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## A morphometric analysis of polymorphism in the pharyngeal dentition of *Cichlasoma minckleyi* (Teleostei: Cichlidae)

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KEYWORDS Cichlids; Dentition; Molarization; Polymorphism; Plasticity; Pharyngeals Summary Dental polymorphism in teleost fishes often involves production of a robust dentition, or "molarization", in one morph. The lower pharyngeals of a sample of wild-caught individuals of the polymorphic Cuatro Cienegas cichlid, Cichlasoma minckleyi (Kornfield and Taylor) (Proc. Biol. Soc. Wash. 96 (1983) 253), were measured to investigate morphological changes associated with molarization. Principal components analysis demonstrates that dental variability in this species increases in larger fish, and that only the molariform morph contributes to this increase. Reduced major axis regression analyses between pairs of variables indicate that the papilliform morph increases both tooth measures and numbers, whereas the molariform morph maintains a relatively constant number of teeth as it produces teeth of progressively larger size. In the papilliform morph, negative allometric scaling between tooth size and dentigerous area is compensated for by addition of teeth. Tooth size variables are isometric in the molariform morph, and tooth numbers are nearly static. These results are consistent with those reported for other polymorphic cichlid species. Further study is required to elucidate the mechanisms whereby tooth form in polyphyodont species may respond to environmental factors (like food hardness), but possibilities include direct mechanical influences or transmission of signals via nerves to developing replacement teeth.

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## Introduction

Polymorphism is the presence of multiple forms among individuals within a single species or population.<sup>1</sup> Polymorphisms in vertebrate dentitions may involve the presence or absence of teeth on different bones, differing numbers of teeth, and differences in tooth shape or size. The toothed tetrapod groups—amphibians,<sup>2-4</sup> reptiles,<sup>5</sup> and mammals,<sup>6,7</sup>—all contain taxa possessing polymorphic dentitions.

However, dental polymorphisms are most common in teleosts<sup>8-11</sup> and chondrichthyans.<sup>12</sup> Teleost

fishes generally possess a combination of two traits: (1) teeth on numerous bones of the oral jaws, palate and tongue, and pharyngo-branchial skeleton; and (2) polyphyodonty (many tooth generations).<sup>13</sup> These traits provide opportunities for variability in locations and numbers of teeth (for examples, see<sup>14</sup>), and—through successive replacement cycles—sizes and shapes of teeth may vary ontogenetically<sup>15–19</sup> or in response to environmental conditions.<sup>20–23</sup>

Study of intraspecific dental variability can shed light on the mechanisms that ultimately lead to the evolution of vertebrate dentitions. Differences between morphs often bear close resemblance to differences between species,<sup>24</sup> and repeated

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evolution of trophic types is a fairly common phenomenon in teleosts.<sup>25-28</sup>

In this study, I focus on one form of dental polymorphism that is found in the pharyngeal bones and teeth of a number of cichlid fishes (Teleostei: Cichlidae) and characterized by two morphotypes. One morph exhibits a gracile dentition with a large number of fine, conical teeth (''papilliform''), while the other morph exhibits a robust dentition with a small number of large, broad and flat teeth ("molariform").<sup>9,20,22</sup> These differences may result from genetic and/or environmental factors, and in some species molarization may be triggered when individuals consume foods of increased hardness (e.g., crushing snails versus eating soft detrital material). In species with molariform and papilliform dental morphs, small individuals usually all appear papilliform. The papilliform morph is often considered a ''default'' phenotype that, if unaltered by genetic or environmental signals, will persist in adult fish. Morphs begin diverging in trophic morphology once the fish pass a threshold size (this is often taken to be the size when hard food consumption becomes feasible), and become increasingly disparate as fish grow larger and undergo successive tooth replacement cycles.

