

Review Paper

Evolution and Development of Complex Biomechanical Systems: 300 Million Years of Fish Jaws

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ABSTRACT

The jaws of teleost fishes are diverse and complex musculoskeletal systems. The focus in this review is on the major biomechanical systems in the teleost head, and the range and interplay of functional, developmental, and genetic influences that shape the modular and integrated evolution of elements. Insights possible from comparative studies are discussed in the context of traditional and new models for studies of craniofacial evolution and development.

INTRODUCTION

THE TELEOST TROPHIC APPARATUS may be the most complex integrated musculoskeletal system in vertebrates. Fish skulls are highly kinetic and frequently contain 20 or more mobile elements that operate during feeding.^{1,2} Also, in contrast to most vertebrates, bony fish generally possess two sets of toothed jaws.^{3,4} Despite these differences, developmental genetics has uncovered numerous pathways responsible for the formation of structures that are shared between species as diverged as humans and fishes. In most vertebrates, changes in immobile craniofacial morphology have uncertain influences on feeding ecology, but changes in the size and shape of particular bones in teleosts almost invariably influence the mechanics of how a fish feeds. The mobility of so many musculoskeletal elements that are coordinately used during teleost feeding provide a unique opportunity to examine how conflict-

ing and complimentary mechanical, developmental, and genetic levels of organization interact.

Biomechanical models of trophic morphology are valuable because they provide testable hypotheses of the relationship between morphology and the kinematics of trophic elements (Figs. 1 and 2). Most vertebrate musculoskeletal systems can be modeled as levers of varying complexity that encapsulate how motion and force are input and output.^{5,6} Simple lever systems, like the lower jaw,^{7,8} generally rotate around a fulcrum and, based on the relative sizes of the input and output links, favor either greater amplification of force or motion. The same is true for more complex lever systems such as four-bar linkages that operate in mechanical modules of the trophic apparatus such as the anterior jaws.⁹ These four-bar linkages have an input as well as an output link, but also incorporate a third link that couples the transmission of motion and force through the other

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elements. Additionally, four-bar linkages include an immobile fixed link about which the other three links rotate in a ringlike configuration. Musculoskeletal lever systems are formulated in a mathematical framework that makes exact predictions about the transmission of motion and force through the trophic apparatus.^{10,11} These predictions facilitate comparative analyses of functional variation and highlight mechanical connections that frequently have nonlinear relationships with feeding abilities.¹² They also identify which phenotypic elements are most likely to evolve in response to particular selection pressures.¹³ However, mechanical models of jaw morphology provide little insight into the developmental and genetic pathways that influence the evolution of form in the trophic apparatus.

Development provides the link between the morphology that ultimately functions in teleost feeding and the genome that encodes trophic phenotypes. Developmental studies of cell fate indicate which musculoskeletal elements share similar developmental origins.¹⁴ Interestingly, the boundaries between adjacent neural crest

populations do not share simple correspondence with most anatomical structures. The sharing of ancestral precursor tissues in cranial bones and muscles^{15,16} suggests the same genes and influences may structure how these elements are formed during ontogeny.

Fish larvae can begin feeding within one week postfertilization,¹⁷ and much of the general morphological organization of the fish trophic apparatus has been structured by this time.^{17,18} Because trophic adaptation frequently involves the modification of existing structures,^{11,19} understanding which ancestral elements are altered during ontogeny is key to understanding how evolutionary novelties arise.¹ Finally, understanding the developmental integration of musculoskeletal elements may provide predictive insight into the directionality of evolutionary change along pathways of least developmental resistance.

Virtually all craniofacial form is genetically encoded, but how genomic changes translate into developmental modifications is complex. Because most modifications to the skull that have functional consequences involve quanti-

FIG. 2. The bones and muscle that operate during an adult teleost feeding strike can be thought of as functioning like a series of fairly modular but inter-connected mechanical systems. A map of the numerous links (*heavy bars*), joints (*open circles*), muscles (*hashed lines*), and the general direction particular elements move (*arrows*) during the strike for several mechanical levers and linkages in the teleost skull are diagrammed. The morphology of the neurocranium (*red*), hyoid (*dark gray*), pectoral girdle (*purple*), opercular series (*orange*), suspensorium (*yellow*), lower jaw (*light blue*), anterior jaw elements (*dark green*), and pharyngeal jaw elements (*light gray*) are depicted in different colors. Although the elements comprising the particular links and muscles are described in more detail in the text, a basic overview of how the lever systems operate is given here. The feeding strike is initiated (**a**) as the skull is pulled posteriorly via the simple lever-like cranial levation system. As the neurocranium rotates upwards it pulls open the hyoid four-bar linkage system, and the sternohyoideus muscle fires resulting in depression of the hyoid linkage. The depression of the hyoid results in expansion of the buccal cavity and in many fishes (**b**) the initiation of the movement in the opercular linkage. As the opercular series is pulled posteriorly, tension is applied to the interopercular ligament connecting the opercular series to the lower jaw. The lower jaw opening lever system is then depressed and rotates ventrally. The rotation of the lower jaw inputs motion into the anterior jaw four-bar linkage (**c**) resulting in maxillary rotation. Maxillary displacement, cranial elevation, and lower jaw rotation combine to protrude the upper jaw in most teleosts. A prey item is frequently taken into the oral cavity coincident with the maximal gape between the rotating lower jaw and protruding upper jaw mechanism. Once the prey is ingested, the upper and lower jaw closing systems are pulled shut (**d**) via the adductor muscles. Relaxation of the hyoid and muscles posterior to the cranium also likely passively decrease buccal volume and facilitate swallowing of prey. Once the prey has been ingested, it is moved to the pharyngeal apparatus for processing (**e,f**). In most fishes, the pharyngeal jaw muscles insert onto and depress the upper pharyngeal jaws through a series of levers and the lower pharyngeal jaw is largely an immobile platform where prey are processed (**e**). However, in the now recognized paraphyletic group known as the Labroidae, several novel muscle insertions allow the lower pharyngeal jaw to be pulled against the upper pharyngeal jaws during prey processing (**f**). After pharyngeal processing, the prey is swallowed and sent to the digestive tract for digestion and absorption. The major bones and muscles that operate in the lever systems during the adult teleost feeding strike include the supracleithrum (SC), coracoid (CO), cleithrum (CL), sternohyoideus muscle (SH), hypohyal (HH), ceratohyal (CH), interhyal (IH), hyomandibula (HM), posttempora (PT), opercle (OP), subopercle (SP), interopercle (IP), quadrate (QT), pterygoids (PG), articular (AR), maxilla (MX), premaxilla (PX), nasal (NA), palatine (PL), dentary (DT), adductor mandibulae muscles (AM), preopercle (PO), neurocranium (NC), levator externus IV muscle (LE), levator posterior muscle (LP), obliquus posterior muscle (OP), pharyngobranchial 3 toothplate (PB), upper pharyngobranchial 4 toothplate (UP), and fifth ceratobranchials (FC). Images in **b** and **d** are used with permission from RC Albertson.

