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SYMPOSIUM

Axial Elongation in Fishes: Using Morphological Approaches to Elucidate Developmental Mechanisms in Studying Body Shape

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Synopsis One of the most notable features in looking across fishes is their diversity of body shape and size. Extant actinopterygian fishes range in shape from nearly spheroidal in pufferfishes to extremely elongate in snipe eels with nearly every shape in-between. One extreme along the body-shape continuum is a highly elongate form, which has evolved multiple times independently in Actinopterygii. Thus, comparison of these separate (independent) radiations provides a unique opportunity for examining the anatomical traits underlying elongation as well as the similarities and differences in the evolutionary pathways followed. Body elongation generally evolves via an increase in region-specific vertebral number, although certain lineages elongate via an increase in vertebral length. In this study, we describe how anatomical characters related to feeding and locomotion are correlated with elongation of the body across Actinopterygii. In addition to modifications of the postcranial axial skeleton, elongation in fishes is often accompanied by an increase in head length, loss of the pelvic fins, reduction of the pectoral fins, and expansion of the median fins. Based on anatomical studies and on recent studies of developmental control of the body axis in different species, we hypothesize how an axial trait might change at the genetic level. Overall, we discuss the evolution of body elongation in fishes in light of an understanding of the underlying anatomical modifications, developmental control, ecology, and locomotion.

Introduction

Fishes come in all shapes and sizes. They range in size over three orders of magnitude from less than 1 cm in *Paedocypris progenetica* (Kottelat et al. 2006) to 1100 cm in *Regalecus glesne* (King of herrings, Eschmeyer et al. 1983). The range in shape is just as dramatic, from nearly spheroidal in *Sphoeroides maculates* (green-spotted pufferfish) to highly snake-like in *Nemichthys scolopaceus* (snipe eel). Body shape and length in fishes, influenced by evolutionary history, has an effect on swimming style, escape response, and microhabitat use. Many studies have focused on the differences in shape either within a single species or among closely related species. In particular, sticklebacks have been a rich system for considering how body shapes diverge within a single species in relation to its ecology and in an evolutionary context (Walker and Bell 2000, Reid and Peichel 2010). In this work, we consider a single body shape across fishes and discuss it in relation to locomotion, ecology, anatomy, and underlying developmental control.

Body elongation in actinopterygian fishes

On one extreme of the body-shape continuum is a highly elongate form, which describes over 15% of extant actinopterygian fishes. Highly elongate species are found in many lineages of fishes although this form is most typical of true eels, Anguilliformes, the clade that gave name to the eel-like or 'anguilliform' body plan. There are two different types of elongation: anguilliform elongation and a stiffer-bodied elongation as observed in barracuda, needlefish,

and trumpetfishes. The eel-like body plan is found in a number of other actinopterygian groups including, but not limited to, Siluriformes (catfish), Stomiiformes (dragonfishes), Ophidiiformes (cusk-eels), Sygnathidae (pipefish), Synbranchiformes (swamp eels), and Zoarcoidei (eelpouts and relatives). As with other body shapes, elongate forms have specific swimming modes and occupy a diversity of habitats.

Locomotion

Many highly elongate fishes move by anguilliform locomotion, which Breder (1926) defined as a serpentine-like motion, indicating that there is at least one wave present on the body. Despite previous descriptions of anguilliform locomotion being inefficient (Webb 1975), Tytell (2004) and Tytell and Lauder (2004) described a locomotory system in American eels (*Anguilla rostrata*) in which efficiencies were within the range of other steadily swimming fish, including mackerel and rainbow trout (Nauen and Lauder 2002a, 2002b). Liao (2002) noted that during slow locomotion in the stiff-bodied needlefish (*Strongylura marina*), the pectoral fins oscillate rapidly, which is likely not producing thrust, but may be aiding in controlling swimming speed. *Erpetoichthys calabaricus* (ropefish) also oscillate the fins rapidly during slow locomotion (Ward, personal observation). Many elongate fishes, including American eels, also locomote terrestrially (Gillis 1998).

While most fishes perform a stereotyped C-start when startled by a potential predator (Eaton et al. 1977), highly elongate fishes perform a retraction response (Meyers et al. 1998, Bierman et al. 2004, Ward and Azizi, 2004). One elongate species that does do a C-start is the muskellunge (*Esox masquinongy*), which is a stiff-bodied elongate species (Hale 2002). A retraction response is quite distinct from a C-start in two main ways. First, there are multiple bends on the body instead of the single bend used for a C-start. It has been assumed that increased vertebral number would also increase flexibility, thereby allowing the fish to have multiple bends in the body (Ward and Azizi 2004). Secondly, the retraction response does not include a propulsive phase, meaning that the fish does not swim away from the stimulus. Instead, the animal flees its predator by retracting back into its environment. The retraction response not only appears to occur in fishes that are considered elongate but these fishes are also found in more structured habitats. In performing the retraction response, an elongate fish retreats into its dense habitat effectively hiding from potential predators. This is distinct from C-starts in which the startled fish outswims the potential predator.

Habitat

Highly elongate fish occupy a number of different habitats, including open water, coral reefs, sand, and sea grass beds. Anguilliform eels are found in a number of different habitats (described in this volume by Mehta et al. 2010). *Moringua edwardsi*, the spaghetti eel, exhibits morphological specializations for burrowing into sediments (DeSchepper et al. 2005). More elongate species of *Luciogobius* are specialists of small-gravel interstitial habitats (Yamada et al. 2009). Many elongate forms tend to be associated with structure (Webb 1982, Ward and Azizi 2004). For example, sargassum pipefish are extremely cryptic among the floating tangle of sargassum while trumpetfish often orient themselves vertically among branches of corals or even tall blades of seagrass.

Anatomical changes associated with elongation of the body

Elongation occurs by lengthening the primary axis of the body relative to the other two body axes (width and depth). Increasing the primary axis could occur by lengthening either the cranial or post-cranial axial skeleton, or a combination of both. These changes would primarily involve skeletal changes although postcranial axial musculature would also be affected in concert with changes in the bony skeleton (Gans 1975, Danos et al. 2008). Highly elongate fishes have a number of modifications of their body plan compared to closely related, stout-bodied species, including modifications to both the paired and median fins.

