

SHORT REVIEW

Genetic and developmental basis of cichlid trophic diversity

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Cichlids have undergone extensive evolutionary modifications of their feeding apparatus, making them an ideal model to study the factors that underlie craniofacial diversity. Recent studies have provided critical insights into the molecular mechanisms that have contributed to the origin and maintenance of cichlid trophic diversity. We review this body of work, which shows that the cichlid jaw is regulated by a few genes of major additive effect, and is composed of modules that have evolved under strong divergent selection. Adaptive variation in cichlid jaw shape is evident early in

development and is associated with allelic variation in and expression of *bmp4*. Modulating this growth factor in the experimentally tractable zebrafish model reproduces natural variation in cichlid jaw shape, supporting a role for *bmp4* in craniofacial evolution. These data demonstrate the utility of the cichlid jaw as a model for studying the genetic and developmental basis of evolutionary changes in craniofacial morphology.

Heredity (2006) **97**, 211–221. doi:10.1038/sj.hdy.6800864; published online 12 July 2006

Keywords: cichlid; adaptive radiation; jaw; *bmp4*; genetic architecture; cranial neural crest

Introduction

Cichlid fishes are an important evolutionary model (Kocher, 2004). Their uncanny ability to exploit new environments by radiating into a wide array of shapes and forms has made them an ideal system to study the factors that underlie morphological diversity. The East African Rift Valley alone contains more than 2000 endemic cichlid species, most of which have evolved in the past 1–2 million years. Several lines of evidence suggest that functional divergence in feeding morphology has contributed to the radiation and maintenance of cichlid species diversity. Thus, adaptive variation in jaw shape is critical to the success of this group.

Cichlids also offer several advantages as an experimental system. The natural variation among species offers an extensive, nonlethal ‘mutant screen’ to assay the molecular cues that underlie morphological variation. Thus, whereas chemical mutagenesis in model laboratory organisms (eg zebrafish) has traditionally been used to assay the molecular basis of jaw morphogenesis, natural selection among cichlids has produced an extensive mutant panel to dissect the molecular cues involved in producing natural variation in jaw morphology. Moreover, because of the recent origin of most cichlid radiations, taxa that differ dramatically in shape can be crossed to produce viable and fertile hybrids. This

offers a unique opportunity to study the genetic basis of characters that afford species an adaptive advantage. There are also a multitude of genomic resources available to cichlid researchers, including genetic linkage maps, physical maps, BAC libraries, and cDNA resources including microarrays (Kocher *et al*, 1998; Watanabe *et al*, 2003; Albertson *et al*, 2003a; Renn *et al*, 2004; Katagiri *et al*, 2005; Lang *et al*, 2005; Lee *et al*, 2005). Importantly, the close evolutionary relationship among African cichlids ensures that genomic resources developed in one species may be used for studies in thousands of related taxa.

For reasons of experimental utility and evolutionary richness, cichlids are an emerging model for studies in evolution and development. Below we review advances made in understanding the genetic and developmental mechanisms that underlie adaptive variation in cichlid jaw shape.

A brief history of cichlid evolution

The ancestors of most East African cichlids can be traced to Lake Tanganyika. At 8–10 million years old, Lake Tanganyika is the oldest of the rift valley lakes. Several lines of evidence suggest that Lake Tanganyika acted as an evolutionary reservoir, seeding the surrounding lakes and river systems to initiate new species flocks within the last 1–2 million years (Meyer *et al*, 1990; Kocher *et al*, 1993, 1995; Salzburger *et al*, 2005). The implications of these findings are twofold. First, it points to a very recent origin for several local cichlid radiations – less than 200 000 years for the 500 cichlid species in Lake Victoria. In addition, these data point to a remarkable degree of convergence among cichlid radiations. Strikingly similar

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Received 22 November 2005; accepted 4 April 2006; published online 12 July 2006