This concise qualitative description belies a serious lack of guantitative understanding about how this pattern develops. How do pharyngeal bones accommodate molarization? Do teeth simply grow bigger through successive generations, or do tooth numbers increase as well? If tooth numbers increase, how and where are additional teeth added? How do tooth sizes scale with the size of the bones and the size of the fish? And how do papilliform and molariform morphs differ in these patterns? The only studies that have addressed these issues rigorously are by Huysseune<sup>29,30</sup> and coworkers,<sup>31</sup> who looked at molarization of the polymorphic African cichlid Astatoreochromis alluaudi. They raised individuals on diets of different hardness and found that soft-food (papilliform) individuals showed continuous increase in tooth numbers but only slight increase in tooth size as they grew, whereas hard-food (molariform) individuals maintained a relatively constant number of teeth, but produced much larger teeth through



**Figure 1** Lower pharyngeals of (A) papilliform (UMMZ 198937-1; S.L. = 112.5 mm) and (B) molariform (UMMZ 198947-1; S.L. = 121.6 mm) *C. minckleyi*. These are the fifth ceratobranchial bones, and are fused at the midline. Close-ups (C): posterior-central portion of (A); and (D): lateral margin of (B).

successive tooth generations. Their most recent study<sup>30</sup> contains data that suggests that molariform fish may actually decrease tooth numbers as they grow to make room for fewer, much bigger teeth.

I have collected data from a relatively large sample of wild-caught individuals of a polymorphic Neotropical cichlid, *Cichlasoma minckleyi*, <sup>33</sup> that permit both comparison with these findings and further consideration of these issues. *C. minckleyi* is endemic to the Cuatro Cienegas Basin (Coahuila, Mexico) and is highly polymorphic in its pharyngeal bones and dentition, though dental intermediates are also present in low frequencies in natural populations (Fig. 1; see  $also^{32,33}$ ). Differences between morphs become more pronounced at larger size; fish less than  $\approx$ 30 mm standard length (S.L.) possess papilliform dentitions.<sup>34</sup>

Recent work has characterized pharyngeal and body-form variability in this species, and has illustrated that both genetic influences and environmental cues play a role in determining trophic morphology.<sup>35,36</sup> Here I characterize morphological variability in the pharyngeal bones and dentition with a multivariate analysis, and then use correlations and bivariate regressions to investigate interrelationships of pharyngeal and dental variables and compare them between morphs. Finally, I speculate on the mechanisms whereby environmental factors like food hardness may influence tooth form in polyphyodont species.

## Materials and methods

Material used in this study consists of 240 wildcaught and preserved individuals of 22–169 mm S.L. (representing juveniles through large adults). All fish were collected from localities within the Cuatro Cienegas Basin, a small (approximately  $30 \text{ km} \times 40 \text{ km}$ ) limestone basin in North-central Mexico,<sup>37</sup> and were obtained from the collections of the University of Michigan Museum of Zoology Fish Division (UMMZ).

Fish were collected between 1939 and 1975; most within a 15-year interval between 1960 and 1975 (Table 1). Relative frequencies of morphs fluctuate over time, consistent with expectation (e.g.,<sup>38</sup>), whereas frequency of intermediates remains approximately constant. It is worth pointing out that more than 83% of the sample used in this study was collected during 1961 or 1975; morph frequency differences between these years are small (Table 1: <2% difference in frequency of papilliform individuals; <3% difference in frequency of molariform individuals). Greater fluctuations in some years may result from sample sizes insufficient to draw conclusions about overall morph frequencies in the population. Moreover, an analysis of museum records of specimens collected between 1960 and 1978 shows relative stability when compared with more recent fluctuations.<sup>39</sup> While relative frequencies have fluctuated, there is no evidence to indicate that the morphology of these fish has changed over this time.