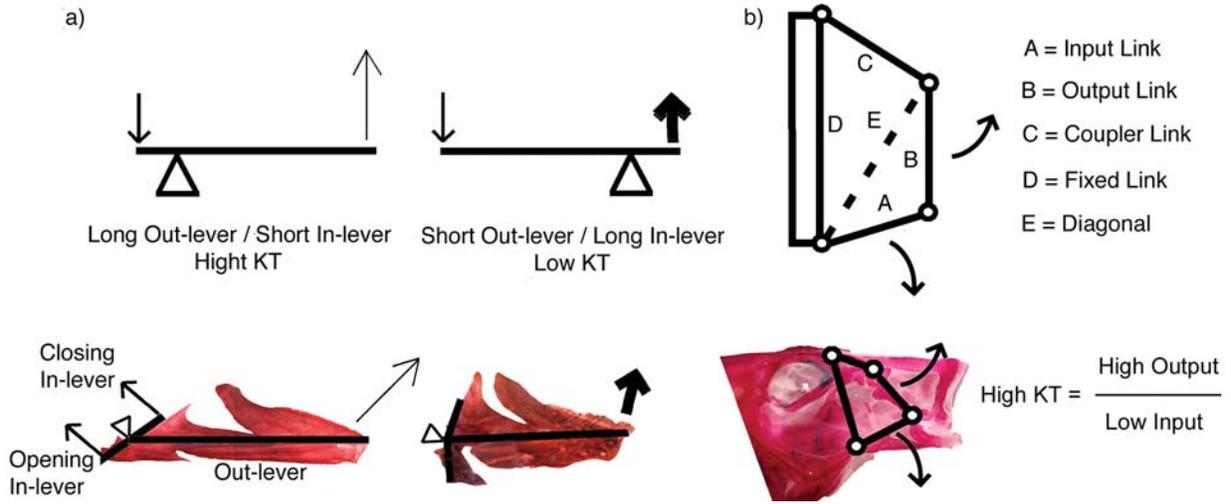
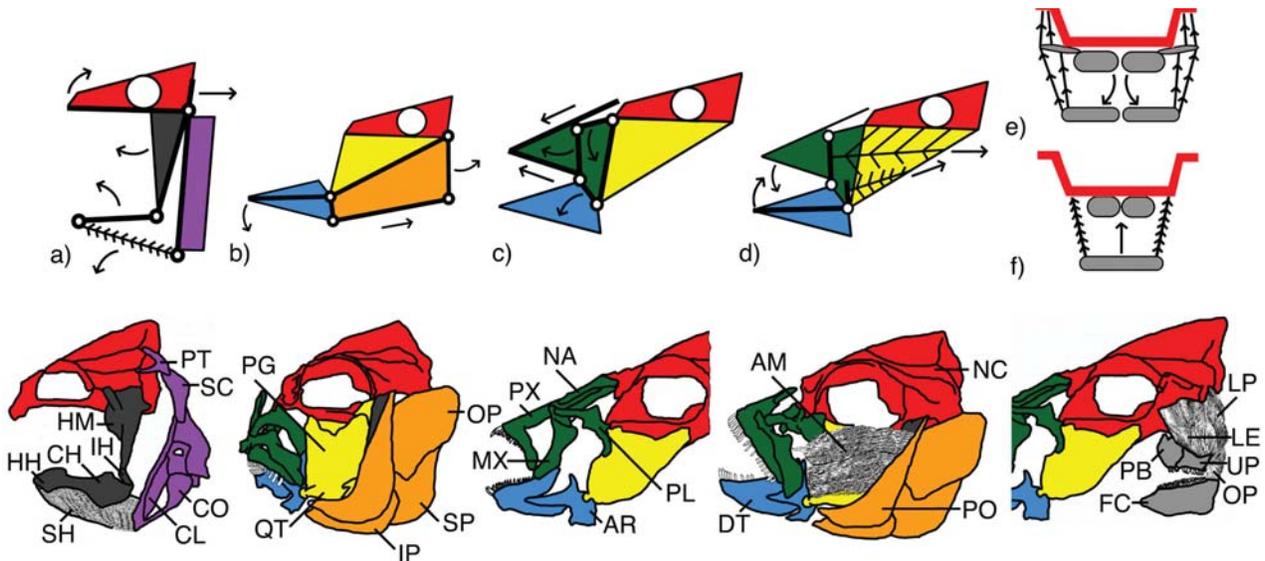


