

The cusp of evolution and development: a model of cichlid tooth shape diversity

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SUMMARY Tooth shape is a hallmark of repeated evolutionary radiations among cichlid fishes from East Africa. Cusp shape and number vary both within populations and among closely related species with different feeding behaviors and ecologies. Here, we use histology and scanning electron microscopy to chart the developmental trajectory of tooth shape differences in fishes from Lake Malawi. We demonstrate that species with bi- or tricuspid adult (replacement) teeth initially possess a first-generation unicuspid dentition. Notably, the timing of turnover from first-generation to replacement teeth

differs among species and is correlated with feeding ecology. Next, we use field data for cichlid species with adult unicuspid, bicuspid, and tricuspid teeth to demonstrate a strong and positive relationship between the number of teeth in a row and tooth shape. We discuss cichlid tooth ontogeny in the context of morphogenetic models designed to explain the developmental basis of tooth shape variation in mammals. We suggest that the dramatic differences in cichlid dentitions can be explained by variation in the expression of common activators and inhibitors acting at multiple stages of odontogenesis.

INTRODUCTION

The shape of teeth occupies a central position in various disciplines, from paleontology to ecology to molecular biology. Comparative odontology is used to identify species or describe new fossils (Carpenter et al. 1998; Sereno et al. 1999), to test biogeographic hypotheses (Krause et al. 1997), and to decipher ancient (MacFadden et al. 1999; Dean et al. 2001) as well as recent (MacLeod 2000) ecologies. Similarly, molecular and developmental biologists consider tooth development a classic example of tissue (Lumsden 1988), cell (Chai et al. 2000), and gene interaction (Peters and Balling 1999). Despite a long tradition of study in mammalian model systems, important questions remain regarding the genetic and developmental basis of differences in tooth shape. Here, we take a different approach to the study of tooth shape. We concentrate on an evolutionary model system that exhibits tremendous tooth shape diversity. We ask how patterns of development observed in the laboratory, coupled with data from natural populations, can help to fill the gaps in our understanding of vertebrate odontogenesis.

East African cichlids represent a striking example of adaptive radiation and concomitant divergence in trophic ecomorphology (Fryer and Iles 1972). Each of the three rift lakes, Tanganyika, Malawi, and Victoria, houses a mono-

phyletic group of cichlids that has evolved convergent feeding morphologies in a short period of evolutionary time (Kocher et al. 1993). For instance, each lake has piscivorous species with long snouts and gracile jaws used to engulf prey, as well as species characterized by short and firmly reinforced jaws used to scrape algae from rock surfaces.

Cichlid teeth are as diverse as their jaws and are key components of the trophic machinery. Tooth morphology ranges from widely spaced sharply pointed unicuspid in zooplanktivorous and insectivorous species (e.g., *Cyanotilapia afra*) to closely packed tricuspid in algal scrapers (e.g., *Labeotropheus fuelleborni*). The shape of cichlid teeth may respond rapidly to selection; tooth shape varies among individuals within populations (Streebman, unpublished data), characterizes diverging morphs (Tichy and Seegers 1999), and evolves replicatively (Ruber et al. 1999). Recently, we demonstrated that shape differences in the first tooth row (bicuspid vs. tricuspid) between *Metriaclima zebra* and *Labeotropheus fuelleborni* are controlled by changes in a small number of genes (Albertson et al. 2003).

A great deal is known about the development of cichlid teeth (Huyseune 1990; Huyseune and Sire 1992a,b). Like most teleost fishes, adult cichlids have (a) multiple rows of teeth on two sets of jaws (oral and pharyngeal), (b) similarly

shaped teeth within a row (homodonty), and (c) tooth replacement throughout life via de novo formation of tooth germs (polyphyodonty). In addition, cichlids (and probably most teleosts) possess a set of first-generation teeth, which can be distinguished from replacement teeth by their small size and rudimentary organization (Huysseune and Sire 1997). Cichlid teeth pass through developmental stages that are similar to those described in mammals (e.g., initiation, bud, cap, bell; Huysseune and Sire 1997; Stock et al. 1997). The stages of mammalian tooth development are elicited by specific combinations of targeted gene expression (Stock et al. 1997; Peters and Balling 1999). It is not known whether the same signaling molecules choreograph tooth development in fishes.

A lot has been learned from mammalian models about the genetic control of tooth initiation. By contrast, less is known about the genes responsible for differences in tooth shape. In the words of Peters and Balling (1999), we know “where and how to make them,” but we are not sure how to explain patterns of variation within a jaw, within a species, or between related species. Few gene knockouts alter tooth shape phenotypes (Pispa et al. 1999), and traditional model organisms (zebrafish, frog, chick, and mouse) exhibit derived dentition patterns characterized by the loss or gross modification of teeth in the oral jaws.

