The development and evolutionary origin of barbels in the channel catfish Ictalurus punctatus (Siluriformes: Ictaluridae)

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THE DEVELOPMENT AND EVOLUTIONARY ORIGIN OF BARBELS IN THE CHANNEL CATFISH *ICTALURUS PUNCTATUS* (SILURIFORMES: ICTALURIDAE)

by

MICHAEL BRENT HAWKINS

B.A., University of Colorado, 2008

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written by Michael Brent Hawkins
has been approved for the Department of Ecology and Evolutionary Biology

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The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.
The development and evolutionary origin of barbels in the channel catfish *Ictalurus punctatus* (Siluriformes: Ictaluridae)

Thesis co-directed by Professor Alexander Cruz and Associate Professor David Stock

Understanding the origin of morphological novelties is an important goal of evolutionary developmental biology. In pursuit of this goal, we have examined the developmental genetic mechanisms that underlie growth and patterning in a largely overlooked group of morphological novelties: the barbels of fishes. Barbels are appendages that project from the head region in a large and disparate assortment of fish taxa, ranging from hagfishes to gobies. They often bear sensory organs and can be supported by a rod of connective tissue, muscle, cartilage, or bone. Considering the scattered distribution of barbels among fishes, along with the variability of barbel position and composition, it is likely that barbels have originated independently in multiple groups. We investigated the roles of genes known to be involved in the development of other appendages in the developing barbels of the channel catfish, *Ictalurus punctatus* (Siluriformes: Ictaluridae). Similar to other appendages, the barbels of *I. punctatus* express members of the Bone morphogenetic protein (Bmp), Distal-less (Dlx), Fibroblast growth factor (Fgf), Hedgehog (Hh), Tumor necrosis factor (Tnf), and Tnf receptor families. Other genes with roles in appendage development were absent from barbels, however, including members of the Dachshund (Dach) and Hox families. Treatment with pharmacological inhibitors of Hh signaling revealed that this pathway is necessary for barbel outgrowth. I conclude that while the barbels of catfishes arose via deployment of a general vertebrate outgrowth mechanism (an Fgf/Hh feedback loop), additional features of the gene regulatory network underlying their development overlap, but are distinct from, those of other appendages.
This thesis is dedicated to my parents, Becky and Gari Hawkins.
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CHAPTER I

INTRODUCTION

The origin of morphological novelties, defined as structures lacking homologs within or between organisms (Müller and Wagner 1991), has long been of interest to evolutionary biologists (Moczek 2008). A major contribution of evolutionary developmental biology to the question of how novelty arises has been the finding that novel morphological structures frequently arise by the co-option of pre-existing gene regulatory networks for their patterning rather than the de novo assembly of such networks (Shubin et al. 2009). Furthermore, in some cases, the same gene regulatory networks have been co-opted in the origin of functionally similar but independently evolved (analogous) structures, as in the appendages of vertebrates and arthropods (Panganiban et al. 1997; Pueyo and Couso 2005). Such comparisons of appendages in distantly related species have emphasized the similarities in the genetic control of their development. However, vertebrates possess a diversity of appendage types, such as paired appendages (limbs and fins), unpaired median fins, genitalia, epithelial appendages (hair, feathers, teeth, and scales), and branchial rays (Kardong 2005), which exhibit developmental differences as well as similarities (Chuong 1998; Freitas et al. 2006; Gillis et al. 2009; Lin et al. 2009; Zeller 2010). A functionally important and phylogenetically widespread group of vertebrate appendages that have yet to be investigated extensively at the developmental genetic level are the barbels of fishes (LeClair and Topczewski 2010).

Barbels (also known as cirri, feelers, tendrils, or tentacles) are appendages that project from the head region of certain fishes, such as the “whiskers” from which catfishes receive their popular name. Despite frequently being sensory in nature and playing a role in food detection,
barbels display extensive variety in their position, composition, developmental stage of appearance, and function among species (Ryder 1883; Sato 1937; Fuiman 1984; Fox 1999). They may exist as either unpaired structures on the midline or paired, bilaterally symmetric appendages, may be located on the top, bottom, and sides of the snout, head, and throat, and may range in number from one to dozens. While their composition is highly variable, they generally consist of an epidermis surrounding a dermal core (Sato 1937). The epidermis is frequently populated by taste buds, but olfactory and mechanosensory organs are also present in some species (Herrick 1903). The dermal layer often contains blood vessels and nerves, and may contain a supporting rod of connective tissue, cartilage, bone, or striated muscle (Sato 1937; LeClair and Topczewski 2010). While most barbels are unbranched, secondary through quaternary branching is found in some species. The point in ontogeny during which barbels develop also varies from embryonic through adult stages. Uses of barbels beyond food detection include luring prey and obtaining oxygen in hypoxic environments (Winemiller 1989; Helfman et al. 2009).

Of the 62 orders of fishes listed by Nelson (2006), my survey of the literature suggests that 27 contain at least one species with barbels (Fig. 1). While once proposed to be homologous structures (Balfour 1880), barbels are most commonly thought to have arisen independently in many of these lineages, based on extensive differences in their position and composition, and their scattered distribution in the fish phylogeny (Ryder 1890; Fox 1999). In many orders, barbels are novel morphological structures by the definition of Müller and Wagner (1991), but exceptions occur, such as the hyoid barbels of goatfishes, which are derived from branchiostegal rays (Starks 1904; McCormick 1993; Kim et al. 2004).
Fig. 1. The phylogenetic distribution of barbels across fishes at the ordinal level. Orders containing one or more fishes with barbels are highlighted in red. Representative species shown at right demonstrate the various positions barbels can occupy. Tree topology adapted from Nelson (2006). Illustrations taken from Dean 1895.
To begin to explore the developmental genetic mechanisms responsible for barbel evolution, we chose to examine barbel development in the channel catfish *Ictalurus punctatus* (Siluriformes: Ictaluridae). This species was selected because its barbels appear early in embryonic development (Northcutt 2005), its embryos are available from commercial sources, and published staging criteria for ictalurid catfishes are available (Armstrong and Child 1962). Like all members of its family, the channel catfish bears four pairs of bilaterally symmetric barbels: the nasal, maxillary, mandibular, and mental pairs (Fig. 2, A-B; Grizzle and Rogers 1976; Nelson 2005). Each of these barbels consists of an epidermis surrounding a dermal core. The epidermis contains many taste buds, which are concentrated along the anterior edge of the barbel and are innervated by rami of the facial nerve (Northcutt 2005). Contained in the dermal layer are blood vessels, connective tissue, pigment cells, nerves, and a supportive rod of “elastic/cell-rich” cartilage that runs the proximal/distal length of each barbel (Joyce and Chapman 1978; Benjamin 1990). This rod of cartilage is located posterior to the central axis of the barbel, and along with the taste bud-dense anterior edge of the epidermis, provides each barbel with distinct anterior/posterior polarity (Fig. 2C).