For each individual, I recorded S.L. and made six measurements on the fused fifth ceratobranchials (lower pharyngeals) in dorsal view, including four linear measures (made to the nearest 0.01 mm with an ocular micrometer) and two tooth counts (Fig. 2). The dentigerous area of the pharyngeals is roughly triangular, and the formula for the area of a triangle ( $[base \times height]/2$ ) was used to estimate total dentigerous area and total number of teeth. Variables analyzed subsequently were: S.L., maximum tooth diameter, dentigerous area (estimated by  $[P1 \times P2]/2$ , number of teeth mediolaterally along the posteriormost tooth row (summed over both sides of the symphysis), number of teeth anteroposteriorly along the symphysis, total number of teeth (estimated by [number of mediolateral teeth  $\times$  number of anteroposterior teeth]/2), and distance covering the four posteriormost teeth along the symphysis; this last measure is between the rostralmost attachment point of the most anterior of these teeth and the caudalmost attachment point of the most posterior tooth (henceforth

Year	Papilliform		Intermediate	2	Molariform	
	Number	%	Number	%	Number	%
1939	1	20.0	1	20.0	3	60.0
1960	0	0.0	2	12.5	14	87.5
1961	74	64.3	7	6.1	34	29.6
1974	6	31.6	2	10.5	11	57.9
1975	53	62.4	9	10.6	23	27.0
Total	134	55.8	21	8.8	85	35.4



**Figure 2** Fifth ceratobranchial measurements: P1, maximum width of dental area; and P2, maximum length of dental area at symphysis; and measurements on inset: mediolateral diameter of largest tooth (maximum tooth diameter—MTD); and Dist4. Numbers of anteroposterior teeth (along the symphysis; dimension P2) and mediolateral teeth (along the back tooth row) were also counted.

referred to as Dist4, this measure is designed to assess changes in the area most affected by molarization). Dist4 was measured on the same side of the symphysis as the tooth with maximum diameter. Redundancy in tooth count variables is deliberate, as I wanted to investigate total numbers of teeth in addition to tooth numbers along each dimension.

To characterize dental variability, I performed a principal components analysis (PCA) on the correlation matrix of log-transformed variables (tooth counts were not transformed, and S.L. was excluded from the analysis) using SYSTAT 10.0. Based on the PCA results, I investigated differences between morphs by removing individuals with intermediate PC I scores (between -0.25 and 0.25; n = 21; rationale provided below), and comparing remaining individuals of either higher (molariform) or lower (papilliform) PC I score. Correlation coefficients and significance values were calculated for each morph for each pair of logged variables (again, tooth counts were not logged). Because I made 21 pair-wise comparisons of the seven variables in this part of the analysis, I used a corrected critical value of P < 0.05/21 = 0.0024 to assess significance of correlations (see<sup>40</sup>).

Reduced major axis linear regression slope coefficients between pairs of variables were also calculated, using the freeware PAST.<sup>41</sup> Reduced major axis regression minimizes error in both x and yvariables, and thus is often more appropriate than least-squares regression for comparison of biological measures. The reduced major axis is equivalent to the first principal component of the correlation matrix of the two variables.<sup>42</sup> Another advantage of reduced major axis regression is that slope coefficients between logged linear variables (in this study, all variables except tooth counts) can be related to scaling relationships between variables. Slopes not significantly different from 1.0 (or 2.0 when other linear measures were compared with dentigerous area) indicated isometric scaling. Slopes significantly greater or less than these values were indicative of positive or negative allometry.

Morphs were compared by looking for overlaps in the 95% confidence intervals of these slope coefficients. Confidence intervals were calculated both from standard error terms and by bootstrapping (2000 replicates);<sup>41</sup> these produced very similar results and only 95% confidence intervals based on standard error are reported here.

## Results

# Characterization of dental variability in C. minckleyi

Principal component factor loadings are shown in Table 2, and scores on the first two components

Table 2Factor loadings on first two principalcomponents.

	PC I (56.0%)	PC II (38.6%)
MTD	0.8979	0.4011
Dist4	0.7726	0.6256
DA	0.4508	0.8780
#ML	-0.5959	0.7035
#AP	-0.8530	0.4193
#Total	-0.8174	0.5657

Percent variance accounted for by each component is in parentheses. These first two components account for 94.6% of variance (the third component accounted for an additional 4.3%). Abbreviations for variables: maximum tooth diameter (MTD), distance from the back of the tooth row to the front of the fourth tooth along the symphysis (Dist4), dentigerous area (DA), number of mediolateral teeth (#ML), number of anteroposterior teeth (#AP), and total number of teeth (#Total).