Post-cranial axial modifications

The most dramatic anatomical changes in an elongate body form occur in the axial skeleton; in particular, the structure of the vertebral column differs significantly between closely related non-elongate and elongate forms. Elongation of the axial skeleton could result from either of two major causes: increase in vertebral number or increase in vertebral length (both are shown in Fig. 1). Overall, elongate fishes tend to have more vertebrae (Fig. 1; *Lepidopus caudatus*). Increases in vertebral length occur in some elongate lineages, but not in all (Ward and Brainerd 2007; Fig. 1: *Sphyraena barrracuda*). Increases in both vertebral number and vertebral

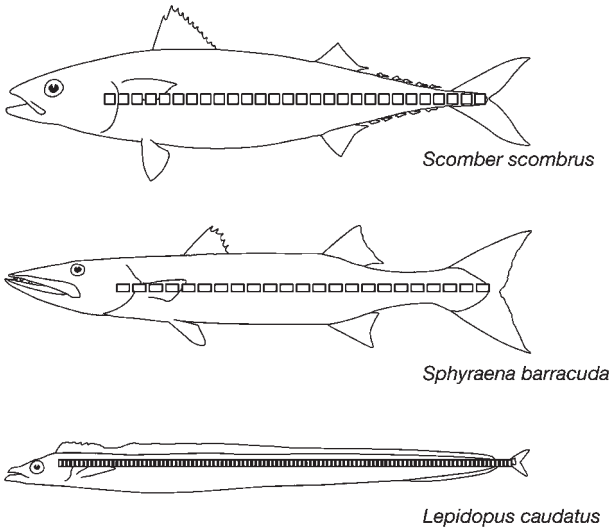

Fig. 1 Elongation of the body due to changes in the axial skeleton. More elongate fishes tend to have more vertebrae or longer vertebrae. Axial elongation in *S. barracuda* occurs by lengthening of the individual centra. Elongation in *L. caudatus* occurs through an increase in overall vertebral number.

length have been observed in other vertebrate groups, including snakes and salamanders (Johnson 1955, Wake 1966, Parra-Olea and Wake 2001, Polly et al. 2001). Previous studies of tetrapods have indicated that vertebral number changes in a region-specific manner (Polly et al. 2001, Müller et al. 2010). In comparison to tetrapods, fishes are considered to have a relatively simplified axial skeleton with only two regions: abdominal (or precaudal) and caudal (Grande and Bemis 1998). Therefore, both vertebral length and vertebral number should be considered in both regions to determine how and where each trait is changing.

Vertebral number in vertebrates has a large range, from eight in some anurans to over 600 in snipe eels (*Nemichthys scolopaceus*; Beebe and Crane 1937, Trueb 1973, Handrigan and Wassersug 2007). In fishes, Tetraodontiformes (pufferfishes and their allies) have the fewest number of vertebrae with 16 in several species including *Carinotetraodon lorteti*, *Sphoeroides dorsalis*, and *Masturus lanceolatus* (Tyler 1980). Vertebral number has long been used as a characteristic in identifying different species as well as a character for systematic analyses (Ford 1937, Tyler 1980, Birdsong et al. 1988); therefore, there is a wealth of information on vertebral number in many species. Using this literature reservoir, Ward and Brainerd (2007) collected abdominal and caudal vertebral numbers from 813 species (encompassing 14 different orders) of chondrichthyan and actinopterygian fishes and found that increases in vertebral number is region-specific for most groups (Fig. 2). It should be noted that this analysis did not include phylogenetic correction due to the lack of known relationships for the species included. In both Chondrichthys and Actinopterygii, there are groups that increase only abdominal vertebral number, groups that increase only caudal vertebral number, and groups that increase equally in both regions.

In a more detailed phylogenetic analysis of selected lineages of actinopterygian fishes, it was found that the largest range in vertebral number occurs in the caudal region. While some groups add abdominal vertebrae, along with slight increases in caudal vertebral number, only Polypteriformes increase the number of abdominal vertebrae without any increase in caudal vertebrae (Ward and Brainerd 2007). Based on this study, vertebral number is modular with respect to the two body regions, indicating that developmental control of abdominal vertebral number differs from control of caudal vertebral number, as shown previously in other vertebrate groups (Polly et al. 2001, Narita and Kuratani 2005, Müller et al., 2010). Changes in somitic level of transition points within the vertebral column are considered to be homeotic changes (see Developmental Control of Elongation of the Body).

Axial elongation could also occur due to an increase in length of the individual vertebrae. The addition of length to the axial skeleton by lengthening the individual vertebrae, rather than by adding vertebral centra, will likely result in decreased body flexibility by having fewer intervertebral joints (Brainerd and Patek 1998, Long et al. 2004). Lengthening of the vertebrae has been shown previously in salamanders and snakes. *Lineatriton lineolus*, a plethodontid salamander, elongates by increasing the length of individual vertebrae (Parra-Olea and Wake 2001). Johnson (1955) demonstrated that axial elongation in vine-like arboreal snakes is generally due to increased vertebral length. This results in greater rigidity of the body and presumably is an adaptation for moving across gaps between small branches (i.e., cantilevering abilities; Lillywhite et al. 2000). Body elongation due to an increase in length of the centrum is not widespread in fishes. For example, *Sphyraena barracuda*, has one of the greatest vertebral aspect ratios (centrum length/width) in fishes (Ward and Brainerd 2007; Fig. 1). When length of the centrum does increase within a lineage, the increase is equivalent in both regions of the axial skeleton, indicating that the control of centrum length is likely different from the control of vertebral number. The number of vertebrae is under regional control