A potential explanation for the seeming ease with which barbels have originated in disparate fish lineages is the co-option of pre-existing developmental genetic mechanisms, such as those used in the development of other vertebrate appendages. To test this hypothesis, we investigated in the developing barbels of *I. punctatus* the expression of a subset of the genes known to control the development of paired appendages, unpaired median fins, epithelial appendages, genitalia, and branchial rays. Specifically, we cloned and examined by *in situ* hybridization the expression of *bmp4, dach1, dlx1a, dlx2a, dlx5a, dlx6a, eda, edar, fgf8a, hoxa13b, hoxd11a*, and *shha*. We also used pharmacological inhibitors of hedgehog signaling to test the role of this pathway in...
barbel development. Our results suggest that barbels have arisen not by the co-option of an elaborated pre-existing vertebrate appendage developmental genetic mechanism, but by deploying an FGF8/SHH positive feedback loop, a general outgrowth program used in the development of other vertebrate appendages.

Fig. 2. Barbels of the channel catfish *I. punctatus*. (A) Schematic diagram of the four barbel pairs in an *I. punctatus* adult as seen in rostral view. (B) Head of *I. punctatus* juvenile cleared and double stained with Alcian Blue (cartilage) and Alizarin Red (mineralized tissue) following immunohistochemistry with anti-calretinin antibody to visualize taste buds. (C) Magnified maxillary barbel of fish in (B) demonstrating morphological differentiation along the anterior-posterior axis, anterior to left, with anterior taste buds, medial nerves, and posterior cartilage rod. (D) Schematic representation of barbel development during embryonic stages in *I. punctatus*. C, cartilage rod; mdb, mandibular barbel; meb, mental barbel; mxb, maxillary barbel; N, nerves; nsb, nasal barbel; TB, taste bud.
Animals

Embryos of *I. punctatus* were purchased from Osage Catfisheries (Osage Beach, MO). Newly fertilized eggs were shipped in chilled water and received in early epiboly stages. Upon arrival, the large mass of adhesive eggs was separated by hand into fragments approximately 3 cm in diameter, each of which was placed in its own container inside a recirculating incubator. Water in the containers (0.0003% Instant Ocean, 0.48 mM sodium bicarbonate, 0.1 mM HCl in distilled water) was maintained at 26°C. Embryos were staged according to the *I. nebulosus* staging table of Armstrong and Child (1962), which has been adapted for *I. punctatus* as well (Northcutt 2005). Certain stages of *I. punctatus* development resembled a combination of stages described for *I. nebulosus*, and these stages are designated here with two numbers (for example, Stage 34/35). Due to imprecise fertilization timing and time spent in chilled water during shipment, it is difficult to ascribe ages to each particular stage. However, the maxillary, mandibular, and mental barbels are visible in Stage 40 embryos at around 4 days post-fertilization (dpf). Embryos were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) pH 7.4 at 4°C, rinsed in PBST (PBS + 0.1% Tween 20), and stored in 100% methanol at -20°C.

Gene cloning and sequence analysis

Total cellular RNA was extracted from 14 dpf *I. punctatus* larvae using an RNAwiz kit (Ambion). Random hexamer primers and Superscript II reverse transcriptase (Invitrogen) were
used to produce cDNA from this RNA. Genes known to be involved in the development of vertebrate appendages were PCR-amplified from cDNA using the following degenerate primer pairs designed using amino acid alignments between zebrafish and other species (added restriction sites are underlined):

\( \text{bmp4} \): GCCGGGATCCATGATHCCNGGNAAYMGNATG, GCCGGAATTCCRCANCCYTNCNACNACCAT

\( \text{dach1} \): GCCGGGATCCCCNCARAAYAAYGARTGYAA, GCCGGAATTTCGCCNKRTTYTTYTGTYTCNAC

\( \text{dlx1a} \): GCCGGGATCCCTTYATGGARTTTYGGNCCNCC, GCCGGAATTCTCATNARYTGNGGTYTGTCAT

\( \text{dlx2a} \): GCCGGGATCCATGACNGGNGTNTTYGAYAG, GCCGGAATTCAADATNGTNCCNGCRCTNAC

\( \text{dlx5a} \): GCCGGGATCCATGCCAYCAYCCNWSNCARGA, GCCGGAATTCTGNGGNSWRTTRCANGCCAT

\( \text{dlx6a} \): GCCGGGATCCATGATGACNATGACNACNAT, GCCGGAATTCTGRTGNGGNGANGARTACCA

\( \text{eda} \): GCCGGGATCCCARGGNCARGARACNACNAT, GCCGGAATTTCCECARNARRAANGTNGTRTGRTT

\( \text{edar} \): GCCGGGATCCAGYGCNGARTAYTCNAGYTG, GCCGGAATTCTCYTCTRCRCTRCDATRCT

\( \text{fgf8a} \): GCCGGGATCCTTYGCNTTYTGTYTAYTAYGC, GCCGGAATTCCGGRTRTARTTDATRAARTCRAA

\( \text{hoxa13b} \): GCCGGGATCCAARGARTTTYGCNTTYTAYCA, GCCGGAATTCTGRAAACADATNGTNACYTG

\( \text{hoxd11a} \): GCCGGGATCCCCNCARGGNTTYGAYCARTT, GCCGGAATTCTGRAACCADATYTNACYTG

\( \text{shha} \): GCCGGGATCCGCNTAYAARCARTTTYATHCC, GCCGGAATTTCGARTACCARTGNAYNCCNTC
The resulting PCR products were cloned into pCR4-TOPO (Invitrogen) and subjected to automated sequencing. Each sequence position was determined from at least three independent clones, together representing both strands. To confirm the identity of clones, sequences were translated and used in a BLAST search on GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Clones were ascribed to specific paralogs based on the top BLAST hits.

**in situ hybridization**

The whole mount *in situ* hybridization protocol used for *I. punctatus* specimens was adapted from that used for zebrafish by Jackman et al. (2004). Proteinase K pre-treatment was carried out at a concentration of 2.5 μg/mL for 30 minutes at room temperature. Gene expression was examined at Stages 29/30, 31, 32/33, 34/35, 36, 37, 38, 39, and 40. Digoxigenin-labeled antisense riboprobes were prepared using the cloned PCR fragments described above as templates. Following *in situ* hybridization, larvae were cleared in 100% glycerol for whole mount observation or dehydrated through a graded ethanol series and embedded in glycol methacrylate (JB-4, Polysciences) for serial sectioning at 4 μm using glass knives. Images of whole mount specimens were captured with a Zeiss Axiocam digital camera mounted on a Zeiss SV11 stereomicroscope. Dissected barbels and sections were visualized with Nomarski differential interference contrast (DIC) optics on a Zeiss Axiovert 135 inverted compound microscope and images captured with a Zeiss Axiocam digital camera.