**Figure 3** (a) Scores on the first two principal components of dental measures in this study. PC I is interpreted as a "molarization" component, with more molariform individuals to the right. PC II is interpreted as a size-related component, with larger individuals towards the top of the plot (see Table 1 for loadings). Vertical lines are at -0.25 and 0.25 on the x-axis, and indicate the range considered "intermediate". (b) Dot-histogram of PC I scores.

(accounting for 94.6% of the variance: 56.0 and 38.6%, respectively) are plotted in Fig. 3a. These loadings indicate that PC I may be interpreted as a "molarization component", with more molariform individuals (possessing fewer, larger teeth, slightly larger dentigerous areas, and larger Dist4) at the right of the plot, and more papilliform individuals (with greater numbers of smaller teeth) at the left. All variables are positively correlated with PC II, many fairly strongly so, and PC II is interpreted as a size-related component. This interpretation is supported by a high correlation between log S.L. and PC II score ( $R^2 = 0.8258$ ).

As expected, dental variability (total range of PC I scores) becomes greater in individuals of larger size (higher PC II score). Morphs are thought to begin differentiating at  $\approx$ 30 mm S.L., when molariform individuals begin eating snails.<sup>34</sup> Those individuals of 30 mm or less in this study (n = 9) express much of the range of variability of the papilliform morph, but only skirt the edge of the molariform field (PC I scores of these individuals range from -0.84 to 0.28). With intermediates excluded as described below, log S.L. correlates with PC I score for molari

form individuals ( $R^2 = 0.5118$ , P < 0.001), but not for papilliform individuals ( $R^2 = 0.0001$ , P = 0.8845).

#### Assignment of individuals to dental morph

Studies comparing teleost dental morphs would ideally use some independent criterion (such as an individual's population membership or the diet an individual was fed in the lab) to classify morphs. In C. minckleyi, both morphs were present at all localities, as were a small number of dental intermediates.<sup>32,33,35</sup> The first principal component represented degree of molarization and largely matched up with qualitative assessments of morph identity. The distribution of PC I scores differed significantly from normality [Fig. 3b; also Kolmogorov-Smirnov one-sample test against a normal distribution with mean of zero and standard deviation of one: P = 0.00001; Lilliefors P < 0.00001]. Mean PC I score is zero, and I removed individuals with intermediate PC I scores, arbitrarily choosing -0.25 and 0.25 as the intermediate range. Individuals with PC I scores >0.25 (n = 85) were classified as molariform, and those with PC scores

<-0.25 (n = 134) were classified as papilliform (Fig. 3).

### Changes in dentition during growth and comparison of morphs

Results of regressions between pairs of variables for each morph are reported in Tables 3 and 4; data are shown in Fig. 4. In the papilliform morph, all variables are positively and significantly correlated. As papilliform individuals grow larger, both pharyngeal dimensions (as indicated by dentigerous area) and tooth numbers increase. Dentigerous area increases isometrically with S.L., but both maximum tooth diameter and Dist4 scale negatively with S.L. (though they are isometric with one another). This negative scaling in tooth size variables relative to dentigerous area is compensated for by addition of teeth in both the anteroposterior and mediolateral directions.

In the molariform morph, pharyngeal dimensions show positive, significant correlations with S.L., but tooth counts do not (with the exception of the number of mediolateral teeth, which has a weak but significant positive correlation). All linear measures exhibit positive allometry with respect to S.L. (though in the case of Dist4 this is marginal). Measures of tooth size are isometric with dentigerous

area. Total numbers of teeth exhibit no significant increase with S.L.; numbers of mediolateral teeth may increase slightly in larger fish, but numbers of anteroposterior teeth are entirely static.