However, recent work allows us to guess the identity of genes that specify differences in tooth shape. Tucker et al. (1998b) engineered a functional knockdown of bone morphogenetic proteins (BMPs) in mice by implanting NOGGIN beads in dental explants. Presumptive incisors treated with NOGGIN acquired cusps and developed as molars; this transformation was accompanied by expanded expression of *Barx1* in dental mesenchyme. Jernvall et al. (2000) showed that correlated gene expression patterns of *Fgf4*, *Lef1*, *p21*, and *Shh* could predict differences in molar cusp shapes between mice and voles. Finally, Jernvall (2000) and Salazar-Ciudad and Jernvall (2002) presented developmental and morphogenetic models to explain the “evolvability” of cusps in mammalian evolution. The models can accurately reproduce the diversity of mammalian tooth shape within and between individuals by varying the concentration of molecular “activators” and “inhibitors” expressed from singular or multiple signaling centers. Taken together, the results of this work are consistent with the hypothesis that tooth shape (i.e., cusp number and morphology) is controlled by antagonistic actions of extracellular signaling ligands (e.g., fibroblast growth factors [FGFs] and BMPs) secreted from transitory enamel knots (EKs) (Jernvall and Thesleff 2000).

In fact, multiple stages of mammalian tooth development can be characterized as the balance between the opponent signaling molecules FGF and BMP. In tooth initiation, the expression of *Bmp4* and *Fgf8* in the epithelium control the mesenchymal expression of *Pax9* and *Msx1*, which direct

tooth formation and position (Neubüser et al. 1997; Tucker et al. 1998a; Peters and Balling 1999). Later, *Bmp4* expression in the mesenchyme may promote formation of the primary EK (Jernvall et al. 1998). Finally, BMPs and FGFs secreted from the EK are candidate activators and inhibitors of cusp development. The expression of *Bmp4* from the primary EK may inhibit secondary EKs from forming (Jernvall and Jung 2000) and/or regulate (induce) the development of subsequent EKs (Salazar-Ciudad and Jernvall 2002). Control of EK number and spacing ultimately determines cusp number and the sharpness of teeth (Jernvall 2000; Salazar-Ciudad and Jernvall 2002). The interplay between BMPs and FGFs as regulators of both tooth initiation and tooth morphogenesis means that, in homodont species, there may be a relationship between the number of teeth per row and the number of cusps per tooth.

We use histology and scanning electron microscopy (SEM) to characterize the developmental trajectory of oral jaw teeth in two cichlid species from Lake Malawi, East Africa. We demonstrate that individuals of both species replace unicuspid first-generation teeth with a multicuspid adult dentition. Interestingly, the timing of turnover from first-generation to replacement teeth differs among species. Next, we use field data for cichlid species with unicuspid, bicuspid, and tricuspid teeth to demonstrate a strong and positive relationship between the number of teeth in a row and tooth shape. Finally, we integrate these results with studies of vertebrate odontogenesis and propose a model to account for cichlid evolutionary and developmental tooth shape variation.

MATERIALS AND METHODS

Tooth development in two study species

Our work on the anatomy, genetics, and development of cichlid craniofacial differences features two species with divergent feeding morphologies (Albertson and Kocher 2001; Albertson et al. 2003a,b). *Metriaclima zebra* (MZ) and *Labeotropheus fuelleborni* (LF) are members of Lake Malawi’s rock dwelling “mbuna” and shared a common ancestor 50,000 to 500,000 years ago (Meyer et al. 1990). These species represent points along a continuum, from ram feeding to suction feeding to biting, which likely reflects early morphological divergence in the rock-dwelling clade (Albertson et al. 1999; Danley and Kocher 2001). MZ is a widespread species characterized as a generalist feeder (McKaye and Marsh 1983). It has a terminal mouth, which it uses to brush diatoms from attached algae and to suck plankton from the water column (Reinthal 1990). By contrast, LF feeds on attached material in the shallows of the surge zone where it uses its inferior-subterminal mouth to bite or scrape algae from rocks (Reinthal 1990).

Both MZ and LF are maternal mouthbrooders that hold embryos and posthatching (3–4 days post fertilization) fishes in the mouth for 21–25 days before release. Newly hatched fry were taken from brooding females at 4 dpf, cultured in 200-ml flasks at 25–26°C, and moved to small aquaria on day 22. Individuals were

fixed on days 7, 10–12, 16–22, 42, 56, and 70 and prepared for SEM or histology. This sampling regime was chosen because we had reason to believe that replacement teeth would not appear until about 6 weeks postfertilization (Huysseune and Sire 1997). There were no significant differences in growth rate between species over this period (data not shown).

SEMs of adult teeth were prepared using skeletonized material. Embryos and juvenile fishes were fixed in 10% formalin in phosphate-buffered saline, dehydrated in an ascending ethanol series, and then critical point-dried out of liquid CO₂. Specimens were sputter coated with Au-Pd alloy, photographed using 4 × 5 Polaroid film, or digitally captured and postprocessed using Adobe Photoshop 4.0 (Adobe Systems, Inc., San Jose, CA, USA). Histological material was prepared by decalcifying whole or partially dissected fish heads in Cal-Ex (Fisher, Hampton, NH, USA), rinsing in fresh water, dehydrating in an ascending ethanol-butyl alcohol series, and then infiltrating and embedding in Paraplast (Fisher). Blocks were sectioned at 8 μm and stained for bone, cartilage, and connective tissue using the HBQ stain (Hall 1986). Material was digitally photographed at 100–600× and images postprocessed using Adobe Photoshop 4.0.