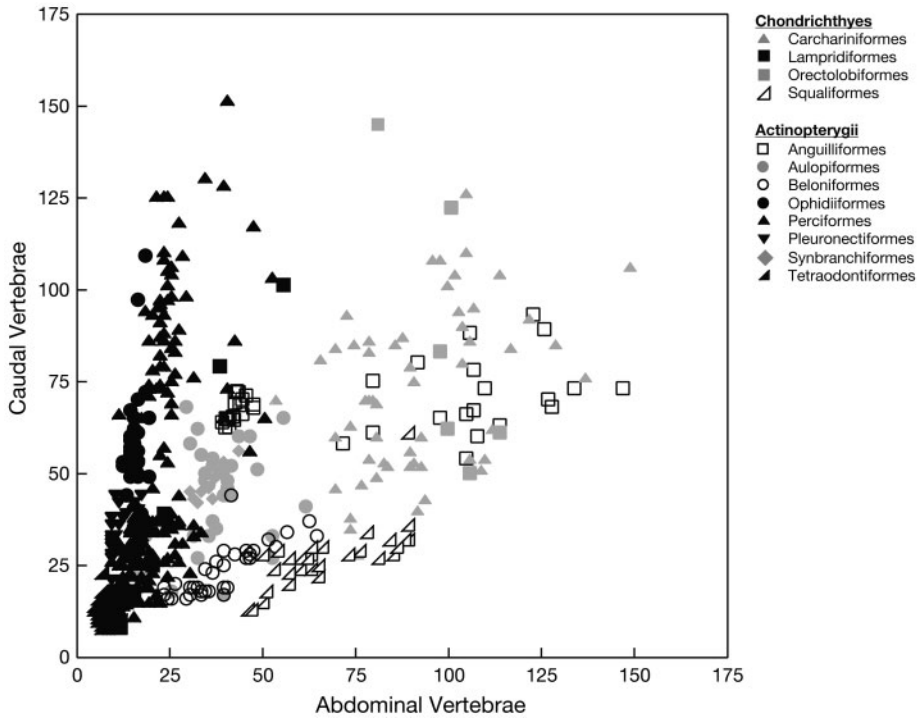

Fig. 2 Regionalization in vertebral number (modified from Ward and Brainerd, 2007). Species were grouped based on their orders. Each point indicates the maximum abdominal and caudal vertebral number for a specific species. Black symbols indicate groups that add caudal vertebrae only ($m > 1$). Gray symbols indicate groups that add equally to both regions ($m = 1$). Open symbols indicate groups that add abdominal vertebrae only ($m < 1$).

whereas changes in centrum length are controlled on a global level, possibly during somitogenesis. However, ossification of the centra occurs later in development and has been shown to be distinct from earlier segmentation in zebrafish and salmon (van Eeden et al. 1998, Grotmol et al. 2003, 2005).

Cranial modifications

Elongation of the body is strongly correlated with length of the head, indicating that more elongate fishes tend to have longer skulls (Fig. 3). There is incredible morphological diversity in the teleost skull; thus, fish have elongated their skulls in many different ways. In this study, we define head length as the distance from the tip of the snout to the posterior edge of the neurocranium. Although elongate bodies typically bear longer skulls, there are many exceptions in teleosts. In Anguilliformes, the overall pattern is that head length is not strongly correlated with body length. However, there are some anguilliform species (see below) that have very long heads as well as long bodies. The congrid, *Heteroconger hassi* and the ophichthid, *Myrophis vafer* are two examples of eels that are highly elongate, but have short skulls (Mehta et al., 2010). Synbranchids (swamp eels), another group that has converged on the elongate eel-like body plan, stichaeids (pricklebacks) and anarhichadids (wolffishes) are other examples of elongate fishes with relatively short heads (Nelson 2006, this study).

What skeletal parts contribute to overall elongation of the head? The teleost skull is comprised of over 20 independently moveable skeletal elements so it is probably not surprising that we find various combinations of skeletal components contributing to elongation of the skull (Westneat 2006). These skeletal combinations change with phylogeny as well as with feeding mode. Here, we discuss taxa that highlight some of the existing cranial diversity in elongate teleosts.

The preorbital region of the skull, the postorbital region, or both, can elongate. In the preorbital region we commonly tend to see elongation of the dentary, nasal, vomers, palatine, pterygoid, and parasphenoid. Of these characters, the most common mode of elongation of the skull is simply addition of length to the upper and lower jaws. Adding length to the upper jaws usually coincides with the reduction, fusion, and reinforcement of bony elements surrounding the upper jaws so that the skull is less kinetic. Two such examples of extreme elongation of the upper and lower jaws (the longest skulls in our

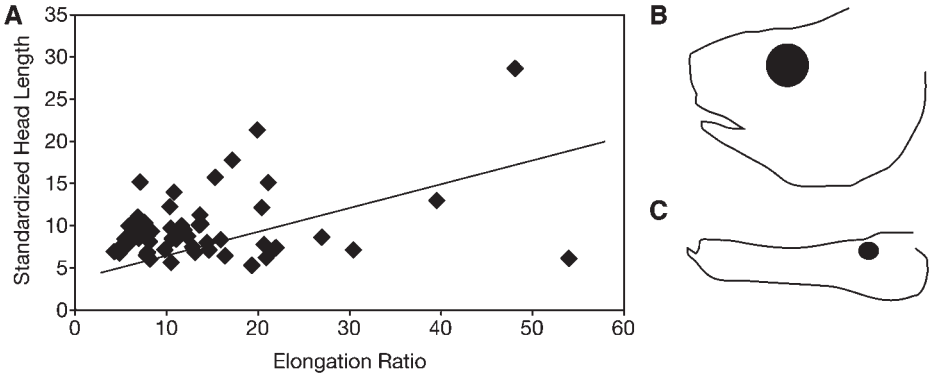

Fig. 3 Length of the head as a factor in elongation of the body. Reduced major-axis regression of head length and elongation ratio in 53 species of actinopterygian fishes (see Appendix 1 for a list of species). Head length was defined as the straight-line distance between the rostrum and the posterior limit of the occipital region of the skull. To correct for differences in size, head length was standardized by the cube root of body mass of that specimen. Elongation ratio was defined as standard length/second largest body axis (Ward and Azizi, 2004). Each measurement was averaged for three individuals of each species studied. Fishes with elongated bodies also tend to have longer heads. Regression statistics: $R = 0.34$, $m = 0.16$, $b = 7.38$. (**B**) Outline of the skull of *Coryphaenoides acrolepis* to show postorbital lengthening. (**C**) Outline of the skull of *Aulostomus maculatus* to show preorbital lengthening.

