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SYMPOSIUM

Biting into the Genome to Phenome Map: Developmental Genetic Modularity of Cichlid Fish Dentitions

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Synopsis Within vertebrates, teleost fishes provide a rich evolutionary context for studying the mechanisms of dental divergence because of the numerous axes along which their teeth have diverged phenotypically and presumably developmentally. Using both a review of teleost *in situ* hybridization and *de novo* transcriptome sequencing in a cichlid fish, we examined whether 341 gene homologs thought to play a role in developing mice teeth are expressed in the tooth-bearing jaws of teleosts. The similarities and putative differences in gene expression documented between the two most commonly used models, zebrafish and cichlids, highlight what can be learned from using a greater diversity of teleost model systems in studies of tooth development. Both types of gene expression analysis also provide substantial evidence for conservation of tooth gene expression from teleosts to mammals as well as between initial and replacement teeth. Additionally, we found that the cichlid oral and pharyngeal jaws share expression for a large percentage of genes that influence tooth development. Our transcriptome analyses also suggest sub-functionalization between gene paralogs expressed in teeth and paralogs expressed in other structures is likely a common pattern across teleost diversity. Teleost dentitions will continue to provide a potent system in which to examine the importance of both gene duplication as well as the conservation of gene expression for phenotypic diversification.

Introduction

Teeth provide a powerful phenotype for integrating across biological disciplines ranging from ecology to genomics. For instance, teeth are used to identify extant and fossil species (Dieleman et al. 2015), to document ancient (Purnell et al. 2007) as well as recent (Cuozzo et al. 2014) ecologies, and to understand tissue (Lumsden 1988; Mitsiadis et al. 1998; Tucker and Sharpe 2004), cell (Jernvall and Thesleff 2000; Sharpe 2001), and gene interactions (Thesleff and Sharpe 1997; Jernvall and Thesleff 2012; Jackman et al. 2013). Because human and teleost fish teeth are homologous and derived from mineralized tooth-like structures present in a common early vertebrate ancestor (Smith and Coates 1998, 2000; Smith 2003; Fraser and Smith 2011; Rasch et al. 2016), teeth provide an ideal organ system for determining how multiple levels of biological complexity have comparatively contributed to vertebrate diversification. Additionally, since a wide array of serially homologous but differentiated tooth phenotypes can co-occur within the same trophic apparatus, we can also assess how independent mechanisms of tooth formation contribute to differentiation within the same individual organism (Fraser et al. 2009; Hlusko et al. 2011; Ellis et al. 2015). Furthermore, because well-studied mammalian dentitions represent only a small subset of vertebrate dental diversity (Stock 2007; Jernvall and Thesleff 2012), comparative studies in new vertebrate models will continue to provide insights into the mechanisms structuring dental diversification (Tucker and Fraser 2014).

Modularity, or the degree to which traits evolve independently, is often invoked as a critical mechanism

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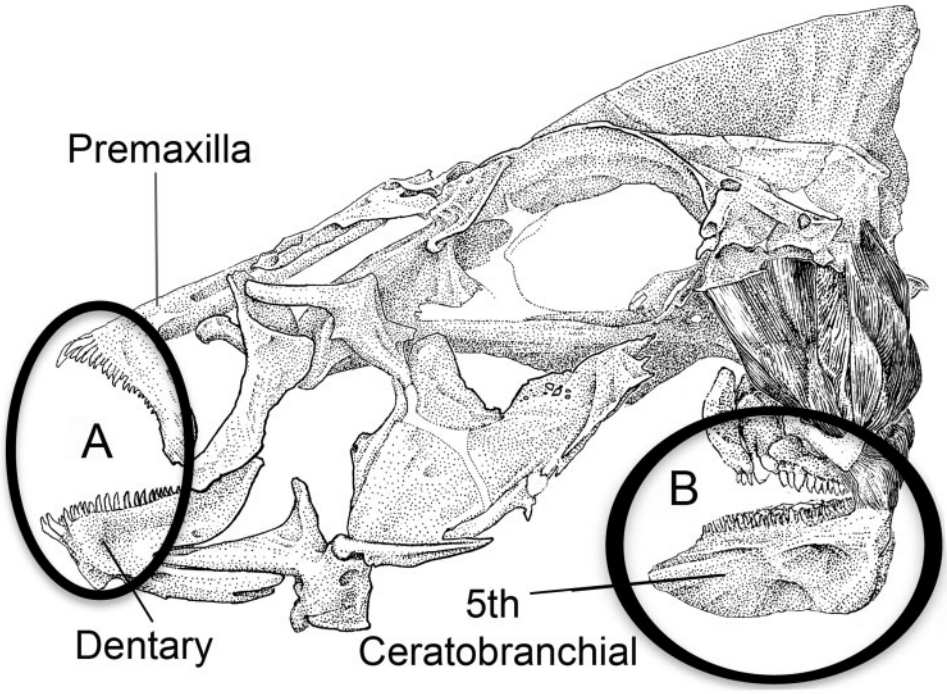

Fig. 1 Cichlids, like most fishes, have two sets of toothed jaws: the oral (**A**) and pharyngeal (**B**) jaws. The oral jaw is fairly homologous to our jaw and the premaxilla and dentary bones are both toothed in cichlids. The pharyngeal jaws are modified gill arches. In cichlids, the fused 5th ceratobranchials form the toothed lower pharyngeal jaw.

during phenotypic diversification. Phenotypic 'modules', units that are semi-autonomous in evolution and potentially so in function, are therefore important to delineate mechanistically (Wagner and Altenberg 1996; Bolker 2000; Hulsey et al. 2005). One potential advantage of unit autonomy is that the pleiotropic effects of change in one component of the genotype to phenotype map, such as the presence or absence of the expression of a particular gene, tend to fall to a greater degree within modules than between modules (Wagner 1996). Generally, the degree to which structural modules like teeth change independently during evolution is thought to be enhanced if there is a corresponding modular organization, a qualitative as well as quantitative difference, in the genetic pathways controlling the development of these structures (Arone and Davidson 1997). Recently, we have come to appreciate that there is a core set of genes that unites the development of all vertebrate teeth that includes members of the *bmp*, *fgf*, *hh*, and *wnt*/β-catenin signaling pathways (Fraser et al. 2009; Rasch et al. 2016). Intriguingly, although every vertebrate tooth likely utilizes this core developmental set of genes, these genes are not uniquely expressed in teeth. Indeed, many other ectodermal appendages in addition to teeth, for example, hair, feathers, scales, and various ectodermal glands develop via signaling interactions that involve these same developmental genes (Wu et al. 2004; Pummila et al. 2007; Sadier et al. 2014). Therefore, a deep developmental homology unites many putative phenotypic modules emerging from the ectoderm that like teeth exhibit reciprocal signaling involving the underlying mesenchymal cells. Understanding what developmental genetic mechanisms allow teeth to phenotypically differentiate during both ontogeny and evolution will demand extending our comparative knowledge of what genes are shared with other ectodermally derived modules as well as what genes are commonly expressed during the formation of different types of vertebrate teeth.

