Integration and evolution of the cichlid mandible: The molecular basis of alternate feeding strategies

R. Craig Albertson*†, J. Todd Streelman‡, Thomas D. Kocher§, and Pamela C. Yelick*

*Department of Cytokine Biology, The Forsyth Institute, and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, MA 02115; ‡School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230; and §Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824

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African cichlid fishes have repeatedly evolved highly specialized modes of feeding through adaptations of their oral jaws. Here, we explore the molecular genetic basis of the opening and closing lever mechanisms of the cichlid lower jaw, which have traditionally been used to describe the mechanics of feeding behavior in bony fishes. Quantitative genetic analyses demonstrate that the opening and closing mechanisms are genetically modular and therefore free to evolve independently. *Bmp4* (*bone morphogenetic protein 4*) is one of two loci that segregate with the mechanical advantage of closing and that together account for >30% of the phenotypic variance in this trait. Species-specific differences in jaw shape are obvious early in cichlid larval development and are correlated with patterns of *bmp4* expression in the mandibular primordium. When *bmp4* is overexpressed in the obligate suction feeder *Danio rerio*, mandibular morphology exhibits specific transformations of opening and closing lever ratios. We conclude that patterns of morphological integration of the cichlid jaw reflect a balance among conflicting functional demands. Further, we demonstrate that *bmp4* has the potential to alter mandibular morphology in a way that mimics adaptive variation among fish species.

adaptive radiation | *bmp4* | jaw shape | morphological integration

A fundamental divergence among bony fishes occurs between species that exploit hard and/or attached prey items and species that feed on highly mobile prey. This divergence is concomitant with the evolution of stereotypical mandibular morphologies that reflect the mechanical properties of the feeding apparatus. Species that prey on hard food evolve short, stout jaws efficient for biting, whereas those that feed on mobile prey often evolve elongate, gracile jaws for suction feeding. This functional dichotomy is exemplified by many percoid groups, where it is strongly correlated with habitat and morphology. For example, shifts along this functional axis are associated with the evolution of North American sunfishes (1, 2). Several coral reef fish lineages exhibit extensive ecological diversity, often associated with elaborate accentuations of biting and suction feeding (3–5). Cichlids have diverged rapidly along this functional axis with the repeated evolution of alternate biting/sucking morphologies that are characteristic of both deep cladogenic events and contemporary fine-scale ecological niche partitioning (6–12). Understanding the molecular basis of changes that differentiate biters from suction feeders will lend significant insight into the adaptive evolution of fish species.

The mechanical implications of jaw shape have been well studied in fishes (4, 5, 11, 13–16). The teleost mandible can be described as two opposing lever mechanisms: one that defines the mechanical advantage of closing and another that defines the mechanical advantage of opening. The closing in-lever is measured as the distance between the jaw joint and the attachment of the adductor mandibulae muscle on the coronoid process (Fig. 1*a*, purple line). The opening in-lever is the distance between the jaw joint and the attachment of the interopercular mandibular ligament on the retroarticular process (Fig. 1*a*, green line). The out-lever is traditionally taken as the distance between the jaw joint and the tip of the anteriormost tooth (this measure has been modified in our study; see *Materials and Methods* for justification and Fig. 1*a*, blue line). The in-lever-to-out-lever ratio is the fraction of force that is transferred from the muscular attachment to the distal-most point of the jaw and is referred to as the mechanical advantage (4, 16). Greater mechanical advantage equals greater force transmission, which is optimal for biting, whereas smaller mechanical advantage translates to greater velocity transfer, which is typical of suction feeders. There is a direct tradeoff between force and velocity, such that species with greater force transmission will have lower velocity transfer and vice versa (16, 17).

The theory of morphological integration postulates that traits that function together will also be inherited together, whereas unrelated traits will be inherited independently (18, 19). Implicit to this theory is the concept of modularity. A module is a complex of traits integrated by pleiotropy and independent of other complexes (20). Thus, morphological integration predicts that functional units will also be genetically modular. Developmental architecture figures prominently in discussions of morphological integration because genetic and functional modularity are mediated by developmental processes (21–25).