FIG. 1. Most musculoskeletal systems can be modeled as levers. All levers exhibit a mechanical tradeoff between the transmission of force and velocity. For example, simple first order levers (a) with a short in-lever and long out-lever on either side of a fulcrum (*triangle*) are able to transmit a lot of motion but not a lot of force. Alternatively, simple levers with long in-levers and short out-levers can transmit a lot of force but not a lot of motion as the *heavy black output arrow* portrays. Levers that transmit a lot of motion are frequently described as having high kinematic transmission (KT) and are often associated with rapid events such as the capture of evasive prey. In the fish trophic apparatus, several more complicated levers known as four-bar linkages (b) also operate. These levers generally have four physical links. All four-bar linkages have an input link (A) in which motion is transmitted into the system, an output link (B) through which motion is transferred out of the linkage, a coupler link (C) that couples motion transmitted through the system to the output link, and an immobile fixed link (D). All of the links swing in a ringlike configuration around the fixed link, and when planar, four-bar linkages have one degree of freedom. If the angle between any of the links is known or the distance (E) between any of the vertices of the links is determined, it completely defines the position and angular relationships among the other links. In the oral jaws of most fishes, the lower jaw can be thought of a simple first order lever system. In the two lower jaws depicted, the first jaw is more extensively modified to transmit motion and the second jaw exhibits a more force modified morphology. The sharing of the out-lever for both lower jaw opening and lower jaw closing highlights the potential for change in one element having pleiotropic effects on two mechanical components of the fish trophic apparatus. The physical elements of the anterior jaw four-bar linkage are diagrammed on a cleared and stained oral jaw. Four-bar linkage systems are more mechanically complex than simple levers, but also exhibit tradeoffs in the transmission of force and velocity.



tative alterations of the shape and size of elements,²⁰ these modifications are not likely the result of simple qualitative changes in the genome.²¹ Most studies of the genetics underlying change in craniofacial form have implicated multiple genes as underlying these modifications.²² We are beginning to learn that genes expressed during development of homologous craniofacial elements are shared not only between evolutionarily disparate vertebrates, but also expressed among different musculoskeletal elements in the teleost skull.^{23,24} Although daunting in scope, general rules and questions are beginning to emerge concerning how teleost genetic architecture is expressed during development and how it is ultimately translated into the mechanics of trophic adaptation.

We first describe the feeding pattern of teleost fish during a generalized feeding strike and enumerate the morphological components that comprise the major biomechanical systems. We then summarize what is known about the genetics and development of the craniofacial skeleton, with special reference to biomechanical linkages. Finally, we highlight a few areas where the study of the developmental genetics of jaw mechanics may provide novel insight. We focus on the biomechanics of jaws to the exclusion of the dentition. Interested readers should see Refs. 25–28 for review (also biteit.helsinki.fi/); and Refs. 29–36 for interesting new data on tooth shape and patterning. Several useful reviews of vertebrate craniofacial development have been published recently.^{37–44}

THE GENERALIZED TELEOST FEEDING STRIKE

Although there is incredible diversity of trophic habits of teleost fish, there is a relatively invariable pattern that characterizes a feeding event.^{1,2} Feeding strikes are generally initiated as the epaxial and hypaxial musculature contracts, resulting in levation of the neurocranium and contraction of the pectoral girdle (Fig. 2). As the neurocranium rotates upwards, the sternohyoideus muscle fires, resulting in depression of the hyoid. The depression of the hyoid causes expansion of the buccal cavity and in some fishes initiation of movement in the opercular linkage. As the opercular series

is pulled posteriorly, this causes tension to be applied to the interopercular ligament that connects the opercle to the lower jaw. The lower jaw is then depressed and rotates downward. The rotation of the lower jaw inputs motion into the anterior jaws linkage and results in rotation of the maxilla. Some combination of rotation of the maxilla coupled to cranial elevation and lower jaw rotation causes upper jaw protrusion in most teleosts. During these kinematic events, the fish creates suction pulling the prey into the oral cavity in close coordination with maximal jaw protrusion and oral jaw gape.

Once the prey is ingested the oral jaw begins to close. The adductor mandibulae are the primary oral jaw closing muscles and they generally attach onto the lower jaw and maxilla and power jaw closing. Relaxation of the hyoid, hypaxials, and epaxial musculature of the head may also be passively involved in the decrease in buccal volume and swallowing of prey. However, once the prey has been ingested it is moved to the pharyngeal apparatus for processing.³ The muscles operating during pharyngeal processing are diverse but include the levator externus IV, levator posterior, and retractor dorsalis. In most perciform fishes, the levator externus IV and levator posterior attach to and depress the upper pharyngeal jaws. In these fishes, the lower jaw is largely immobile during processing and provides a stable substrate for pharyngeal processing. However, in the now recognized paraphyletic group known as the Labroidei⁴⁵ several of these muscles attach onto the lower pharyngeal jaw, allowing the lower pharyngeal jaw to be pulled against the upper pharyngeal jaws during prey processing. After processing, prey are swallowed and sent to the digestive tract for digestion and absorption.

MECHANICAL SYSTEMS: MORPHOLOGY IN MOTION

Neurocranium rotation

The epaxial musculature transfers power to the neurocranium through the midpoint of its attachment on the neurocranium that can be modeled as the in-lever in this system⁴⁶ (Fig.

2a). As this muscle contracts, the neurocranium rotates around a joint formed between the post-temporal and supracleithrum in many fish. The out-lever to this system can be modeled as the length of the neurocranium. As the neurocranium is elevated, it functions to increase the buccal volume of the fish, initiate upper jaw protrusion, and provide input to the hyoid linkage system.