dataset) are Bean's sawtoothed eel, *Serrivomer beanii* (Anguilliformes: Serrivomeridae) and the bobtail eel, *Cyema atra* (Saccopharyngiformes: Cyematidae). In both of these species, the jaws are extremely long and narrow. In the case of *C. atra*, the jaws are quite delicate and the overall jaw length is greater than half of the length of the skull. In sphyraenids (Grubich et al. 2008) and trichiurids (DeSchepper et al. 2008) the head is also elongated by a lengthening of the upper and lower jaws (Vertebral morphology shown in Fig. 1). The bristlemouth, *Gonostoma elongatum* (Stomiiformes: Gonostomatidae) exhibits an elongate skull by way of the oral jaws and also by elongation of the palatine, parasphenoid, and pterygoid series. The Pacific Grenadier, *Coryphaenoides acrolepis* (Gadiformes: Macrouridae; Fig. 3B) exhibits a flattened and enlarged opercular series which provide length to the postorbital region of the skull. The opercular series also contributes to lengthening the postorbital region of the skull in the Korean sand eel, *Hypoptychus dybowskii* (Gasterosteiformes: Hypoptychidae); these fishes also have longer dentaries.

Extension of the preorbital region can occur by elongation of the jaws or by lengthening the lacrimal and frontal bones. A good example of a species with a long preorbital region and surprisingly short jaws is the tube snout, *Aulostomus maculatus* (Gasterosteiformes: Aulorhynchidae; Fig. 3C). Tube snouts have a very small gape and short jaws but a very elongate quadrate bone that lies in the preorbital region of the skull. The interopercle and preopercle are also unusually elongate and although their posterior connections with the hyomandibula and the opercle are maintained in the postorbital portion of the skull, their articulation with the articular is well in front of the orbit (Kaufman 1976, Hey-Aronson 1983).

There are several elongate deep-sea species, about which we know very little, that appear to possess short heads. However, their lower jaws are angled upwards making the dentary very long as in viperfish, *Chauliodus* and scaly dragon fish, *Stomias* (Stomiiformes: Stomiidae). Although we see the dentary angled dorsally in some Osteoglossmorphs such as in arowanas and arapaimas, the head is lengthened due to enlargement of the circumorbital plates, resulting in elongation of the postorbital region of the skull (Nelson 2006).

As exemplified by the elongate taxa in this dataset, there are many different ways of lengthening the skull. Why some lineages simply elongate the jaws while other lineages appear to undergo radical enlargement of other skeletal regions, aside from the jaws, is completely unknown. For example, is postorbital elongation of the skull more common in those species that rely on jaw protrusion for feeding? The fish skull accomplishes several critical functions, only one of which is capturing prey (Liem 1980). Future research examining how feeding mode, sensory modality, and respiration factor into this observed diversity will enable us to better understand the patterns of cranial evolution within and across different elongate groups.

Modifications of fins

While not actually contributing to elongation of the body, reduction and loss of limbs are the anatomical

correlates most often associated with body elongation in tetrapods. Loss and reduction of limbs has occurred both within amphibians and lepidosaurs, including *Siren*, caecilians, amphisbaenians, and snakes (Gans 1975, Lande 1978, Greer 1991). Elongate squamates have smaller limbs regardless of whether they had relatively shorter or longer tails (Wiens et al. 2006).

Fewer investigators have focused on the relationship between size or presence of the paired fins and body shape in fishes. Nelson (1989–90) described loss of pelvic fins in fishes, noting that there did not appear to be a phylogenetic component to such loss. Instead, loss of fins occurs at multiple points throughout the evolutionary history of fishes. The pelvic fins are not present in species that demonstrate a wide range of body shapes, although more elongate fish tend to have lost the pelvic fins. Members of both the Anguilliformes and Tetraodontiformes lack pelvic fins; these two groups demonstrate the greatest range in body shape of any group of fishes (Winterbottom 1974, Tyler 1980, Belouze 2002, Nelson 2006). Lack of the pectoral fins occurs in members of a few lineages including Heterenchelyidae (mud eels) and Ophichthidae (and worm eels). The anguilliform group, Muraenidae (moray eels), is the only clade whose members have universally lost the pectoral fins (Bohlke et al. 1989). At least one species is known to vary intraspecifically in presence/absence of the pectoral fins (*Channallabes apus*, a clariid catfish; Adriaens et al. 2002).

Fewer actinopterygian fishes have lost the pectoral fins than have lost the pelvic fins and many highly elongate actinopterygian species tend to have smaller pectoral fins than do non-elongate species (Fig. 4A and B). We measured dimensions of the body and fins from one representative of each of the 53 different families that Nelson (2006) indicated as having at least some elongate representatives (see Appendix 1 for a list of species). This analysis was not meant to show diversity of fin shape within a closely related group (here defined as the family level), but instead to show fin diversity across fishes. Length of the pectoral and pelvic fins was defined as the length of the marginal fin ray. Fin widths were defined as maximal span of the fin rays. We found a significant negative correlation between elongation of the body and both the length and width of the pectoral fin (Fig. 4A and B), but no significant correlation with length and width of the pelvic fin (data not shown). More elongate fishes tend to have smaller pectoral fins, but the relationship between size of the pelvic fin and body shape is not as clear. This is likely due to the widely divergent functions of the pelvic fins in different fishes, ranging from tactile organs in gouramis to stabilization of the body in rainbow trout (Barton 2007, Standen 2008).

Documenting a negative relationship between elongation of the body and the size of the pectoral fins is a small step towards understanding the connection between body shape and size of the paired fins in actinopterygian fishes from anatomical, functional, and developmental perspectives. Little is known about how the musculoskeletal anatomy of the pectoral fin is modified in species with relatively smaller fins. One change to the pectoral girdle in elongate fishes involves the posttemporal, which connects the girdle to the skull. The posttemporal has either lost its connection with the skull or is absent in at least three different lineages of elongate fishes: Anguilliformes (true eels), Mastacembeloidei (spiny eels), and in *Ptilichthys goodei*, a member of Zoarcoidei (Travers 1984, Belouze 2002, Hilton and Kley 2005). To the best of our knowledge, no investigators have examined the functional consequences of fin reduction in fishes.