Previous studies have demonstrated that aspects of the cichlid feeding apparatus are genetically correlated and have evolved in response to strong directional selection (26, 27). Here, we test the specific hypothesis that the cichlid lower jaw is a morphologically integrated structure by tracking the segregation of functional morphology in an F_2 mapping population derived from two cichlid species that employ different modes of feeding. We also explore the potential role of *bmp4* (*bone morphogenetic protein 4*), a candidate gene for the evolution of craniofacial diversity, in regulating lever ratios in the teleost jaw. We show that patterns of integration and modularity are consistent with the theory of morphological integration but that they also underscore the notion that modularity is a matter of degrees (24); we also show that the underlying genetic architecture of the cichlid jaw reflects the functional and developmental complexity of the anatomy it encodes. In addition, we demonstrate that *bmp4* is associated with and has the potential to alter adaptive shape differences of the teleost mandible.

Materials and Methods

Species Rearing and Morphology. We compared two Lake Malawi rock-dwelling cichlid species with distinct feeding behaviors and jaw morphologies (28). *Labeotropheus fuelleborni* (LF) is a specialized biting species, characterized by a short, stout lower jaw with high mechanical advantage. LF feeds by cropping

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Abbreviations: Bmp4, bone morphogenetic protein 4; QTL, quantitative trait loci; dpf, days postfertilization; LF, *Labeotropheus fuelleborni*; MZ, *Metriaclima zebra*.

†To whom correspondence should be addressed at: Department of Cytokine Biology, The Forsyth Institute, 140 The Fenway, Boston, MA 02115. E-mail: calbertson@forsyth.org.