**Pharmacological inhibition of hedgehog signaling**

Two pharmacological inhibitors of the hedgehog signaling pathway, cyclopamine (Chen et al. 2002a) and SANT-1 (Chen et al. 2002b), were applied to *I. punctatus* embryos at various
stages of development. Cyclopamine (LC Labs) and SANT-1 (Tocris Bioscience) were dissolved in 100% ethanol and DMSO, respectively, to obtain 10 mM stock solutions. Treatments were carried out at 26°C in Nunclon 24-well plates, with one individual in 1 mL of medium per well. Cyclopamine experiments were conducted with treatment concentrations of 50 and 100 μM in 30% Danieau’s medium + 1% ethanol, and SANT-1 experiments were conducted with treatment concentrations of 10 and 20 μM in 30% Danieau’s medium + 1% DMSO. Vehicle-only control groups for cyclopamine and SANT-1 treatments were raised in 1% ethanol and 1% DMSO, respectively. Chorions were left intact for treatments beginning prior to hatching. Individuals were kept in the same medium from the onset of treatment until being sacrificed at the end of the experiment.

**Immunohistochemistry and skeletal staining**

Taste buds were visualized in 14 dpf *I. punctatus* larvae using anti-calretinin primary antibody (Swiss Antibodies) following the protocol described by Northcutt (2005). After developing the antibody stain, specimens were stained for bone and cartilage following the acid-free double stain method described by Walker and Kimmel (2007). While the immunohistochemistry protocol alone stains taste buds brown, the skeletal staining protocol causes this brown color to turn blue. Skeletal staining alone does not label taste buds.
CHAPTER III

RESULTS

Formation of barbels during embryonic stages

During embryonic stages following somitogenesis, the barbels of *I. punctatus* appear in the order of the maxillary pair at Stage 31, followed by the mandibular pair at Stage 37, and finally the mental pair at Stage 38 (Fig. 2D; Northcutt 2005). This pattern of appearance is consistent with other catfishes (Ryder 1883). Maxillary barbels are first visible at Stage 31 as lateral protrusions of the head that are located immediately posterior to the eye and immediately anterior to the midbrain-hindbrain boundary. As the maxillary and mandibular processes of the first pharyngeal arch become apparent at Stage 32, the maxillary barbel buds are positioned on the former processes (Fig. 3B). Throughout embryonic development the maxillary barbels migrate anteriorly with the mouth but retain their position at the margin of the upper jaw. Given their location, it is likely that maxillary barbel mesenchyme is composed of first arch neural crest cells.

The lower jaw barbels (mandibular and mental barbels) first appear several stages after the maxillary barbels. At Stage 37, the mandibular barbels are first apparent as lateral buds of the lower jaw. While referred to as mandibular barbels, close examination revealed that these barbels are not derived from the mandibular pharyngeal arch but are outgrowths of the hyoid arch. Mental barbels appear at Stage 38 and are also outgrowths of the hyoid arch, first appearing as small buds that project ventrally from the lower jaw. The nasal barbels only appear later in larval development, and were not examined in the present study.
In the maxillary, mandibular, and mental barbel pairs, outgrowth at first precedes cartilage formation but later in development these processes are concurrent. Cartilage is visible in the maxillary barbel by Stage 40, well after the barbel has grown past the lower jaw, and Alcian Green stains cartilages in the maxillary and mandibular barbel pairs by Stage 42.

Expression of *bmp4*, *fgf8a* and *shha* in developing barbels

FGF8 and SHH play important roles in the development of several vertebrate appendage types including the paired appendages, unpaired median fins, branchial rays, and genitalia. In the developing paired appendages, the expression of FGF8 in the distal apical ectodermal ridge (AER) induces expression of SHH in the zone of polarization activity (ZPA) located in the posterior appendage bud mesenchyme (Lewandoski et al. 2000). Together, FGF8 and SHH form a positive feedback loop that is necessary for proper outgrowth throughout development of the fin/limb (Laufer et al. 1994; Niswander et al. 1994). SHH also acts as a morphogen and is involved in the anterior/posterior patterning of fins/limbs (Riddle et al. 1993). A similar mechanism of outgrowth has also been proposed for the development of the unpaired median fins (Freitas et al. 2006; Abe et al. 2007), chondrichthyian branchial rays (Gillis et al. 2009), and the genital tubercle of the mouse (Cohn 2004). The latter two structures differ somewhat from limbs and fins in the details of the mechanism, however. Developing branchial rays express SHH in a distal thickened epithelial ridge, rather than in a ZPA-type signaling center in the mesenchyme (Gillis et al. 2009). In genital tubercle development, the FGF8 signaling center (the urethral epithelium) is derived from endoderm rather than ectoderm, and no AER is present (Liu et al. 2009). BMP4 also plays an important role in the development of the paired appendages and the genital tubercle, where BMP signaling inhibits FGF signaling in the absence of BMP
inhibitors (Zuniga et al. 1999; Perriton et al. 2002). In the limb bud, BMP signaling plays roles in outgrowth and proximal-distal patterning, and BMP4 is expressed in the AER and the anterior, posterior, and distal bud mesenchyme (Pueyo and Couso 2005).

To investigate the involvement of BMP4, FGF8, and SHH in *I. punctatus* barbel development, we analyzed the expression of *bmp4*, *fgf8a*, and *shha* via *in situ* hybridization (Fig. 3). At Stage 29/30, prior to any morphological indication of the maxillary barbel, *bmp4* and *fgf8a* are expressed in epithelium of first branchial arch where the maxillary barbel bud will first appear. *shha* is expressed in the midline of the embryo at this stage, but is not in the region of the future maxillary barbel. Expression patterns similar to that observed at Stage 29/30 are also observed in Stages 31 and 32/33, with *bmp4* and *fgf8a* expressed in the maxillary barbel bud epithelium and *shha* expression absent from this barbel (Fig. 3, A-C).

Expression of *shha* is first observed in the maxillary barbel bud at Stage 34/35 (Fig. 3, F, I). At this stage, both *fgf8a* and *shha* are expressed in the maxillary barbel bud epithelium in a domain that spans the anterior/posterior length of the bud (Fig. 3, E-F), but expression is only found in the ventral-most two-thirds of the bud (Fig. 3, H-I). *bmp4* is also expressed at Stage 34/35 in the maxillary barbel bud epithelium in a domain across the anterior/posterior length of the bud (Fig. 3D), but this *bmp4* expression domain is limited to the dorsal two-thirds of the bud epithelium (Fig. 3G). Patterns of *bmp4*, *fgf8a*, and *shha* expression similar to those described for Stage 34/35 are observed at Stage 36 as well (data not shown).