The median fins are known to be propulsive in South American knifefish (Gymnotiformes) (Kasapi et al. 1993, Lauder et al. 2002). Based on our study, the median fins show a strong positive relationship with elongation of the body (Fig. 4C and D). More elongate fishes tend to have relatively longer dorsal and anal fins, with some elongate fishes having dorsal fins that are confluent, running the length of the body from just posterior to the skull to the caudal fin. The expansion of the median fins in other elongate species may indicate that the median fins are important for propelling an elongate body or that elongation of the median fins are genetically linked to elongation of the body.

Developmental control of elongation of the body

With the increasing number of studies of how morphological changes in shape develop, information has become available for generating hypotheses about the molecular control of anatomical modifications during evolution. For example, Darwin's finches have long been known to vary beak shape in ways that are critical for selection of food and choice of mates (Podos 2001, Grant and Grant 2006). Recently, Abzhanov et al. (2004, 2006) described several molecular pathways leading to changes in beak shape. Deep and broad beaks (as seen in *Geospiza magnirostris*) result from a change in *Bmp4* expression (Abzhanov et al. 2004). The long and narrow

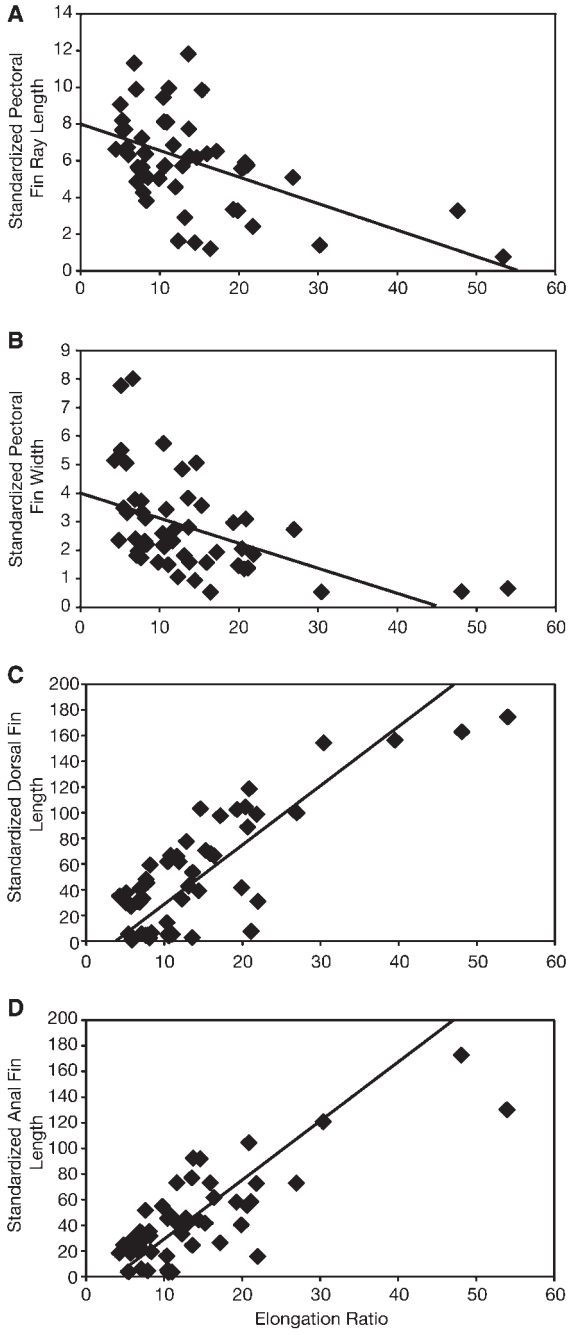

Fig. 4 Paired fin reduction and median fin expansion in elongate fishes. Reduced major-axis regression of fin size and body shape in 53 species of actinopterygian fishes (see Appendix 1 for a list of species). Length of the pectoral fin ray was measured as the length of the marginal fin ray; width of the pectoral fin was the span of a splayed fin. Lengths of the dorsal and anal fins were measured at the base of the fin along the intersection between fin and body. Measurements of fins were standardized by the cubic root of mass and regressed against elongation ratio (defined by Ward and Azizi, 2004, as standard length/second major body axis). Each measurement was averaged for three individuals of each species studied. Elongate fishes tend to have smaller pectoral fins and longer median fins. (**A**) Length of pectoral fin ray: $R = -0.51$, $m = -0.14$, $b = 7.96$. (**B**) Width of pectoral fin: $R = -0.49$, $m = -0.09$, $b = 3.98$. (**C**) Length of the dorsal fin: $R = 0.81$, $m = 5.33$, $b = -23.04$. (**D**) Length of the anal fin: $R = 0.79$, $m = 4.40$, $b = -15.19$.

beaks of the cactus and large cactus finches (*G. scandens* and *G. conirostris*, respectively) have an increase in calmodulin (CaM) expression (Abzhanov et al. 2006). As molecular techniques extend beyond traditional model systems, functional and evolutionary morphologists will be able to utilize these techniques to identify the genetic control of a trait of interest.

Regionalization of the body

Previous morphological studies have shown that there appears to be uncoupling in control of vertebral number in the different regions of the vertebral column (Fig. 2; Asano 1977, Polly et al. 2001, Ward and Brainerd 2007, Müller et al. 2010). This has been demonstrated in all groups of vertebrates by a change in vertebral number in one region with little or no change in the other regions. For example, otophysan fishes have a wide range in the number of caudal vertebrae (15–230+), but show little variation in abdominal vertebrae (13–25, Ward and Brainerd 2007). Contrarily, *Erpetoichthys calabaricus* has approximately twice as many abdominal vertebrae as members of its sister genus, *Polypterus*, while only having one to two fewer caudal vertebrae (Ward and Brainerd 2007).

Hox genes have long been known to determine vertebral identity (Krumlauf 1994). Burke et al. (1995) demonstrated that there are distinct boundaries of *Hox* expression in chicks and mice that corresponded with transition points in the vertebral column. Knockout mice have demonstrated that loss of an entire *Hox* paralogous group has a dramatic effect on patterning of the region of the vertebral column with which the *Hox* group is associated (Wellik and Capecchi 2003, McIntyre et al. 2007, Wellik 2007). For example, if the *Hox11* paralogous group is non-functional, the sacral vertebrae take on the identity of lumbar vertebrae with a loss of sacral lateral extensions. However, in comparing across species, there have been inconsistencies in the functioning of individual Hox genes. *Hox10* genes inhibit the formation of rib-bearing vertebrae (Carapuço et al. 2005) although *Hox10* is expressed in rib-bearing somites of corn snakes and caecilians (Woltering et al. 2009). Therefore, it is important to test gene function in additional taxa to determine whether it is constant across different vertebrate species.