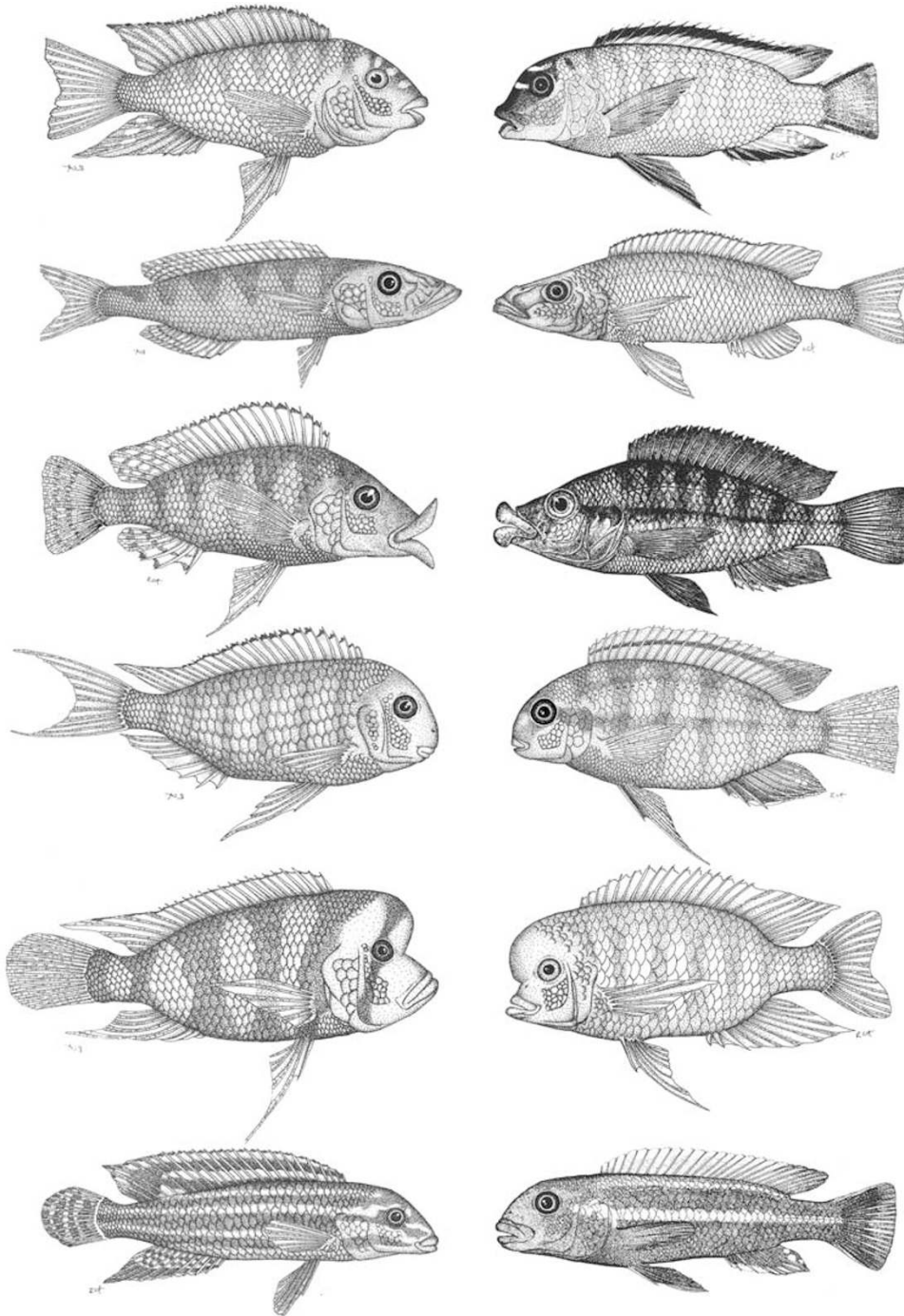


Figure 1 Cichlids exhibit remarkable evolutionary convergence. Similar ecomorphs have evolved repeatedly within different cichlid assemblages. All of the cichlids in the left-hand column are from Lake Tanganyika. All of the cichlids in the right-hand column are from Lake Malawi, and are more closely related to one another than to any species within Lake Tanganyika. Note the similarities among color patterns and trophic morphologies.

ecotypes have evolved repeatedly in several lineages, both within and between lakes (Figure 1). The observation that unrelated cichlids have solved ecological problems in a similar fashion suggests there may be constraints on the direction of cichlid evolution. Whether morphological convergence equals molecular convergence remains an important question to be addressed.

The molecular basis of alternate feeding strategies

On biters and suckers

Cichlids partition their foraging habitat both by what they eat and how they gather prey. A fundamental divergence among cichlids occurs between species that

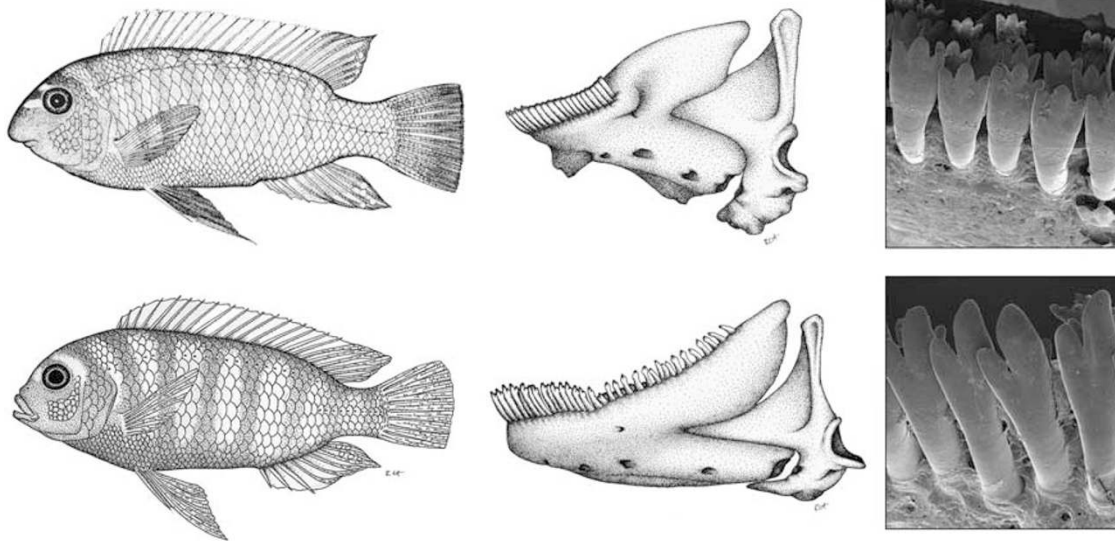


Figure 2 Biting and sucking species exhibit distinct morphologies. *Labeotropheus fuelleborni* (top) is a specialized biting species characterized by a short, robust lower jaw, and an outer row of closely spaced tricuspid teeth. *Metriaclima zebra* (bottom) forages with a sucking mode of feeding, and has a more elongate jaw and an outer series of larger bicuspid teeth.

forage on hard and/or attached prey items (eg snails and filamentous algae), and those that feed on mobile prey (eg plankton and other fishes). This functional divergence is accompanied by the evolution of stereotypical jaw morphologies, which reflect the mechanical properties of the feeding apparatus. Species that strip attached algae from the rocky substratum evolve short, stout jaws efficient for biting, whereas pelagic feeders evolve more elongate, gracile jaws for suction feeding. This divergence has occurred repeatedly during East African cichlid evolution. Variation along this functional axis is also associated with the evolution of several other fish taxa, including North American sunfishes as well as several lineages of coral reef fishes (Motta, 1982; Wainwright and Shaw, 1999; Wainwright *et al*, 2004). Thus, understanding the molecular basis of this functional divergence will have broad implications for understanding the evolution of bony fishes.

Several studies have demonstrated the linkage between functional morphology of the teleost jaw and differences in feeding performance (Liem, 1974, 1980; Otten, 1983; Reinthal, 1990; Bouton *et al*, 1997, 1998). From a mechanical perspective the cichlid mandible can be described as two opposing lever systems. The first defines the mechanics of jaw opening, and is measured as the ratio between the depth of the retroarticular process (opening in-lever) and the length of the jaw (out-lever). The second describes the mechanics of jaw closing, and is taken as the ratio between the height of the coronoid process (closing in-lever) and the length of the jaw (out-lever). These ratios are formally referred to as mechanical advantage, as they define the fraction of force that is translated from the muscular attachments on the retroarticular and coronoid processes to the tip of the mandible. High mechanical advantage predicts powerful jaw rotation, which is typical of biting species. Low mechanical advantage predicts weak but rapid jaw rotation, typical of sucking species. Dentition is also an excellent predictor of foraging niche. Biting species typically possess an outer row of small, closely spaced

teeth with multiple cusps. Suction feeders, on the other hand, are characterized by an outer row of large, intermittently spaced teeth with fewer cusps. Like jaw morphology, cichlid dental patterning has evolved rapidly and replicatively, and is associated with both ancient and contemporary trophic evolution (Ruber *et al*, 1999; Huysseune *et al*, 2002; Streelman *et al*, 2003).