Between Stages 36 and 37, *bmp4* expression switches from an epithelial domain to the anterior mesenchyme of the maxillary barbel, while *fgf8a* and *shha* maintain patterns of epithelial expression similar to that observed at Stages 34/35 and 36 (Fig 3, J-L). However, the intensity of staining for *fgf8a* expression in the maxillary barbel diminishes substantially from
Fig. 3. *in situ* hybridizations demonstrating expression of *bmp4*, *fgf8a*, and *shha* during *I. punctatus* barbel development. Dorsal views with anterior to the left in (A-F, J-L); lateral views with anterior to the left in (M, P-R); Rostral views with dorsal to left in (N-O). At Stage 32/33 (A-C), *bmp4* and *fgf8a* are expressed in maxillary barbel epithelium (A-B, arrowheads) and *shha* is absent from this tissue (C, arrowhead). *bmp4* and *fgf8a* expression patterns at Stage 34/35 are similar to that in Stage 32/33 (D, E), but *shha* is now expressed in maxillary barbel epithelium at Stage 34/34 (F, arrowhead). (G-I) Transverse sections of maxillary barbels demonstrating epithelial expression of *bmp4*, *fgf8a*, and *shha* at Stage 34/35; section plane indicated by red line in (D). *bmp4* is expressed in the dorsal two-thirds of the epithelium (G, arrow) while *fgf8a* is expressed in the ventral two-thirds of the epithelium (H, arrow), and *shha* is expressed in the distal tip of the barbel (I, arrow). At Stage 37 (J-L), *bmp4* expression switches to the maxillary barbel mesenchyme (J) while *fgf8a* and *shha* continue to be expressed in the epithelium (K-L). *bmp4* continues to be expressed in the anterior maxillary barbel mesenchyme (M, arrow) in Stage 38, while *fgf8a* expression is not detected in any of the barbel pairs (N), and *shha* is expressed in the anterior epithelium of the maxillary (R, arrow) and mandibular barbels (R, arrowhead). e, eye; mhb, midbrain-hindbrain boundary.
Stage 36 to Stage 37. From Stage 38 onward, \textit{fgf8a} is not expressed in any of the barbel pairs (Fig 3, N, Q).

At Stage 38 the maxillary barbel \textit{shha} expression domain is confined to the anterior epithelium (with the exception of the distal tip, where \textit{shha} is expressed throughout the epithelium), and this pattern continues throughout the remainder of maxillary barbel outgrowth (Fig. 3 O, R), as does the expression of \textit{bmp4} in the anterior mesenchyme (Fig. 3, M, P). Expression of \textit{bmp4} and \textit{shha} are first observed in the mandibular barbel buds at Stage 38, with \textit{shha} expressed throughout the bud epithelium (Fig. 3O) and \textit{bmp4} expressed in the bud mesenchyme (Fig. 3P).

The \textit{shha} expression domain in the mandibular barbels is confined to the anterior epithelium beginning at Stage 39 (Fig. 3R). \textit{shha} expression is also first seen in the mental barbel bud epithelium at this stage. It is not until Stage 40 that \textit{bmp4} is expressed in the mesenchyme of the mental barbel buds. In contrast to the maxillary barbel buds, there is no stage of epithelial \textit{bmp4} expression of in the developing mandibular and mental barbel buds. While attempts to amplify \textit{shhb} from \textit{I. punctatus} were unsuccessful, expression analysis in the cuckoo catfish, \textit{Synodontis multipunctatus}, suggests that this paralog is not expressed in the developing barbels (unpublished data).

These results demonstrate that barbels are similar to paired appendages, unpaired median fins, branchial rays, and genitalia in that each of these appendage types express FGF8 and SHH during outgrowth. However, extensive differences exist in the expression domains of these genes between barbels and other appendages, including expression in different tissue layers, expression position, and duration of expression.
Expression of Dlx gene family members in the developing barbels

Members of the Dlx gene family are expressed during development in a variety of vertebrate appendage types, and are also involved in the dorsoventral patterning of the pharyngeal arches (Depew et al. 2002; Shigetani et al. 2005). In the paired appendages, Dlx genes are expressed in the AER epithelium of the fin/limb buds (Thomas et al. 2000; Panganiban and Rubenstein 2002). Dlx genes are also expressed in the developing unpaired median fins (Akimenko et al. 1994; Ellies et al. 1997; Freitas et al. 2006; Abe et al. 2007). Both epithelial and mesenchymal Dlx expression is observed in the teeth of fishes and mammals (Thomas et al. 1995; Zhao et al. 2000; Jackman et al. 2004; Stock et al. 2006). Dlx5 and Dlx6 are expressed in the developing genital tubercle of mice (Robledo et al. 2002). In mouse, nested expression of Dlx genes acts to pattern the dorsoventral identity of the pharyngeal arch skeleton: Dlx1/2 are expressed throughout the dorsal-ventral axis of the pharyngeal arches and specify dorsal identity, Dlx5/6 are expressed in a ventrally-restricted domain and specify a ventral identity, and Dlx3/4 expression is restricted more ventrally still (Depew et al. 2002). Similarly, studies in zebrafish have revealed that dlx1a/2a are expressed throughout the dorsoventral axis of the arches, dlx5a/6a are expressed in a ventrally restricted domain nested within the dlx1a/2a domain, and dlx3b/4a/4b are expressed in a ventrally restricted domain nested within the dlx5a/6a domain (Talbot et al. 2010).

The number of Dlx homologs in I. punctatus is unknown, but the closely related zebrafish possesses eight Dlx gene family members (Stock et al. 1996). Six of these eight Dlx genes form three bi-gene clusters (dlx1a/dlx2a, dlx3b/dlx4b, and dlx5a/dlx6a), and the two genes within each cluster are closely co-expressed (Ellies et al. 1997; Qiu et al. 1997). We successfully cloned and analyzed in I. punctatus the expression of four genes comprising two of the three bi-gene clusters: dlx1a, dlx2a, dlx5a, and dlx6a.
If the expression patterns of Dlx genes in the barbels followed that of the pharyngeal arches, \textit{dlx1a}/2a would be expressed in the maxillary and lower jaw barbels, and \textit{dlx5a/6a} would be expressed in the lower jaw barbels only. Instead, we found \textit{dlx1a}, \textit{dlx2a}, and \textit{dlx5a} to be expressed in the developing maxillary barbels (Fig. 4, A-C), while lower jaw barbels express only \textit{dlx2a} and \textit{dlx5a} (Fig. 4, I-J). In contrast to these genes, \textit{dlx6a} is not expressed in any barbels at any stage examined (Fig. 4, D).