Tooth shape and tooth number in nature

To test for an association between cusp number and number of teeth per tooth row, 10–15 individuals of species from natural

populations, sampled in 2001, were assayed. Species included *Cyanotilapia afra* (unicuspid teeth in the first tooth row), MZ (bicuspid), and LF (tricuspid). The number of teeth in the first tooth row was determined for both the upper and lower jaws (premaxillae and dentaries). Here, we concentrate on counts for the dentaries (premaxillae counts exhibited the same trend). Because jaw size differs among these species, we measured jaw width (to the nearest 0.01 mm) for all individuals and express number of teeth in the first tooth row as the number of teeth per millimeter of jaw width. Analysis of variance (ANOVA) and regression were performed in Microsoft Excel.

RESULTS

The adult teeth of MZ are bicuspid (with a larger medial cusp) in the outer-most row and tricuspid (even cusp heights) in two to three posterior rows on both the dentaries and premaxillae (Stauffer et al. 1997). LF adults are characterized by three to five rows of closely spaced tricuspid teeth (with even cusp heights) on the oral jaws. Notably, F₁ of LF and MZ have teeth intermediate in morphology (Fig. 1, D–F).

By 7 dpf, MZ and LF both possess a single row of first-generation unicuspid teeth (Figs. 1A and 2, A and E). In LF,

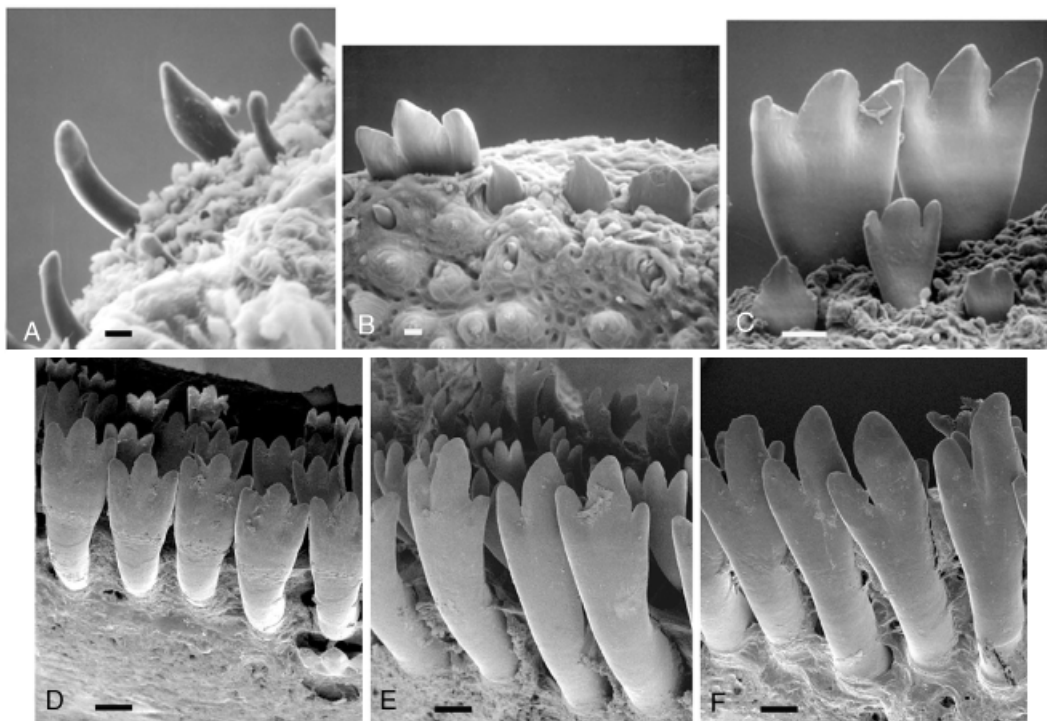


Fig. 1. SEM of first-generation and replacement teeth in the lower jaw of *Metriacilima zebra* (MZ) and *Labeotropheus fuelleborni* (LF). (A) Lingual view of unicuspid first-generation teeth in LF, 11 dpf (scale bar = 7.5 μm). (B) Lingual view of spatulate (transitional) and tricuspid replacement teeth in LF, 17 dpf (scale bar = 7.5 μm). (C) Lingual view of multiple rows of replacement tricuspid teeth in LF, 70 dpf (scale bar = 25 μm). D, E, and F are facial views of adult teeth in LF, the F₁ hybrid of LF and MZ, and MZ, respectively (scale bars in D, E, and F = 100 μm).