What follows is an overview of progress made toward understanding the molecular basis of alternate feeding strategies. Three questions will be addressed. First, what is the number and effect of genes that underlie adaptive shape differences among cichlid species? Second, how has genetic variation for these traits evolved among cichlids? Finally, how does genetic variation manifest itself during development?

The number and effect of genes that distinguish feeding morphologies

Reverse genetics is a classic approach that has been successfully employed to dissect the genetic basis of complex traits in both laboratory and natural populations. This approach was recently taken for studying the feeding apparatus in East African cichlids. Two Lake Malawi cichlid species that forage in distinct ways and possess disparate jaw morphologies were crossed to produce an F₂ mapping population (Figure 2) (Albertson *et al*, 2003a,b). *Labeotropheus fuelleborni* (LF) is a specialized biting species with a short, robust jaw that it employs to crop filamentous algae from the rocky substratum. LF possesses an outer row of closely spaced tricuspid teeth on both the upper and lower jaws, which resemble the cutting edge of shearing scissors. *Metriaclima zebra* (MZ) is a generalist feeder with a more elongate jaw that it uses to brush loose plant matter from algae beds, and pluck plankton from the water column with a sucking mode of feeding. MZ's dentition resembles the teeth of a comb, with taller intermittently spaced bicuspid teeth on both jaws. Oral jaw morphologies of LF and MZ reflect different modes of food

collection, and place them on opposite ends of the biting/sucking continuum.

Analysis of line crosses enables one to estimate the minimum number of genetic factors that underlie a particular trait, as well as the relative contribution of additive, dominance, and epistatic effects to the inheritance of that trait. The Castle-Wright estimator compares variances between F_1 and F_2 generations to estimate the number of genes that contribute to a phenotypic difference, while a joint-scaling test can be used to assess genetic effects (reviewed in Lynch and Walsh (1998)). The application of these techniques in the $LF \times MZ$ cross provided the first insights into the genetic architecture of cichlid feeding morphology (Albertson *et al.*, 2003b). Between one and 10 genes were estimated to affect the shape of each bony element in the head. Furthermore, there was little support for nonadditive genetic effects on trophic morphology. These data support a relatively simple additive genetic basis for cichlid trophic shape differences. Of particular interest, the distribution of tooth shape among the F_2 was tri-modal, suggesting that cusp number is largely determined by a single genetic factor. This finding has important implications for the evolution of dentition in cichlids.

Evolution and development of cichlid tooth shape

Dentition figures prominently in discussions of adaptive radiation in groups as distinct as hominids and teleosts (McCollum and Sharpe, 2001; Sire and Huisseune, 2003). The observation that differences in tooth shape may be controlled by changes at a small number of loci suggests that this character has the potential to respond rapidly to selection. Integrating knowledge of vertebrate tooth development with patterns observed among natural cichlid populations, Streelman *et al.* (2003) proposed a simple morphogenetic model to explain the development basis of variation in cichlid dentition.

Implicit to Streelman's model is the observation that a common set of activators and inhibitors are iteratively expressed at multiple stages of vertebrate tooth development (reviewed by Thesleff and Sharpe (1997, 1999); Cobourne and Sharpe (2003)). For instance, BMPs and FGFs are initially expressed in the oral epithelium, and specify the position of tooth formation. Later, these same signaling molecules are secreted from the enamel knot, a putative epithelial signaling center that regulates the development of dental cusps (Jernvall and Thesleff, 2000). The iterative effects of a common suite of signaling molecules during both tooth initiation and morphogenesis predicts an association between the number of teeth within the jaw and the number of cusps on a tooth. Natural variation in cichlid dentition provided the perfect opportunity to explore this association.

A strong positive relationship between tooth and cusp number was observed among cichlid species (Streelman *et al.*, 2003; Streelman and Albertson, 2006). *Cyanotilapia afra* is a planktivore with few, large, distantly spaced unicuspid teeth in the outer tooth row on both the upper and lower jaw. *Metriaclima* species are generalized feeders that forage in the water column and from the rocky substrate, and have an outer row of significantly more bicuspid teeth. *LF* feeds exclusively from rocks, and has many, smaller tricuspid teeth. This trend is likely applicable to a much broader sampling of cichlid species.

In general, species with larger, unicuspid teeth (suction feeders) have fewer teeth, whereas species with smaller, multicuspid teeth (biters) have many more teeth in their jaws (Ribbink *et al.*, 1983).

This association could be explained by different concentrations of a single inhibitory molecule at two distinct odontogenic stages (Streelman *et al.*, 2003). At the initiation stage, the local concentration of a putative inhibitor within the oral epithelium will establish where a tooth forms, and the relative distance between adjacent tooth buds (ie how many teeth will develop within a single tooth row). High levels will establish a relatively broad inhibitory field, which will result in the formation of large, distantly spaced teeth. A decrease in concentration will produce smaller inhibitor fields, leading to a greater number of teeth. During morphogenesis, the level of the same inhibitor secreted from the enamel knot will affect cusp development. Higher levels will lead to fewer cusps, whereas lower concentrations will lead to more cusps. Given its role during mammalian tooth initiation and morphogenesis, *bmp4* has been posited as a candidate for the coordinated evolution of cichlid dentition (Streelman *et al.*, 2003; Streelman and Albertson, 2006). While allelic variation in *bmp4* does not segregate with tooth shape in $LF \times MZ$ F_2 hybrids (Albertson *et al.*, 2003a), it is divergently expressed during tooth development in cichlid species with different dental patterns (Streelman and Albertson, 2006) (Figure 3). Thus, BMP4 signal may be modulated by an as-of-yet unidentified *trans* acting molecule during cichlid odontogenesis. The recent characterization of two signaling molecules (at least one of which is a known BMP4 antagonist, reviewed by Streelman and Albertson, 2006) that exhibit coordinated effects on tooth and cusp number offers additional support for Streelman's model (Kangas *et al.*, 2004; Kassai *et al.*, 2005).

Morphological integration of the cichlid jaw

The theory of morphological integration postulates that genetic correlations will evolve among traits that function together (Olson and Miller, 1958; Wagner, 1996). The mechanical properties of the cichlid jaw are well understood, providing an opportunity to test this hypothesis in an evolutionary model system. The geometry of the cichlid mandible can be reduced to three vectors of functional relevance: the length of the jaw (out-lever), the height of the coronoid process (closing in-lever), and the depth of the retroarticular process (opening in-lever) (Figure 4). Coordinated changes of in- and out-lever lengths will effect the mechanical properties of jaw rotation. Thus, we predicted that the in- and out-levers of the cichlid jaw would be integrated, whereas the two in-levers would be genetically decoupled.