In zebrafish there are a number of distinct *Hox* boundaries as previously seen in amniote model systems (Prince et al. 1998). Beyond this initial study, few investigators have examined the role of *Hox* genes in patterning of the vertebral column in

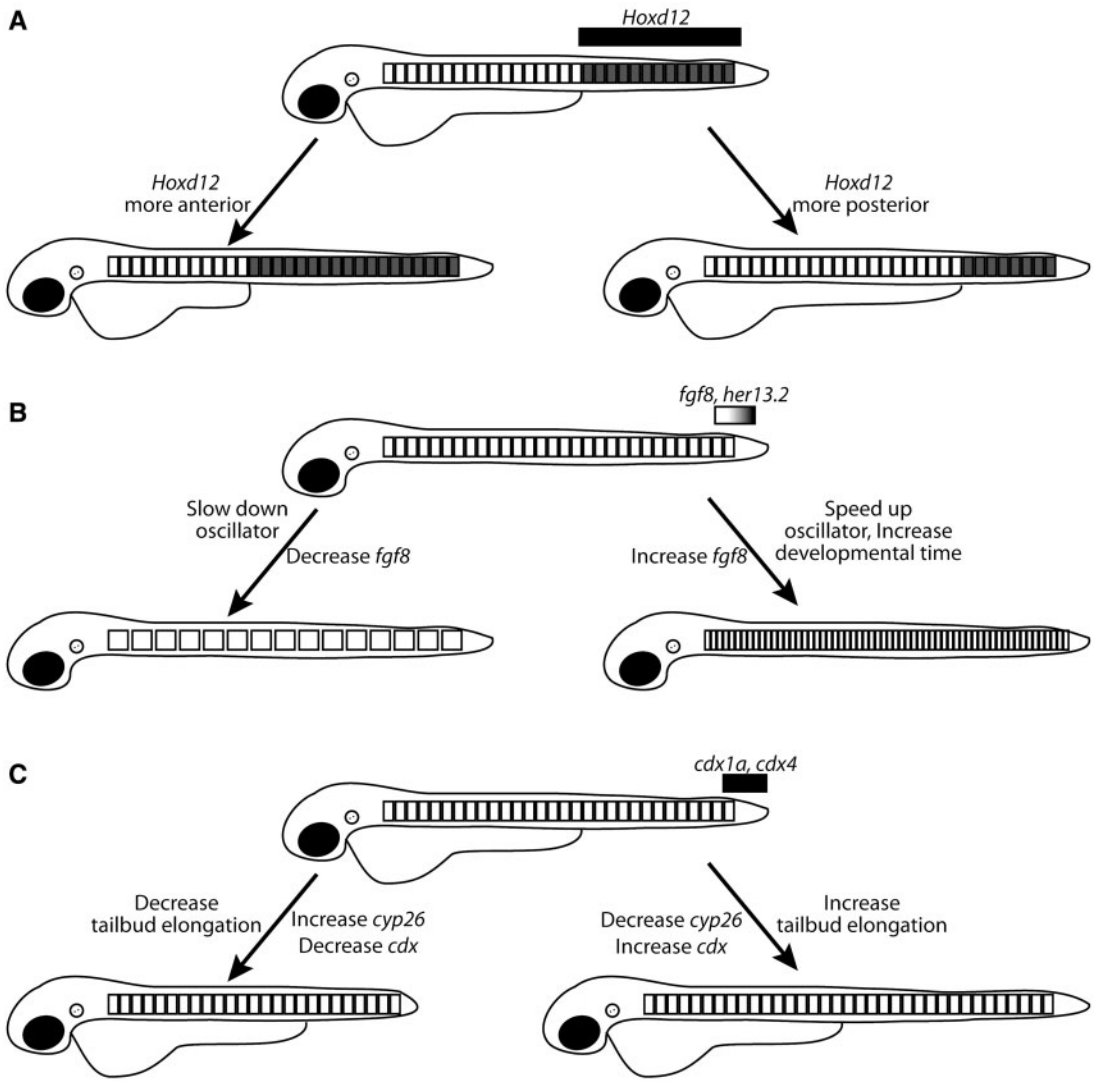

Fig. 5 Developmental mechanisms underlying body elongation in fishes. See text for more details. (**A**) Homeotic changes resulting in a change in the number of abdominal vertebrae. (**B**) Trade-off between somite length and number. (**C**) Increase in axial elongation resulting in an overall increase in vertebral number.

fishes (Ahn and Gibson 1999a, 1999b). As in caecilians and corn snakes, *Hoxa10* is expressed in rib-bearing somites in sticklebacks and zebrafish (Prince et al. 1998, Ahn and Gibson 1999a, 1999b). Therefore, it is likely that the function of this gene has changed during evolution of the vertebrates. In zebrafish, the anterior limit of *Hoxd12a*[1] expression occurs at the transition point between the abdominal and caudal regions (van der Hoeven et al. 1996). The transition between posterior regions in the vertebral column are correlated with expression boundaries of *Hox10*, *Hox11*, and *Hox12* genes in corn snakes and lizards as well; cloacal vertebrae in corn snakes are found in segments that express *Hoxa11* which is also correlated with the transition between in lumbar and sacral in whiptail lizards (Di-Poi et al. 2010). Given the expression boundary seen in zebrafish, it is possible that manipulating the anterior expression of *Hox12* may lead to a change in the number of abdominal vertebrae in fishes; a more anterior expression limit would lead to fewer abdominal vertebrae and a greater number of caudal vertebrae (Fig. 5A). Retinoic acid (RA) is known to cause anterior limits of hox expression to change resulting in changes in vertebral identity (Kessel 1992, reviewed by Alexander et al. 2009). Modification of *Hox* expression domains would result in homeotic transitions within the vertebral column.