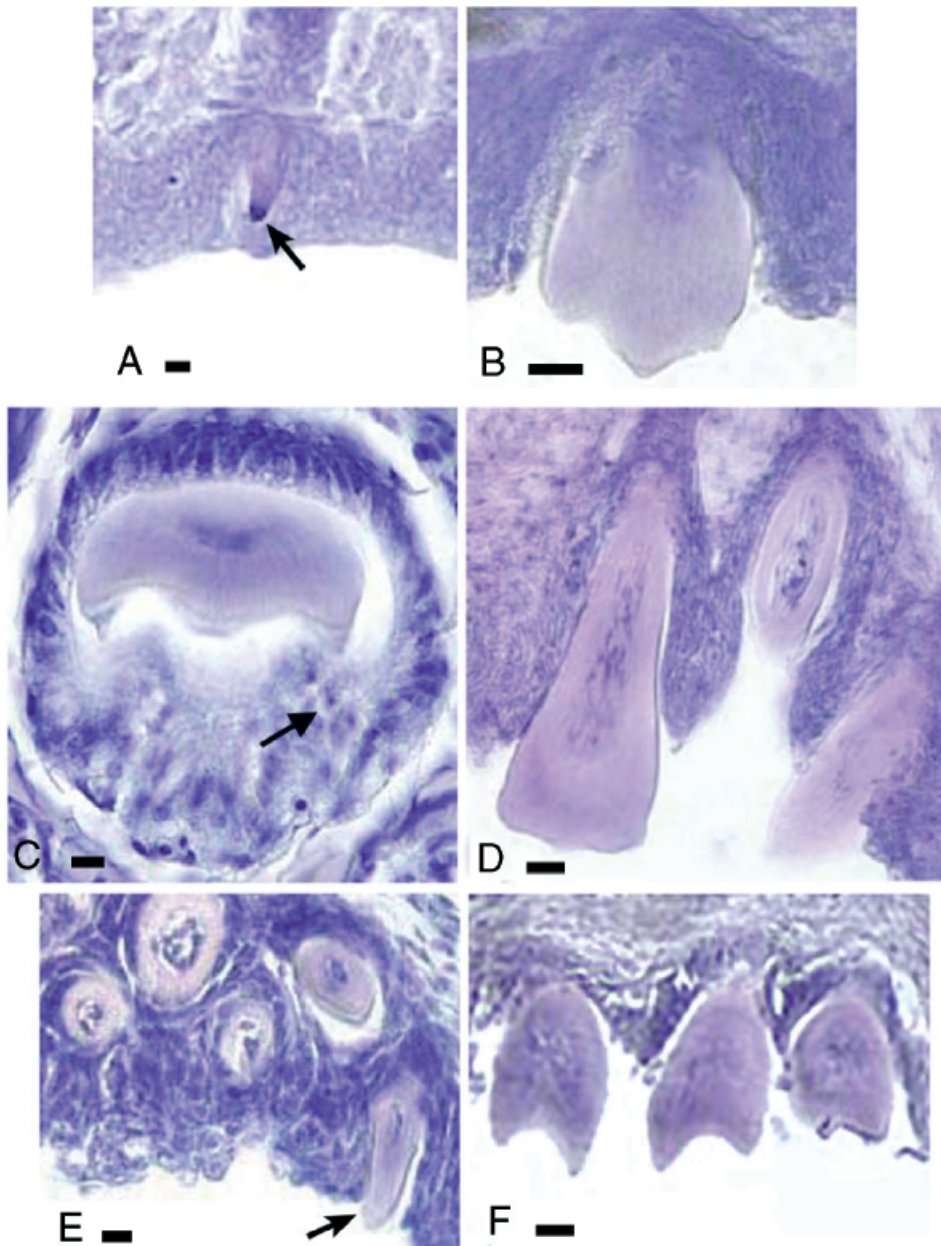


Fig. 2. Histological sections of developing teeth in the upper jaw of *Metriaclicma zebra* (MZ) and *Labeotropheus fuelleborni* (LF). (A) Unicuspid first generation tooth (arrow) in LF, 11 dpf (scale bar = 10 μ m). (B) Tricuspid replacement tooth in LF, 17 dpf (scale bar = 10 μ m). (C) Oblique cross-section through tricuspid tooth, showing surrounding odontoblasts (arrow) in LF, 20 dpf (scale bar = 10 μ m). (D) Tricuspid tooth in LF, 42 dpf (scale bar = 20 μ m). (E) Cross-section through unicuspid first-generation tooth (arrow) in MZ, 22 dpf (LF already has tricuspid teeth at this age) (scale bar = 15 μ m). (F) Bicuspid teeth in MZ, 70 dpf (scale bar = 40 μ m).

first-generation teeth are replaced by tricuspid teeth beginning at day 17 (Figs. 1B and 2, B–D). However, in MZ, bicuspid replacement teeth are not apparent until day 42, approximately 3 weeks later than in LF (Fig. 2F). In both species, the complete adult dentition appears as waves of replacement teeth. For instance, in LF, unicuspid teeth are first replaced by tricuspid teeth with three sharply pointed cusps, followed by wider spatulate teeth with rounded cusps. Replacement teeth erupt first at the midline of the first tooth row and fill in laterally. By 70 dpf in LF, tricuspid teeth appear in multiple rows (Fig. 1C).