Serially, homologous systems such as the leaves of plants, arthropod limbs, or vertebrate teeth clearly contribute to organismal diversification, and the degree of genetic independence among these iterative structures is likely to have substantial evolutionarily consequences (Bateson 1894; Wagner 1989; Streelman and Albertson 2006; Smith et al. 2009). The teeth of teleost fish provide a rich evolutionary system for understanding how the independence of developmental genetic modules contributes to phenotypic divergence. There are numerous axes along which teleost teeth have diverged phenotypically and presumably developmentally to meet the astonishing array of trophic challenges their prey presents in aquatic environments (Figs. 1 and 2). For instance, many teleost fishes can exhibit a large number of teeth in multiple rows on two independent sets of jaws (oral and pharyngeal),

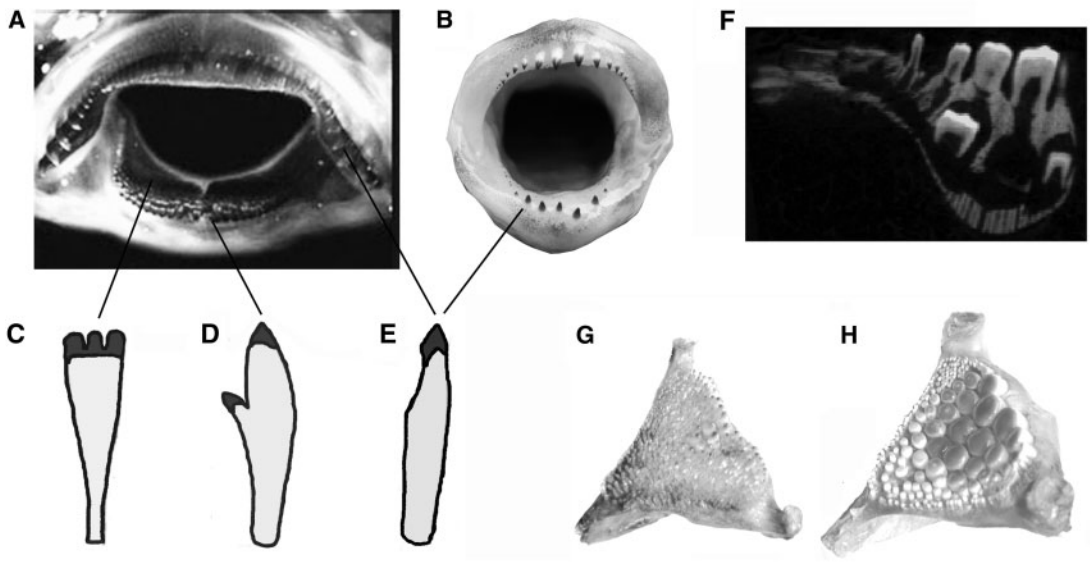

Fig. 2 Axes of cichlid fish tooth diversity. The dentition of different cichlid species varies extensively in whether it is heterodont (**A**), with variation in tooth shape and number in the many rows that can occur on the same jaw, or homodont (**B**), fairly uniformly shaped teeth throughout a jaw. Cichlids commonly vary in whether their teeth or tricuspid (**C**), bicuspid (**D**), or unicuspid (**E**). The lines depict where teeth with these shapes are located in the heterodont and homodont cichlid dentitions. Cichlids also vary extensively in patterns of tooth replacement (**F**) As is shown in the lateral CT scan of a cichlid lower pharyngeal jaw. Substantial variation in cichlid tooth morphology that is only seen after several rounds of tooth replacement can also occur within populations as well as in radiations of species that have diverged over very short timeframes. For instance, the papilliform (**G**) and molariform (**H**) lower pharyngeal jaw dental phenotypes depicted represent morphological variants that interbreed within populations of the cichlid *Herichthys minckleyi*.

differentially shaped teeth within a row (heterodonty), and the production of replacement tooth germs throughout life (polyphyodonty) (Fryer and Illes 1972; Motta 1984; Huysseune and Thesleff 2004; Huysseune 2006; Zhang et al. 2009; Fraser et al. 2009).

Among the many lineages of teleosts, cichlid fishes likely represent one of the best groups for examining modularity in the dentition. Cichlids, like most fish, have two toothed jaws (Fig. 1). They have oral jaws that are largely homologous to our jaws and are used primarily to capture prey, and they also have pharyngeal jaws, modified gill arches, that process prey (Schaeffer and Rosen 1961; Liem 1973). However, unlike any other group of fish, cichlids exhibit an incredible amount of divergence in tooth morphology, and the putative functional independence of their two toothed jaws could have promoted both their trophic divergence as well as their unparalleled species richness (Fryer and Iles 1972; Liem 1973; Hulsey et al. 2006).

Generally, the degree that teeth in different regions of the teleost trophic apparatus are evolutionarily or developmentally decoupled remains unclear. However, several aspects of tooth morphology are conserved between vertebrates as divergent as cichlids and humans (Kerr 1960; Sire et al. 2002). Additionally, tooth number is correlated on the oral and pharyngeal jaws of cichlids, tooth size is associated with variation in tooth number on their pharyngeal jaws, and the teeth on the two jaws of cichlids do share a core network of gene expression (Fraser et al. 2009; Hulsey et al. 2015; Fig. 3). Cichlid tooth phenotypes could therefore be highly integrated at multiple levels of biological design and constrained to diverge in concert. Alternatively, the capacity of the cichlid dentition to diversify independently could be substantial as their oral and pharyngeal jaw mechanics have been shown to diverge in a completely independent fashion (Hulsey et al. 2006). Furthermore, cypriniform fish such as *Danio rerio*, the most commonly used genetic model system the zebrafish, have lost their oral jaw dentition while retaining teeth on only their lower pharyngeal jaw (Huysseune and Sire 1998; Stock 2001; Aigler et al. 2014). Teeth on the two jaws of fish can also diversify independently within populations. In cichlids, single polymorphic species like *Herichthys minckleyi* show no apparent variation in their oral jaw teeth but are highly polymorphic even among interbreeding individuals in the size and number of their pharyngeal jaw teeth (Hulsey et al. 2005, 2015; Fig. 2G, H). Therefore, the developmental genetic systems underlying the formation of teeth on the two jaws of teleosts might be expected to be highly distinct modules and often diverge independently during evolution.