Patterns of genetic covariation of the cichlid jaw offer strong support for the theory of morphological integration (Albertson *et al.*, 2003a,b, 2005) (Figure 4). For example, the closing in-lever and the out-lever share two of three quantitative trait loci (QTL), and are inherited together in the F_2 of the $LF \times MZ$ cross. The opening in-lever and the out-lever share only one of five QTL, but still segregate together. Alternatively, the closing and opening in-levers share none of eight QTL, and are not inherited together. These data suggest different levels of integration for different regions of the cichlid mandible,

and support the idea that modularity is not an all-or-nothing concept, but rather a matter of degrees (Klingenberg, 2004).

The jaw closing mechanism is integrated to a greater extent than the mechanism for jaw opening (Albertson *et al*, 2005). We have argued that this disparity reflects differences in the evolutionary, functional, and developmental complexity of these two linkage systems. The jaw closing mechanism, for example, appeared early in actinopterygian evolution and has remained relatively unaltered (reviewed in Liem *et al*, 2000). Jaw closing in teleosts is also a relatively simple process that involves the coordinated action of the upper and lower jaws (Barel, 1983; Otten, 1983; Liem *et al*, 2000; Westneat, 2003). Jaw opening, on the other hand, has been extensively modified during the evolution of ray-finned fishes (reviewed in Liem *et al*, 2000), and is a more complex process that involves the synchronized action of several biomechanical systems defined by the oral jaws, hyoid, operculum, skull and pectoral girdle (Liem, 1978; Westneat, 1994; Wainwright *et al*, 2004). From a developmental perspective, most of the vertebrate mandible originates from cranial neural crest cells derived from the

presumptive midbrain. In contrast, the retroarticular process develops from a heterogeneous population of crest cells derived from up to three hindbrain segments (Köntges and Lumsden, 1996). Thus, greater levels of integration were observed among traits with a more static evolutionary history, simple function, and homogeneous developmental origin. These observations support the hypothesis that the cichlid mandible is a morphologically integrated structure (Olson and Miller, 1958; Wagner, 1996).

How broadly applicable are the patterns of integration observed in this laboratory cross? If LF and MZ mark opposite ends of a putative biting/sucking functional continuum, then a multitude of species occupy a place somewhere along this axis. Clear expectations were established from the LF × MZ cross about which traits should co-vary among natural populations. Members of the *Pseudotropheus tropheops* species complex are characterized by jaw morphologies that are roughly intermediate between LF and MZ. These species also tend to partition their foraging habitat by time spent feeding on loose or attached algae (Albertson, 2002). Patterns of phenotypic integration of jaw characters across twelve *P. tropheops* species are strikingly similar to those observed among the LF × MZ F₂, suggesting that a common molecular mechanism may underlie variation along this axis (Figure 5).

The evolution of genetic variation

The QTL sign-test has been used to assay the role of directional selection in phenotypic divergence (Orr, 1998). The null hypothesis is that traits diverge in response to 'neutral' processes (eg genetic drift). The

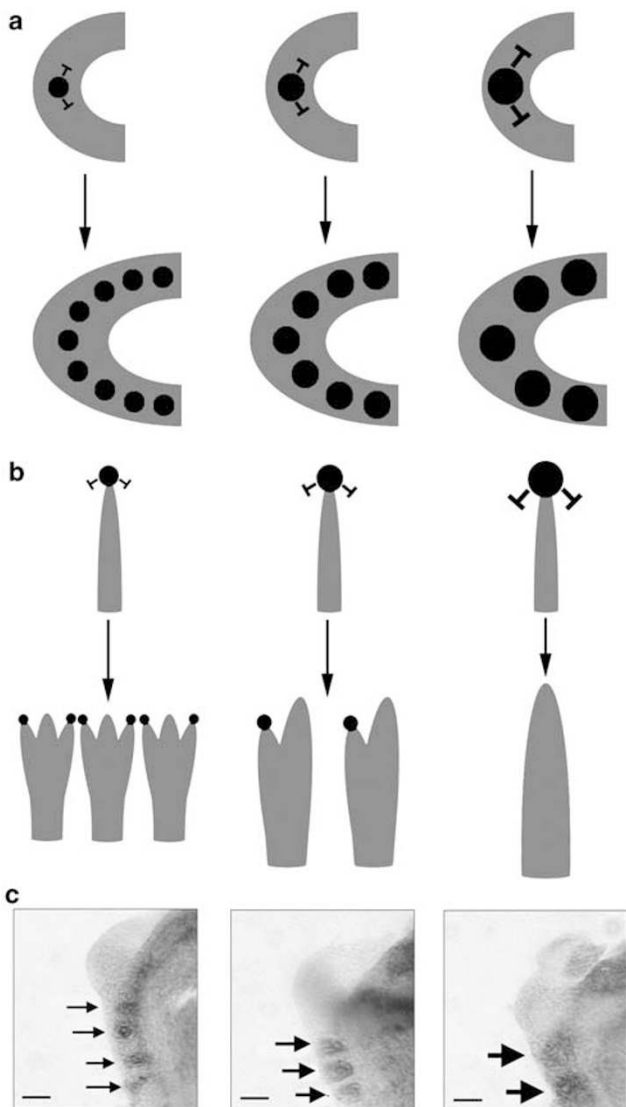


Figure 3 Coordination of tooth size and number in the cichlid jaw (adapted, with permission, from Strelman *et al*, 2003). A positive association between tooth size, tooth number and tooth cusp number was identified among Lake Malawi cichlid species (Strelman *et al*, 2003). This relationship could be explained by the concentration of a common local inhibitor at two distinct ontogenetic stages. (a) At the initiation stage, expression of an inhibitory molecule within the oral epithelium (black dot) will determine both where a tooth will form and how close to it the next tooth will be. A smaller inhibitory field will lead to the development of many small teeth, whereas a larger inhibitory focus will produce fewer large teeth. (b) At morphogenesis stages, expression of the same inhibitory molecule from the primary enamel knot (black dots, upper panel) will inhibit the formation of secondary enamel knots (black dots, lower panel), and thus the formation of multiple cusps. Lower concentrations of the inhibitor will lead to more cusps; higher concentrations will result in fewer cusps. The net result of a and b is many, small multicuspid teeth when inhibitory concentrations are low, and few, large unicuspid teeth with higher concentrations (lower panel, b). (c) Divergent expression of *bmp4* in the developing oral jaw of cichlids with different dental patterns (Strelman and Albertson, 2006). *Bmp4* is expressed in many small tooth buds in the developing jaw of *Labeotropheus fuelleborni* (left panel), which, as an adult, has many small tricuspid teeth. *Metriaclima benetos* has fewer, bicuspid teeth, and *bmp4* is expressed in larger tooth buds (middle panel). *Cyanotilapia afra* is characterized by even fewer large unicuspid teeth, and *bmp4* is expressed within few, large tooth buds during odontogenesis (right panel). Interestingly, at this early developmental stage all three species have unicuspid teeth. Thus, these data show divergent expression of *bmp4* associated with dental patterning (not shape). It remains to be seen whether variation in *bmp4* expression is also associated with differences in tooth shape (see Strelman and Albertson, 2006 for a review). Scale bars equal 20 μm.

