to wider, both depending on the dosage (Fig. 4; Pispa et al. 2004; Tucker et al. 2004). In addition, people with the EDAR 370 A variant, which induces a higher signaling activity of the Eda pathway *in vitro* (Bryk et al. 2008; Mou et al. 2008), exhibit a higher prevalence of additional cusps on their molars as well as a double shoveling phenotype in their upper incisors (Park et al. 2012). Together, these results suggest that the Eda pathway regulates tooth traits including cusp number and tooth size and/or width.

Other genetically modified mice, for example, those misexpressing *Fgf20*, *Barx1*, *Lrp4*, *Fgf3* (Fig. 4), *Sostdc1*, or *Wnt10a*, also exhibit changes in the patterning, number, or size of teeth (Yamashiro et al. 2007; Charles et al. 2009a; Cho et al. 2011; Miletich et al. 2011; Häärä et al. 2012; Ahn et al. 2017). Within teeth, cusp number has also been shown to be regulated by Sostdc1-mediated cross-talk between Wnt and Shh signaling (Kim et al. 2019) downstream of EK formation. Single and combined modification of the HH, Activin, and Eda pathways during mouse development can alter molar size and complexity (Harjunmaa et al. 2012, Fig. 4). In addition, variation in the temporal, spatial, and functional differences in tooth signaling center activities has been shown to be linked to variation of tooth length in the upper molars of mice (Hayden et al. 2020).

Comparative studies across rodent strains and species also provide support for the existence of peripheral, sub-modules of the tooth GRN that control the variation of specific morphological traits. For example, *in silico* manipulation of single or multiple signaling pathways within a computational model of mouse molar development (ToothMaker: Salazar-Ciudad and Jernvall 2010; Salazar-Ciudad 2012; Harjunmaa et al. 2014) can generate morphologies that mirror those observed across living and extinct rodents (Harjunmaa et al. 2014). *In vivo* comparisons of two strains of mice also have linked variation in the timing, localization, and level of EK or pre-EK gene expression to variation in tooth length within and among murine species (Hayden et al. 2020). These comparative studies suggest that the balance of activator and inhibitor levels and their regulators (e.g., *Edar*, BMP4, Activin, and/or *Shh* pathways and their regulation) within the tooth GRN are critical to shaping the variation within and among rodent species (Laugel-Haushalter et al. 2013 and Fig. 4).

In the hierarchical and modular framework proposed in our article, each of the pathways or circuits that control an aspect of tooth shape would represent a sub-module, that is, a sub-circuit of one or multiple pathways and their regulators, associated with one given trait (see examples above). Intriguingly, the comparative studies discussed here demonstrate that simple tweaking of existing GRN sub-modules within a single species can generate a pattern of morphological variation that mirrors that observed among species. At least for rodent teeth, this finding is consistent with the evolution of morphology occurring along "developmental lines of least resistance." However, additional comparative research in model and non-model vertebrates is necessary to fully characterize the sub-modules, their regulatory loops, and their impact on morphological variation within and among species.

Evolutionary implications of core and sub-modules in the tooth GRN

A major goal of modern research on tooth diversity is to assemble a comprehensive picture of GRNs

regulating tooth shape. This goal is complicated, in part, by the fact that many pathways with roles in tooth development are pleiotropic and expressed at multiple developmental stages (see Fig. 3A for an example of a theoretical GRN), and likely has roles in multiple GRN modules. For example, *Edar* is not only expressed in EKs but also more diffusely at other stages of tooth development (Tucker et al. 2000; Sadier et al. 2019). Similarly, *Shh* is expressed at different stages of tooth development and is both a critical gene for tooth initiation (*Shh* KO leads to the arrest of tooth development at early stages, Fig. 3B) and subsequent morphogenesis (Dassule et al. 2000). However, while many genes and pathways may have pleiotropic roles in tooth development, the circuit of their interactions with other genes and pathways within a given developmental stage is often unique from that in other stages (see Box 1 and Fig. 3A). Therefore, the topology of gene interactions (i.e., the interaction map between developmental genes, TFs, and CREs) can be used to identify GRN sub-circuits that represent modules and regulate specific traits (see Box 1 and Fig. 3A; see Peter and Davidson 2009, "4.1 Subcircuits and their biological 'jobs'") (Fig. 4). Given this, modules are often most easily identified when a combination of techniques are used to resolve genes' roles in tooth development and their interactions with other genes (e.g., genetic manipulation in animals, experimental manipulation *in vitro*, comparative expression assays, TF/CRE interaction assays, computational modeling).