In adult fishes collected in the field, there is a significant difference among species in the mean number of teeth per millimeter of jaw width (Fig. 3; ANOVA $P < 0.0001$). This difference is positively correlated with tooth shape (i.e., cusp number; $r^2 = 0.995$, ANOVA $P < 0.0001$). The mean number of unicuspid teeth in the first tooth row, per millimeter of jaw width, for *C. afra* specimens was 1.3 ± 0.20 (means \pm SD). The mean number of bicuspid teeth, per millimeter of jaw width, in the first row of MZ individuals was 3.3 ± 0.52 ; LF individuals averaged 4.8 ± 0.74 tricuspid teeth per millimeter of jaw width, in the first tooth row. This trend is probably

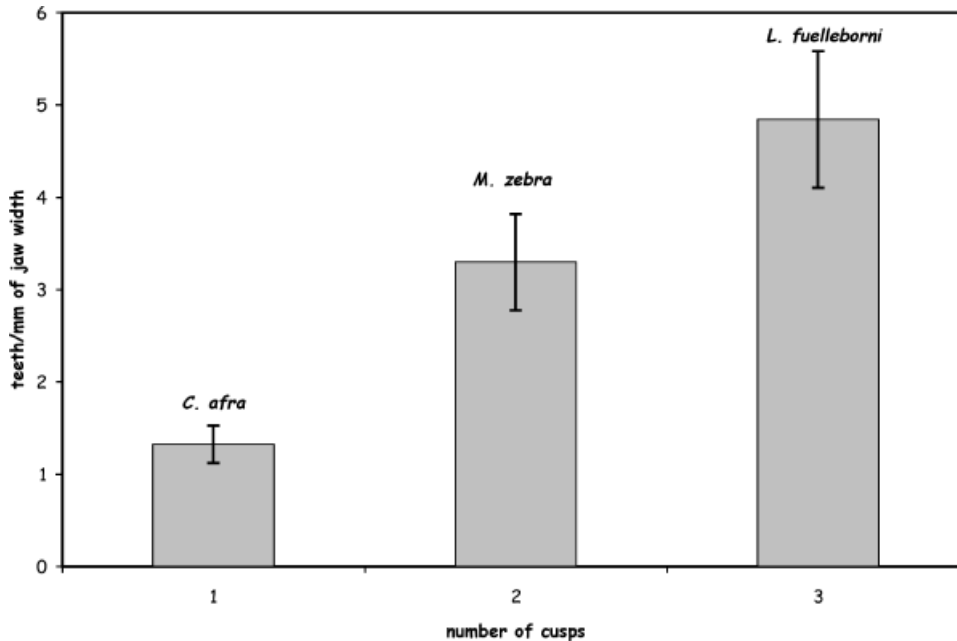


Fig. 3. Tooth shape (cusp number) is associated with the number of teeth in the first tooth row, per millimeter of jaw width, for three species of cichlid fish from Lake Malawi, East Africa ($r^2 = 0.995$; ANOVA $P < 0.0001$). Bars represent the means \pm SD ($n = 10$ – 15 individuals per species).

caused by both differences in tooth size and in tooth spacing. For example, individual unicuspid teeth of *C. afra* are easily visible with the naked eye, as are spaces between teeth. By contrast, teeth of LF are tightly packed in the jaw and cannot be perceived without magnification.

DISCUSSION

Despite the tradition of study in mammals, there are good reasons to study tooth diversity and development in other vertebrates (Huysseune and Sire 1998; Stock 2001). Fishes, in particular, provide a rich evolutionary context in which to view the highly derived mammalian dentition. Most teleosts exhibit (a) many teeth in multiple rows on two independent sets of jaws (oral and pharyngeal), (b) similarly shaped teeth within a row (homodonty), and (c) the production of replacement tooth germs throughout life (polyphyodonty). Fishes display a dazzling range of tooth shapes and often have multiple rows of differently shaped teeth (e.g., MZ). Polyphyodonty conceivably means that patterns of gene expression, which determine tooth initiation and morphogenesis, can be studied at any developmental stage. Here, we examined the trajectory of cichlid fish tooth development through the larval and juvenile periods.

Diverse adult cichlid dentitions share a common embryonic “ground state”

We demonstrated that two species (MZ and LF) with a multicusped adult dentition have unicuspid first-generation

teeth. A unicuspid first-generation dentition is probably the norm for most cichlids regardless of the shape of replacement teeth. Such a dentition has been observed in other cichlids: *Hemichromis bimaculatus* (unicuspid replacement teeth; Huysseune and Sire 1997) and two other Lake Malawi species, *Melanochromis auratus* and *Pseudotropheus tropheops* (replacement dentitions like MZ; unpublished observation). In fact, this may be a more general pattern for teleosts (Stock 2001; Lo Galbo et al. 2002) and other nonmammalian groups (Sire et al. 2002).

Furthermore, we showed that LF replaces first-generation unicuspid teeth with tricuspid teeth up to 21 days before MZ undergoes a similar transition to bicuspid teeth. Our sampling strategy for SEM and histology precludes an exact timing of replacement teeth in MZ. However, cleared and stained specimens of MZ time series (data not shown) supported the notion that turnover of first-generation to replacement teeth occurs during the sixth week (40+ days) postfertilization. The timing of replacement in MZ is similar to that in *H. bimaculatus*, where replacement teeth appear 1 month after initial resorption of Meckel’s cartilage (approximately 44 dpf; Huysseune and Sire 1997). Likewise, both *M. auratus* and *P. tropheops* possess unicuspid first-generation teeth at 23 dpf, 1 week after LF has developed tricuspid teeth (unpublished observation).