Studies of gene expression during the formation of teeth in cichlids and other teleost fishes have

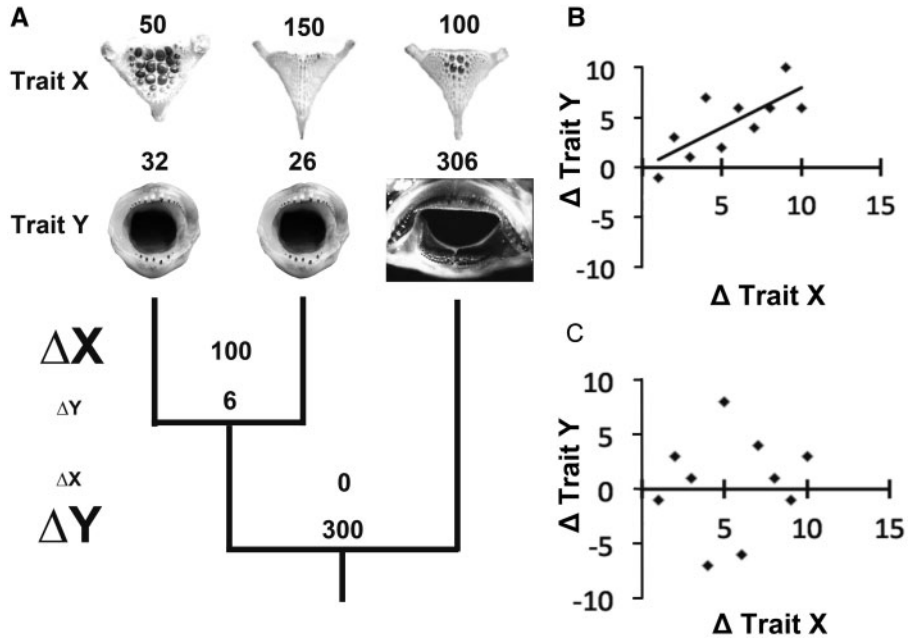

Fig. 3 Testing for evolutionary independence of phenotypes among species. The evolutionary independence of any two phenotypes (**A**) can be tested explicitly using phylogenies and correlations of independent contrasts. In the example shown, the number of teeth on the pharyngeal jaw (Trait X) and the number of teeth on the oral jaw (Trait Y) are evolving independently. Effectively, when there is lots of change in the pharyngeal jaw teeth number, there is very little change in oral jaw tooth number. Conversely, when there is lots of change in the oral jaw tooth number there is very little change in pharyngeal jaw tooth number. This is the kind of macro-evolutionary change we would expect if these traits evolve independently during evolution (**B**). If trait evolution is alternatively highly correlated, we would expect change in trait X and trait Y to change in concert and show a correlation (**C**). In Malawi cichlids at least, changes in tooth number on the two jaws evolve in a surprising integrated manner. These phenotypic correlations characterizing this classic adaptive radiation suggest there are likely shared mechanistic forces, such as the shared presence of the same tooth genes, structuring phenotypic evolution of teeth on the two distinct jaws.

produced at least two generalizable results. First, an extensive number of genes are conserved in their expression during the formation of teeth from fish to tetrapods (Stock 2001; Fraser et al. 2006; Wise and Stock 2006; Cleves et al. 2014). Although these findings have not been extensively reviewed, many genes like *bmp2, bmp4, fgf8, pitx2, shh, dlx2*, as well as *runx2* are all present during tooth development in cichlids as well as in mice (Fraser et al. 2008, 2009). Second, there is likely substantial conservation in the presence of the same basic set of genes wherever teeth are formed in the trophic apparatus (Fraser et al. 2009). Both of these results support the ideas that all vertebrate teeth are evolutionarily homologous structures, that they are ancient in origin, and that they only evolved once (Smith and Johanson 2003; Ellis et al. 2015). Therefore, much of the genome-to-phenome map governing tooth diversification in one clade of vertebrates or in one part of the trophic apparatus could provide insight into how teeth have diverged in other vertebrate lineages.

Yet, one of the problems with using the mouse, the most ubiquitously used vertebrate genetic model, and its dental developmental network as a standard for all vertebrate teeth is that unlike both humans and cichlid fishes, mice do not replace their teeth (Fraser et al. 2004). Therefore, we know relatively little about whether the genes responsible for phenotypic differentiation of vertebrate replacement teeth are generally the same genes utilized in the formation of the initial dentition (Fraser et al. 2013). An example of differential expression between first generation and the replacement dentition is that of the single gene sonic hedgehog (*shh*). It appears that *shh* is necessary for tooth initiation and the establishment of the odontogenic band in vertebrate dentitions, but is not redeployed to initiate the replacement dentition across vertebrate taxa ranging from fish (Fraser et al. 2006, 2013) to reptiles (Handrigan and Richman, 2010). Thus, there could be substantial differences in the genes generating replacement teeth as first generation cichlid teeth are generally homogenous, simple, and are not generally as phenotypically differentiated as replacement teeth (Fryer and Iles 1972; Streelman et al. 2003). Importantly, unlike mammals that replace their teeth at most a single time, cichlids and most teleost fishes can replace their teeth once every 100 days repeatedly

throughout their life (Tuisku and Hildebrand 1994; Huysseune and Sire 1997; Stock et al. 1997; Streelman et al. 2003). Much of the phenotypic diversity in the teleost dentition is also set-up during the time between when tooth replacement begins and the onset of reproductive activity (Ellis et al. 2015; Hulsey et al. 2015). Therefore, teleost fish, including cichlids offer a system that could be used to determine what genes are conserved not only during initial vertebrate tooth formation, but also what genes are expressed as these structures are replaced and differentiate phenotypically into adult dentitions.

The developmental genetic redundancy that follows whole genome duplication has potentially played a major role in vertebrate diversification (Ohno 1970; Braasch et al. 2016). Genome duplication could also have been fundamental to the diversification of teeth because as compared to their distant relatives like tunicates or amphioxus, the clade uniting jawed vertebrates from sharks to tetrapods have had two rounds of genome duplication (Van de Peer and Meyer 2005). These genome duplications effectively gave organisms like mice and humans four paralogous copies of many important craniofacial genes that play a role in fundamental processes such as tooth development (Sharpe 2001). Additionally, following their split from other vertebrate groups, the ancestor of most teleost fishes underwent another round of genome duplication approximately 350 million years ago that gave them an additional copy of many genes when compared to tetrapods (Amores et al. 1998; Wittbrodt et al. 1998; Meyer and Schartl 1999; Taylor et al. 2001; Braasch et al. 2006, 2007; Arnegard et al. 2010; Opazo et al. 2013). When contrasted with their sister group that contains only the seven species of gar and one species of *Amia*, the success and unparalleled adaptive divergence of the over 28,000 teleost species is thought to be partly a consequence of this further genome duplication (Taylor et al. 2003; Santini et al. 2009). However, the mechanistic significance of this teleost-specific genome duplication during ontogeny and across phylogeny is only now being fully appreciated as a diversity of fish species like the Mexican tetra (*Astyanax mexicanus*), medaka (*Oryzias latipes*), pufferfishes (Tetraodontidae), stickleback (*Gasterosteus aculeatus*), and cichlids have had their whole genome sequenced (Jones et al. 2012; Hulsey 2009; McGaugh et al. 2014; Brawand et al. 2014; ; Braasch et al. 2016). It is exciting that the genomic resources are now available to allow us to examine the role of processes like gene duplication in the adaptive diversification of a species rich group like teleosts.

One of the most widely proposed mechanisms whereby duplicate genes, or paralogs, might contribute to diversification is through a process known as sub-functionalization (Force et al. 1999; Yu et al. 2003; Postlethwait et al. 2004). Sub-functionalization occurs when a gene that was ancestrally expressed in a number of tissues is duplicated, and then over time, the functions of these paralogs evolve to become subdivided in where or when they are expressed. For instance, immediately following duplication both paralogs might be expressed in all tissues (i.e., both the oral and pharyngeal jaw teeth) where the originally unduplicated gene was expressed. But, subsequently these paralogs could evolve to become narrowly expressed in a complementary subset of the tissues in which they were originally found (i.e., one paralog present only in oral teeth and one paralog present in only pharyngeal teeth). This subdivision of gene function could thereby reduce pleiotropy between gene expression modules and facilitate adaptive divergence in different tissues without the potentially constraining effects of shared gene expression (Force et al. 1999; Guilllaume and Otto 2012).