Previous results suggest that the HH, Activin, and Eda pathways and some of their regulators together form a sub-module that controls the number of cusps and therefore, to some degree, dental complexity (Fig. 4; Harjunmaa et al. 2014). Analysis of Eda pathway variants further suggests that the Eda pathway, at least, is also implicated in regulating overall tooth size and tooth width (see above). These findings, and others, support the hypothesis that individual pathways can regulate different dental traits depending on the spatiotemporal context in which they are deployed. Thus, a given pathway could be part of multiple sub-modules, including, for example, the core module and sub-modules that are active at different stages during development (Fig. 3). From an evolutionary perspective, the incorporation of the same pathway in multiple modules could help explain instances in which some traits (e.g., tooth length and width) co-vary.

The existence of a hierarchical and modular structure for tooth GRNs can also help explain the repeated, independent evolution of similar tooth traits in multiple lineages. As an example of this, the molars of transgenic mice overexpressing *Eda* and *Edar* display a stephanodont-like phenotype, which is characterized by longitudinal crests (Mustonen et al. 2003; Kangas et al. 2004; Tucker et al. 2004; Harjunmaa et al. 2012). Researchers have proposed that a similar modulation of *Edar* signaling drove the independent evolution of stephanodonty in the *Apodemus* and *Stephanomys* rodent lineages (Rodrigues et al. 2013; Fig. 4). This example illustrates how, in theory, the simple tweaking of a single, potential tooth sub-module could drive the repeated evolution of a complex phenotype such as stephanodonty. In a more complex example, the regulatory circuit that links *Shh*, *Wnt*, and *Sostdc1* (Cho et al. 2011) has been proposed to serve as a key coordinator of dental evolution in Murinae by regulating the number and distance among cusps, and their shape (Kim et al. 2019). Molars of *Shh*-activity-suppressed-*Sostdc1* null mice exhibit extra cusps and overall shorter distance among cusps, a morphology that is rarely seen in the genus *Mus* but has been documented in other murine genera. Thus, the *Shh*, *Wnt*, and *Sostdc1* regulatory circuit could be part of a module that controls differences in molar morphology across species via changes in cusp number and spacing (Kim et al. 2019).

Reiteration of the GRN core and sub-modules during vertebrate tooth development and evolution

An important feature of mammalian tooth development is the reuse of the same signals for the formation of the tooth itself (during the bud and early cap—pEK stage) and its cusps (during the early bell—sEK stage; Fig. 2). As outlined in the examples presented in this article, mutations that affect tooth number generally also affect cusp numbers. This suggests that the same mechanisms are required for tooth and cusp induction (Harjunmaa et al. 2012; Kim et al. 2019) and that the part of the tooth GRN that is active for the formation of a tooth is reiterated for the formation of the cusps (Jernvall and Thesleff 2000; Cho et al. 2007; Kim et al. 2019; Fig. 3). Applying the definition of GRN modules presented here, we suggest that, in mammals, cusp formation involves reiteration of the core and sub-modules, with the core module being responsible for the formation of each cusp at earlier developmental stages and the sub-modules being responsible for determining the shape of cusps at later developmental stages. Modification of the sub-modules over evolutionary time would explain differences in cusp shape among species (Figs. 2 and 4).

While the mechanisms behind the morphogenesis of multicuspid teeth (e.g., sEKS, etc.) are well understood in mammals, the situation in other vertebrates

Box 1 Definitions and putative list of core module genes

Developmental GRN: Circuits of genes and TFs that govern the development of an anatomical element (e.g., a molar) or an organism.

GRN module: A sub-circuit of the GRN (comprising genes and their TFs and regulatory interactions). The output of a module executes a given developmental function (e.g., initiate a tooth; controlling the height of a cusp), see Fig. 3A.

Core module: Module of the GRN that is essential for the induction of the development of an anatomical element (e.g., setting up the initial domain).