Hyoid linkage

The pectoral girdle of the fish forms the generally immobile fixed link in the hyoid linkage.¹⁰ As the neurocranium is elevated it pulls the hyomandibula dorsally and outwards operating as the input link for the system. The interhyal that connects the hyomandibula with the hypohyal then rotates anteriorly coupling the motion of the neurocranium to the output link. The input from this rotation that is augmented with contraction of the sternohyoideus muscle depresses the sternohyoideus and the urohyal that forms the tip to the output link of the linkage system.

Opercular linkage

Movement in the opercular linkage is initiated with contraction of the levator operculi muscles that rotates the opercle, the input to the opercular linkage^{5,47} (Fig. 2b). The linkage has a fixed link that extends from the quadrate-articular joint to the hyomandibula-opercle joint and is bordered by the pterygoids. Opercular rotation transfers a posteriorly directed force to the interopercle that functions as the coupler link in this four-bar mechanism. The output to this system is the posterior portion of the lower jaw that is depressed as the opercle rotates.

Lower jaw opening

The lower jaw is composed of the angular, articular, and dentary. As the interopercular ligament tightens its attachment on the descending process of the lower jaw, the generally fused articular and dentary swing open on the quadrate-articular joint (Fig. 2c). The length between this ligament and the joint generally serves as the opening in-lever to the lower jaw.⁷ In some groups of fishes such as parrotfish (*Scaridae*) and angelfish (*Pomacanthidae*), the

connection between the articular and dentary form a novel joint.^{6,48} However, in most teleosts, the dentary forms the tip of an out-lever that extends from the quadrate joint and this bone is the toothed portion of the lower jaw.

Anterior jaw four-bar linkage

As the lower jaw swings open, the other mobile elements in the anterior jaw four-bar linkage, the maxilla and nasal rotate⁹ (Fig. 2c). As the lower jaw rotates down, the maxilla, which serves as the output link, swings out and this motion is coupled via the rotation of the nasal, the coupler link, to the suspensorium. The suspensorial region between the quadrate-articular joint and the point that the nasal attaches on the neurocranium acts as the fixed link in this skeletal four-bar crank chain. The maxilla in many groups of fishes is toothed, but in most fishes the upper jaw teeth are confined to the premaxilla.¹

Jaw protrusion

The premaxilla is the toothed element that forms the upper jaw and it is frequently protruded during teleost feeding⁴⁹ (Fig. 2c). The premaxilla is pushed out as the maxilla swings outwards, when the neurocranium rotates posteriorly, and as the lower jaw is depressed. The ascending process of the premaxilla extends posteriorly and dorsally and frequently runs along the center of the cranium between the eyes. The descending process of the premaxilla is often ligamentously connected to the maxilla and is the toothed portion of this bone.

Lower jaw closing

The closing lever of the lower jaw is formed largely from the same bony elements as the opening lower jaw system⁷ (Fig. 2d). Although the out-lever is generally modeled as the same length of the articular and dentary as in the opening system, the closing lever can be roughly conceptualized as the length of the ascending arm of the articular.

However, this lever link is functionally modeled as the distance between where the adductor mandibulae attaches on the ascending arm to the quadrate-articular joint. The adductor mandibulae are the muscles that function to close the oral jaws. Most teleosts generally ex-

hibit three adductor mandibulae muscles that attach on several bones in the oral jaw apparatus.⁵⁰ These muscles have been repeatedly duplicated and lost during teleost evolution, making generalizations of how individual adductor mandibulae operate difficult.

A crushing jaw in the throat

Many teleosts exhibit a pharyngeal bite that involves the upper jaw being compressed against the lower pharyngeal arches through a series of lever systems incorporating the upper pharyngobranchials³ (Fig. 2e and f). In teleosts such as cichlids and other labroids, the upper pharyngeal jaws are composed of the second, third, and fourth pharyngobranchials. The upper pharyngeal jaws in these fish are buttressed by synovial joints on the neurocranium and serve as a platform against which the lower pharyngeal jaw applies forces. These elements are toothed in many groups of teleosts.

The lower pharyngeal jaw in most perciformes is composed of fifth ceratobranchials that are modified to varying degrees in different lineages of fishes³ (Fig. 2a). In most teleosts the paired fifth ceratobranchials are toothed but are separated structures. In the highly successful group of labroid fishes, these fifth ceratobranchials are fused either through suturing of the bone, as in cichlids, or the formation of a single bone in labrids. Muscles that normally insert on the upper pharyngobranchials in most teleosts insert on the fused fifth ceratobranchials in labroids.⁴ The size and shape of teeth on this lower pharyngeal jaw generally match the teeth on the upper pharyngeal jaws.

Independence of trophic mechanical systems

The mechanical modules described here are generally conceptualized as largely independent of one another.^{5,6} However, these systems are neither functionally nor anatomically modular (Fig. 2). For instance, the lower jaw functions in multiple linkage systems (*see* b–d) and the various functional lengths of this element must trade-off in complex ways accordingly. Although these biomechanical systems can be modeled to function in isolation, it is important to understand that they evolve as related parts of an integrated craniofacial skeleton.