Only a few studies of gene expression during the development of teleost teeth have examined gene expression in paralogous duplicates (Wise and Stock 2006; Gibert et al. 2015). Importantly, sub-functionalization of gene duplicates could occur in a number of ways spatially between different phenotypic modules. Each complementary paralog could be differentially expressed in one of the two original structures as suggested above. For instance, one paralog of a duplicated *wnt10* gene could retain its expression in both structures, while the complementary paralog becomes sub-functionalized to a single structure. Alternatively, expression of *wnt10a* might be isolated to the pharyngeal jaw teeth, while its paralog *wnt10b* might be isolated to the cichlid oral jaws. Another possibility is that only one paralog, *wnt10a*, could be isolated to all forms of a particular structure such as teeth on both the oral and pharyngeal jaws, while *wnt10b* could be isolated to another deeply homologous structure such as the scales that cover the fish externally (Fraser et al. 2010). The teeth on the two jaws of cichlids represent a set of serially homologous but evolutionarily divergent structures that could provide a rich system for investigating the role of gene sub-functionalization during vertebrate phenotypic divergence.

Using both a review of *in situ* hybridization studies in teleosts as well as transcriptome sequencing of the oral and pharyngeal jaws of a cichlid, we examined several questions concerning the conservation and independence of gene expression in teleost

dentitions. First, we detailed a large number of genes expressed during tooth development that are conserved in tooth bearing regions from mice to teleosts. Then, we investigated the overlap of tooth gene expression between the oral and pharyngeal jaws. Finally, we documented patterns of sub-functionalization in gene paralogs to understand how this process might be generally playing a role in differentiating teleost oral and pharyngeal dentitions.

Methods

To determine which genes have previously been found to show expression in teleost teeth, we reviewed the literature and web-based resources (e.g., www.zfin.org) for studies of *in situ* hybridization, the primary method used prior to RNA-seq to establish localization of gene expression. We tabulated the gene name, taxon of teleost fish used in the study, whether the *in situ* hybridization was performed on initial or replacement teeth, and if oral or pharyngeal teeth were examined. We also compared these studies to our analyses of tooth gene expression in the transcriptomes of juvenile cichlid oral and pharyngeal jaws.

To further explore the mouse tooth gene homologs expressed in teleost tooth-bearing regions, we separately assembled two transcriptome libraries: one for the oral and one for the pharyngeal jaws of the cichlid *Herichthys cyanoguttatus*. This cichlid was utilized because it belongs to the sister group of the endangered and polymorphic cichlid *Herichthys minckleyi* (Hulsey et al. 2010, 2016), that shows substantial phenotypic divergence in teeth on the pharyngeal jaws but little variation in oral jaw teeth (Hulsey et al. 2005, 2006, 2015). To generate the oral jaw library, we dissected the toothed premaxilla and dentary from an ontogenetic series of 65 fish ranging in size from 20 mm to 70 mm standard length and pooled their jaws. Using these same individuals, we removed the toothed lower pharyngeal jaw to generate a single pharyngeal jaw transcriptome. Because these species are polyphyodont with tooth replacement continuously occurring at these sizes and since teeth should be one of the most transcriptionally active structures in these bony regions (Schneider et al. 2014), we assumed that we would be capturing primarily RNA that is expressed in developing cichlid replacement teeth. In the closely related species *H. minckleyi*, tooth numbers are generally not increasing at the body sizes examined (Hulsey et al. 2015). Although we cannot rule out that some initial teeth are forming in the sizes of fish examined here, this suggests that the teeth forming in the fish we examined were likely primarily replacements for teeth lost from previously formed tooth crypts.

Once the jaws were dissected, we placed these tissues immediately into RNAlater and shipped them on dry ice to LC Sciences (Houston, TX, USA) for sequencing. Our two RNA-seq libraries were generated using Illumina Truseq RNA Sample Preparation Kits. Sequencing of the resulting cDNA libraries was carried out with an Illumina HiSeq 2000. The resulting Illumina libraries were then filtered and only paired-end reads were used for further assembly. *De novo* transcript assembly was conducted using Trinity release_20130216 that consists of three successive software programs: Inchworm, Chrysalis, and Butterfly (Grabherr et al. 2011).

We utilized a custom comparative genomics pipeline to isolate putative tooth genes from the transcriptome of the cichlid *H. cyanoguttatus*. To isolate these loci, we first documented all the genes and their paralogs that have been examined in teleost tooth *in situ* hybridization studies (Table 1). Then, we augmented this list with genes annotated in the "bite-it" tooth gene expression database (http://bite-it.helsinki.fi/) that catalogues genes that have been screened for roles in mouse tooth development. From this database, we isolated 268 genes and their currently accepted abbreviations. Individual gene abbreviations were then queried against the annotated Tilapia (*Oreochromis niloticus*) ensembl genome database (Cunningham et al. 2015) resulting in 341 cichlid homologs to genes known to be expressed in mouse teeth. For these loci, 146 genes, or 73 pairs, represented two duplicated paralogs.

The transcript sequence for each gene from Tilapia was then used to query an un-annotated transcriptome database for the model Central American cichlid *Amphilophus citrinellus* using "blastn" algorithms run using default parameters as implemented in Viroblast (Deng et al. 2007). The transcriptome and genome of this cichlid have been well-characterized using genomic and transcriptomic analyses of multiple life-stages and multiple tissue types (Henning et al. 2013; Elmer et al. 2014; Franchini et al. 2014; Kratochwil et al. 2015), and the species is relatively closely related to *H. cyanoguttatus* (Hulsey et al. 2010, 2016). Only Tilapia tooth gene sequences that returned an unambiguous single best match and *A. citrinellus* sequences that subsequently generated a reciprocal best blast hit to the same gene in Tilapia were used in further analyses.