Putative core module genes list: *B-catenin*, *Bmp4*, *Fgf3*, *Fgf10*, *Lef1*, *Pitx1*, *Pitx2*, *Shh*, and *Sox2* (as identified in Rasch et al. 2016). Given their central role in tooth signaling centers and tooth patterning, and their potentially conserved evolutionary role, other possible candidates could include but are not restricted to *Eda*, *Edar* and *Edaradd*, *Sostdc1*.

Sub-module: Module at the periphery of the GRN that has secondary functions in the development of an anatomical element (e.g., patterning one part of the element or controlling one axis of variation), downstream of the core module.

is less clear. In particular, the existence in other vertebrates of structures homologous to mammalians EKs is debated, in particular, in other vertebrates with multicuspid teeth. While EKs are thought to be a mammalian innovation (Weeks et al. 2013), cell division, histology, and gene expression data suggest that "EK-like" structures might also parti cipate in the formation of cusps in non-mammalian species with multicuspid teeth. In cat-sharks (Rasch et al. 2016), a signaling center that expresses *Bmp4*, *Fgfs 3-10*, *Ptc2*, and *Shh* and bears non-dividing cells have been observed at the apex of each cusp in multicuspid teeth, a finding that suggests homology with EKs. In cichlid fishes, EK-like structures have also been observed (in particular, regarding the restricted expression patterns of signaling center genes, see Fraser et al. 2008, 2013) and are thought to arise through mechanisms resembling those seen in mammals (Streelman et al. 2003). In squamates, the existence of "EK-like" structures is more equivocal. In the ball python, bearded dragon, leopard gecko, and alligator, the same set of genes is expressed during tooth or cusp induction, but their expression does not follow the spatially-restricted pattern that characterizes mammalian EKs (Handrigan and Richman 2011; Richman and Handrigan 2011; Weeks et al. 2013), suggesting that reptiles do not possess structures homologous to mammalian EKs. However, more recent studies in anole and chameleon have revealed that their tooth cusps are formed via folding of the inner epithelium, a process that is reminiscent of the manner in which mammalian cusps are formed (Zahradnicek et al. 2014). Further, "EK-like" structures complete with EK-like apoptotic and molecular signatures have been characterized in the veiled chameleon (Landova Sulcova et al. 2020). Altogether, previous work suggests that EK-like structures for cusp formation may be conserved across vertebrates, with extreme specialization in mammals, and might have been lost in some lineages (e.g., some squamates). Studies of such structures and their characteristics (e.g., expression patterns, apoptosis) in a wider range of taxa are needed to confirm this idea. If EK-like structures are widely present across vertebrates, this would provide an important line of evidence that the core and sub-modules are active in a reiterative manner for the formation of multicuspid teeth in vertebrates.

Conclusion

Investigations of the role of GRN modules in shaping the pattern of morphological evolution have remained largely theoretical (reviewed in Schlosser and Wagner 2004; Klingenberg 2010, 2014; Deline et al. 2018; Uller et al. 2018). Given the current knowledge of vertebrate tooth development, we propose that this organ provides an excellent system with which to test this hypothesis from developmental and evolutionary points of view. Investigating the factors that underlie tooth development and evolution from a modular perspective holds great potential to fill many important knowledge gaps. First, it could help explain why patterns of expression of tooth GRN genes are restricted and highly conserved at early stages of tooth development (such as bud and early cap), and less constrained and more variable at later stages (cap/late cap). Second, this framework could explain why tooth morphology at bud/early cap stages is generally more conserved among species, whereas tooth morphology at the cap stage—when adult tooth shape starts to establish—varies highly among species. Ultimately, a full understanding of tooth GRN components, their links, and spatial and temporal variation will contribute to defining the intrinsic factors and mechanisms that underlie the high morphological diversity of vertebrate dentitions.

Acknowledgments

We thank Helder Gomes Rodrigues for his helpful comments on the manuscript and Suzanne Miller for her help during the review process. We also thank Gareth Fraser and Darrin Hulsey for organizing the 2020 SICB Symposium S3 Biology at the Cusp: Teeth as a Model Phenotype for Integrating Developmental Genomics, Biomechanics, and Ecology in which this review article was presented, as well as the Society for Integrative and Comparative Biology.