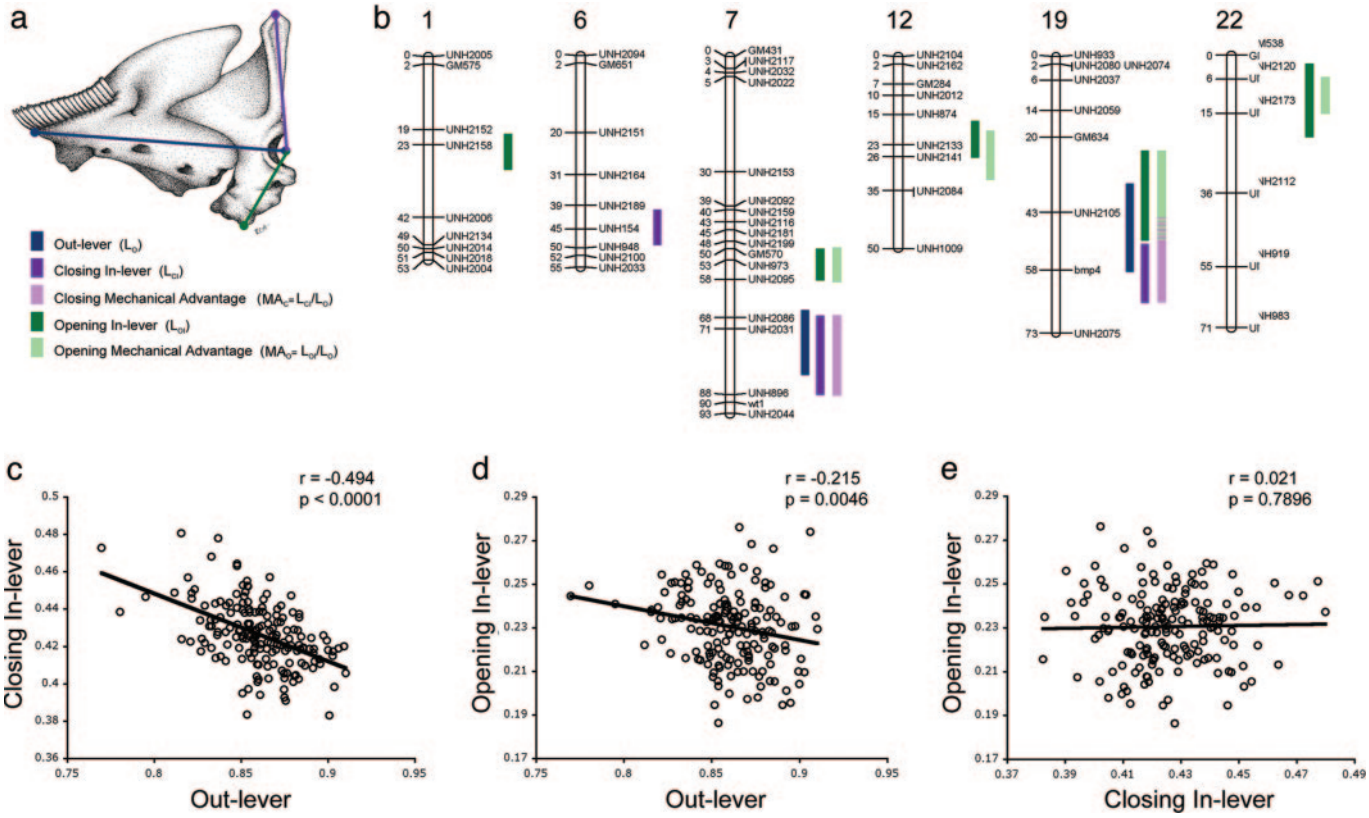

Fig. 1. Genetic basis of jaw opening and closing in cichlids. (*a*) Lever systems of the lower jaw. The out-lever is shown in blue, the closing in-lever is shown in purple, and the opening in-lever is shown in green. (*b*) Cichlids linkage groups that show significant associations with functional morphology of the jaw. Bars indicate regions exceeding the 95% genome-wide significance threshold for the corresponding QTL. Colors represent QTL affecting different traits. (*c–e*) Genetic correlation of traits in the F_2 population. (*c*) The out-lever is negatively correlated with the closing in-lever. (*d*) The out-lever is also negatively correlated with the opening in-lever, although the correlation coefficient is significantly less than that for the closing in-lever ($q = 4.21$; $P < 0.01$; ref. 40). (*e*) Closing and opening in-levers are not correlated in the F_2 population.

attached algae from the substrate. *Metriaclima zebra* (MZ) is a more generalized feeder, characterized by a more gracile elongate lower jaw with relatively low mechanical advantage. MZ is one of a few rock-dwelling species that actively feeds on plankton in the water column with a suction mode of feeding. The breeding and rearing of cichlids for quantitative trait loci (QTL) analyses has been described in refs. 26–28.

Oral jaw dentition in African cichlids is characterized by multiple rows of often flexible teeth that vary in length and shape. Furthermore, a significant proportion of our F_2 mapping population exhibited aberrant patterning and/or loss of dentition in their oral jaws. For these reasons, we felt that measuring the out-lever to the tip of the mandible (instead of the tip of the tooth) would produce the most reliable estimates of force transmission.

LF and MZ embryos were obtained for developmental studies by natural matings in 40-gallon tanks. Because Lake Malawi rock-dwelling cichlid females incubate their clutch in their mouths, brooding females were easily identified by an enlarged buccal cavity. Embryos were stripped from females' mouths and incubated externally in 1-liter Erlenmeyer flasks containing 600 ml of tank water and 200 ml of egg water (29). An aeration stone was placed at the bottom of the flask to provide enough air to keep the embryos vigorously swirling at the bottom of the flask. Embryo medium was changed every 2–3 days. Embryos were incubated at 25–26°C and staged according to refs. 30 and 31.

Zebrafish were maintained and bred at 28.5°C in a 14-h light/10-h dark cycle at The Forsyth Institute Zebrafish Facility. Embryos were collected by natural mating of pairwise crosses and maintained at 28.5°C as described in ref. 29.

Linkage Analysis. A Lake Malawi cichlid linkage map was constructed by using JOINMAP 3.0 (32). The locus file consisted of genotypes for 173 F_2 hybrid progeny at 170 marker loci. The grouping module of JOINMAP assigned 152 of 170 loci to 25 linkage groups using a logarithm of odds (LOD) score threshold of 4.0. The mapping module of JOINMAP built the genetic map for each linkage group by using the Kosambi mapping function, a LOD threshold of 1.0, a recombination threshold of 0.450, and a jump threshold of 5.0. A ripple function was performed after each locus was added to ensure optimal marker order. Compared with an earlier report (26), cichlid linkage groups have been renamed according to a more complete cichlid linkage map for the Nile Tilapia (33) and a comparative map of East African cichlids (J.T.S., unpublished data).

QTL Analysis. Multiple QTL mapping (MQM) analysis was performed in MAPQTL 4.0 (34) as described in ref. 26.

Cartilage and Bone Staining. Cartilages and bone of larval fish were stained with alcian blue and alizarin red as described in ref. 35, with slight modification. Larvae were fixed overnight in 4% paraformaldehyde (PFA) in phosphate-buffered solution containing 0.1% Tween 20 (Sigma-Aldrich) (PBT). Larval cartilages were stained with 20 mg of alcian blue (8XG, Sigma-Aldrich) in 65 ml of glacial acetic acid and 35 ml of absolute ethanol. After staining, specimens were enzymatically cleared by using a trypsin

solution containing 1 g of trypsin powder (Sigma-Aldrich) in 65 ml of distilled H_20 and 35 ml of sodium borate-saturated distilled H_2O. In older fish [>7 days postfertilization (dpf)], bone was stained with alizarin red. All cleared and stained specimens were stored in 80% glycerol. Cartilage and bone preparations were photographed with a Zeiss Axiocam digital imaging system and processed in PHOTOSHOP 7 (Adobe Systems, San Jose, CA).