expectation under the null hypothesis is that individual populations will accumulate alleles of antagonistic (+ and -) effect. The alternate hypothesis is that traits have diverged under directional selection. Under this hypothesis individual populations are expected to accumulate alleles of consistently positive or consistently negative effect. The crux of the QTL sign-test is the ratio between loci that exhibit a consistent directional effect and the total number of QTL. A QTL ratio of 0.500 is observed when half the alleles from species 'A' have a positive effect and half have a negative effect, consistent with the null hypothesis. A QTL ratio of 1.000 is observed when all the alleles from species 'A' have a positive effect, and all the alleles from species 'B' have a negative effect, consistent with the alternate hypothesis.

A survey of allelic effects for QTL that underlie the shape of the cichlid feeding apparatus produce a QTL

ratio of 0.949 ($n = 39$ QTL) (Albertson *et al*, 2003a). When considering just the opening and closing lever mechanisms of the lower jaw, a QTL ratio of 1.000 is obtained ($n = 10$ QTL) (Albertson *et al*, 2005). These quantitative genetic data offer the first empirical evidence that the cichlid feeding apparatus diverged in response to strong and/or consistent directional selection.

Exploring patterns of natural variation in jaw morphogenesis

An emerging challenge for evolutionary biologists is to understand how genetic variation at functional loci is translated to adaptive variation in adult form. In previous sections, we reviewed progress made toward understanding the genetic basis of differences in cichlid jaw shape. Below we present an overview of recent work that examines the developmental origin of adaptive variation in cichlid jaw shape. An important first question along these lines is, when are shape differences first evident among cichlid species?

By 7 days post-fertilization (dpf), LF and MZ exhibit clear differences in mandibular morphology (Figure 6a). The presumptive mandible is not ossified at this stage, but the cartilaginous precursor, Meckel's cartilage (Mk), exhibits discrete differences in lever ratios. Thus, developmental methodologies may be employed to study the evolution of cichlid jaw shape. To understand the developmental basis of variation in adult skeletal morphology, we examined patterns of natural variation in cichlid jaw morphogenesis.

The progenitor cells of most of the craniofacial skeleton, including the jaw, are the cranial neural crest (CNC), a 'key innovation' contributing both to the origin and evolutionary success of vertebrates (Gans and Northcutt, 1983; Helms and Schneider, 2003; Trainor *et al*, 2003). Evolutionary change in CNC-derived structures may occur as a result of changes in crest cells themselves, or their environment (reviewed in Gerhart and Kirschner, 1997). Development of the CNC has been well studied in several model organisms (reviewed in Hall, 1999). In bony fishes, the CNC migrate in three distinct streams from the putative mid- and hindbrain into a bilateral series of endodermal pouches, called pharyngeal arches. The iterative segmentation of the posterior-most arch into four additional arches is achieved through a complex morphogenic process that involves coordinated signaling from the pharyngeal endoderm and the CNC (Crump *et al*, 2004). CNC cells within each pharyngeal arch condense and differentiate to form the skeletal anatomy of the head.

Patterns of CNC migration and segmentation were examined in cichlid species with distinct jaw morphologies. While the timing of CNC migration was relatively conserved among species, differences were observed in CNC segmentation. Species with elongate, gracile jaws possessed thin, elongate CNC segments, whereas species with short, robust jaws had short, robust CNC masses (Figure 7). Thus, differences in cichlid CNC development predict adaptive variation in adult morphology. Since pharyngeal arch morphogenesis involves reciprocal signaling from the CNC and the surrounding epithelia, it is unclear whether this difference is due to changes in crest cells or their environment (Schneider and Helms, 2003).

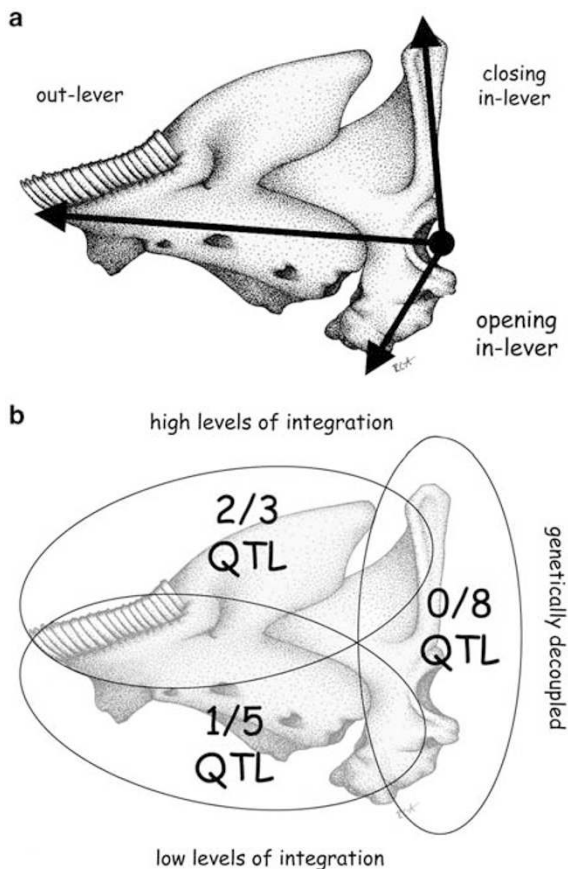


Figure 4 Patterns of morphological integration in the cichlid jaw. (a) The geometry of the cichlid jaw can be described as two opposing lever systems. The first defines the mechanics of jaw closing and is taken as the ratio between the closing in-lever and the out-lever. The second describes the mechanics of jaw opening and is the ratio between the opening in-lever and the out-lever. The ratio between in- and out-levers is formally referred to as mechanical advantage. Greater mechanical advantage translates to greater force during jaw rotation, whereas lower mechanical advantage translates to weaker but more rapid jaw rotation. Thus, there is a tradeoff between powerful and rapid jaw movement. (b) The theory of morphological integration predicts that traits that function together will also be genetically modular. Quantitative genetic analyses in cichlids support this theory. Higher levels of integration were observed among the in- and out-levers, traits that function together. In contrast, the closing and opening in-levers, traits that participate in distinct functions, were genetically decoupled.