\textit{dlx2a} is the first Dlx gene detected in the barbels, and is expressed in the maxillary barbel bud epithelium at Stage 31, followed by \textit{dlx1a} in the mesenchyme at Stage 32/33, and \textit{dlx5a} in the maxillary barbel epithelium and mesenchyme at Stage 34/35 (Fig. 4, A-C; data not shown). The tissue layer in which these genes are expressed was verified by sectioning (Fig. 4, E-G). Once initiated, expression of \textit{dlx1a} and \textit{dlx5a} continues in the maxillary barbel mesenchyme throughout outgrowth of the structure (Fig. 4, H, J). \textit{dlx2a}, on the other hand, follows a pattern of expression similar to \textit{bmp4} in that \textit{dlx2a} expression shifts from the epithelium to the mesenchyme between Stages 36 and 37. From Stage 37 onward, \textit{dlx2a} is expressed in the maxillary barbel mesenchyme (Fig. 4I).

The first Dlx gene to be expressed in the mandibular barbel mesenchyme is \textit{dlx5a} at Stage 38 and is followed by \textit{dlx2a} at Stage 39 (Fig. 4, I-J). Expression of \textit{dlx2a} and \textit{dlx5a} in the mental barbel bud mesenchyme begins at Stage 40, and expression of \textit{dlx2a} and \textit{dlx5a} in the lower jaw barbel mesenchyme continues throughout barbel outgrowth. \textit{dlx1a}, however, is not expressed in the lower jaw barbels in any of the stages examined (Fig. 4H). These results demonstrate that the developing barbels of \textit{I. punctatus} deviate from Dlx expression patterns in the pharyngeal arches as well as expected expression patterns based on Dlx bi-gene cluster co-expression.
Fig. 4. Dlx gene expression in the developing barbels shown by in situ hybridization. Dorsal views with anterior to the left in (A-D). Rostral views with dorsal to left in (H-J). At Stage 34/35 (A-D), *dlx1a*, *dlx2a*, and *dlx5a* are expressed in the maxillary barbels (A-C, arrows), while *dlx6a* expression is not detected in the barbels (D, arrow) but is present in the gill cover (D, arrowhead). (E-F) transverse sections of Stage 34/35 specimens to determine tissue layers of Dlx gene expression. *dlx1a* is expressed in the maxillary barbel mesenchyme (E, arrow), while *dlx2a* is expressed in the dorsal epithelium of the maxillary barbel (F, arrow), and *dlx5a* is expressed in the mesenchyme (G, arrow) and a small portion of the dorsal maxillary barbel epithelium (G, arrowhead). (H-J) Dlx genes are differentially expressed in the maxillary and lower jaw barbels. At Stage 38, *dlx1a* is expressed in the maxillary barbel mesenchyme but is absent from the lower jaw barbels (H, arrow), while *dlx2a* and *dlx5a* are expressed in the mesenchyme of the maxillary and mandibular barbels (I-J, arrows).
Expression of \textit{eda} and its receptor in the developing barbels

The ectodysplasin signaling pathway includes the extracellular signaling ligand \textit{eda}, its receptor \textit{edar}, and the receptor adaptor protein \textit{edaradd}, which together are required for the development of epithelial appendages (teeth, scales, hair, and feathers; Mikkola and Thesleff 2003). Zebrafish with mutations in \textit{eda} or \textit{edar} fail to develop teeth, scales, or fin rays (Harris et al. 2008).

To determine if the ectodysplasin signaling pathway has a role in barbel development, we examined the expression of \textit{eda} and \textit{edar}. Whole mount \textit{in situ} hybridization for \textit{eda} produced ambiguous results, as most specimens displayed low levels of staining throughout the body that may have been artifactual. Low levels of \textit{eda} expression in the posterior maxillary barbel bud mesenchyme were observed at Stage 34/35 (Fig. 5, A, C), but it is not clear that this staining represents actual localization of the transcript.

Analysis of \textit{edar} expression proved more successful. At Stages 34/35 and 36, \textit{edar} is expressed in the ventral maxillary barbel bud epithelium (Fig. 5, B, C) in a domain similar to \textit{fgf8a}. \textit{edar} continues to be expressed in the maxillary barbel epithelium at Stage 37, but in a domain that is restricted to the distal portion of the barbel. Expression of \textit{edar} in the mandibular barbel bud epithelium is also observed at this stage, and epithelial expression can be observed in the mental barbel bud by Stage 39 (data not shown).

The expression of \textit{eda} in the barbel mesenchyme and \textit{edar} in the barbel epithelium correspond to the tissue layers of \textit{eda} and \textit{edar} expression in developing cichlid teeth and chicken feathers (Houghton et al. 2005; Fraser et al. 2009). \textit{edar} is also expressed in the mouse limb bud AER and developing fin rays of zebrafish paired appendages (Pispa et al. 2003; Harris et al. 2008).
Fig. 5. Barbel expression of *ectodysplasin* and its receptor demonstrated by *in situ* hybridization. Dorsal views with anterior to the left in (A-B). At Stage 34/35, *eda* appears to be expressed at low levels in the barbel mesenchyme (A, C, arrows), but this result is questionable due to similar levels of staining throughout specimens. *edar* is expressed in the ventral maxillary barbel epithelium at Stage 34/35 (B, D, arrows).

**Other vertebrate appendage development genes not expressed in the barbel**

Dach proteins are transcription factors involved in patterning the proximal/distal axis of the paired appendages (Kida et al. 2004). 5’ members of the HoxA and HoxD complexes play roles in both the proximal/distal and anterior/posterior patterning of paired appendages (Zakany and Duboule 2007). *dach1, hoxa13b*, and *hoxd11a* were not expressed in the developing barbels of *I. punctatus* (Fig. 6, D-F). Detection of *dach1, hoxa13b*, and *hoxd11a* expression in the pectoral fins and *dach1* expression in the gill filaments demonstrates that these negative results are unlikely to be an artifact of the *in situ* hybridization protocol (Fig. 6, A-C). These results show that certain developmental genetic mechanisms used to pattern the proximal/distal and anterior/posterior axes of the paired appendages are not deployed in the developing barbels.
**Fig. 6.** *in situ* hybridizations demonstrating the absence of *hoxa13b, hoxd11a*, and *dach1* from the developing barbels. Rostral views with dorsal at top in (D-E). Lateral views with anterior to the left in (F). At Stage 38, *hoxa13b* and *hoxd11a* are expressed in the pectoral fin mesenchyme (A, B, arrows) while *dach1* is expressed in the developing gill filaments (C, arrow). Expression of none of these genes is detected in the barbels during any of the stages examined, including Stage 38 (A-C, arrows indicate maxillary barbel).