Whole-Mount *in Situ* Hybridization (WISH) Analysis. WISH analyses were performed on staged embryos by using an adapted protocol (36, 37). Embryos were collected at desired stages, fixed in 4% PFA in PBT, and dehydrated in methanol. Embryos were rehydrated, digested with 10 μg/ml proteinase K, and refixed in 4% PFA. Prehybridization was performed at 65°C for 3 h in 50% formamide/5× standard saline citrate (SSC)/0.1% Tween 20/4.6 mM citric acid/50 μg/ml heparin/500 μg/ml tRNA. Digoxigenin-labeled antisense riboprobes (Roche Applied Science) were added directly to prehybridization mix and allowed to incubate overnight at 65°C. Riboprobes were synthesized from cichlid and zebrafish *bmp4* cDNAs (refs. 26 and 28, respectively). Washes were performed the next day at 65°C in graded solutions from 100% hybridization mix to 100% 2× SSC and then in 0.2× SSC. Embryos and α-dig antibody were preblocked at room temperature for at least 3 h in a solution containing one part 10% Boehringer blocking reagent dissolved in 1 M maleic acid, one part lamb serum, and three parts filtered maleic acid buffer (MAB) (100 mM maleic acid/150 mM NaCl/0.1% Tween 20, pH adjusted to 7.5 with NaOH). After preblocking, embryos were transferred to the α-dig antibody solution and blocked overnight at 4°C. The next morning, embryos were washed first in MAB and then in AP buffer solution (60 mM Tris·HCL, pH 9.5/60 mM NaCl/30 mM $MgCl_2$/0.1% Tween 20). Staining was achieved with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate in AP buffer. After staining, embryos were refixed in 4% PFA and dehydrated in 100% methanol overnight to remove background staining. Embryos were then either stored in 80% glycerol as whole mounts or embedded in JB-4 medium (Canemco and Marivac, Canton de Gore, QC, Canada) and serially sectioned at 5 μm.

***Bmp4* mRNA Injection.** Full-length mRNA was transcribed from linearized pBluescript plasmid containing zebrafish *bmp4* cDNA (38) by using the T3 mMessage Machine and Poly(A) Tailing kits (Ambion, Austin, TX). One-cell stage wild-type embryos were injected with 50, 100, 150, and 200 ng/μl of polyadenylated *bmp4* mRNA (1- to 3-nl volume). Larvae injected with 50 ng/μl *bmp4* showed little effect (n = 43). Conversely, 200 ng/μl-injected embryos were severely ventralized and exhibited early lethality (n = 42). Larvae injected with 100 and 150 ng/μl *bmp4* exhibited the most consistent effects, as presented below (n = 97). *gfp* mRNA (150 ng/μl) was injected as a negative control (n = 18). To ensure the specificity of *bmp4* overexpression, left–right asymmetric heart development was examined in 100 and 150 ng/μl-injected embryos. *Bmp4* overexpression has been shown to randomize asymmetric positioning of the heart (39). At 24 h postfertilization, *gfp*-injected zebrafish exhibited a normal cardiac "jog" to the left of the dorsal midline (n = 18). Approximately 40% of embryos injected with either 100 or 150 ng/μl *bmp4* (n = 48 and 49, respectively) exhibited randomized (i.e., no jog or right jog) heart development, consistent with previously reported results (39). Thus, injection of exogenous *bmp4* mRNA elicits a specific response in zebrafish embryos.