Molariform and papilliform morphs are significantly different in all but two of the 21 bivariate comparisons (this includes instances of no relationship in the molariform morph). The relationships between numbers of mediolateral teeth and S.L. are not significantly different between morphs, though the slope is greater (and the relationship stronger) in the papilliform morph. Likewise, the confidence intervals of the regression slopes of maximum tooth diameter and Dist4 overlap slightly; this is a reflection, if anything, of similarity in relative proportions (the ratio of anteroposterior to mediolateral dimensions of the largest teeth).

## Discussion

Multivariate results confirm both that small C. minckleyi individuals are morphologically papilliform and that subsequent changes occur mostly in molariform individuals. Of the nine individuals of 30 mm S.L. or less, six plotted as papilliform, two plotted as intermediates and only one was on the lower edge of the molariform range; these results,

	S.L.	MTD	Dist4	DA	#ML	#AP	#Total
S.L.		0.6873	0.8947	0.9815	0.5615	0.4099	0.6285
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MTD	0.7282		0.7614	0.6989	0.3393	0.1994	0.3306
	0.8043		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	0.8805						
Dist4	0.7729	0.9327		0.9275	0.5961	0.2865	0.5371
	0.8178	1.0168		<0.0001	<0.0001	<0.0001	<0.0001
	0.8627	1.1009					
DA	2.0023	2.3115	2.4478		0.5942	0.3914	0.6295
	2.0495	2.5481	2.5061		<0.0001	<0.0001	<0.0001
	2.0967	2.7848	2.6204				
#ML	13.624	16.452	16.745	6.6795		<0.0001	<0.0001
	15.345	19.078	18.764	7.4871		0.2105	0.6391
	17.066	21.704	20.783	8.2947			
#AP	9.3317	12.306	11.241	4.5427	1.2153		0.8056
	10.727	13.337	13.117	5.2341	1.4305		<0.0001
	12.122	15.358	14.993	5.9255	1.6457		
#Total	180.34	215.38	217.57	88.004	11.772	17.347	
	201.09	250.01	245.89	98.116	13.105	18.746	
	221.84	284.65	274.22	108.23	14.438	20.146	

Table 3 Results of correlation and regression analyses for tooth variables for papilliform ( mincklevi

Correlation coefficients and P values are above the diagonal; slopes (middle value) and upper and lower 95% confidence intervals of slopes (upper and lower values, respectively) are below the diagonal. Slopes in bold indicate that the slope for papilliform individuals was significantly greater than for molariform individuals. Variables are abbreviated as in Table 2.

	S.L.	MTD	Dist4	DA	#ML	#AP	#Total
S.L.		0.7803	0.8364	0.9590	0.2473	0.0029	0.0822
		<0.0001	<0.0001	<0.0001	<0.0001	0.6234	0.0078
MTD	1.1059		0.9460	0.8792	0.0740	-0.0325	-0.0001
	1.2283		<0.0001	<0.0001	0.0118	0.0987	0.9188
	1.3507						
Dist4	1.0130	0.8577		0.9256	0.1067	-0.0352	0.0002
	1.1083	0.9023		<0.0001	0.0023	0.0852	0.8955
	1.2036	0.9469					
DA	2.2055	1.7378	1.9590		0.4081	-0.0022	0.0345
	2.3048	1.8764	2.0796		<0.0001	0.6665	0.0890
	2.4041	2.0150	2.2002				
#ML	9.8902	*	8.7435	4.2644		0.0416	0.4269
	12.127		10.942	5.2619		0.0613	<0.0001
	14.364		13.141	6.2594			
#AP	*	*	*	*	*		0.7552
							<0.0001
#Total	*	*	*	*	6 5220	12 923	
					7 7729	14 447	
					9 0239	15 961	

 Table 4
 Results of correlation and regression analyses for tooth variables for molariform C. minckleyi.

Notation as in Table 3. (\*) indicates correlations were not significant at P < 0.0024. Slopes in bold indicate that the slope for molariform individuals was significantly greater than for papilliform individuals.