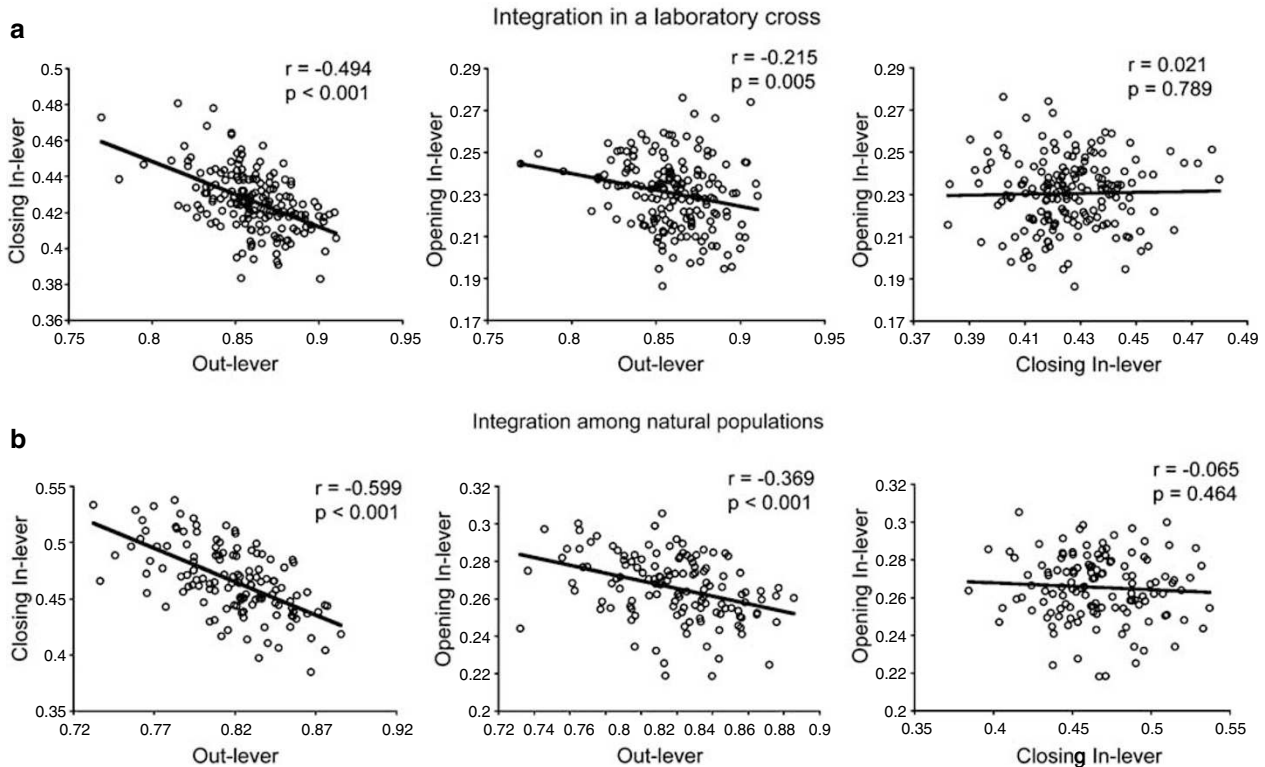


Figure 5 Patterns of integration are similar in a laboratory cross and natural populations. (a) The length of the in- and out-levers were inherited together in the F_2 from the LF \times MZ cross. The closing and opening in-levers, on the other hand, did not segregate together. (b) Similarly, the in- and out-levers co-vary among natural populations of *Pseudotropheus tropheops*, and the closing and opening in-levers do not. Differences in mechanical advantage among *P. tropheops* species correspond to differences in habitat preference and feeding performance (Albertson, 2002; Albertson, unpublished data).

Condensation and differentiation of the CNC are also important stages in jaw morphogenesis (Hall and Miyake, 2000). Work in several vertebrate models has provided a wealth of information regarding the signaling molecules that regulate these events. *Bmps*, *fgfs*, and an array of homeobox genes (eg *msx*, *otx*, *dlx*) have been of particular interest, because they regulate various aspects of mesenchymal condensation and differentiation. BMP levels, for example, determine condensation size by the recruitment and expansion of precursor cells (eg CNC) (Goldring *et al*, 2005). BMPs also regulate the transition from condensation growth to overt differentiation of chondroblasts (Hall and Miyake, 2000; Pizette and Niswander, 2000; Goldring *et al*, 2005). We cloned and examined the expression of several genes involved in CNC condensation during cichlid embryogenesis (Albertson *et al*, 2005). In general, local expression domains were consistent with those reported for other animal models. Furthermore, relatively conserved patterns of *fgf8*, *bmp2*, *dlx2* and *msx d/e* expression were observed during cichlid jaw morphogenesis. However, striking differences were observed in *bmp4* expression (Figure 6b). During the pharyngula period (CNC condensation stages), LF and MZ exhibited marked differences in the level of *bmp4* transcripts present within the mandibular arch. While expression of *bmp4* mRNA was largely restricted to the distal end of the first arch in MZ embryos, *bmp4* was ubiquitously expressed throughout the mandibular mesenchyme in LF embryos. This difference in *bmp4* expression is correlated with differ-

ences in larval jaw morphology; specifically, differences in the height of the coronoid process.

To explore the potential role of *bmp4* in regulating adaptive variation in jaw morphogenesis, its expression and function was examined in the experimentally tractable zebrafish, *Danio rerio*. Zebrafish are obligate suction feeders characterized by thin elongate mandibles, used to feed on small aquatic insects, crustaceans and plankton (see Hernández *et al*, 2002 for characterization of zebrafish feeding kinematics). At pharyngula stages of development, little *bmp4* was found in the zebrafish mandibular arch (Figure 6c). Likewise, zebrafish larvae develop a thin elongate mandible with a very short coronoid process. Thus, in zebrafish and cichlids *bmp4* levels are correlated with the height of the coronoid process. Modulation of *bmp4* levels in zebrafish embryos led to a quantitative transformation of mandibular morphology. Specifically, embryos injected with translationally competent *bmp4* mRNA exhibited expanded growth of both the retroarticular and coronoid processes. In the lexicon of functional morphology, *bmp4* overexpression resulted in an increase in mechanical advantage of the jaw, and reproduced natural variation in cichlid jaw shape.