DEVELOPMENTAL MODULES: MIGRATING CREST CELLS AND GENETIC MUTANTS

The head skeleton of vertebrates mainly comprises derivatives of the cranial neural crest, a transient migratory population of cells that are essentially unique to vertebrates.^{51–54} Seven pharyngeal arches exist in teleost fish, while mammals have lost or grossly modified pharyngeal arches 5, 6, and 7.⁵⁵ The neurocranium is derived from both cranial neural crest cells (CNCCs) and mesoderm, while the pharyngeal skeleton, which includes the jaws and branchial arches, derives solely from the CNCCs.⁵⁶ These CNCC populations originate and migrate from the developing anterior neural tube (neuroepithelium) in three major streams, the trigeminal (mandibular), hyoid, and branchial (postotic)⁵⁴ that target specific regions of the face and branchial arches.^{14,57}

The streams of CNCCs are generated from the midbrain and specific corresponding segments of the hindbrain, known as rhombomeres¹⁴ numbered from 1–7 (r1–7, Fig. 3a). These segments in turn, through CNCC contributions, correspond to the development of the anterior neurocranium and a segmented pharyngeal arch skeleton that develop originally as cartilages—from the oral jaws (pharyngeal arch 1) and supportive elements to the hyoid (pharyngeal arch 2) and posterior pharyngeal arches (pharyngeal arches 3–7). CNCCs not only contribute to the branchial and facial skeletal, but also adopt muscular, neuronal, glial, and pigment cell fates.^{14,54,56,58} Notably, Hox genes likely play a major role in the morphogenesis of the pharyngeal skeleton.⁵⁵ CNCCs that target the first arch of jawed vertebrates do not express Hox genes, whereas those that contribute to all posterior arches do.⁵² This has led to the controversial hypothesis that the origin of oral jaws was dependent on a Hox-negative environment.^{40,59}

Skeletal derivatives of the first arch include Meckel's cartilage (presumptive lower jaw) and the palatoquadrate (upper jaw). The second arch forms structures that include the ceratohyal, basihyal, interhyal cartilages and the hyosymplectic cartilages (i.e., hyoid skeleton). The posterior arches (3–7) form the remaining branchial