The assembled oral jaw transcriptome was composed of 182,230 contigs and had a mean contig size of 657 base pairs. The assembled pharyngeal jaw transcriptome was composed of 156,892 contigs

Table 1 Gene expression studies of mouse tooth genes using *in situ* hybridization in teleost teeth

	in situ				Transcriptome	
Genes	**O**	**P**	**Taxa**	**Citation**	**O**	**P**
axin2	R		C	Fraser et al. 2013	*axin2a, axin2n2*	NP
acrvr1l		I	Z	Payne et al. 2001	*acrvr1l*	*acrvr1l*
aldh1a2		I	Z	Gibert et al. 2010, 2015	*aldh1a2*	*aldh1a2*
atp2b1a		I	Z	Go & Korzh 2013	*atp2b1a*	*atp2b1a*
barx1		I	CZ	Sperber and Dawid 2008, Fraser et al. 2009	*barx1*	NP
bmi1	R		C	Streelman et al. 2015	*bmi1a*	NP
bmp2a	I	I	MZ	Wise and Stock 2006	***	***
bmp2b	I	I	ACMTZ	Fraser et al. 2004, 2009, 2013; Wise et al. 2006;	*bmp2b*	NP
bmp4	I	I	ACMPZ	Wise and Stock 2006; Fraser et al. 2009, 2012, 2013	*bmp4*	*bmp4*
bmp6		I	S	Cleves et al. 2014	*bmp6*	*bmp6*
bmpr2	I/R		C	Bloomquist et al. 2015	*bmpr2a, bmpr2n2*	*bmpr2n2*
col1a1a		I	Z	Kawasaki 2009	*col1a1n1, col1a1b*	*col1a1n1, col1a1b*
ctnnb1	I/R		C	Fraser et al. 2013; Streelman et al. 2015; Bloomquist et al. 2015	*ctnnb1, ctnnbl1*	*ctnnb1, ctnnbl1*
cx43		I	Z	Ablooglu et al. 2007; Wiweger et al. 2012	*cx43*	*cx43*
cyp26b1		I	Z	Gibert et al. 2015	*Cyp26b1*	*Cyp26b1*
dkk1b		I/R	Z	Huysseune et al. 2014	***	***
dlx2a	I/R	I	ZC	Jackman et al. 2004; Borday-Birraux et al 2006; Wiweger et al. 2012; Gibert et al. 2010; Fraser et al. 2009, 2013	*dlx2*	NP
dlx2b		I	Z	Jackman et al. 2004; Borday-Birraux et al 2006; Ablooglu et al. 2007; Gibert et al. 2010; Wiweger et al. 2012; Go et al. 2013	***	***
dlx3b		I	Z	Borday-Birraux et al. 2006	*dlx3bn1, dlx3bn2*	*dlx3bn2*
dlx4a		I	Z	Borday-Birraux et al. 2006	*dlx4a*	NP
dlx4b		I	Z	Borday-Birraux et al. 2006	*dlx4b*	NP
dlx5a		I	Z	Borday-Birraux et al. 2006	*dlx5*	*dlx5*
eda	R		C	Fraser et al. 2013	*eda*	NP
edar	I		C	Fraser et al. 2009; Bloomquist et al. 2015	*edar*	NP
eve1	I	I	MZ	Laurenti et al. 2004; Debiais-Thibaud et al. 2007	NP	NP
fgf3	R	I	Z	Jackman et al. 2004, Fraser et al. 2013	*fgf3*	NP
fgf4		I	Z	Jackman et al. 2004	*fgf4n1*	*fgf4n1*
fgf8		I	Z	Jackman et al. 2004	NP	NP
fgf10	R	I	CZ	Gibert et al. 2010; Fraser et al. 2013	*fgf10a*	NP
fgfr1	R		C	Bloomquist et al. 2015	*fgfr1b*	*fgfr1b*
fgfr2	R		C	Fraser et al. 2013	*fgfr2*	*fgfr2*
foxa2	R		C	Streelman et al. 2015	*foxa2n1*	*foxa2n1, foxa2n2*
fsta	R		C	Fraser et al. 2013	*fsta*	NP
gsc	R		C	Bloomquist et al. 2015	*gsc*	NP
hopx	R		C	Streelman et al. 2015	*hopx*	*hopx*
hoxa2b		I	C	Fraser et al. 2009	NP	NP
hoxa5a		I	C	Fraser et al. 2009	NP	NP
hoxd4a		I	C	Fraser et al. 2009	NP	NP
itga5		I	Z	Ablooglu et al. 2007	*Itga5n1, itga5n2*	*Itga5n1, itga5n2*
itgb3		I	Z	Ablooglu et al. 2007	*itgb3a, itgb3b*	*itgb3a, itgb3b*

(continued)

Table 1 Continued

	in situ				Transcriptome	
Genes	**O**	**P**	**Taxa**	**Citation**	**O**	**P**
irx1b	R		C	Fraser et al. 2013	*irx1b*	NP
irx2	R		C	Fraser et al. 2013	*irx2*	NP
jag2	R		C	Fraser et al. 2013	*jag2n1, jag2b*	*jag2n1*
lef1	R		C	Fraser et al. 2013; Bloomquist et al. 2015	*lef1*	NP
lgr4	R		C	Streelman et al. 2015	*lgr4*	NP
lgr6	R		C	Streelman et al. 2015	*lgr6*	*lgr6*
lhx6		I	Z	Jackman et al. 2004	*lhx6*	NP
lhx7		I	Z	Jackman et al. 2004	***	***
mcam	R		C	Streelman et al. 2015	*mcam*	*mcam*
msx1	R		C	Bloomquist et al. 2015	*msx1b*	NP
notch1	R		C	Fraser et al. 2013	*notch1a, notch1b*	NP
odam		I	Z	Kawasaki 2009	*odam*	*odam*
osr2	R		C	Fraser et al. 2013	*osr2*	NP
pax9	I	I	CZ	Jackman et al. 2004; Fraser et al. 2009	*pax9*	*pax9*
pitx2	I	I	CPTZ	Fraser et al. 2004, 2009, 2012; Jackman et al. 2004	*pitx2*	*pitx2*
ptch1	R		C	Fraser et al. 2013	*ptch1*	NP
ptch2	R		C	Fraser et al. 2013	*ptch2*	NP
raraa		I	Z	Gibert et al. 2015	*raraa*	*raraa*
rarab		I	Z	Gibert et al. 2015	*rarab*	*rarab*
runx2	I/R	I	C	Fraser et al. 2009, 2013	*runx2*	NP
scpp1		I	Z	Kawasaki 2009	***	***
scpp5		I	Z	Kawasaki 2009	NP	NP
scpp9		I	Z	Kawasaki 2009	***	***
sfrp5	R		C	Bloomquist et al. 2015	*sfrp5*	NP
shha	I	I	CPTZ	Fraser et al. 2004, 2009, 2012, 2013; Stock et al. 2006; Jackman et al. 2010	*shha*	*shha*
smo	R		C	Bloomquist et al. 2015	*smo*	NP
sostdc	R		C	Fraser et al. 2013	*sostdc1a, sostdc1b*	NP
sox2	R		C	Fraser et al. 2013	*sox2*	*sox2*
sox9a	R		C	Streelman et al. 2015	*sox9a*	*sox9a*
sp7		I	Z	Wiweger et al. 2012	*sp7*	*sp7*
sparc		I	Z	Kawasaki 2009	*sparc*	*sparc*
spry4	R		C	Fraser et al. 2013; Bloomquist et al. 2015	*spry4*	*spry4*
tbx	R		C	Bloomquist et al. 2015	*tbx1*	NP
wnt10a	R		C	Fraser et al. 2013; Bloomquist et al. 2015	*wnt10b*	*wnt10a*
wnt5a	R		C	Fraser et al. 2013	*wnt5a*	*wnt5a*
wnt7b	R		C	Fraser et al. 2008; Bloomquist et al. 2015	*wnt7bb*	NP