**Hedgehog signaling is required for barbel outgrowth**

To test the role of hedgehog signaling in barbel development we exposed *I. punctatus* to cycloamine and SANT-1, two pharmacological inhibitors of the hedgehog pathway (Chen et al. 2002a; Chen et al. 2002b). Treatment with 50 μM cycloamine beginning at Stage 25 blocked
maxillary barbel initiation in all treated individuals (n = 12; Fig. 7A), while control embryos raised in vehicle alone (1% EtOH) exhibited normal barbel outgrowth (n = 12; Fig. 7B) (p = 7.4 x 10^{-7}, 2-tailed Fisher’s exact test). Embryos were sacrificed once control specimens reached Stage 36. Identical results were obtained after treatment with 100 μM cyclopamine, 10 μM SANT-1, and 20 μM SANT-1.

It is not clear if the negative effect of Hh signaling inhibition on maxillary barbel outgrowth is a direct effect on the barbels themselves or an indirect effect due to the failure to develop derivatives of the maxillary processes of the mandibular arch. Zebrafish embryos mutant for shha or treated with hedgehog signaling inhibitors at stages similar to Stage 25 in I. punctatus fail to form condensations that give rise to the upper jaw (Wada et al. 2005; Eberhart et al. 2007; Schwend and Ahlgren 2009).

We therefore tested the requirement of hedgehog signaling for barbel outgrowth at later stages by treating embryos with 50 μM cyclopamine beginning at Stages 35, 37, and 39, and sacrificing specimens once controls reached Stage 42. Embryos beginning treatment at Stage 35 possessed only diminutive maxillary barbels (n = 19, data not shown), while controls exhibited well-developed maxillary, mandibular, and mental barbels. Similarly, embryos treated beginning at Stage 37 had smaller maxillary and mandibular barbels, with the mental pair entirely lacking (n = 13, data not shown). Embryos beginning treatment at Stage 39 possessed maxillary, mandibular, and mental barbels that were all of reduced size (n = 22, data not shown) compared to controls. These results demonstrate the requirement for hedgehog signaling in the initial growth and continued outgrowth of the maxillary and lower jaw barbels. Such a requirement is similar between barbels and other vertebrate appendage types (Gillis et al. 2009).
Fig. 7. Treatment with pharmacological inhibitors of hedgehog signaling block barbel outgrowth. Lateral views with anterior to the left in (A-B). Embryos treated with 50 μM cyclopamine beginning at Stage 25 fail to develop barbels (B, arrowhead) while control embryos display normal barbel outgrowth at Stage 36 (A, arrow).
CHAPTER IV

DISCUSSION

As shown by the results above, the barbels of *I. punctatus* express genes involved in the development and patterning of other vertebrate appendage types. Of the 12 genes examined, eight are expressed in the developing maxillary barbels, and only six of these eight genes are expressed in the mandibular and mental barbels. The vertebrate appendage development genes that are expressed in the barbels exhibit both similarities and differences in their expression domains between the developing barbels and other vertebrate appendage types. The differences suggest either that barbels did not evolve by the co-option of a complete appendage development mechanism or that divergence in gene expression occurred following such co-option. As an example of incomplete co-option, it is possible that barbels arose through the deployment of a general vertebrate outgrowth program – an FGF8/SHH positive feedback loop.

**Maxillary barbels, paired appendages, and branchial rays**

Based on gene expression data presented here, barbel development proceeds in a manner that is more similar to the development of the paired appendages and branchial rays than that of other appendage types (Fig. 8). FGF8 semi-orthologs are expressed in the ectoderm prior to and during early outgrowth of the barbels, paired appendages, and branchial rays (Mahmood et al. 1995; Gillis et al. 2009); however, expression of *fgf8a* in the maxillary barbels is not confined to an AER-like pseudostratified ridge of epithelium as it is for the latter two appendage types. Instead, *fgf8a* is expressed in the basal portion of a multi-layered epithelium that caps the maxillary
Fig. 8. Schematic diagram comparing gene expression between paired appendages and early and late maxillary barbels. Colored lines to the left of each black and white outline indicate epithelial expression, while those to the right indicate mesenchymal expression. Gene expression in a generalized paired appendage bud in (A), early maxillary barbel bud in (B), and a late stage maxillary barbel in (C). Epi., epithelium; Mes., mesenchyme.
barbel bud mesenchyme like an umbrella. An additional discrepancy is that expression of $fgf8a$ in the maxillary barbels disappears prior to the complete outgrowth of the appendages, while in the paired appendages and branchial rays FGF8 is expressed throughout outgrowth (Crossley et al. 1996; Gillis et al. 2009). Barbels, paired appendages, and branchial rays also express SHH semi-orthologs during development, although this expression is ectodermal in the case of barbels and branchial rays, but is in lateral plate mesoderm-derived mesenchyme of the developing paired appendages (RIDDLE et al. 1993; Gillis et al. 2009). Polarization of SHH semi-ortholog expression along the anterior/posterior axis is observed in barbels, paired appendages, and branchial rays; although SHH is anteriorly restricted in barbels and posteriorly restricted in paired appendages and branchial rays (RIDDLE et al. 1993; Gillis et al. 2009). While the data presented here demonstrate the expression of $fgf8a$ and $shha$ in the maxillary barbels, and the requirement of hedgehog signaling for barbel outgrowth, future studies are needed to assess the functional interactions of $fgf8a$ and $shha$ in the maxillary barbel bud. The existence of a FGF8-SHH positive feedback loop is a hallmark of paired appendage and branchial ray development (Crossley et al. 1996; Gillis et al. 2009), and investigation into the presence of such a loop in the maxillary barbel is an important next step in understanding the evolution of barbels and the developmental genetic mechanisms of barbel outgrowth. *I. punctatus* is limited in terms of the experimental tools available to manipulate gene function, but cyclopamine and SU-5402, a pharmacological inhibitor of FGF signaling (Mohammadi et al. 1997), could be used for such a study.

Another interesting similarity between the maxillary barbels and paired appendages is the expression of BMP4 during development. In the early limb bud, BMP4 is expressed in the anterior, posterior, and distal mesenchyme, as well as the AER (Pueyo and Couso 2005). BMP
signaling would inhibit FGF signaling from the AER if it were not for the presence of SHH-induced inhibitors of BMP signaling (Zuniga et al. 1999). In the maxillary barbel bud, epithelial \textit{fgf8a} expression disappears shortly after \textit{bmp4} expression switches from the epithelium to the anterior mesenchyme. It would be interesting to functionally investigate this correlated gene expression in \textit{I. punctatus} using the BMP signaling inhibitor dorsomorphin (Yu et al. 2008).