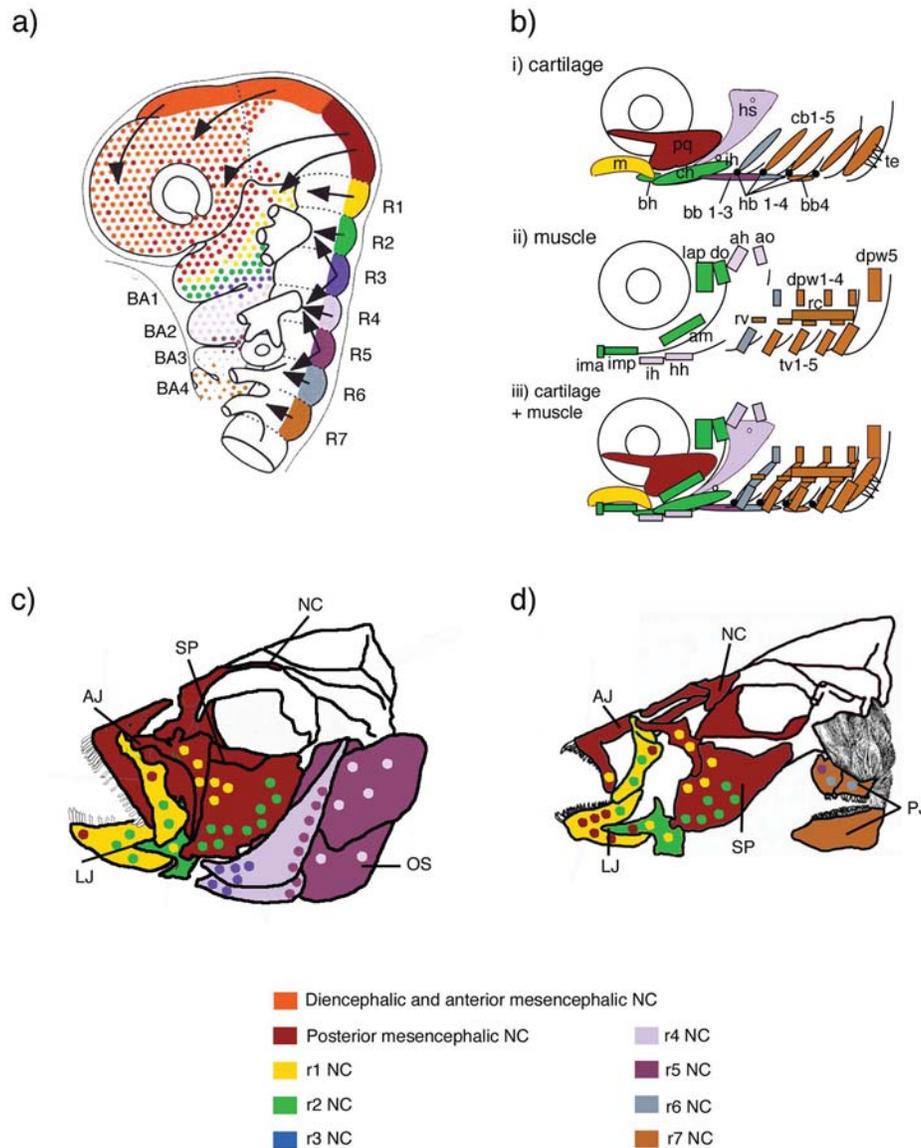


FIG. 3. Rhombomeric origins of cranial neural crest (CNCC) subpopulations in an embryo are depicted (a). Segmentation of the CNCC populations from the midbrain and rhombomeres 1–7 (r1–r7) target specific branchial arches (BA1–BA4 as shown) and aid in the formation of the craniofacial and branchial skeletal components [14, 52]. The diagram in (a) was modified from Ref. 52. The common segmental rhombomeric neural crest origins in cranial cartilages and muscles of the zebrafish larva are illustrated (b). The left side view of the craniofacial and branchial skeleton at 96 hpf (i), cranial and branchial muscles (ii), and the skeleton and muscles combined (iii) are shown. The colors indicate putative rhombomere specific CNCC involvement. The modified diagrams (i–iii) in (b), nomenclature and abbreviations are from Schilling and Kimmel.⁶⁰ Skeletal abbreviations: bb, basibranchial; bh, basihyal; cb, ceratobranchial; ch, ceratohyal; hb, hypobranchial; hs, hyosymplectic; ih, interhyal; mc, Meckel’s cartilage; pq, palatoquadrate; te, teeth. Muscle abbreviations: ah, adductor hyoideus; am, adductor mandibulae; ao, adductor operculi; do, dilator operculi; dpw, dorsal pharyngeal wall; hh, hyohyoideus; ih, interhyoideus; ima, intermandibularis anterior; imp, intermandibularis posterior; lap, levator arcus palatine; rc, rectus communis; rv, rectus ventralis; tv, transversus ventralis. Craniofacial skeletal morphology of two cichlid species (c, d) depicting putative skeletal fates of segmented CNCC subpopulations from the midbrain and r1–r7. Individual skeletal elements within mechanical systems and morphological modules are likely to be derived from multiple rhombomeric segments. Morphological modules: AJ, anterior jaw; SP, suspensorium; NC, neurocranium; LJ, lower jaw; PJ, pharyngeal jaw. Colored dots reflect the putative CNCC contributions to the specific skeletal elements in (c) and (d). The posterior elements of the neurocranium in (c) and (d) are not colored due to uncertainty of developmental origin.

skeleton, including the pharyngobranchials, epi-branchials, hypobranchials, basibranchials, and the ceratobranchial cartilages that are associated with the formation of the pharyngeal jaws in teleost fish (see Fig. 3b–d). Muscle and other connective tissues that originate from specific rhombomeres attach to corresponding skeletal elements of the same derivation, throughout the head and pectoral region.^{14,58,60}

Neurocranium

The teleost neurocranium originates from the collaboration of a number of cell types, which are mainly CNCCs and mesodermal cells. The skeletal elements (cartilage and bone) that form the anterior neurocranium (i.e., vomerine region) are derived from CNCCs that migrate from the posterior midbrain and r1. Less is known about the developmental origin of the posterior neurocranium.⁵⁶ Many mutations affecting the developing neurocranium of zebrafish (*D. rerio*) were reported by Schilling et al.⁶¹ and by Piotrowski⁶² (Table 1). A number of other genes have a major influence on the development of the neurocranium in vertebrates including *Fgf8*,^{63,64} *Dlx5*,⁶⁵ and *Shh*.⁶⁴

The hyoid elements and the opercular series

The CNCCs that migrate to the second pharyngeal arch generate the skeletal elements of the hyoid and opercular series. CNCCs that target the second pharyngeal arch originate from r4, with some contributions of crest cells originating from r3 and r5 (Fig. 3).^{56,60,66} Genes implicated in the development of the hyoid and opercular skeleton include *Otx2*,⁶⁷ *Hox-a1*,⁶⁸ *Hox-a2*,⁶⁹ *Endothelin-1 (Edn1)*,⁷⁰ *Pbx1*,⁷¹ and *Fgf8*,⁶³ along with a number of genetic mutations that affect the development of the hyoid elements in zebrafish^{61,62} (see Table 1).

Oral jaws

The CNCCs that migrate to the first pharyngeal arch produce the upper and lower jaw cartilages (Fig. 3). These crest cells originate from the combined levels of the posterior midbrain, r1 and r2.¹⁴ In the zebrafish embryo, the CNCCs that contribute to the formation of the

mandibular cartilages derive from rhombomeres 1–3.⁷² Numerous genes are implicated in the development of the oral jaw elements. These include (but are not limited to) *Dlx* genes,^{73,74} *Msx1* and *Msx2*,^{75–77} *Pax9*,⁷⁸ *gooseoid*,⁷⁹ *Fgf8*,⁶³ *Barx1*,⁸⁰ *Bmp2* and *Bmp4*,⁸¹ and *Pitx1*,⁸² along with a number of genetic mutations which cause defects of the oral jaw elements, identified in *D. rerio*^{61,62} (see Table 1).

Pharyngeal jaws

The pharyngeal jaws are located in the posterior-most regions of the pharyngeal skeleton. The CNCCs that contribute to this jaw are thought to arise at the level of r7 (see Fig. 3a, b, and d). Genes involved in the formation of these elements include *Fgf3*,⁸³ *Hox-a1*,⁶⁸ and a number of genetic mutants described by Schilling et al.⁶¹ and Piotrowski et al.⁶² (see Table 1).

INDEPENDENCE OF DEVELOPMENTAL MODULES

The most striking feature of the genetic and developmental data we have reviewed is the mixture of both modularity and integration in the construction of the vertebrate head. Loss of function mutations for various genes invariably affects multiple structures derived from different pharyngeal arches. For example, Piotrowski et al.⁶² identified only five of 100 zebrafish mutants defective in anterior arches with no posterior arch phenotype. Similarly, although CNCCs migrate from the hindbrain according to well-defined pathways, mixing of crest from different axial origin in the development of the embryonic skeleton is apparent.^{14,84} Despite the pervasive trend of genetic and developmental integration, there is also evidence of compartmentalized effects on skeletal elements. Mutants for *Pax9*, *Bapx1*, *Dlx1* and *Dlx2*, and *Fgf8* target proximal first arch derivatives while those for *Msx1* and *Msx2*, *Fgf4*, and *Otx2* target distal elements. Similarly, *Dlx* molecules and *Edn1* are involved in patterning of dorsal versus ventral structures of the pharyngeal skeleton. A shift in focus toward regulatory mutations, such as those segregating in