Ultimately, for an increase in axial elongation, the total number of body segments needs to increase. For example, the total number of thoracolumbar vertebrae in many mammals is set at 19 (Narita and Kuratani 2005). The number of thoracic vertebrae

decreases with a requisite increase in lumbar vertebrae in certain lineages resulting in the same overall number of thoracolumbar vertebrae. This change is likely due to a shifting of the *Hox* boundary that separates the thoracic and lumbar regions. Therefore, in examining elongation of the body and the changes in vertebral number, we also need to consider how total vertebral number increases.

Increasing axial elongation

Highly elongated species tend to have more vertebrae, regardless of the group (Wake 1966, Richardson et al. 1998, Polly et al. 2001). The vertebrae are derived from the somites, segmented structures that form very early in development; therefore, species with more vertebrae should also have an increased number of somites. It has been unclear exactly how the number of somites increases in certain lineages; two hypotheses have recently been developed based on findings from both the morphological and developmental fields. The first hypothesis states that the time for each somite to form decreases while the overall time of somitogenesis stays the same. This would result in a higher number of somites, but the length of each somite would be shorter. The second hypothesis states that maintaining the posterior growth of the embryo will result in more similarly sized somites (Ward and Brainerd 2007, Gomez and Pourquié 2009). Recent study of somitogenesis in corn snakes (*Pantherophis guttatus*) in comparison to other vertebrate models indicates that its greater somite number is likely due to a combination of these two hypotheses. Snakes form more somites by increasing the rate of somite formation coupled with a slowing of the overall developmental rate. *Aspidoscelis uniparens* (desert grassland whiptail lizard) has the same overall developmental rate as seen in corn snakes. However, *A. uniparens*, with only approximately 90 somites, takes four hours to form each somite pair, in contrast to corn snakes that form a greater number of somites due to somite pairs forming every 100 min (Gomez et al. 2008, Gomez and Pourquié 2009).

The control of overall developmental rate is not currently understood. However, developmental studies of model systems have pointed to a few ideas about the control of outgrowth of the tailbud or axial elongation. Somite boundaries form when oscillating genes are expressed in the same cells as the wavefront gene. In zebrafish a number of genes in the notch pathway oscillate including *her1* and *her7* (Holley et al. 2000, Sawada et al. 2000, Henry et al. 2002, Gajewski et al. 2003, reviewed by Holley 2007). Genes in the wavefront in zebrafish include *fgf8*, *her13.2*, and *fss/tbx24* (Holley et al. 2000, Sawada et al. 2001, Kawamura et al. 2005, reviewed by Holley 2007). During somitogenesis, the anterior limit of the wavefront expression retreats posteriorly. *Fgf8* and *her13.2* are expressed in the tailbud and are considered to be wavefront genes (Holley et al. 2000, Sawada et al. 2000). *Fgf8* maintains the cells in an immature, stem-cell-like state (reviewed by Holley 2007). When FGF8 is applied to an embryo, it forms more, smaller somites. When *fgf8* expression is blocked, fewer larger somites are formed (Dubrulle et al. 2001, Sawada et al. 2001). This manipulation indicates that modification of the somite clock and/or wavefront does not necessarily result in axial elongation, instead we would expect to see a tradeoff between somite length and number of somites (Fig. 5B). This is similar to the previously described tradeoff of abdominal and caudal vertebrae with no change in overall vertebral number.

Somite number, and therefore, vertebral number, is strongly related to axial elongation, or how long the tailbud grows posteriorly. Studies using the mouse model system have indicated a role of cdx genes in controlling axial elongation. The *cdx* genes are homologous to caudal (*cad*) in *Drospholia*, a gene required for posterior body specification (Mlodzik et al. 1985). Specifically, *cdx2* has been shown to be required for proper vertebral development. *Cdx2* null mutants have approximately half the number of somites as found in wildtype mice (Savory et al. 2009). Savory et al. (2009) demonstrated that *cdx2* functions by initiating *cyp26a1* expression. *Cyp26* genes are known to degrade RA (reviewed by White and Schilling 2008); degradation of RA appears to keep the tailbud outgrowing. Additionally, *cdx2* controls the continued expression of *Wnt3a* and *T*, two genes known for their role in patterning of the posterior body (Faas and Isaacs 2009, Savory et al. 2009). Further study of *cdx* genes in *Xenopus tropicalis* has shown the importance of these genes in proper elongation of the axial skeleton. There is significant truncation of the body in *X. tropicalis* embryos when *cdx* gene expression is reduced using morpholinos, in particular when all three *cdx* genes are knocked-down (Faas and Isaacs 2009).

Work on *cdx* genes in zebrafish has also demonstrated a critical role in anteroposterior patterning. Reduced expression of *cdx4* due to mutation or knockdown by morpholino results in a truncated body (Davidson et al. 2003, Shimizu et al. 2005). *Cdx1a* is also expressed in the tailbud, but knockdown of *cdx1a* alone does not result in a truncated body, likely due to redundant functions between

cdx1a and *cdx4* (Shimizu et al. 2005). When both genes are knocked down, the number of somites at 17hpf is decreased from 17-18 (wildtype condition) to just over 10 somites. After this point, number of somites does not change (Shimizu et al. 2005). It is likely that highly elongated species will have modifications of the expression of cdx genes (Fig. 5C).

Future directions

The elongate body form is pervasive throughout Actinopterygii; it has evolved in most major groups of fishes. As we have demonstrated, body elongation is associated with specific changes in morphology, including reduction and loss of the paired fins and lengthening of the median fins. In addition, body elongation is strongly correlated with changes to the axial skeleton that may include lengthening of the head, vertebral centra, and/or an increase in the number of vertebrae (Fig. 1). Based on comparative analyses from major groups of vertebrates, it has been shown convincingly that the numbers of vertebrae in a given region of the vertebral column change region-specifically (Polly et al. 2001, Narita and Kuratani 2005, Ward and Brainerd 2007, Müller et al. 2010). This information on evolution of a trait, coupled with the understanding of the different developmental processes occurring during regionalization of the body, gives a basis for determining how vertebral number varies developmentally.