The role of *bmp4* in trophic evolution

In recent years, BMP4 has garnered significant attention as a candidate for craniofacial evolution in vertebrate. This growth factor is known to regulate embryonic

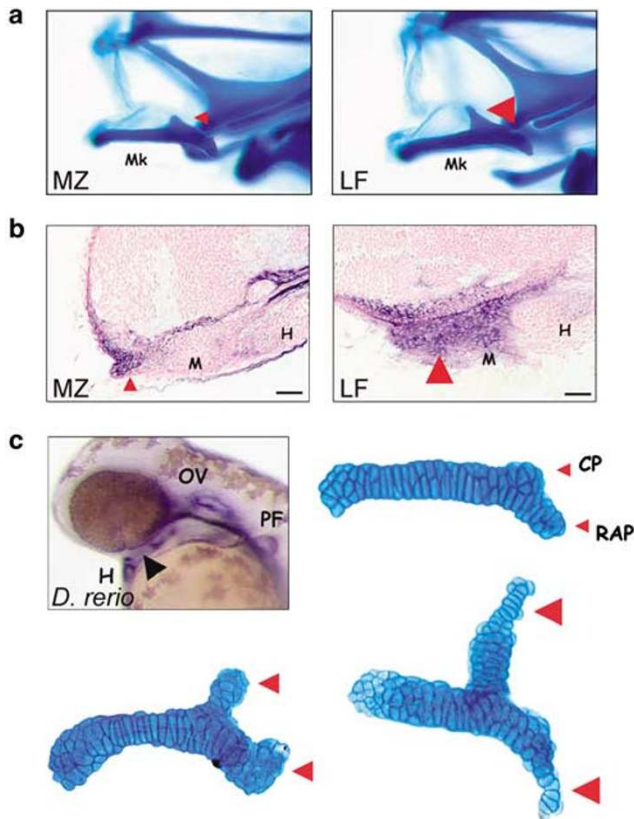


Figure 6 Differences in the height of the coronoid process are evident early in development and correlated with levels of *bmp4*. (a) By 7 days post-fertilization LF and MZ exhibit discrete differences in the height of the presumptive coronoid process (red arrowheads). Mk, Meckel's cartilage. (b) This morphological difference is associated with differences in the level *bmp4* expression in the mandibular mesenchyme (red arrowheads). M, mandibular arch; H, hyoid arch. (c) The zebrafish pharyngula (shown here at 32 h) shows little *bmp4* expression within the mandibular arch (black arrowhead, top left panel), which is associated with the development of a mandible with a very short coronoid process (cp, top right panel). When *bmp4* was overexpressed in the zebrafish embryo, expanded growth of both the coronoid (cp) and retroarticular (rap) processes were observed. In other words, over-expression of *bmp4* led to an increase in the mechanical advantage of the developing mandible. H, heart; OV, otic vesicle; PF, pectoral fin.

cartilage development (Monsoro-Burq *et al*, 1996; Shum *et al*, 2003), shows restricted patterns of expression during vertebrate jaw morphogenesis (Semba *et al*, 2000), and is involved with regional patterning of the mandibular arch (Haworth *et al*, 2004). BMP4 also regulates mesenchymal condensation size, and treatment with exogenous BMP results in an increase in size and ectopic formation of skeletal elements (Langille, 1994; Hall and Miyake, 1995; Barlow and Francis-West, 1997; Goldring *et al*, 2005). In cichlids, BMP4 exhibits accelerated rates of amino-acid substitute compared with other growth factors involved in craniofacial development (eg BMP2, DLX, PAX, OTX) (Terai *et al*, 2002). BMP4 amino-acid evolution was restricted to the prodomain, which supports the hypothesis that regulation of BMP4 may underlie at least some aspect of cichlid diversity (Terai *et al*, 2002). In Galapagos finches there is a strong correlation between beak morphology and *bmp4* expression (Abzhanov *et al*, 2004). Thick-beaked ground finches

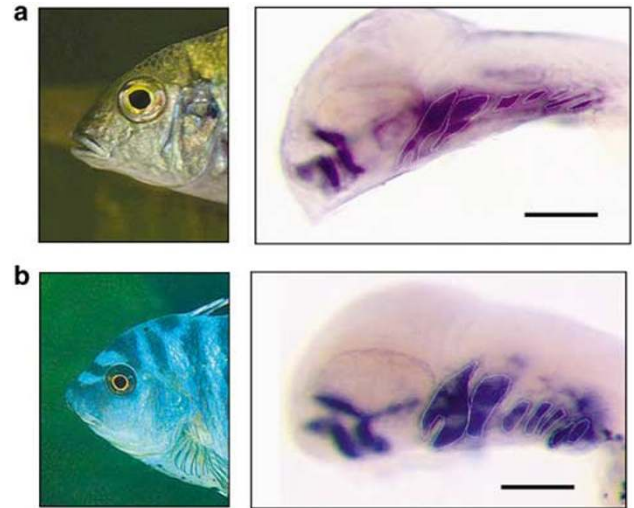


Figure 7 Differences in cranial neural crest segmentation predict differences in adult feeding morphology. Cranial neural crest cell masses were visualized via whole-mount *in situ* hybridization using an antisense cichlid *dlx2* probe. (a) The obligate suction feeder, *Tramitichromis intermedius*, possesses a gracile feeding apparatus. Likewise, cranial neural crest segments in *T. intermedius* embryos are long and thin. (b) Alternatively, the biting species, *Labeotropheus fuelleborni*, has a short, robust feeding apparatus, which resembles the thick cranial neural crest cell masses seen in *L. fuelleborni* embryos. This difference is evident throughout the pharyngula period, and is therefore not stage dependent (data not shown). Thus, differences in adult feeding morphology are determined early in embryogenesis. Scale bars equal 200 μ m.

exhibit broad *bmp4* expression in the beak prominence, whereas thin-beaked cactus finch species show little *bmp4* expression in the developing beak. Moreover, modulating BMP4 levels within the frontonasal process mesenchyme of chick embryos altered beak morphology in a way that approximated natural variation among Galapagos finch species (Abzhanov *et al*, 2004).

The beak of the fish?

On several levels, the beak of the finch is the terrestrial analogue to the cichlid jaw. Both have evolved to exploit specific trophic niches, exhibit tremendous morphological diversity, and are characterized by rapid and replicative evolution. Remarkably, it now appears that variation in these two traits shares a molecular basis. In both systems, expanded *bmp4* expression is associated with a biting/crushing morphology. These are among the first studies to link a genetic change with differences in feeding performance.