**Figure 4** Bivariate relationships between variables for papilliform (above diagonal) and molariform (below diagonal) individuals of *C. minckleyi*. For ease of comparison, axes are of the same length and scale for each bivariate relationship.

though based on a small number of individuals, are supportive of earlier work on development of trophic morphology in this taxon.<sup>34</sup> Total range of PC I scores increased in larger individuals; S.L. was not correlated with PC I score in papilliform individuals, whereas there was a correlation in molariform individuals.

The results of the regression analyses presented here are largely consistent with those presented by Huysseune<sup>29,30</sup> and coworkers<sup>31</sup> for *A. alluaudi*. In both species, the papilliform morph increases both tooth measures and numbers, whereas the molariform morph appears to maintain a relatively constant number of teeth as it produces teeth of progressively larger size. The scaling relationships presented in this study illustrate how this is accomplished in *C. minckleyi*. Negative allometric scaling between tooth size and dentigerous area is compensated for by addition of mediolateral and anteroposterior teeth in the papilliform morph. Tooth size variables are isometric in the molariform morph, and tooth numbers are nearly static.

One interesting difference between C. minckleyi and A. alluaudi is that tooth numbers in the hardfood (molariform) morph of A. alluaudi may actually decrease as the fish grow larger, as documented in both wild-caught and laboratory-raised individuals.<sup>30</sup> Huysseune<sup>30</sup> states that this decrease probably occurs at the edges of the ceratobranchials and away from the symphysis, where teeth are least affected by molarization. A decrease in tooth numbers might be due to one-for-two tooth replacement, cessation of tooth replacement, or both, as well as suppression of addition of teeth. Teasing these possibilities apart requires documentation of the history of discrete tooth families (a "tooth family" is defined as a functional tooth and its successors<sup>43</sup>) throughout the life of the animal; this is difficult in cichlid pharyngeals because of the large numbers of teeth and the necessity of documenting the nature and continuity of the dental lamina.<sup>29,30</sup> From a morphological/histological perspective, recognition of tooth families may be hindered by the lack of distinct crypts for replacement tooth development in some teleosts.<sup>44</sup>

However, in *C. minckleyi* neither morph appears to reduce numbers of teeth. In the molariform morph, tooth size variables scale approximately isometrically with dentigerous area. It would be interesting to investigate how the largest teeth in *A. alluaudi* scale. All other things being equal, isometric increase in tooth diameter should represent the upper limit of size increase possible without tooth reduction.

A maintenance of constant tooth number is normally considered reflective of one-to-one tooth replacement. However, it is also possible that apparent one-for-one replacement can result when some teeth are not replaced and new tooth positions are added in approximately equal numbers. Increasing tooth numbers indicate production of new tooth families; the data indicate that as papilliform fish grow, tooth numbers increase along both mediolateral and anteroposterior dimensions, though mostly in the former. Molariform fish maintain approximately constant tooth numbers; what little increase there may be occurs in the mediolateral dimension. It is perhaps worth noting that teeth are often more crowded mediolaterally than anteroposteriorly, as can be seen in Figs. 1 and 2. I agree with Huysseune<sup>30</sup> that addition of tooth families probably occurs on the edges of the dentigerous area (points of the triangle), which possess many small teeth in all forms, rather than in the center area most affected by molarization.

#### Mechanisms of molarization in teleosts

In other polymorphic cichlids,<sup>9,20,22</sup> tooth form seems largely under the control of diet. But in *C. minckleyi*, it has been demonstrated that both genetics and diet work together to produce the phenotype.<sup>35</sup> Odd and seemingly conflicting results of earlier experiments with *C. minckleyi* (e.g., development of ''molars'' in fish not fed snails;<sup>45</sup> molariform parents producing papilliform off-spring<sup>34</sup>) make sense in this context. Tooth form responds to food hardness, but only within the range of variability allowed by an individual's genetic background.