Funding

A.S. and K.E.S. are supported by NSF IOS Award 2017803, and S.E.S. is supported by NSF IOS Award 2017738.

References

Abzhanov A, Kuo WP, Hartmann C, Grant BR, Grant PR, Tabin CJ. 2006. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. Nature 442:563–7.

Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ. 2004. Bmp4 and morphological variation of beaks in Darwin's finches. Science 305:1462–5.

Ahn Y, Sims C, Murray MJ, Kuhlmann PK, Fuentes-Antrás J, Weatherbee SD, Krumlauf R. 2017. Multiple modes of Lrp4 function in modulation of Wnt/β-catenin signaling during tooth development. Dev Camb Engl 144:2824–36.

Brakefield PM. 2006. Evo-devo and constraints on selection. Trends Ecol Evol 21:362–8.

Bryk J, Hardouin E, Pugach I, Hughes D, Strotmann R, Stoneking M, Myles S. 2008. Positive selection in East Asians for an EDAR allele that enhances NF-κB activation. PLoS One 3:e2209.

Buchtová M, Handrigan GR, Tucker AS, Lozanoff S, Town L, Fu K, Diewert VM, Wicking C, Richman JM. 2008. Initiation and patterning of the snake dentition are dependent on Sonic Hedgehog signaling. Dev Biol 319:132–45.

Busey HA, Zattara EE, Moczek AP. 2016. Conservation, innovation, and bias: embryonic segment boundaries position posterior, but not anterior, head horns in adult beetles. J Exp Zoolog B Mol Dev Evol 326:271–9.

Catón J, Tucker AS. 2009. Current knowledge of tooth development: patterning and mineralization of the murine dentition. J Anat 214:502–15.

Charles C, Lazzari V, Tafforeau P, Schimmang T, Tekin M, Klein O, Viriot L. 2009a. Modulation of Fgf3 dosage in mouse and men mirrors evolution of mammalian dentition. Proc Natl Acad Sci U S A 106:22364–8.

Charles C, Pantalacci S, Tafforeau P, Headon D, Laudet V, Viriot L. 2009b. Distinct impacts of Eda and Edar loss of function on the mouse dentition. PLoS One 4:e4985.

Cheng Y, Gao B, Wang H, Han N, Shao S, Wu S, Song G, Zhang YE, Zhu X, Lu X, et al. 2017. Evolution of beak morphology in the Ground Tit revealed by comparative transcriptomics. Front Zool 14:58.

Cho S, Lee H, Cai J, Lee M, Kim J, Ohshima H, Jung H. 2007. The primary enamel knot determines the position of the first buccal cusp in developing mice molars. Differentiation 75:441–51.

Cho S-W, Kwak S, Woolley TE, Lee M-J, Kim E-J, Baker RE, Kim H-J, Shin J-S, Tickle C, Maini PK, et al. 2011a. Interactions between Shh, Sostdc1 and Wnt signaling and a new feedback loop for spatial patterning of the teeth. Development 138:1807–16.

Clune J, Mouret J-B, Lipson H. 2013. The evolutionary origins of modularity. Proc Biol Sci 280:20122863.

Cobourne MT, Hardcastle Z, Sharpe PT. 2001. Sonic hedgehog regulates epithelial proliferation and cell survival in the developing tooth germ. J Dent Res 80:1974–9.

Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. 2000. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 127:4775–85.

Davidson EH. 2010. Emerging properties of animal gene regulatory networks. Nature 468:911–20.

Davidson EH, Erwin DH. 2006. Gene regulatory networks and the evolution of animal body plans. Science 311:796–800.

Davidson EH, Levine MS. 2008. Properties of developmental gene regulatory networks. Proc Natl Acad Sci U S A 105:20063–6.

Debiais-Thibaud M, Chiori R, Enault S, Oulion S, Germon I, Martinand-Mari C, Casane D, Borday-Birraux V. 2015. Tooth and scale morphogenesis in shark: an alternative process to the mammalian enamel knot system. BMC Evol Biol 15:292.

Deline B, Greenwood JM, Clark JW, Puttick MN, Peterson KJ, Donoghue PCJ. 2018. Evolution of metazoan morphological disparity. Proc Natl Acad Sci U S A 115:E8909–18.