Whether the oral (O) or pharyngeal (P) teeth were examined is noted. Also, whether an *in situ* hybridization analysis was performed on initial (I), or replacement (R) teeth is indicated. The teleost taxon (A = Mexican tetra, C = cichlid, M = medaka, T = trout, P = pufferfish, S = stickleback, and Z = zebrafish) examined is also provided. The citations for these studies are given. The presence of genes expressed in the oral and pharyngeal transcriptomes, including both paralogs if present, are shown. If a gene was not present in the transcriptome, it is demarcated as not present (NP). If the homology of the paralogs with zebrafish genes is known, the paralog generally ends with a or b. If the homology is not known, paralogs end in n1 or n2. The gene *scpp5* was not found in the *Amphilophus citrinellus* genome. Six genes screened in zebrafish for tooth development have no homolog in the Tilapia or Medaka genome and are noted below (***).

and had a mean contig size of 585 base pairs. Subsequently, all *H. cyanoguttatus* transcriptome contigs produced for each jaw were aligned against individual *A. citrinellus* transcripts of each gene. Using the program Sequencher 4.8 (Genecodes, Ann Arbor, MI, USA), we isolated tooth gene homologs in the *H. cyanoguttatus* transcriptome using an initial cutoff of 90% sequence similarity that permitted large alignment gaps. This sequence similarity ensured that homologs would align but paralogs that diverged before the last common ancestor with Tilapia would not align. We constrained the searches to only return sequences with a minimum alignment of 40 nucleotides with *A. citrinellus* genes. Then, the alignments for these genes were individually inspected visually to ensure protein-coding alignment of at least 200 base pairs thereby providing high confidence in the homology of our annotations.

Genes recovered were sorted into four categories: (1) those that appeared in the transcriptome of both jaws, (2) the transcriptome of the oral jaw only, (3) the transcriptome of the pharyngeal jaw only, and (4) putative tooth genes that were not present in either transcriptome. We also annotated the 73 pairs of paralogs based on three potential kinds of differential expression and putative sub-functionalization. The first group contained one tooth gene paralog that was expressed in both jaws but another paralog that was isolated to a single jaw. The second group examined was complementary paralogs that were alternatively expressed in the two jaw transcriptomes. The third group we demarcated contained genes that have one paralog expressed in the jaws but another paralog presumably expressed in other tissues since the protein retains an open reading frame in the cichlid genomes.

Results and discussion

We documented several general patterns concerning the presence and absence of teleost tooth gene expression. Both *in situ* hybridization and RNA-seq transcriptomes provided substantial evidence for conservation of tooth gene expression from teleosts to mammals and between initial and replacement teeth. Additionally, we found that the oral and pharyngeal jaws share expression in a substantial percentage of genes that influence tooth development indicating that the dentitions on these two jaws are not exceptionally independent at the level of the presence or absence of genes expressed. Our transcriptome analyses of paralog expression also suggest sub-functionalization between gene paralogs expressed in teeth and paralogs expressed in other structures is likely a common pattern across teleost diversity.

Teleost teeth and *in situ* hybridization

There are 76 genes that have been implicated in mouse tooth development that have also been verified via *in situ* hybridization to play a role in the formation of teleost dentitions (Table 1). The reviewed studies further support the idea that there is extensive conservation in the genetic underpinnings of tooth development from mice to teleosts. Additionally, eleven of these genes have been shown via *in situ* hybridization to be expressed in both the oral and pharyngeal teeth of teleosts suggesting there might be substantial similarity in the developmental genetic basis of tooth formation on both jaws (Fraser et al. 2009). However, 34 of the tooth markers have only been studied in the oral jaws and 31 genes have been exclusively examined in the pharyngeal dentition. Therefore, whether the proportion of genes shared between the dentition on the two jaws is as low as 10% or is much greater is unclear from the *in situ* hybridization studies. Because most pharyngeal tooth gene expression has been performed in zebrafish, which only houses teeth on their lower fifth ceratobranchial element (Stock et al. 2006; Stock 2007) and because most of the remaining studies have examined expression in cichlid teeth but on only the oral jaw, the degree of developmental genetic independence of the dentitions on these two jaws requires further investigation.

The examination of multiple lineages of teleosts can clearly provide interesting insight into the conservation and divergence of dental developmental networks. For instance, six orthologous genes that are shared during dental development between zebrafish and mouse (*bmp2a*, *dkk1b*, *dlx2b*, *lhx7*, *scpp1*, and *scpp9*) have likely been lost from the genomes of cichlids and medaka (Table 1). In some cases, paralogs of these genes are known to be involved during tooth development and this developmental redundancy leading to loss of paralogs might be a general feature of teleost evolution. However, only the paralogs of *bmp2, dlx2, dlx4*, and *rara* have been documented through *in situ* hybridization to both be expressed in teleost teeth. Additionally, only for *bmp2* in medaka have the two paralogs of any duplicated gene been recorded from both the oral and pharyngeal dentitions (Wise et al. 2006). Interestingly, the Tilapia genome appears to have lost the *bmp2a* paralog making the redundancy in *bmp2* ortholog expression for cichlids likely dispensable as has been suggested for *bmp2* paralogs in zebrafish (Wise and

Stock 2010). Although teleosts such as the Mexican tetra, medaka, pufferfishes, and stickleback have only been used in a comparatively few studies, more extensive examinations of tooth gene expression in these and additional lineages of fish will likely continue to shed important light on the conservation and divergence of vertebrate dental development. It is also clear that many studies of *in situ* hybridization have not adequately detailed which paralog of duplicated genes they have studied during tooth development (Table 1). Further analyses of the presence and absence of paralogs within the developing dentitions of teleosts could provide a more general understanding of the importance of redundancy, neo-functionalization, and sub-functionalization, as well as whether the same genes are involved in forming teeth during different stages of ontogeny.

Our understanding of the genes involved in teleost tooth replacement is primarily confined to studies of the teeth on the oral jaws of cichlids. There are only seven genes that teleost *in situ* hybridization studies have shown to be involved in both initial tooth formation as well as tooth replacement (Table 1). However, because we know that a substantial number of genes are involved in tooth initiation from *in situ* studies and that many of these genes are present in the transcriptomes analyses of primarily replacement teeth examined here, the combination of these two techniques suggest the majority of genes that are involved in the formation of initial teeth are likely to be involved in the formation of replacement teeth (Table 1). A total of 91% of the genes that have been examined in teleost *in situ* studies and that are present in the Tilapia genome are present in at least one of the cichlid jaw transcriptomes. Some notable exceptions include *eve1* and several *hox* genes. These genes have been implicated in the formation of initial teeth in the oral and pharyngeal jaws (Laurenti et al. 2004; Debiais-Thibau et al. 2007; Fraser et al. 2009), but they are absent from the transcriptome of the jaws. Combining single gene studies using methods such as *in situ* hybridization with high throughput analyses of expression as provided via RNA-seq will continue to provide synergistic insight into the genes underlying dental diversification.