TABLE 1. LINKING MECHANICS, MORPHOLOGICAL MODULES, AND PUTATIVE LOCI

<i>Mechanical system</i>	<i>Morphological modules</i>	<i>Genes</i> ^{a,b}
<i>Neurocranium lever</i>	NC	a) <i>Bab</i> [61], <i>Cod</i> [61], <i>Dlx1</i> [73], <i>Dlx2</i> [73, 74], <i>Dlx5</i> [65, 74], <i>Dlx6</i> [74], <i>Dol</i> [62], <i>Fgf8</i> [63, 64], <i>Fla</i> [61], <i>Gsc</i> [79], <i>Ham</i> [62], <i>Hen</i> [62], <i>Her</i> [61], <i>Hoxal</i> [68], <i>Msx2</i> [103, 104], <i>Pax9</i> [78], <i>Pek</i> [62], <i>Ser</i> [61], <i>Shh</i> [64, 105], <i>Tfap2a</i> [61, 106], <i>To259b</i> [62], <i>Twist</i> [104], <i>Vgo</i> [62]
	NC	a)
<i>Hyoid linkage</i>	HY	b) <i>Bab</i> [61], <i>beta-cat</i> [107], <i>Cod</i> [61], <i>Dlx1</i> [73], <i>Dlx2</i> [73], <i>Dul</i> [61], <i>Her</i> [61], <i>Edn1</i> [62], <i>Fgf8</i> [63], <i>Fla</i> [61], <i>Foxl1</i> [108], <i>Hot</i> [62], <i>Hoxa2</i> [52], <i>Moz</i> [109], <i>Otx2</i> [67], <i>Pbx1</i> [71], <i>Ppt</i> [62], <i>Prx1</i> [110], <i>Prx2</i> [110], <i>Ser</i> [61], <i>She</i> [62], <i>Tfap2a</i> [61, 106], <i>To259b</i> [62], <i>Tq5</i> [61], <i>Vgo</i> [62]
	PG	<i>Emx2</i> [58], <i>Jef</i> [58], <i>Pbx1</i> [58], <i>Pax1</i> [58]
	OS	c) <i>Edn1</i> [70], <i>Hoxal</i> [68], <i>Hoxa3</i> [52], <i>Hoxb4</i> [52]
<i>Opercular linkage</i>	SP	d) <i>Fgf8</i> [64], <i>Gsc</i> [79], <i>Hoo</i> [62], <i>Ham</i> [62], <i>Moz</i> [109], <i>Otx2</i> [67], <i>She</i> [62], <i>Shh</i> [62], <i>Tfap2a</i> [61, 106], <i>To259b</i> [62], <i>Tq5</i> [61], <i>Tx224</i> [62]
	AJ	e) <i>Bab</i> [61], <i>Bapx1</i> [111, 112], <i>Barx1</i> [80, 113], <i>beta-cat</i> [107], <i>Bmp2</i> [81, 114], <i>Bmp4</i> [24, 81, 114], <i>Bmp4</i> [24, 81, 114], <i>Cod</i> [61], <i>Dul</i> [61], <i>Dlx1</i> [73], <i>Dlx2</i> [73, 74, 115], <i>Dlx3</i> [73], <i>Dlx5</i> [65, 73, 74, 115], <i>Dlx6</i> [73, 74], <i>Dol</i> [62], <i>Fgf4</i> [116], <i>Fgf8</i> [63], <i>Hot</i> [62], <i>Ham</i> [62], <i>Hoo</i> [68], <i>Hoxal</i> [68], <i>Gsc</i> [79], <i>Jef</i> [62], <i>Lhx6</i> [117], <i>Lhx7</i> [117], <i>Otx2</i> [67], <i>Pax9c</i> [78], <i>Pitx1</i> [82], <i>Pitx2</i> [118], <i>Ppt</i> [62], <i>Prx1</i> [110], <i>Prx2</i> [110], <i>She</i> [62], <i>Suc</i> [62], <i>Stu</i> [62], <i>Tfap2a</i> [61, 106], <i>To259b</i> [62], <i>Tx224</i> [62], <i>Vgo</i> [62]
	OS	c)
	SP	d)
	LJ	e)
<i>Lower jaw opening lever</i>	LJ	e)
	AJ	f) <i>Barx1</i> [80, 113], <i>beta-cat</i> [107], <i>Bmp2</i> [81, 114], <i>Dlx2</i> [74, 115], <i>Dlx3</i> [73], <i>Dlx5</i> [73, 74], <i>Dlx6</i> [73, 74], <i>Dol</i> [62], <i>Fgf4</i> [116], <i>Fgf8</i> [63, 64], <i>Gsc</i> [79], <i>Ham</i> [62], <i>Hen</i> [62], <i>Hoxal</i> [68], <i>Jef</i> [62], <i>Lhx6</i> [117], <i>Lhx7</i> [117], <i>Pek</i> [62], <i>Pitx1</i> [82], <i>Pitx2</i> [118], <i>Prx1</i> [110], <i>Prx2</i> [110], <i>Shh</i> [64]
	NC	a)
	SP	c)
	LJ	e)
<i>Anterior jaw linkage</i>	AJ	f) <i>Barx1</i> [80, 113], <i>beta-cat</i> [107], <i>Bmp2</i> [81, 114], <i>Dlx2</i> [74, 115], <i>Dlx3</i> [73], <i>Dlx5</i> [73, 74], <i>Dlx6</i> [73, 74], <i>Dol</i> [62], <i>Fgf4</i> [116], <i>Fgf8</i> [63, 64], <i>Gsc</i> [79], <i>Ham</i> [62], <i>Hen</i> [62], <i>Hoxal</i> [68], <i>Jef</i> [62], <i>Lhx6</i> [117], <i>Lhx7</i> [117], <i>Pek</i> [62], <i>Pitx1</i> [82], <i>Pitx2</i> [118], <i>Prx1</i> [110], <i>Prx2</i> [110], <i>Shh</i> [64]
	NC	a)
<i>Jaw protrusion</i>	AJ	f)
	NC	a)
<i>Lower jaw closing lever</i>	LJ	e)
	LJ	e)
<i>Pharyngeal jaw</i>	LJ	<i>Bab</i> [61], <i>Cod</i> [61], <i>Dul</i> [61], <i>Fac</i> [61], <i>Fla</i> [61], <i>Fgf3</i> [83, 119], <i>Hoxal</i> [68], <i>Jef</i> [62], <i>Ser</i> [62], <i>Suc</i> [62], <i>Tfap2a</i> [61, 106], <i>Tq5</i> [61], <i>Vgo</i> [62]