Erwin DH, Davidson EH. 2009. The evolution of hierarchical gene regulatory networks. Nat Rev Genet 10:141–8.

Espinosa-Soto C, Wagner A. 2010. Specialization can drive the evolution of modularity. PLoS Comput Biol 6:e1000719.

Evans AR, Wilson GP, Fortelius M, Jernvall J. 2007. High-level similarity of dentitions in carnivorans and rodents. Nature 445:78–81.

Hulsey CD, Cohen KE, Johanson Z, Karagic N, Meyer A, Miller CT, Sadier A, Summers AP, Fraser GJ.. 2020. Grand challenges in comparative tooth biology. Integr Comp Biol (doi: 10.1093/icb/icaa038).

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

Fraser GJ, Bloomquist RF, Streelman JT. 2013. Common developmental pathways link tooth shape to regeneration. Dev Biol 377:399–414.

Fraser GJ, Graham A, Smith MM. 2004. Conserved deployment of genes during odontogenesis across osteichthyans. Proc Biol Sci 271:2311–7.

Fraser GJ, Graham A, Smith MM. 2006. Developmental and evolutionary origins of the vertebrate dentition: molecular controls for spatio-temporal organisation of tooth sites in osteichthyans. J Exp Zool B Mol Dev Evol 306B: 183–203.

Fraser GJ, Hulsey CD, Bloomquist RF, Uyesugi K, Manley NR, Streelman JT. 2009. An ancient gene network is co-opted for teeth on old and new jaws. PLoS Biol 7:e1000031.

Häärä O, Harjunmaa E, Lindfors PH, Huh S-H, Fliniaux I, Åberg T, Jernvall J, Ornitz DM, Mikkola ML, Thesleff I.

2012. Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. Development 139:3189–99.

Handrigan GR, Richman JM. 2010. Autocrine and paracrine Shh signaling are necessary for tooth morphogenesis, but not tooth replacement in snakes and lizards (Squamata). Dev Biol 337:171–86.

Handrigan GR, Richman JM. 2011. Unicuspid and bicuspid tooth crown formation in squamates. J Exp Zool B Mol Dev Evol 316B:598–608.

Harjunmaa E, Kallonen A, Voutilainen M, Hämäläinen K, Mikkola ML, Jernvall J. 2012. On the difficulty of increasing dental complexity. Nature 483:324–7.

Harjunmaa E, Seidel K, Häkkinen T, Renvoisé E, Corfe IJ, Kallonen A, Zhang Z-Q, Evans AR, Mikkola ML, Salazar-Ciudad I, et al. 2014. Replaying evolutionary transitions from the dental fossil record. Nature 512:44–8.

Hayden L, Lochovska K, Sémon M, Renaud S, Delignette-Muller M-L, Vilcot M, Peterkova R, Hovorakova M, Pantalacci S. 2020. Developmental variability channels mouse molar evolution. Elife 9:e50103.

Hendrikse JL, Parsons TE, Hallgrímsson B. 2007. Evolvability as the proper focus of evolutionary developmental biology. Evol Dev 9:393–401.

Hinman VF, Jarvela AMC. 2014. Developmental gene regulatory network evolution: insights from comparative studies in echinoderms. Genesis 52:193–207.

Hulsey CD, Fraser GJ, Meyer A. 2016. Biting into the genome to phenome map: developmental genetic modularity of cichlid fish dentitions. Integr Comp Biol 56:373–88.

Jernvall J, Aberg T, Kettunen P, Keränen S, Thesleff I. 1998. The life history of an embryonic signaling center: BMP-4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. Development 125:161–9.

Jernvall J, Keränen SVE, Thesleff I. 2000. Evolutionary modification of development in mammalian teeth: quantifying gene expression patterns and topography. Proc Natl Acad Sci U S A 97:14444–8.

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

Jernvall J, Thesleff I. 2012. Tooth shape formation and tooth renewal: evolving with the same signals. Development 139:3487–97.

Jia S, Zhou J, Gao Y, Baek J-A, Martin JF, Lan Y, Jiang R. 2013. Roles of Bmp4 during tooth morphogenesis and sequential tooth formation. Development 140:423–32.