Cichlid oral and pharyngeal jaw transcriptomes

Using transcriptome sequences, we were able to more than double the list of genes expressed in mouse teeth that are also expressed in the toothed jaws of teleosts. Approximately 80% of the genes we screened are present in the oral and/or pharyngeal jaw tooth transcriptomes. This supports the idea that a substantial number of the genes that function to generate vertebrate tooth phenotypes are likely to be conserved in that role in the over 60,000 vertebrates descended from the last common ancestor of mammals and teleosts. This extensive conservation in gene expression might represent a general pattern for many types of organismal structures like eyes and hearts that have a single ancient origin but have been maintained across much of vertebrate diversity (Meng et al. 2013; Richards et al. 2013; McGaugh et al. 2014).

The oral and pharyngeal jaw transcriptomes indicate that there is shared expression for a large number, 137, of the tooth genes between the two jaws of cichlids. Although there are a number of interesting exceptions, many of the genes that have only been examined in one jaw using *in situ* hybridization tended to also be present in the transcriptomes from both jaws (Table 1). This sharing of over one-third of the genes examined between both toothed components of the cichlid trophic apparatus indicates that pleiotropy could commonly constrain tooth differentiation on the two jaws of cichlids. The morphological correlations that have been observed among species in phenotypes like oral and pharyngeal jaw tooth number could well be a result of this substantial sharing of conserved gene expression during tooth formation (Fraser et al. 2009).

We recovered a higher proportion of the mouse tooth genes homologs from the oral jaw transcriptome (Table 2). There were 136 genes, almost the same number that present in both jaw transcriptomes, which were recovered exclusively from the oral jaw transcriptome. Howver, only 11 genes were isolated exclusively from the pharyngeal transcriptome. This bias between the two jaws in observed expression could be due in part to the fact that mouse tooth development takes place on one of the same bones, the dentary, that is toothed in the oral jaws of cichlids (Smith and Coates 1998; Fraser et al. 2004, 2008). However, this pattern could also be due to the vagaries of RNA-seq or the fact that only the lower pharyngeal jaw was examined whereas both the upper as well as the lower jaw were analyzed in the oral jaw transcriptome. However, if the tooth genes shared across vertebrates do show a bias towards expression only in the oral jaw, then teleost fishes like cichlids, that do have teeth on their oral jaws, might provide greater insight into human and mammalian tooth development when compared to teleosts such as zebrafish that only have teeth on their lower pharyngeal jaw (Stock 2007; Fraser et al. 2009). These data also suggest that cichlids with

Table 2 Tooth genes expressed in the oral and pharyngeal jaw transcriptomes of *Herichthys cyanoguttatus*

Location	Genes
Oral and pharyngeal jaw	*acrvr1l, aldh1a2, atp2b1a, alpl#, arnt, axin1n1#, baxa†, bglap, bmp4, bmp6, bmp7b#, bmpr2n2†, cdkn1a, clu, cmyb, col1a1b, col1a1n1, col4a1, col4a2, col4a5, col5a1, col5a2b, crabp1a†, creb3l1, ctgf, ctnnb1, ctnnbl1, cx43, cyp26b1, cyp26c1, dcn, dlx1, dlx3bn2†, dlx5, egfra, egfrn2, egr1, erbb2, erbb3a†, fadd, fbln1, fgf1a#, fgf4n1#, fgfr1b#, fgfr2, fgfr3, fn1b†, foxa2n1†, foxo1a†, gll3, hip1, hopx, hsp6, irx3, itga4, itga5n1, itga5n2, itgb3a, itgb3b, jag1, jag2n1†, krt18, krt8, lama3, lama5, lamb3, lgr6, lum, mcam, mdkb, mfng, mmel1, mmp13b, mmp2, mmp20, mmp9, mycn, ndrg1a#, nfkbiaa, nfkbiab, notch3, oclnb†, odam, odc1, pax9, pcna, pitx2, pstpip1b, pstplp1a, ptprz1b, pvlr1b#, rab23, raf1b, raraa, rarab, rarga, runx3, rxra, rxrgb, sdc2, sdc3, sdc4, sema3ab†, sema3c, sema3fb, shha, slit1n2#, slit3, snal1a, sox2, sox9a, sp4, sp6, sp7, sparc, spock1, spp1, spp2, spry4, srgap1, srgap2a#, tfap2a, tgfbr2n1#, timp2a, timp2b, timp3, tjp1a, tjp1b, tjp2a, tjp3, tncn1†, tnfrsf19, traf3, tuft1a, tuft1n2, wnt5a†,vcan*
Oral jaw specific	*axin2a, axin2n2, barx1, bcann1, bcann2, bcpg5a, bmi1a, bmp2b, bmp3, bmpr2a†, ccnd1, cluap, col2a1b*, col4a3, col5a2n1, col5a3a, col5a3b, col6a2n1, crabp1b†, cspg4, dlx2, dlx3bn1†, dlx4a, dlx4b, dlx6, dspp, eda, edar, edaradd, edn3b, epha7, erbb3b†, erbb4a, errb4novel, faslg, fgf3, fgf10a, fgfr4, fmoda, fmodb, foxn1, foxo1b†, fsta, fus, fzr1a#, gas1a, gas1b, gdnfa, gdnfn2, gfra1a#, gl12n2, gll1, gll2b, gpc1a, gpc1b, gpc2, gpc5a, gpc5b, gsc, hand1, hand2, has1, hgfa, hgfb, igf, inhbaa, irx1a, irx1b, irx2, itgb6, jag2b†, jupa, lama2, lama4, lef1, lfng, lgr4, lhx6, lhx8b, met, mme, msxa, msxe, msx1b, msxn2, netrin1b, netrin1n2, ngf, nogginn1, notch1a, notch1b, nrg1, nrp1a, nrp1b, ntf3, ntf4, osr2, p4ha3, prrx1, ptch1, ptch2, pth1ra, pthlha, pthlhn2, ptn, ptprz1a, reln, ret, ror1, ror2, runx1, runx2, rxrba, sema3aa†, sema3b, sema3fa, sfrp5, slit2, slitrk6, smo, snal1b, sostdc1a, sostdc1b, tbx1, tgfb2, tgfb3, tncn2†, traf4, traf6, viml, wnt10b*, wnt3, wnt4a#, wnt5b†, wnt6, wnt7bb#*
Pharyngeal jaw specific	*baxb†, col2a1a*, fas, fn1a†, foxa2n2†, gpc4, isl1, oclna†, ofd1, tgfb1, wnt10a**
Not recovered from either jaw	*Alpnovel#, alpp, axin1n2#, bcl2n1, bcl2n2, bgn, bmp5, bmp7a#, col6a2n2, col7a1l, cspg5a, cspg5b, dab1a, dab1b, edn2, egf, eve1, fgf1b#, fgf2, fgf4n2#, fgf7, fgf8, fgfr1a, foxf2a, foxf2n1, foxj1a, foxj1b, fzd6, fzr1b#, gfra1b#, hoxa2b, hoxa5a, hoxd4a, hnf1a, hspg2, jupb, lrrn3n1, lrrn3n2, ndrg1b#, ngfrn1, ngfrn2, nrp2b, nrp2n2, ntrk1, ntrk3n1, ntrk3n2, pvrl1a#, rarb, scpp5, slit1b#, srgap2b#, tgfbr2n2#, tlx1, wnt4b#, wnt7ba#, wt1a, wt1b*