A final similar feature between maxillary barbels and paired appendages is the expression of \textit{edar} in the epithelium of the developing appendages. \textit{edar} is expressed in the ventral epithelium of the maxillary barbel (Fig. 5D) and the AER of the mouse limb bud (Tucker et al. 2000; Pispa et al. 2003). However, the role of ectodysplasin signaling in paired appendage development is not well understood (Harris et al. 2008), and it is not clear if the shared \textit{edar} expression in developing barbels and paired appendages is functionally significant.

Major points of divergence among barbel and paired appendage development are the differences in Dlx gene expression patterns between these two appendage types, and the absence of Dach and 5’ HoxA/HoxD complex genes in the developing barbels. All vertebrate Dlx genes are expressed in the AER of the paired appendages (Panganiban and Rubenstein 2002), while Dlx genes are expressed in the epithelium (early stage \textit{dlx2a} and \textit{dlx5a}) and the mesenchyme (\textit{dlx1a}, late stage \textit{dlx2a}, \textit{dlx5a}) of the barbels. In paired appendages, Dach1 and the 5’ HoxA/HoxD complex genes are involved in proximal distal patterning, and the 5’ HoxA/HoxD complex genes have additional roles in patterning the anterior/posterior axis (Kida et al. 2004; Zakany and Duboule 2007). The absence of such patterning genes might be expected, given the lack of extensive morphological differentiation along these axes in barbels, and indicates major differences between the developmental genetic mechanisms that pattern paired appendages and those that pattern barbels. Thus, while barbel development shares certain features with the
development of other vertebrate appendage types, there are also substantial differences. Taken altogether, these data suggest that *I. punctatus* barbels may develop by deploying a general vertebrate outgrowth program, an FGF8/SHH positive feedback loop, that is found in the developing paired appendages, unpaired median fins, genitalia, and branchial rays.

The deployment of a general vertebrate outgrowth mechanism to produce a morphological novelty has been proposed to explain the evolution of genitalia (Liu et al. 2009). According to this hypothesis, external genitalia have evolved not by co-opting the full limb development program, but by deploying an AER-like outgrowth genetic cassette in the urethral epithelium. Evidence for the ability of such a program to drive the outgrowth of head appendages is provided by FGF8/SHH overexpression experiments in chick embryos and neural crest cultures (Abzhanov and Tabin 2004). In experiments *in vivo*, groups of cells co-expressing endogenous or ectopic FGF8 and SHH produced numerous cartilaginous protrusions on the head, and groups of neural crest cells in culture which co-expressed FGF8 and SHH were able to form chondrogenic nodules. The ability to produce outgrowths whenever FGF8 and SHH are both expressed in the same tissue might be a general feature of the vertebrate head, and would provide a mechanism to explain the ease with which barbels appear to arise in evolution.

**Divergent Dlx gene expression patterns between barbels and pharyngeal arches**

Similar to other vertebrate appendages and the pharyngeal arches, barbels were found to express members of the Dlx gene family. However, Dlx gene expression patterns in the barbels diverge from that observed in pharyngeal arches and expectations based on Dlx bi-gene cluster co-expression in the pharyngeal arches and non-barbel vertebrate appendages (Ellies et al. 1997; Qiu et al. 1997; Depew et al. 2002; Shigetani et al. 2005; Talbot et al. 2010). Based on their
positioning along the dorsoventral axis, \textit{dlx1a} and \textit{dlx2a} would be expected to be expressed in the maxillary, mandibular, and mental barbel pairs, if the developing barbels followed the same Dlx patterning mechanism as the pharyngeal arches (Shigetani et al. 2005). This expectation is congruent with Dlx bi-gene cluster co-expression, wherein \textit{dlx1a/2a} are expected to be closely co-expressed (Ellies et al. 1997; Qiu et al. 1997). However, the developing barbels deviate from both of these expectations, as \textit{dlx1a} is expressed in the maxillary barbel mesenchyme but entirely absent from the lower jaw barbels, while \textit{dlx2a} is expressed at first in the maxillary barbel epithelium, then switches to the maxillary barbel mesenchyme, and is expressed in the mesenchyme of the lower jaw barbels. Barbel \textit{dlx5a} and \textit{dlx6a} expression patterns also deviate from expectations based on Dlx bi-gene cluster co-expression. \textit{dlx5a} is expressed in the epithelium and mesenchyme of the maxillary barbels and the mesenchyme of the lower jaw barbels, while \textit{dlx6a} is not expressed in any of the barbel pairs. These findings reveal that the regulation of Dlx genes, which is conserved between mouse and zebrafish, has diverged in \textit{I. punctatus} from the time Siluriformes and Cypriniformes split approximately 250 million years ago (Peng et al. 2006). It will be of interest to examine Dlx gene expression in other catfishes to investigate whether this change in Dlx gene regulation is specific to \textit{I. punctatus} or if it is found across siluriform fishes. If the latter situation is supported, then changes in Dlx gene regulation might have played an important role in the origin of barbels in catfishes.

Divergent gene expression between maxillary and lower jaw barbels

While six of the genes examined are expressed in the developing maxillary, mandibular, and mental barbel pairs, two additional genes are only expressed in the maxillary barbels during development. \textit{dlx1a} and \textit{fgf8a} are both expressed in the developing maxillary barbels, but are not
expressed in either pair of developing lower jaw barbels. Due to gene duplication events in the evolution of teleosts (Amores et al. 1998), both Dlx and Fgf gene families display a high degree of paralogy, and these paralogs may play functionally redundant roles in barbel development. Therefore, it is possible that another FGF ligand plays the same role in lower jaw barbels that \textit{fgf8a} does in the maxillary barbels. Similarly, it is also possible that one of the Dlx family genes not examined in this study is expressed in the developing lower jaw barbels. However, we cannot rule out that the lower jaw barbels do not use FGF signaling during development.

Genes that are expressed in both the maxillary and lower jaw barbels exhibit different expression domains in the different barbel pairs. \textit{bmp4} and \textit{dlx2a} are expressed in the maxillary barbel epithelium up through Stage 36, and expression of both genes transitions to the mesenchyme by Stage 37. In contrast, these genes are expressed only in the mesenchyme of the mandibular and mental barbels. Thus, gene expression patterns in the developing lower jaw barbels resemble those observed in the maxillary barbels at Stage 38, but have no expression patterns equivalent to the maxillary barbels at Stage 37 or earlier. The unique expression patterns found in the maxillary barbel are therefore not required in the development of the lower jaw barbels.