Replacement of first-generation teeth occurs earlier in LF than in the other species we examined. This equips LF fry with the adult tricuspid dentition about a week before release from oral incubation. In contrast, MZ individuals possess a unicuspid dentition for 2–3 weeks after release. This may reflect different feeding demands for each species. Individuals

of LF have a subterminal mouth with a strongly overslung upper jaw and may be morphologically incapable of feeding by suction. Alternatively, MZ juveniles probably feed on plankton from the water column, a predominant mode of feeding for larval and juvenile fishes (Liem 1991) and a preferred mode of MZ adults given an abundance of plankton (Reinthal 1990).

Both MZ and LF replace a unicuspid first-generation dentition (one row) with multiple rows of multicusped adult teeth. This suggests that, for these species, patterns of gene expression governing both tooth initiation and morphogenesis vary through ontogeny. Moreover, once the pattern of gene expression corresponding to the adult dentition is generated, it must be replicated with each wave of replacement to faithfully maintain adult dental patterns. It has been estimated that the functional life of an adult tooth is 101 days in the cichlid *Tilapia mariae* (Tuisku and Hildebrand 1994). Thus, a 5-year-old individual will have replaced its adult dentition at least 18 times. Additional changes in gene expression during adult tooth replacement should result in ontogenetic variation in tooth shape and patterning.

Ontogenetic shifts in tooth morphology are known for a few cichlids for both oral (*Chilotilapia rhoadesii*) and pharyngeal (*Haplochromis incola*) teeth (Fryer and Iles 1972). These changes are usually associated with differing feeding demands for juveniles versus adults (e.g., soft vs. hard prey). Since the 1960s, biologists have known that certain cichlid species (e.g., *Astatoreochromis alluaudi*, *Cichlasoma citrinellum* and *managuense*) have distinct adult morphs with divergent pharyngeal jaw shapes and dentition (Greenwood 1965; Meyer 1987). This phenomenon likely has a strong environmental component and may be induced by compressive forces on the pharyngeal bones themselves (Huyseune et al. 1994).

It is not known whether phenotypic plasticity plays a role in generating different oral jaw tooth shapes. Tuisku and Hildebrand (1994) demonstrated that the development of replacement tooth germs in the cichlid lower jaw is dependent on mandibular innervation. It is possible that such neural input directs not only the process of replacement but, in certain cases, the shape of new teeth as well. This might result in rapid changes in dentition given changing feeding behaviors. Polyphyodonty, coupled with the potential to modulate gene expression over ontogeny, may provide cichlids with the means to alter tooth shape in the face of fluctuating environmental conditions.

A model of cichlid tooth shape diversity

The developmental trajectories and evolutionary diversity of cichlid teeth raises several questions. How are the shapes of first-generation versus replacement teeth specified? How do polyphyodont species maintain the fidelity of multiple generations of replacement teeth? How do we explain the

presence of multiple rows of differently shaped teeth within the same jaw? In what follows, we offer a model to account for the diversity of cichlid tooth shapes. The model assumes that the molecules governing tooth development in mammals have similar functions in fishes. We acknowledge that there is no direct evidence of this. However, despite the differences discussed above, the structural basis of tooth organ development is similar between fishes and mammals (Huyseune and Sire 1998). The developmental blueprint of other structures, involving the same molecular players (e.g., FGFs, BMPs, Wnts, Shh) is conserved across vertebrate classes (Jung et al. 1999; Poss et al. 2000; Capdevila and Izpisua Belmonte 2001). Our model borrows heavily from other conceptions of reaction-diffusion systems to describe periodic patterning of feather primordia (Jung et al. 1998), tongue papillae (Jung et al. 1999), and primate molars (Jernvall and Jung 2000).

The simplicity of our model is motivated by the positive relationship between number of teeth in the first tooth row and tooth shape, maintained in natural cichlid populations (Fig. 3). This association, coupled with the iterative role of certain signaling molecules (e.g., BMPs, FGFs) in mammalian tooth development, suggests that variation in a common set of these factors might explain differences in both aspects of the cichlid dentition.

Tooth initiation (Fig. 4, A–C) results from interactions between one or more factors of competence (e.g., *Pax9*, *Fgf8*) and local inhibitors (Peters and Balling 1999). In our model, the factor of competence defines the field in which teeth can develop. However, teeth will not form in regions where the factor of competence is antagonized by discrete foci of inhibitor. The concentration of inhibitor from any single focus and the distribution of foci vary in a coordinated manner. In this scenario, the expression of inhibitor from a single focus controls tooth development, via antagonistic interactions with the factor of competence, and tooth position (i.e., the distance of nearby, inhibitor-expressing foci) by self-inhibition.