NC, neurocranium; HY, hyoid; PG, pectoral girdle; OS, opercular series; SP, suspensorium; LJ, low jaw; AJ, anterior jaw; PJ, pharyngeal jaw.

^aSubscripts are citations to primary literature.

^bGenes putatively influencing a particular morphological module are indicated with a letter if the module is used in multiple mechanical systems.

natural populations,^{24,85,86} may greatly improve our understanding of modular effects on craniofacial phenotypes.

FUTURE DIRECTIONS: INTEGRATING BIOMECHANICS AND EVOLUTIONARY DEVELOPMENT

The teleost craniofacial skeleton is a unique system in which to study the evolution and development of functional morphology. Jaws (i) have been modeled as biomechanical systems for decades, (ii) are well studied in terms of genetics and development, and (iii) exhibit tremendous functional diversity in lineages amenable to genetic and developmental analysis. Despite these advantages, few studies have addressed the genetic and developmental basis of biomechanical design. The reasons for this are obvious, but not intractable: functional morphologists and developmental biologists speak different languages but are both interested in the evolution of biological form. Functional morphologists who study biomechanics are interested in the component links of adult functional anatomies; developmental geneticists are interested in discrete skeletal elements of developing embryos. For instance, it would not be apparent to a developmental biologist to study the fixed link of the anterior four-bar linkage model (D in Fig. 1b), because this length is comprised of multiple skeletal elements. Similarly, it is not conventional for a functional morphologist to atomize the lower jaw out-lever into its component parts (Fig. 1a) although the dentary and articular are likely under distinct genetic and developmental control. Genetic and developmental studies of explicitly functional systems are likely to provide novel insights concerning phenotypic evolution. We highlight three future research directions of interest.

The genetic basis of simple versus complex lever systems

Understanding the evolution of complex systems is a major objective of contemporary research in biology and related sciences.⁸⁷⁻⁸⁹ It is believed that complexity is a general emergent

property of design principles in evolution and engineering, and that complex systems are qualitatively different from simpler ones.⁸⁹ The distinction between complex and simple systems has been illustrated using biomechanical models that predict force and kinematic transmission (KT) in the oral jaws of bony fishes⁹⁰ (Fig. 1). Simple lever models of the lower jaw exhibit a one-to-one relationship between form and function; proportional differences in the lengths of component bones produce proportional differences in KT. By contrast, more complex models of anterior jaw mechanics show a nonlinear, many-to-one mapping of form to function. Four-bar configurations with different shapes can yield the same KT.⁹⁰ Knowledge of how form maps to function is a first step to understand how simple and complex biomechanical systems evolve, yet we know almost nothing about the genetic and developmental control of these systems. Albertson and colleagues demonstrated that the simple biomechanical system of the cichlid lower jaw is surprisingly complex at the genetic level.²⁴ Applying similar approaches to study four-bar linkage systems in the same skull (Fig. 2), one can ask if the genetic architecture of simple mechanical systems is distinct from that of more complex ones.

Modular and integrated evolution of the trophic apparatus

The degree to which elements of functional systems evolve independently (modularity) or in concert (integration) affects the 'evolvability' of lineages.^{91,92} For instance, the 'decoupling' of oral and pharyngeal jaws has been implicated in the species richness of certain teleost groups.⁹³ Explicit evolutionary tests of modularity can now be performed using quantitative genetic^{24,94} and phylogenetic comparative methods (e.g., Hulsey, unpublished). Developmental studies of the independence of jaw elements can be explored to provide a mechanistic understanding of these patterns.⁴⁰

The mutational spectrum and rapid correlated evolution

Recent research has changed the way that evolutionary biologists think about the molec-

ular basis of functional diversification and adaptation.^{85,95} By contrast to mutations in amino acid sequence with large pleiotropic effects (e.g., Table 1), it is reasoned that rapid microevolutionary divergence probably occurs by noncoding mutations that target tissue specific regulatory modules.⁹⁶ This idea is so compelling that it has gained popular attention in publications such as *The New Yorker* (October 24, 2005). And yet, the patterns of pleiotropy for genes involved in craniofacial development correspond well with divergent phenotypes related to feeding biology in teleost lineages (e.g., the cichlids of East Africa). Sets of genes (e.g., Bmp, Dlx, Msx, Pax, Wnt, Shh, Fgf, and Barx) have concerted effects on jaws, muscles, brains, dentitions, guts, and limbs/fins.^{97–100} These concerted changes tend to mimic the very morphologies that differentiate closely related cichlid species with contrasting feeding strategies.^{11,24,30,101,102} Pleiotropic mutations to genes in key molecular pathways, or patterns of tight linkage, might therefore explain the correlated evolution of phenotypes in explosively radiating lineages. This hypothesis is readily tested by (i) broad surveys of character correlation among trophically diverse but related species, (ii) genetic crosses among species segregating differences in brain, jaw, dental and fin morphology, and (iii) analyses of candidate gene expression in embryos.

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