A potential explanation for the differences in gene expression between the developing maxillary and lower jaw barbels are the differences in developmental timing and position between the maxillary, mandibular, and mental barbels. The maxillary barbels are derived from the mandibular arch, while the lower jaw barbels grow from the hyoid arch. These two arches have divergent transcriptional profiles, including the presence of Hox complex genes in the hyoid that are absent from the Hox-default mandibular arch (Shigetani et al. 2005). Such
differences in the transcriptional background in which the maxillary and lower jaw barbels develop may account for the observed differences in gene expression between the barbel pairs.

**The origin and evolution of barbels in fishes**

Maxillary barbels are invariably present across catfishes, but species may possess from one to three additional barbel pairs (Burgess 1993; Diogo 2005). The plesiomorphic condition for catfishes is to possess only the maxillary barbel pair, such as in *Diplomystes*, and the presence of nasal or lower jaw barbels in catfishes are derived features (Diogo 2005). While nasal barbels have been gained and lost numerous times, the presence and number of lower jaw barbels follows a clear phylogenetic pattern. In the lineage leading to ictalurid catfishes, lower jaw barbels first appear in the node leading to the non-diplomystid/non-loricarioid clade, where species transition from having no lower jaw barbels to having both mandibular and mental barbel pairs (Diogo 2005). In this sense, the order of appearance of barbel pairs during *I. punctatus* development roughly recapitulates the order of appearance of barbels throughout catfish evolutionary history. From this condition of having maxillary, mandibular, and mental barbel pairs, certain lineages have independently lost one or both pairs of lower jaw barbels (Diogo 2005).

While the specific genetic events and selective pressures leading to the origin of maxillary barbels in the siluriform common ancestor are not known, a plausible scenario can be proposed. The presence of barbels in a species frequently correlates with feeding strategies in which gustatory cues play a critical role in food detection, such as in benthic habitats where vision is impeded by dark or turbid conditions (Helfman et al. 2009). In such circumstances, additional sensory cells could provide a selective advantage. External taste buds are present on the lips and
heads of many fishes (Herrick 1903; Hansen et al. 2002), and an increase in the surface area of this epithelium could increase the number of taste buds. The barbels of catfishes may have at first only been present as smaller, less patterned protrusions of the upper jaw that expanded the taste bud-bearing epithelium, and were only subsequently modified to become the elaborated structures observed in modern catfishes. This hypothesis could be tested by examining barbel gene expression in a catfish group exhibiting the plesiomorphic barbel condition. It may be the case that the developing barbels of these catfishes display only a subset of the patterning genes identified in *I. punctatus* barbels. Contrary to this hypothesis is the observation that *Diplomystes* catfishes exhibit the highest density of taste buds along the anterior edge of their maxillary barbels (Arratia and Huaquin 1995), suggesting that some anterior/posterior patterning mechanism is already in place in these catfishes.

Given the structural and developmental similarities between the maxillary barbels and the lower jaw barbels, one potential explanation for the origin of the lower jaw barbels is that they evolved by co-opting portions of the maxillary barbel developmental program. Indeed, as shown above, the maxillary and lower jaw barbels share similar gene expression patterns. Interestingly, several loricarioid lineages have evolved a single pair of lower jaw barbels independently from the non-diplomystid/non-loricarioid clade, indicating that lower jaw barbels are not historically homologous across all catfishes (Diogo 2005). One such genus, *Corydoras*, is available in the pet trade and has been spawned in captivity by hobbyists. It would be interesting to examine the development of lower jaw barbels in species of this genus to see if they have evolved by co-opting similar or different portions of the maxillary barbel development program as the lower jaw barbels of the non-diplomystid/non-loricarioid catfishes.
One of the first hypotheses concerning the evolutionary relationships between the barbels of fishes was proposed by Francis Balfour (1880). After describing developing sturgeon barbels and gar adhesive organs (the latter of which he believed to be barbel rudiments), Balfour concluded, “…the barbels of fishes must be phylogenetically derived from the papillae of a suctorial disc adjoining the mouth (p. 89).” In a summary at the end of the chapter, Balfour went on to surmise that such a disc was “a very primitive vertebrate organ, which has disappeared in the adult state of almost all the Vertebrata (p. 98),” but still persists in certain bony fishes. Therefore, according to Balfour’s hypothesis, the barbels of fishes are historically homologous structures inherited from the vertebrate common ancestor, which have been subsequently lost repeatedly in non-barbelled species.

John Adams Ryder (1890) rejected Balfour’s barbel homology hypothesis, favoring instead a hypothesis that barbels are independently derived on the basis that the extensive differences in position, composition, and developmental timing indicate “a want of community of descent” of these structures. This is in agreement with the modern consensus, which is based on the scattered phylogenetic distribution of barbeled species along with Ryder’s arguments. Thus, the barbels of fishes from different orders are likely to be homoplastic structures produced through convergent evolution. Given the many independent origins of barbels, we might not expect to observe barbeled fishes from different orders recruiting the same genes to pattern their barbels during development.

Based on our current understanding of barbel development, however, we cannot distinguish between Ryder’s independent origin hypothesis and the reversal hypothesis put forth by H. B. Pollard (1894; 1895). Similar to Balfour, Pollard proposed that the vertebrate common ancestor possessed barbels. However, rather than believing that barbels were passed down in an unbroken
chain to contemporary barbelled vertebrates, Pollard thought that modern vertebrates with barbels represent evolutionary reversals to the ancestral condition (Pollard 1895). If this were to be the case, we would expect barbeled fishes from different orders to use the same ancestral barbel developmental genetic mechanism. Such a result would be difficult to distinguish from the parallel evolution of barbels. Only by examining barbel development in non-siluriform fishes can we begin to investigate these ideas.

We have presented here the first study of gene expression during barbel development in fishes. In the words of John Ryder, “The most interesting feature of the developmental evolution of the young catfishes is the early appearance of the barbels (1883).” The more interesting work is certainly yet to come, when barbel development can be compared and contrasted between fishes from different orders that display differences in barbel position, composition, and developmental timing. Loaches (order Cypriniformes), and sturgeons and paddlefish (order Acipenseriformes), develop barbels early in development and can be spawned in the aquarium or aquaculture facilities, making them tractable non-catfishes in which to study barbel development (Bemis and Grande 1992; Snyder 2002; Fujimoto et al. 2006). Recent studies have advanced our understanding of zebrafish “barbology” (LeClair and Topczewski 2010). Zebrafish barbels do not appear until juvenile stages, making developmental studies difficult, but certain insights may be obtained by examining adult barbel morphology in different mutant and transgenic lines. Such investigations into the evolution and development of fish barbels holds promise to increase our understanding of the developmental genetic mechanisms underlying morphological novelty and convergence.