This analysis has shown that molarization in C. minckleyi occurs through development of larger replacement teeth, accompanied by maintenance of approximately constant tooth numbers. It is not clear whether one of these factors (tooth size or tooth numbers) is a cause and the other an effect, or whether cause and effect are not altered when the influence is primarily genetic versus primarily environmental. However, in most dental pattern formation models (e.g.,  $^{46-48}$ ) any gaps between teeth above a threshold size would be filled in by additional teeth. Changes in tooth size (in relation to growth of the dentigerous area) could therefore control production of additional tooth families. This would be true regardless of whether gradients in tooth size are genetically-determined (e.g.,<sup>49</sup>) or imposed by diet.

A direct relation between force exerted on food and degree of molarization has not been documented in any fish. Nonetheless, mechanical influences resulting from hard food consumption seem the most likely candidate for an environmental mechanism for molarization.<sup>50</sup> However, while bone may respond continuously to external factors through the coordinated action of osteoblasts and osteoclasts,<sup>51</sup> and even through alterations of gene expression patterns,<sup>52</sup> teeth cannot grow or alter their morphology once they have mineralized. Changes may only be achieved during successive tooth generations.

The mechanism whereby mechanical influences alter size and shape of replacement teeth may be direct or indirect. Prior to mineralization of a teleost tooth, the epithelial enamel organ folds into a form prefiguring final tooth shape, and the entire thickness of cap enameloid is deposited as a largely collagenous matrix.<sup>53,54</sup> Forces might be transmitted directly to developing tooth germs during these early stages. However, unlike many other teleosts, developing replacement teeth in cichlids are in crypts within the bone<sup>55</sup> (rather than in soft epithelial tissue outside the bone), and mechanical stresses would need to be transferred through the bone to affect them. Also, if mechanical forces are operating directly on developing replacement tooth germs, they may do so by increasing the width of the enameloid cap, but not its thickness, to match with histological observation (e.g.,<sup>29</sup>).

Forces might also be transmitted indirectly from functional teeth and surrounding tissues to developing replacement teeth. Functional and replacement teeth are linked by the dental lamina.<sup>56</sup> In addition, functional and replacement teeth may share innervation. Dental innervation in teleosts has been all but ignored (rare exceptions include  $^{57-61}$ ), but pharyngeal teeth are innervated through their attachment (which is usually collagenous and may be mineralized or unmineralized<sup>62</sup>) by the branch of the vagal (X) ganglion that serves each branchial arch (pers. obs. and<sup>63,64</sup>). Pharyngeal teeth may also be located near taste buds and other sensory structures, <sup>65,66</sup> and it is likely that the functions of teleost dental innervation include mechanoreception.

Much attention has been paid to mechanoreceptors in mammalian periodontal ligament, but presence of pulpal mechanoreceptors has also been suggested.<sup>67,68</sup> Teleost teeth lack a periodontal ligament (but see<sup>69,70</sup>) so, if present, mechanoreceptors would more likely be found in attachment or pulp. The presence of neurochemical markers (including some associated with mammalian mechanoreceptors under certain circumstances; e.g., neuropeptide Y<sup>71</sup>) in axons innervating teleost teeth<sup>72–74</sup>may lend support to this hypothesis.

Signals transmitted via any of these mechanisms may affect timing and location of cell proliferation in various regions of the developing tooth, as is the case in the mammalian enamel knot<sup>75,76</sup> (though

enamel knots have not yet been documented in teleosts). Subsequent enameloid mineralization and dentinogenesis<sup>54,77,78</sup> will reflect the size and shape thus specified at earlier stages (see also<sup>79</sup>).

Studies by Hildebrand and coworkers<sup>60,61,72–74</sup> provide the baseline for investigating these issues; what must be added to their approach is a comparative and evolutionary perspective. Whether or not it provides the mechanism for molarization of dentitions in polymorphic species, dental innervation and its relationship to tooth development, replacement, and morphology in teleosts clearly warrants further study.

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