Jussila M, Thesleff I. 2012. Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harb Perspect Biol 4:a008425 (doi:10.1101/cshperspect.a008425).

Kangas AT, Evans AR, Thesleff I, Jernvall J. 2004. Nonindependence of mammalian dental characters. Nature 432:211–4.

Kavanagh KD, Evans AR, Jernvall J. 2007. Predicting evolutionary patterns of mammalian teeth from development. Nature 449:427–32.

Kavanagh KD, Shoval O, Winslow BB, Alon U, Leary BP, Kan A, Tabin CJ. 2013. Developmental bias in the evolution of phalanges. Proc Natl Acad Sci U S A 110:18190–5.

Keränen SVE, Kettunen P, Åberg T, Thesleff I, Jernvall J. 1999. Gene expression patterns associated with suppression of odontogenesis in mouse and vole diastema regions. Dev Genes Evol 209:495–506.

Kim J, Ahn Y, Adasooriya D, Woo EJ, Kim HJ, Hu KS, Krumlauf R, Cho SW. 2019. Shh plays an inhibitory role in cusp patterning by regulation of Sostdc1. J Dent Res 98:98–106.

Klingenberg C. 2010. Evolution and development of shape: integrating quantitative approaches. Nat Rev Genet 11:623–35.

Klingenberg CP. 2014. Studying morphological integration and modularity at multiple levels: concepts and analysis. Philos Trans R Soc Lond B Biol Sci 369:20130249.

Kouvaris K, Clune J, Kounios L, Brede M, Watson RA. 2017. How evolution learns to generalise: using the principles of learning theory to understand the evolution of developmental organisation. PLOS Comput Biol 13:e1005358.

Lacquaniti F, Ivanenko YP, D'avella A, Zelik K, Zago M. 2013. Evolutionary and developmental modules. Front Comput Neurosci 7: 61.

Laffont R, Renvoisé E, Navarro N, Alibert P, Montuire S. 2009. Morphological modularity and assessment of developmental processes within the vole dental row (*Microtus arvalis*, Arvicolinae, Rodentia). Evol Dev 11:302–11.

Landova Sulcova M, Zahradnicek O, Dumkova J, Dosedelova H, Krivanek J, Hampl M, Kavkova M, Zikmund T, Gregorovicova M, Sedmera D, et al. 2020. Developmental mechanisms driving complex tooth shape in reptiles. Dev Dyn 249:441–64.

Laugel-Haushalter V, Paschaki M, Thibault-Carpentier C, Dembelé D, Dollé P, Bloch-Zupan A. 2013. Molars and incisors: show your microarray IDs. BMC Res Notes 6:113.

Lesot H, Vonesch JL, Peterka M, Turecková J, Peterková R, Ruch JV. 1996. Mouse molar morphogenesis revisited by three-dimensional reconstruction. II. Spatial distribution of mitoses and apoptosis in cap to bell staged first and second upper molar teeth. Int J Dev Biol 40:1017–31.

Levine M, Davidson EH. 2005. Gene regulatory networks for development. Proc Natl Acad Sci U S A 102:4936–42.

Lipson H, Pollack JB, Suh NP. 2002. On the origin of modular variation. Evolution 56:1549–56.

Liu M, Zhao S, Wang X-P. 2014. YAP overexpression affects tooth morphogenesis and enamel knot patterning. J Dent Res 93:469–74.

Mallarino R, Campàs O, Fritz JA, Burns KJ, Weeks OG, Brenner MP, Abzhanov A. 2012. Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs. Proc Natl Acad Sci U S A 109:16222–7.

Mallarino R, Grant PR, Grant BR, Herrel A, Kuo WP, Abzhanov A. 2011. Two developmental modules establish 3D beak-shape variation in Darwin's finches. Proc Natl Acad Sci U S A 108:4057–62.

Miletich I, Yu W-Y, Zhang R, Yang K, Andrade SD, Pereira SDA, Ohazama A, Mock OB, Buchner G, Sealby J, et al. 2011. Developmental stalling and organ-autonomous regulation of morphogenesis. Proc Natl Acad Sci U S A 108:19270–5.