Results and Discussion

We identified two QTL that affected the mechanical advantage of closing and that together accounted for 33% of the phenotypic variance in the F_2 population (Table 1, which is published as supporting information on the PNAS web site). Four QTL were identified that affected the mechanical advantage of opening and that together accounted for 47% of the variance (Table 1). The mechanical advantage of opening and closing mapped to distinct regions of the cichlid genome (Fig. 1*b*), suggesting that these biomechanical traits are genetically modular. We next explored the genetic control of each lever system. Two QTL were identified that affected out-lever length (Fig. 1*b*) and accounted for 25% of the phenotypic variation in this trait (Table 1). Three QTL were identified for the closing in-lever (Fig. 1*b*) and accounted for 34% of the variance (Table 1). Two of three closing in-lever QTL mapped to intervals that overlapped out-lever QTL on linkage groups 7 and 19. Closing in-lever and out-lever were also inherited together in the F_2 population (Fig. 1*c* and ref. 26), suggesting that these functionally related traits are genetically integrated. Five QTL were identified that affected the length of the opening in-lever and accounted for 47% of the phenotypic variance (Fig. 1*b* and Table 1). Four of five of these QTL mapped to chromosomal positions that did not overlap with out-lever QTL. Although the opening in-lever and out-lever were inherited together in the F_2 population (Fig. 1*d*), the correlation coefficient was significantly less than that for the closing in-lever and out-lever ($q = 4.21$; $P < 0.01$; ref. 40). Taken together, these results imply that the opening lever system is integrated to a lesser extent than that of the closing lever system. Complete decoupling of genetic control was observed for the opening and closing in-levers. All QTL for these traits mapped to discrete regions of the genome, and they were not inherited together in the F_2 population (Fig. 1 *b* and *e*). Thus, critical aspects of the cichlid jaw opening and closing mechanisms are genetically decoupled and therefore free to evolve independently. These data are consistent with recent findings in the family Labridae, where modifications of opening and closing mechanical advantage occurred independently during the evolution of this group (41).

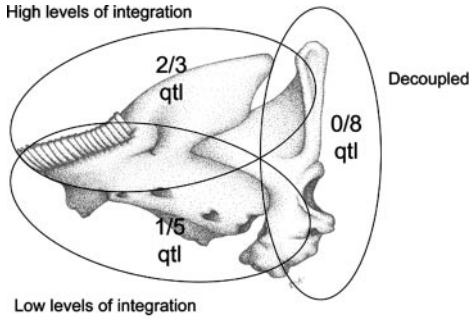

Fig. 2. Venn diagram depicting regional differences in the level of integration of the cichlid mandible. The out-lever and closing in-lever share 2/3 QTL and exhibit high levels of integration. The out-lever and opening in-lever only share 1/5 QTL and exhibit low levels of integration. The closing and opening in-levers have no QTL in common (0/8) and are genetically decoupled.

Morphogenesis of the teleost mandible is defined by three basic axes of growth: one rostrally giving rise to the out-lever, another dorsally producing the closing in-lever (coronoid process), and a third ventrally generating the opening in-lever (retroarticular process). Our genetic data suggest that this geometry exhibits different levels of integration (Fig. 2). For example, the rostral and dorsal dimensions shared 2/3 QTL (67%), the rostral and ventral axes shared 1/5 QTL (20%), and the dorsal and ventral axes shared 0/8 QTL (0%). In other words, the out-lever and closing in-lever exhibited relatively high levels of integration, the out-lever and opening in-lever exhibited lower levels of integration, and the closing and opening in-levers displayed no overlapping genetic control. This trend is also supported by differences in the slopes of our genetic correlations (Fig. 1 *c–e*).

Differences in the degree of integration are consistent with regional differences in the functional and developmental complexity of the jaw. For example, jaw closing is a relatively simple process that involves the coordinated action of elements pertaining mainly to the oral jaws (11, 14, 42). The jaw closing mechanism also emerged early among ray-finned fishes (actinopterygians) and has remained relatively unchanged (reviewed in ref. 43, pp. 540–549). Alternatively, jaw opening is a more complex process that has been extensively modified over actinopterygian evolution (43). In advanced teleosts, this function is achieved by means of the synchronized action of several biomechanical systems defined by the jaws, hyoid, operculum, skull, and pectoral girdle (4, 16, 44). Thus, higher levels of integration were observed among traits intimately associated by function, whereas traits that are part of a more complex functional network were integrated to a lesser extent. Differences in integration might also reflect differences in developmental complexity. Most of the mandible originates from cranial neural crest cells that migrate from the embryonic midbrain (45). In contrast, the retroarticular process develops from a heterogeneous population of crest cells derived from up to three hindbrain segments (45). Thus, lower levels of integration were observed among traits with different developmental origins. These observations are also consistent with the theory of morphological integration (16, 19).