Tooth shape is controlled by the frequency and timing of EK development (Jernvall 2000). During the cap stage (Peters and Balling 1999), BMPs and FGFs are expressed in the EK(s) and probably function as cusp activators and inhibitors to direct the formation of subsequent and adjacent EKs (Jernvall 2000; Jernvall and Jung 2000; Salazar-Ciudad and Jernvall 2002). The specific identity of activator and inhibitor in cusp development is debated (i.e., compare the role of BMPs in Jernvall 2000 and Jernvall and Thesleff 2000 vs. Salazar-Ciudad and Jernvall 2002). We focused on the net amount of inhibitor to demonstrate how varying concentrations of the factor can generate diverse tooth shapes (Fig. 4D). Note that different effective concentrations of inhibitor could be elicited by regulation of the inhibitor itself, regulation of activator, or a combination of the two; what

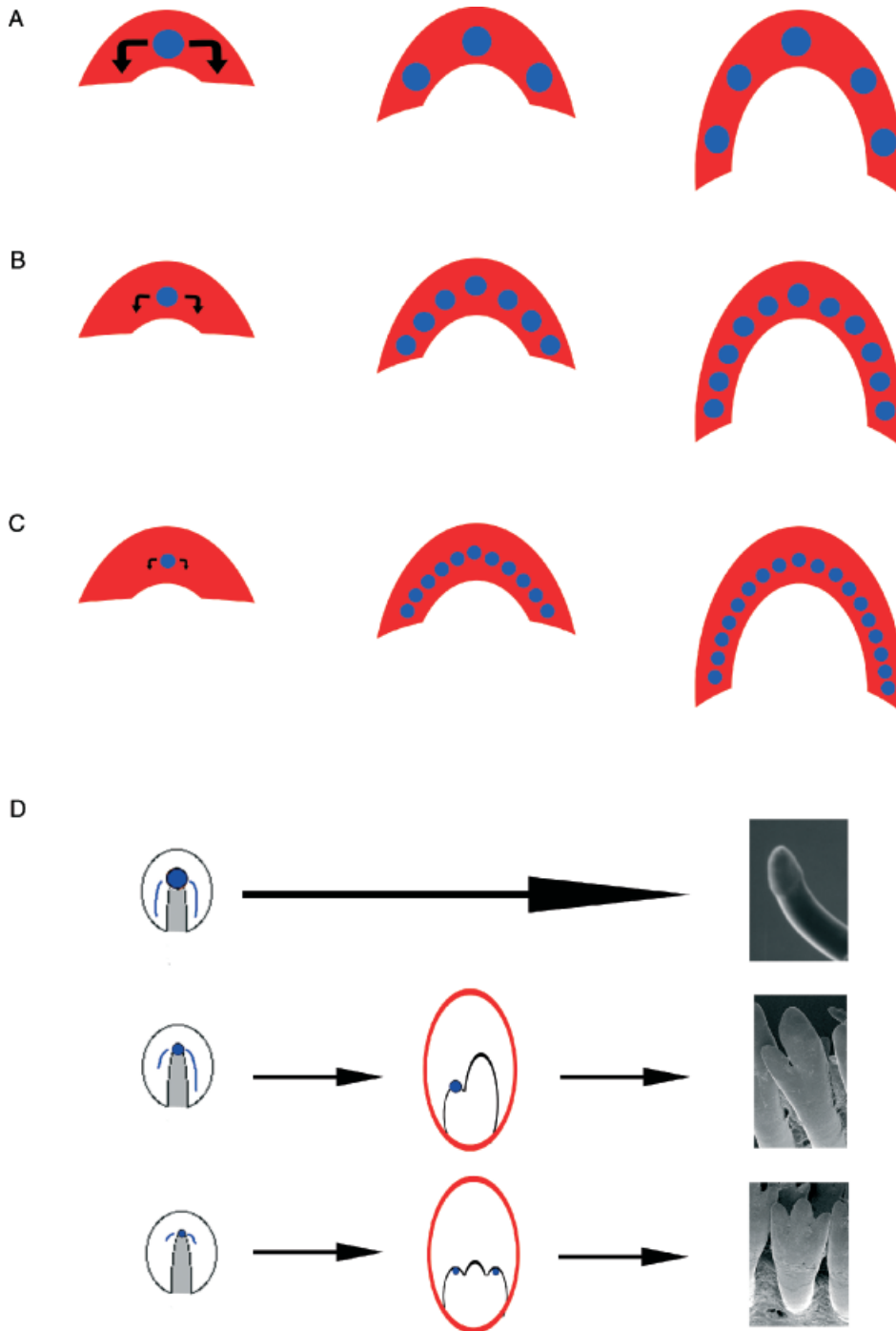


Fig. 4. Variation in cichlid tooth number and shape can be explained by gradients in the net concentration of a single inhibitory factor during two stages of tooth development. (A–C) Tooth development at the initiation stage; (D) tooth development at the time of morphogenesis. (A–C) Cross-section through the first tooth row with the tip of the jaw toward the top of the page. (D) Inhibitory signal from the primary enamel knot (EK) and resulting tooth morphologies. (A–C) Placement of teeth is determined by a factor(s) of competence that defines the area in which teeth can develop (in orange), as well as localized foci of inhibitor (blue). Teeth develop in areas of orange that are not affected by areas of blue. (D) Likewise, the development of secondary EKs, and thus cusps, is dependent on the inhibitory signal from the primary EK. In all panels, the size of blue circles represents the concentration (strength) of inhibitor. The first-generation dentition of all cichlids (A) is characterized by a single row of widely spaced uni-cuspid teeth, resulting from high concentrations of inhibitor from distantly spaced foci and a high concentration of inhibitor from the EK (D, top). In species like *C. afra*, with a unicuspid adult dentition, the concentration of inhibitor does not change with tooth replacement. A second row of uni-cuspid teeth is initiated by a replicated stripe of competence and inhibitory factors. In species with multicuspid replacement teeth, the concentration of inhibitor decreases with tooth turnover (B and C). This decrease is greater in posterior tooth rows for MZ, setting the stage for an outer row of bicuspid teeth with inner rows of greater numbers of tricuspids. Note that the adult bicuspid tooth of MZ is asymmetric (D, middle), implying an asymmetric field of inhibition (stronger at the midline). In LF adults (C and D, bottom) the decrease in concentration is strong and uniform, generating multiple rows of tightly packed tricuspid teeth.