Mou C, Thomason HA, Willan PM, Clowes C, Harris WE, Drew CF, Dixon J, Dixon MJ, Headon DJ. 2008. Enhanced ectodysplasin-A receptor (EDAR) signaling alters multiple

fiber characteristics to produce the East Asian hair form. Hum Mutat 29:1405–11.
Moustakas JE, Smith KK, Hlusko LJ. 2011. Evolution and development of the mammalian dentition: insights from the marsupial *Monodelphis domestica*. Dev Dyn 240:232–9.
Mustonen T, Pispa J, Mikkola ML, Pummila M, Kangas AT, Pakkasjärvi L, Jaatinen R, Thesleff I. 2003. Stimulation of ectodermal organ development by Ectodysplasin-A1. Dev Biol 259:123–36.
Nakatomi M, Hovorakova M, Gritli-Linde A, Blair HJ, MacArthur K, Peterka M, Lesot H, Peterkova R, Ruiz-Perez VL, Goodship JA, et al. 2013. Evc regulates a symmetrical response to Shh signaling in molar development. J Dent Res 92:222–8.
Ochoa-Espinosa A, Yucel G, Kaplan L, Pare A, Pura N, Oberstein A, Papatsenko D, Small S. 2005. The role of binding site cluster strength in Bicoid-dependent patterning in Drosophila. Proc Natl Acad Sci U S A 102:4960–5.
O'Connell DJ, Ho JWK, Mammoto T, Turbe-Doan A, O'Connell JT, Haseley PS, Koo S, Kamiya N, Ingber DE, Park PJ, et al. 2012. A Wnt-Bmp feedback circuit controls intertissue signaling dynamics in tooth organogenesis. Sci Signal 5:ra4 (doi:10.1126/scisignal.2002414).
Pantalacci S, Guéguen L, Petit C, Lambert A, Peterkovà R, Sémon M. 2017. Transcriptomic signatures shaped by cell proportions shed light on comparative developmental biology. Genome Biol 18: 29 (doi:10.1186/s13059-017-1157-7).
Park J-H, Yamaguchi T, Watanabe C, Kawaguchi A, Haneji K, Takeda M, Kim Y-I, Tomoyasu Y, Watanabe M, Oota H, et al. 2012. Effects of an Asian-specific nonsynonymous EDAR variant on multiple dental traits. J Hum Genet 57:508–14.
Peter IS, Davidson EH. 2009. Modularity and design principles in the sea urchin embryo gene regulatory network. FEBS Lett 583:3948–58.
Peter IS, Davidson EH. 2010. The endoderm gene regulatory network in sea urchin embryos up to mid-blastula stage. Dev Biol 340:188–99.
Pispa J, Mikkola ML, Mustonen T, Thesleff I. 2003. Ectodysplasin, Edar and TNFRSF19 are expressed in complementary and overlapping patterns during mouse embryogenesis. Gene Expr Patterns 3:675–9.
Pispa J, Mustonen T, Mikkola ML, Kangas AT, Koppinen P, Lukinmaa P-L, Jernvall J, Thesleff I. 2004. Tooth patterning and enamel formation can be manipulated by misexpression of TNF receptor Edar. Dev Dyn 231:432–40.
Polly PD. 2007. Development with a bite. Nature 449:413–4.
Raff RA. 2007. Written in stone: fossils, genes and evo–devo. Nat Rev Genet 8:911–20.
Rasch LJ, Martin KJ, Cooper RL, Metscher BD, Underwood CJ, Fraser GJ. 2016. An ancient dental gene set governs development and continuous regeneration of teeth in sharks. Dev Biol 415:347–70.
Raup DM. 1966. Geometric analysis of shell coiling: general problems. J Paleontol 40:1178–90.
Renaud S, Auffray J-C, Michaux J. 2006. Conserved phenotypic variation patterns, evolution along lines of least resistance, and departure due to selection in fossil rodents. Evolution 60:1701–17.
Renaud S, Pantalacci S, Auffray J-C. 2011. Differential evolvability along lines of least resistance of upper and lower molars in island house mice. PLoS One 6:e18951.
Reno PL, McCollum MA, Cohn MJ, Meindl RS, Hamrick M, Lovejoy CO. 2008. Patterns of correlation and covariation of anthropoid distal forelimb segments correspond to Hoxd expression territories. J Exp Zool B Mol Dev Evol 310B:240–58.
Richman JM, Handrigan GR. 2011. Reptilian tooth development. Genesis 49:247–60.