The mechanical advantage of closing segregates with allelic variation at *bmp4* on LG19. In recent years, Bmp4 has emerged as an attractive candidate for the evolution of craniofacial diversity in vertebrates. The suspected importance of this molecule stems from several observations. From a developmental perspective, *bmp4* exhibits distinct mandibular expression patterns during craniofacial development (46). Levels of *bmp4* expression in the frontonasal prominence have also been correlated with adaptive variation in beak shape among diverse avian species (47, 48), and misexpression of Bmp4 in the beak primordium of the developing chick has been shown to modulate shape in a way that approximates natural variation among birds (47, 48). From a genetic perspective, East African cichlids exhibit a rate of Bmp4 amino acid evolution that is significantly greater than that of other craniofacial genes (49). Furthermore, the accumulation of amino acid substitutions in cichlid Bmp4 was restricted to the prodomain, with little variation found in the signal peptide or mature domain, suggesting that regulation of Bmp4 might underlie some aspect of cichlid evolution. Our genetic data offer evidence that a causative mutation affecting functional morphology is linked to *bmp4* in cichlids. To further investigate the potential role of *bmp4* in regulating adaptive variation of the teleost mandible, we examined its expression and function during jaw morphogenesis.

Cichlids that employ alternate feeding strategies exhibited differences in mandibular morphology by 7 dpf. The pharyngeal skeleton is not mineralized at this stage, but differences in lever ratios were obvious in the cartilaginous precursor of the mandible, Meckel's cartilage (Fig. 3*c*). LF larvae exhibited a relatively high mechanical advantage of closing, whereas MZ larvae showed a much lower closing mechanical advantage. The mechanical advantage of opening was also significantly different at this stage, although the difference was far less pronounced. Thus, at least some part of the patterning mechanism that establishes alternate feeding morphologies appears to be acting early in cichlid development.

Differences in larval lever ratios were correlated with patterns of *bmp4* expression in the mandibular pharyngeal arch. LF and MZ embryos showed little difference in the expression of *dlx2*, *bmp2*, *msxD/E*, and *fgf8* during segmentation and pharyngula stages of development, and differences at later stages were mainly associated with variation in the timing of development of bone and teeth (unpublished data). In contrast, dramatic differences in *bmp4* expression were observed in the first arch of LF and MZ embryos at the high-pec stage (late pharyngula period) of embryonic development (Fig. 4). At this stage, *bmp4* is expressed in a variety of tissues, including ear, nose, brain, heart, and fins. *Bmp4* transcripts were also detected in the mesenchyme of the mandibular arch in both LF and MZ embryos. Although expression of *bmp4* mRNAs in MZ embryos was restricted to the distal-most portion of the first arch (Fig. 4*a*, red arrowhead), *bmp4* was ubiquitously expressed throughout the mandibular mesenchyme in LF embryos (Fig. 4*b*, red arrowhead). To assess the potential of *bmp4* in regulating functional shape differences among fish species, we manipulated levels of *bmp4* mRNA during embryonic development in the experimentally tractable zebrafish, *Danio rerio*.

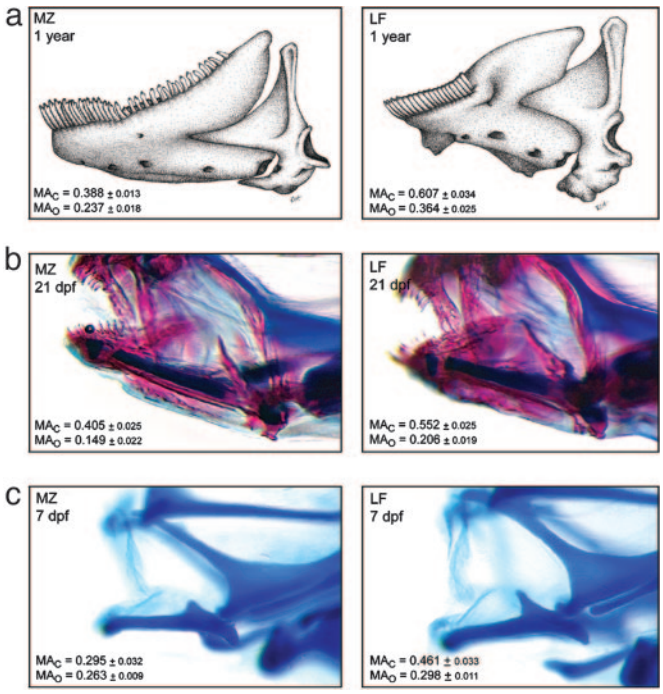

Fig. 3. Ontogeny of biomechanical lever systems in cichlids. (*a*) Adult LF and MZ (>1 year) show significant differences in the mechanical advantage of closing and opening ($n = 29$). (*b* and *c*) This difference is evident in juveniles at 21 dpf ($n = 6$) (*b*) and in larvae as early as 7 dpf ($n = 10$) (*c*).