The presence and absence of 341 tooth genes are grouped as (1) present in both the oral and pharyngeal jaws, (2) the oral jaw alone, (3) pharyngeal jaw alone, or (4) not present in either jaw. When available in the Tilapia ensmbl genome database, paralogs are indicated as ending with *a* or *b*. When a gene was referred to as number 1 or number 2 in the Tilapia database presumably because of ambiguity about their homology, n1 or n2 respectively were added here to the end of the gene name. Complimentary paralogs expressed in alternative jaws (*), gene pairs that have one paralog expressed in both jaws and one expressed in only one jaw (†), as well as genes with paralogs not expressed in either jaw (#) are indicated.

their two toothed jaws could provide a framework in which to uncover developmental discrepancies between teeth from what are seemingly the disparate structural units of the oral and pharyngeal jaws (Fraser et al. 2009). Because distinct developmental programs could even define anterior (incisors) to posterior (premolars) teeth in the oral jaw of mammals (Hlusko et al. 2011), expression differences among tooth bearing regions like the jaws of cichlids could provide intriguing insights into the origins and evolution of the vertebrate dentition.

A substantial number of mouse tooth genes were not recovered in either cichlid jaw transcriptome. Of the 57 genes that we screened that were not recovered in the transcriptomes of cichlid tooth-bearing regions, 20 of these genes were represented by the paralogs of the genes *bcl2*, *cspg5*, *dab1*, *foxf2*, *foxj1*, *lrrn3*, *ngfr*, *nrp2*, *ntrk3*, and *wt1*. Although all of these genes could be absent from developing teeth, caution might be warranted in completely excluding their presence from developing cichlid dentitions. As in any transcriptome study, genes that show low transcript abundance, as important morphogens and transcription factors often do, could have been missed (García-Ortega and Martínez 2015). Additionally, many of these genes might be expressed only in the formation of first generation teeth that develop during the first few weeks following hatching. The transcriptomes presented here were generated from fish that ranged from a month to several months old making our inferences about gene expression primarily relevant to the formation of replacement teeth (Fraser et al. 2009; Kratochwil et al. 2015). The absence of many of these genes during the development of teeth in cichlids could also reflect a lack of conservation across vertebrate tooth development. Because of their morphological differentiation, mammalian teeth as represented by the mouse dentition could readily have a suite of genes that are not expressed in the teeth of other vertebrate groups. The monophyodont mouse dentition is also unusual compared to most mammals that possess a diphyodont dentition characterized by a round of tooth replacement. Furthermore, gene expression from the mouse dental model has been predominantly compiled from their non-replacing molars (Miletich and Sharpe 2003). As gene expression is investigated in more non-model organisms, the presence and absence of genes unique to the teeth of particular lineages will undoubtedly become apparent (Rasch et al. 2016).

Tooth gene paralog expression

The expression patterns of paralogs provide several interesting insights into the potential role of gene duplicates in dental diversification. In approximately 12% of the paralogs examined, both paralogs were conserved and expressed in both the oral and pharyngeal jaw transcriptomes. The retained duplicates included the paralogs of *col1an1*, *col4a*, *ctnnb1*, *nfkbia*, *pstpip1*, *timp2*, *tjp1*, and *tuft1*. In all these cases where both paralogs are present, it would be interesting to know if the duplicates have somehow diverged in function in time or space among different morphological components of individual teeth. It is also possible that the co-expression of the duplicates might have been conserved simply to ensure functional redundancy in critical aspects of tooth development (Wagner 2008; Chen et al. 2013). Cichlid teeth could provide a powerful replicated framework on multiple levels to examine how co-expressed paralogs become temporally or spatially differentiated within serially homologous structures.

Sub-functionalization of putative tooth gene paralogs has occurred in a number of ways in the jaws of cichlids. Notably, in about 16% of the paralogs examined, one paralog was present in both jaw transcriptomes but the other paralog appeared to be sub-functionalized to a particular jaw. Examples of this included *crabp1b*, *jag2b*, and *sema3aa* in the oral jaw transcriptome as well as *baxb*, *fn1a*, and *oclna* that were found in the pharyngeal jaw transcriptome. There were only a few genes that displayed a pattern of alternative transcription with one paralog expressed exclusively in the oral jaw and one paralog expressed exclusively in the pharyngeal jaw (Table 2). The paralogs of *col2a1* as well as *wnt10* exhibited this pattern. In the oral jaws, *col2a1b* and *wnt10b* were recovered, but in the pharyngeal jaws *col2a1a* and *wnt10a* were expressed. Complementary sub-functionalization is clearly not a major axis of developmental genetic divergence of the tooth genes examined. Interestingly, approximately 20% of the genes we screened and were not recovered in either transcriptome did have paralogs that were expressed in at least one of the jaw transcriptomes. Some notable examples of this type of sub-functionalization included the paralogs of *bmp7*, *fgf1*, and *ndrg1*. Importantly, these tooth genes that show jaw specific expression could provide candidate loci for the dental divergence of polymorphic cichlid species like *Herichthys minckleyi* that show exceptional phenotypic differentiation in teeth on only one jaw (Hulsey and García de León 2013; Hulsey et al. 2015).

Gene duplication is a common phenomenon and appears to be playing a substantial role in developmental differentiation of cichlid teeth. Importantly, whole genome duplications are only the most obvious and large-scale manifestation of genetic duplication. Gene copy number variation is now recognized as ubiquitous in most populations and its influence on micro-evolutionary divergence is receiving increasing attention (Cheng et al. 2005; Hastings et al. 2009). This potential for individual genes to duplicate means that for many of the genes examined we cannot unambiguously ascribe their duplication to the initial telelost whole-genome duplication event. Detailing the patterns and timescale over which tooth genes become sub-functionalized will demand a much better understanding of the homology and origin of many of these genes. As our knowledge of teleost genomics and gene duplication increases, it will be interesting to evaluate whether gene expression changes in structures such as teeth following macro-evolutionary events like whole-genome duplication mirror those consequences found on a more micro-evolutionary level when individual genes are duplicated.

Future directions

The presence and absence of particular tooth genes as we examined here only provides an initial window into the qualitative divergence that characterizes the developmental genetics of dental diversity of cichlids and other vertebrates. Quantitative variation in many layers of developmental genetic mechanisms are critical to how phenotypes are shaped and undoubtedly are playing a large role in cichlid dental modularity. For instance, alternative enhancers on the same gene that influence the abundance of gene transcripts, the presence of alternative transcripts of the same proteins, as well as the timing and patterning of micro-RNAs are all likely to be modified substantially during the differentiation of serially homologous structures like teeth (Jackman and Stock 2006; Kratochwil and Meyer 2015). With the ever-increasing availability of genomic resources, it is now also feasible to extensively manipulate gene expression and perform functional assays to experimentally test the independence of gene networks in different structures like the jaws of cichlids. Coupling these experimental approaches with modeling of the potential interactions among genes will further allow us to test the distinctiveness of individual dental modules. As our understanding of the genome to phenome map continues to expand for conserved structures like teeth, we will be able to increasingly

appreciate how the organization of developmental genetic modules influences vertebrate phenotypic diversification.

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