Rodrigues H, Renaud S, Charles C, Poul Y, Solé F, Aguilar J-P, Michaux J, Tafforeau P, Headon D, Jernvall J, et al. 2013. Roles of dental development and adaptation in rodent evolution. Nat Commun 4:2504.
Sadier A, Jackman W, Laudet V, Gibert Y. 2020. Vertebrate tooth row: is it initiated by a single organizing tooth? Bioessays 42:1900229.
Sadier A, Twarogowska M, Steklikova K, Hayden L, Lambert A, Schneider P, Laudet V, Hovorakova M, Calvez V, Pantalacci S. 2019. Modeling Edar expression reveals the hidden dynamics of tooth signaling center patterning. PLoS Biol 17:e3000064.
Salazar-Ciudad I. 2012. Tooth patterning and evolution. Curr Opin Genet Dev 22:585–92.
Salazar-Ciudad I, Jernvall J. 2010. A computational model of teeth and the developmental origins of morphological variation. Nature 464:583–6.
Schlosser G, Wagner G. 2004. Modularity in development and evolution. Chicago (IL): The University of Chicago Press.
Schluter D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–74.
Smith JM, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L. 1985. Developmental constraints and evolution: a perspective from the mountain lake conference on development and evolution. Q Rev Biol 60:265–87.
Stock DW. 2001. The genetic basis of modularity in the development and evolution of the vertebrate dentition. Philos Trans R Soc Lond B Biol Sci 356:1633–53.
Streelman JT, Webb JF, Albertson RC, Kocher TD. 2003. The cusp of evolution and development: a model of cichlid tooth shape diversity. Evol Dev 5:600–8.
Thesleff I, Jernvall J. 1997. The enamel knot: a putative signaling center regulating tooth development. Cold Spring Harb Symp Quant Biol 62:257–67.
Tucker A, Sharpe P. 2004. The cutting-edge of mammalian development; how the embryo makes teeth. Nat Rev Genet 5:499–508.
Tucker AS, Headon DJ, Courtney JM, Overbeek P, Sharpe PT. 2004. The activation level of the TNF family receptor, Edar, determines cusp number and tooth number during tooth development. Dev Biol 268:185–94.
Tucker AS, Headon DJ, Schneider P, Ferguson BM, Overbeek P, Tschopp J, Sharpe PT. 2000. Edar/Eda interactions regulate enamel knot formation in tooth morphogenesis. Development 127:4691–700.
Tucker AS, Sharpe PT. 1999. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. J Dent Res 78:826–34.
Uller T, Moczek AP, Watson RA, Brakefield PM, Laland KN. 2018. Developmental bias and evolution: a regulatory network perspective. Genetics 209:949–66.
Ungar P. 2015. Mammalian dental function and wear: a review. Biosurf Biotribol 1:25–41.

Ungar PS. 2010. Mammal teeth: origin, evolution, and diversity. Baltimore (MD): JHU Press.

Vaahtokari A, Aberg T, Thesleff I. 1996. Apoptosis in the developing tooth: association with an embryonic signaling center and suppression by EGF and FGF-4. Development 122:121–9.

Wagner A. 2011. The origins of evolutionary innovations: a theory of transformative change in living systems. New York: Oxford University Press.

Weeks O, Bhullar B-AS, Abzhanov A. 2013. Molecular characterization of dental development in a toothed archosaur, the American alligator Alligator mississippiensis. Evol Dev 15:393–405.

Yamashiro T, Zheng L, Shitaku Y, Saito M, Tsubakimoto T, Takada K, Takano-Yamamoto T, Thesleff I. 2007. Wnt10a regulates dentin sialophosphoprotein mRNA expression and possibly links odontoblast differentiation and tooth morphogenesis. Differentiation 75:452–62.

Zahradnicek O, Buchtova M, Dosedelova H, Tucker AS. 2014. The development of complex tooth shape in reptiles. Front Physiol 5: 74 (doi:10.3389/fphys.2014.00074).

Zeller R, López-Ríos J, Zuniga A. 2009. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. Nat Rev Genet 10:845–58.