Zebrafish are obligate suction feeders with mandibular morphology that is characterized by relatively low mechanical advantage at all stages of development. Very little *bmp4* was detected in the mandibular arch of the zebrafish pharyngula, and by 6 dpf, Meckel's cartilage is little more than rod with no coronoid process (closing in-lever) (Fig. 5). Observations in both cichlids and zebrafish suggest that levels of *bmp4* expression in the mandibular arch are associated with growth of the coronoid process. We tested this hypothesis by overexpressing *bmp4* in the zebrafish embryo. We found that *bmp4* mRNA-injected embryos exhibited specific differences in jaw shape compared with those injected with *gfp* mRNA (Fig. 6). Specifically, *bmp4* overexpression elicited growth of a coronoid-like process. This phenotype is consistent with observations in other vertebrates, where the application of BMP in the mandibular arch induced ectopic cartilage formation (50, 51). Interestingly, zebrafish injected with *bmp4* also exhibited attenuated growth along the rostral axis of Meckel's cartilage, as well as increased growth of the retroarticular (Fig. 6). In other words, zebrafish treated with *bmp4* displayed a greater mechanical advantage of opening and closing.

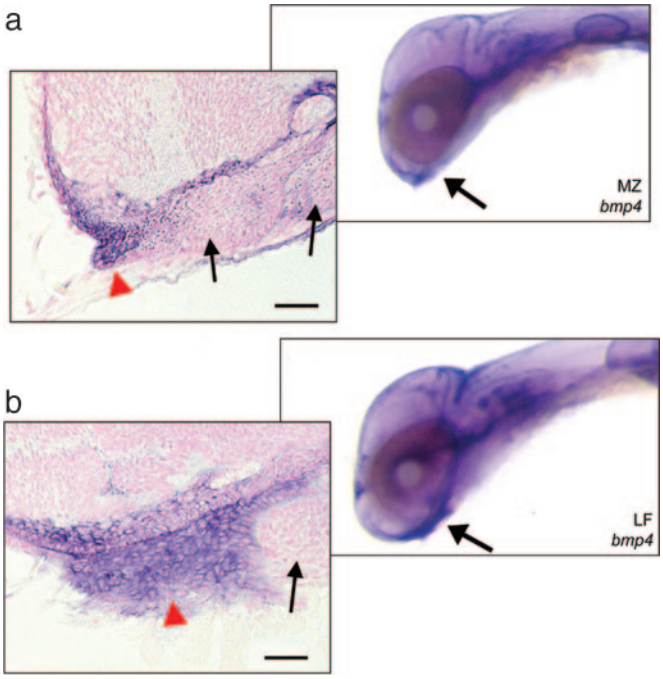

Fig. 4. LF and MZ embryos exhibit different levels of *bmp4* expression in the mandibular arch. (*a*) At the high-pec stage, MZ pharyngula express *bmp4* at the distal tip of the first arch (red arrowhead). In *Left*, the black arrows indicate the first and second pharyngeal arches. In *Right*, the black arrow indicates the mandibular arch. (*b*) Similarly staged LF embryos express *bmp4* throughout the mandibular mesenchyme (red arrowhead). In *Left*, the black arrow indicates the second arch. In *Right*, the black arrow indicates the mandibular arch. Differences in *bmp4* expression were not size-dependent. At no stage did MZ embryos show a level of mandibular *bmp4* expression comparable to what was observed in LF. (Scale bars, 20 μm.)

These results are consistent with recent findings that suggest that Bmp4 signaling may have contributed to the evolution of beak shape in Darwin's finches (47). In both fish and finches, increased levels of *bmp4* were associated with biting/crushing morphologies, demonstrating a remarkable consistency of effects. Expanded *bmp4* expression was also associated with increased proliferation in the frontonasal mass in ducks (48). Given the extensive diversity in jaw shape exhibited by birds and fishes, the idea that *bmp4* might underlie adaptive morphological transformations in these two groups implicates this molecule as a key player in vertebrate diversity. It remains to be seen, however, whether *bmp4* is associated with only certain types of

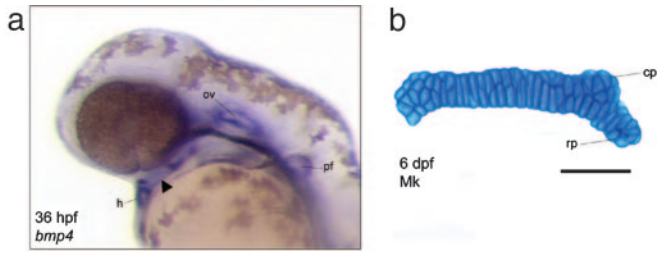

Fig. 5. *Bmp4* expression and jaw morphogenesis in the zebrafish, *D. rerio*. (*a*) Expression of zebrafish *bmp4* at 36 h postfertilization. Throughout much of the pharyngula period of embryonic development, *bmp4* was expressed in the developing heart (h), ear (otic vesicle, ov), and pectoral fin (pf). However, very little *bmp4* was detected in the mandibular arch (black arrowhead). (*b*) In 6-dpf wild-type embryos, Meckel's cartilage (Mk) possessed a distinct retroarticular process (rp) but lacked a discernable coronoid process (cp).