The notion that *bmp4* might underlie adaptive variation in disparate vertebrate classes (birds and fishes), implicates this molecule as a major factor contributing to vertebrate diversity, and raises several important questions. For one, are the effects of *bmp4* limited to specific morphological transformations (eg gracile to robust jaws), or does *bmp4* participate in a wider array of craniofacial diversity? BMP4 levels have recently been correlated with differences in proliferation zones in the embryonic beaks of ducks and chickens, implicating BMP4-mediated cell proliferation as a determinant of jaw width (Wu *et al*, 2004). Given the broad and iterative role of BMPs during jaw morphogenesis, *bmp4* has the potential to mediate variation along several axes of diversity.

What other genes might underlie trophic diversity? Several vertebrate craniofacial mutants exhibit phenotypes that resemble variation in cichlid jaw shape. Mice deficient in *otx2*, for example, possess a shortened mandible (Hide *et al*, 2002). This gene is expressed in the neural crest-derived mandibular mesenchyme (Kimura *et al*, 1997), and acts to pattern the rostral portion of the embryonic head (Matsuo *et al*, 1995). Furthermore, duplication and functional diversification of the ancestral *otx* gene has been implicated in the evolutionary success of vertebrates (Matsuo *et al*, 1995; Suda *et al*, 1999). In mice lacking *pax9*, jaw length is relatively unaffected, however, the coronoid process is missing (Peters *et al*, 1998). This is a very specific defect, probably related to the role of *pax-9* in promoting mesenchymal condensation (Peters *et al*, 1999) and the observation that the coronoid process develops from a distinct CNC condensation (Atchley and Hall, 1991). In both mice and zebrafish, loss of *bapx1* activity leads to a loss of the retroarticular process (Miller *et al*, 2003; Wilson and Tucker, 2004). Local *bapx1* expression is restricted by FGFs and BMPs (Wilson and Tucker, 2004), and positively regulated by *edn1* within the developing mandible (Miller *et al*, 2003). Furthermore, experimental manipulation of *bapx1* expression leads to transformations in the shape of the developing mandible (Wilson and Tucker, 2004). These defects resemble variation in cichlid feeding morphology, and could provide the basis for future investigation into the molecular basis of trophic diversity.

Fish 'n chips

A series of recent experiments have identified genes that are divergently expressed during cichlid jaw morphogenesis using microarray analyses (Kijimoto *et al*, 2005; Kobayashi *et al*, 2006). DNA chips were generated from total RNA isolated from the Lake Victorian cichlid, *Haplochromis chilotes*. Target cDNA was generated from larval jaw explants of Victorian species with different jaw morphologies: *Haplochromis* sp. 'red tail sheller' is a crushing species that feeds on mollusks and is characterized by a short stout jaw; *H. parvidens* is a streamlined piscivorous species characterized by an elongate feeding apparatus; *H.* sp. 'rockkribensis' is a generalist feeder that forages on loose algae from the rocky substratum; and *H. chilotes* is an insectivorous species characterized by a narrow head and enlarged fleshy lips. In one experiment, microarray analysis revealed three clones that were differentially expressed in the jaws of *Haplochromis* sp. 'red tail sheller' and *H. parvidens* (Kijimoto *et al*, 2005). Among them was *cimp1*, a novel metalloproteinase from the astacin family of proteins. The biological function of astacins in vertebrates is poorly understood, but what little is known suggests they participate in a variety of processes including morphogenesis and pattern formation (Mohrlen *et al*, 2003; Goldman and Shalev, 2003). Divergent expression of *cimp1* among cichlids was confirmed via *in situ* hybridization and quantitative PCR analyses (Kijimoto *et al*, 2005). In both cases, species with a shorter feeding apparatus showed higher levels of *cimp1* expression in the developing jaws and head, whereas *H. parvidens* showed lower levels. In a similar experiment, a gene encoding microfibril-associated glycoprotein 4 (*magp4*)

was found to be divergently expressed in the developing jaws of *H. chilotes* and *Haplochromis* sp. 'rockkribensis'. Interestingly, *magp4* haploinsufficiency has been implicated in the inherited human disease, Smith-Magenis syndrome (SMS) (Zhao *et al*, 1995). Patients with SMS have a microdeletion of chromosome 17p11.2, and exhibit distinctive facial features including accelerated mandibular growth and wide mouths with full fleshy lips (Allanson *et al*, 1999). SMS is also associated with severe behavioral phenotypes including extreme irritability and aggression (Sarimski, 2004). Quantitative PCR confirmed divergent expression of *magp4* during cichlid jaw morphogenesis, with *Haplochromis* sp. 'rockkribensis' exhibiting consistently higher levels of expression. Collectively, these experiments suggest that *cimp1* and *magp4* are associated with the development of species-specific jaw morphologies, and demonstrate that DNA chips may be used to identify genes that are differentially regulated during cichlid jaw development.

Microarrays are an important addition to the growing number of genomic resources used to study the evolution of cichlid trophic morphology. The use of microarrays will compliment the genetic methodologies reviewed above. For example, genetic analyses will point to the causative mutations that result in divergent jaw shapes. These loci will be targets for natural selection, and therefore their evolutionary history may be studied in the broader context of cichlid evolution. Alternatively, microarray experiments will facilitate an understanding of how genetic variation at functional loci will alter downstream regulatory networks. In this way, DNA chips will provide the opportunity to study the evolution of developmental pathways among closely related species.

Conclusions

Questions related to the development and evolution of complex morphologies will benefit from integrating studies in natural populations and laboratory models. Forward genetic approaches (eg chemical mutagenesis) in laboratory models (eg zebrafish) have provided significant insights into the molecular pathways that regulate morphogenesis of the embryonic jaw. Unfortunately, because most craniofacial mutants exhibit severe defects and die early in development, significantly less is known about the regulation of the jaw morphogenesis beyond embryonic stages (reviewed by Albertson and Yelick, 2004). We view the extensive variation among cichlid species as a mutant panel generated by natural selection. These natural variants can be used to assay a broad range of traits that develop and are remodeled throughout the life of the animal. With this resource, we have the opportunity to complement work in laboratory models by identifying the genetic factors and molecular pathways that regulate morphogenesis beyond the embryo. Continued work in cichlids, and other evolutionary models (Abzhanov *et al*, 2004; Kimmel *et al*, 2005), will provide critical insights into the mechanisms that regulate variation in jaw shape.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant Nos. 9905127

and 0445212 awarded to TDK, and by the National Institutes of Health through an Institutional Postdoctoral Training Grant (T32 DE08327) awarded to the Forsyth Institute, which supports RCA. The authors wish to thank JT Strelman for permission to adapt and reproduce Figure 3, and for insightful discussions on much of the material presented here. This manuscript was also much improved by the thoughtful comments of two anonymous reviewers.

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