matters in the model is that the ratio of activator to inhibitor is dynamic.

All cichlids examined to date have widely spaced, unicuspid, first-generation teeth (Huysseune and Sire 1997). According to our model, this ground state is produced by relatively few foci expressing inhibitor at relatively high levels. For species with unicuspid adult teeth (*C. afra*), the concentration of inhibitor does not change with tooth replacement. Alternatively, in species replacing unicuspid with multicusped teeth, (e.g., MZ and LF, Fig. 4, B and C), the concentration of inhibitor from single foci, and likewise the number of foci, should change with tooth turnover. A decrease in the concentration of inhibitor from any single focus will remove the self-inhibitory field toward neighboring foci and reduce the inhibitory signal toward the factor(s) of competence. In cichlids and most teleosts, teeth near the midline develop before those in lateral and posterior positions. In the context of our model, this means that the position of any tooth is most likely regulated by foci located medial to the site of tooth development.

A similar decrease in the concentration of inhibitor from the primary EK removes the inhibitory field toward subsequent EKs (Fig. 4D). Therefore, lower concentrations of inhibitor during both tooth initiation and morphogenesis would result in more replacement teeth per row with more cusps. A direct prediction of the model, then, is that the number of first-row teeth per millimeter of jaw width should increase through ontogeny in species like MZ and LF. A second related prediction is that the cusps of tricuspid teeth should be closer to one another than the cusps of bicuspid teeth. In fact, this seems to be borne out by inspection of the distance between cusps in the adult dentition of LF vs. MZ (Fig. 1, D and F).

Most adult cichlids have teeth in multiple rows. This means that a second (and sometimes third, fourth, or fifth) region of the jaw gains odontogenic potential. In species like *C. afra*, this second row is patterned like the first (e.g., Fig. 4A); the result of another wave of inhibitor in relatively high concentration, which generates well-spaced foci. In species like MZ, with adult bicuspid teeth in the first row and multiple rows of tightly packed tricuspid teeth behind, a gradient of inhibitor along the facial-lingual axis can explain the variation in tooth shape and spacing among rows. Similarly in LF, our model would predict that multiple rows of closely spaced tricuspid teeth are generated by a consistent decrease in levels of inhibitor across each tooth row.

Given the role of *Bmp4* in both tooth initiation and tooth morphogenesis, coupled with the inhibitory action of this signaling ligand in other reaction-diffusion models (Jung et al. 1998, 1999; Jernvall and Jung 2000), it is tempting to speculate that this is the molecule responsible for the coordinated changes (i.e., tooth placement and tooth shape) in the cichlid dentition. If so, our model would help to resolve an apparent

paradox in vertebrate tooth development. Stock (2001) observed that in the mouse, *Bmp4* has the opposing functions of inhibiting tooth initiation (Neubüser et al. 1997) and specifying incisor identity (Tucker et al. 1998b). We suggest a different interpretation: BMP4 plays an ancestral inhibitory role in *both* tooth initiation and tooth morphogenesis. The molecule does not “specify” incisor identity per se but rather inhibits secondary cusp development. Secondary cusps do not develop either because *Bmp4* is an inhibitor as modeled by some (Jung et al. 1998; Jernvall and Thesleff 2000; Jernvall and Jung 2000) or because the molecule is an activator (Salazar-Ciudad and Jernvall 2002) that induces a particularly large primary EK. Testing the role of putative inhibitors and activators, during various stages of tooth development, may be informative in a system in which more than two tooth types occur.

Our model should direct research aimed to explain the genetic basis of differences in cichlid dentitions. For instance, we demonstrated that tooth shape differences have a simple genetic basis (Albertson et al. 2003a,b). Tooth shape in LF × MZ F₂ does not associate with RFLP variation in the *Bmp4* gene (Albertson et al. 2003). This suggests that the genetic factor(s), which hypothetically modulate BMP4 concentrations in the developing dentition, operate in *trans* (i.e., the hypothesized differences in BMP4 concentration are not caused by mutations in the *Bmp4* gene). Despite evidence for genetic control of tooth shape, we need a better understanding of the heritability of dentition patterns in cichlids and the environmental conditions that might induce plasticity. Finally, the ability to culture cichlid dental explants (Koumans and Sire 1996) provides a means to directly evaluate the role of candidate factors in cichlid tooth initiation and morphogenesis.

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