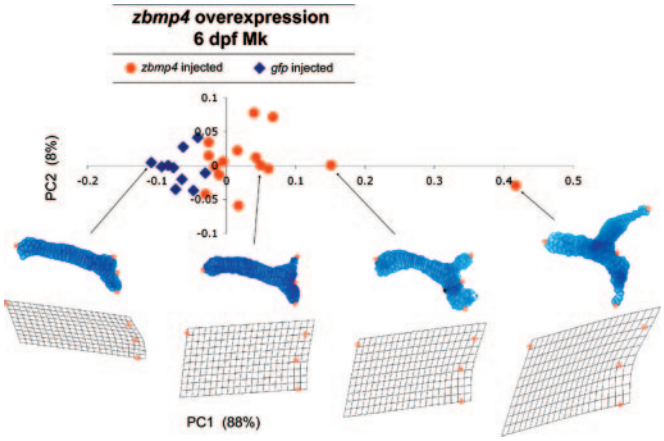

Fig. 6. Distinct morphological transformation of Meckel's cartilage (Mk) by *bmp4* overexpression. Landmark-based morphometric analysis revealed concomitant growth of the coronoid and retroarticular processes in 6-dpf zebrafish larvae when embryos were injected with 100 or 150 ng/μl translation-competent *bmp4* mRNA at the one- to two-cell stage, compared with *gfp*-injected negative control embryos.

transformations (e.g., gracile to robust jaws) or a wider array of craniofacial diversity.

Our work demonstrates the potential of integrating studies in natural populations and model organisms to address questions relating to evolution and development. Quantitative genetic analyses in cichlids show that the lower jaw is a morphologically integrated structure. They also implicate *bmp4* as a candidate for differences in the mechanical advantage of closing. Functional analyses in zebrafish substantiate a role for *bmp4* in regulating the closing lever system, but they also show that *bmp4* has the potential to stimulate growth of both the coronoid and retroarticular processes, traits that were decoupled in cichlids.

This study takes important steps toward understanding the molecular changes that have accompanied morphological evolution in cichlids, but several important questions remain to be addressed. For one, what other genes might be involved in the evolution of cichlid trophic diversity? Several vertebrate mutants exhibit mandibular phenotypes that resemble variation in cichlid feeding morphology. Mice deficient of *otx2* exhibit a shortened mandible (52), whereas mice lacking *pax9* are missing the coronoid process (53), and both chickens and zebrafish lacking *bapx1* are missing the retroarticular process (54, 55). In addition, it remains unclear whether differences in *bmp4* expression in the cichlid mandible are due to divergence in protein structure or regulatory elements. High rates of amino acid substitution in the prodomain of East African cichlid Bmp4 implicate posttranslational processing as a possible mechanism of diversity (49); however, accumulating empirical and theoretical evidence in a variety of organisms posits a significant role for the evolution of cis-regulatory systems in generating morphological diversity (reviewed in ref. 56). It is also interesting to speculate about the evolutionary importance of variances that cannot be explained by genetic effects. Several researchers have recently argued that phenotypic plasticity is an important factor underlying cichlid trophic evolution (57, 58). We present evidence for genetic control of cichlid jaw shape acting early in development; however, our data do not discount the possibility that phenotypic plasticity could reshape the mandible at later stages of development. Understanding how the interaction between genetic and environmental effects might influence the evolution of cichlid feeding morphology will be an important topic to explore. Finally, it is important to note that our interpretation of differences in cichlid feeding performance was based on variation in bone shape. Studies that take into consideration additional mechanical param-

eters, including muscle mass, origin, and insertion (as described in ref. 42), could help us gain a more comprehensive understanding of the genetic basis of alternate feeding strategies. Identifying the causative mutations and characterizing the molecular pathways that contribute to adaptive morphological transformations remains an ambitious task that may be facilitated by continued work in evolutionary and developmental fish models.

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