There are a number of potential avenues for future research on the genetic or developmental control of axial elongation in fishes. Given the increased ease with which various molecular techniques can be carried out, it is now possible to determine how certain traits have evolved in specific lineages. While this has been examined in tetrapod species, less attention has focused on developmental evolution of the axial skeleton in non-model fish species. Given the extraordinary range in anatomical diversity and the wide range of body shapes of fishes, this group provides a wealth of potential developmental studies. One crucial question to address is: what ultimately controls axial elongation? It is possible to increase the number of vertebrae in a given region at the expense of the other region. It is also possible to increase vertebral number at the expense of vertebral length. Some highly elongate species have increased vertebral number as well as vertebral length. To do this, outgrowth of the tailbud must be increased. This could occur via a number of mechanisms but does require maintenance of the stem-cell-like population present in the tailbud for a longer time relative to overall developmental time (Fig. 5C).

The study of elongation of the body provides a framework for examining the relationships between genotype, phenotype, function, and selection. The effects of anatomy on function have long been shown to be important for selection (Arnold 1983). In particular, variation in vertebral number has been shown to have effects on locomotor performance both in sticklebacks and garter snakes (Arnold and Bennett 1988, Swain 1992a, 1992b, Kelley et al. 1997). Given the dramatic variation that is seen in vertebral number in fishes, it is likely that functional consequences of increased vertebral number in different regions of the axial skeleton will be important to consider when studying anatomical diversity in fishes.

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Appendix 1

Museum numbers of individuals examined for Figs 3 and 4. The number in parentheses is the number of individuals examined from a particular lot. (AMNH: American Museum of Natural History; ABW: personal collection of the primary author.)

Ammodytidae: *Ammodytes americanus* AMNH 223129 (3)
Anarhichadidae: *Anarhichas lupus* AMNH 49682 (2)
Anarhichadidae: *Anarrhichthys ocellatus* AMNH 37417 (1)
Ateleopodidae: *Ateleopus japonicus* AMNH 90099 (1), AMNH 243584 (1), AMNH 242625 (1)
Balitoridae: *Barbatula barbatula* AMNH 10338 (3)
Blenniidae: *Petroscirtes mitratus* AMNH 31459 (3)
Carapidae: *Carapus bermudensis* AMNH 43239 (3)
Cepolidae: *Cepola macrophthalma* AMNH 49647 (2), AMNH 22675 (1)
Chaenopsidae: *Chaenopsis limbaughi* AMNH 241431 (2), AMNH 33570 (1)
Channidae: *Channa asiatica* AMNH 12140 (3)
Chaudhuriidae: *Pillaia indica* ABW uncatalogued (2)
Chlopsidae: *Kaupichthys nuchalis* AMNH 238956 (1), AMNH 247641 (1), AMNH 248213 (1)
Clariidae: *Channallabes apus* AMNH 6516 (3)
Clinidae: *Ophiclinus gracilis* AMNH 37641 (2)
Cobitidae: *Misgurnus anguillicaudatus* AMNH 11130 (3)
Congridae: *Heteroconger longissiumus* AMNH 75332 (2)
Cryptacanthodidae: *Cryptacanthodes maculatus* AMNH 221689 (2)
Cyematidae: *Cyema atrum* AMNH 36471 (1)
Derichthyidae: *Derichthys serpentinus* AMNH 44192 (1), AMNH 44193 (1), AMNH 44195 (1)
Eurypharyngidae: *Eurypharynx pelecanoides* AMNH 8809 (1)
Gempylidae: *Gempylus serpens* AMNH 8261 (1)
Gobiidae: *Gobius fluviatilis* AMNH 20820 (3)
Gonostomatidae: *Gonostoma elongatum* AMNH 240642 (3)
Halosauridae: *Halosaurus guentheri* AMNH 84385 (1), AMNH 84357 (1)
Heteropneustidae: *Heteropneustes fossilis* AMNH 1908 (3)
Hypopomidae: *Brachyhypopomus pinnicaudatus* AMNH 39848 (3)
Hypoptychidae: *Hypoptychus dybowskii* AMNH 49686 (2)
Liparidae: *Liparis callyodon* AMNH 12469 (3)
Macrouridae: *Coryphaenoides acrolepis* AMNH 12862 (2)
Mastacembelidae: *Caecomastacembelus taiaensis* AMNH 215705 (3)
Merlucciidae: *Merluccius bilinearis* AMNH 59343 (3)
Microdesmidae: *Microdesmus longipinnis* AMNH 86439 (3)
Microstomatidae: *Nansenia groenlandica* AMNH 58098 (2), AMNH 58085 (1)
Muraenesocidae: *Gavialiceps taeniola* AMNH 242702 (1)
Notacanthidae: *Notacanthus abbottii* AMNH 243567 (3)
Notosudidae: *Scopelosaurus hamiltoni* AMNH 58048 (1)
Ophichthidae: *Ophichthus gomesii* AMNH 87074 (3)
Ophidiidae: *Ophidion holbrookii* AMNH 87135 (3)
Pholidae: *Pholis gunnellus* AMNH 221788 (1), AMNH 36868 (1), AMNH 221859 (1)
Pholidichthyidae: *Pholidichthys leucotaenia* AMNH 50382 (1)
Phycidae: *Urophycis regia* AMNH 82304 (3)
Plesiopidae: *Notograptus guttatus* AMNH 211804 (3)
Plotosidae: *Plotosus lineatus* AMNH 78544 (2), AMNH 242114 (1)
Scytalinidae: *Scytalina cerdale* AMNH 3253 (1)
Serrivomeridae: *Serrivomer beanii* AMNH 29746 (3)
Siluridae: *Siluris glanis* AMNH 36490 (3)
Sphyraenidae: *Sphyraena barracuda* AMNH 243798 (3)
Stichaeidae: *Anoplarchus purpurescens* AMNH 49654 (1), AMNH 19659 (1)
Synaphobranchidae: *Synaphobranchus kaupii* AMNH 84649 (3)
Synbranchidae: *Synbranchus marmoratus* AMNH 37670 (2), AMNH 215245
Trichiuridae: *Trichiurus lepturus* AMNH 20735 (2), AMNH 49706 (1)
Trichomycteridae: *Vandellia beccarii* AMNH 55625 (3)
Zoarcidae: *Zoarces viviparous* AMNH 36853 (3)