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Cite this article: Stewart TA. 2015 The origin of a new fin skeleton through tinkering. *Biol. Lett.* 20150415.
http://dx.doi.org/10.1098/rsbl.2015.0415

Received: 17 May 2015
Accepted: 18 June 2015

Subject Areas:
evolution, developmental biology

Keywords:
development, evolution, novelty, saltation, siluriformes

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Evolutionary developmental biology

The origin of a new fin skeleton through tinkering

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Adipose fins are positioned between the dorsal and caudal fins of many teleost fishes and primitively lack skeleton. In at least four lineages, adipose fins have evolved lepidotrichia (bony fin rays), co-opting the developmental program for the dermal skeleton of other fins into this new territory. Here I provide the first description of lepidotrichia development in an adipose fin, characterizing the ontogeny of the redtail catfish, *Phractocephalus hemioliopterus*. Development of these fin rays differs from canonical lepidotrich development in the following four ways: skeleton begins developing in adults, not in larvae; rays begin developing at the fin's distal tip, not proximally; the order in which rays ossify is variable, not fixed; and lepidotrichia appear to grow both proximally and distally, not exclusively proximodistally. Lepidotrichia are often wavy, of irregular thickness and exhibit no regular pattern of segmentation or branching. This skeleton is among the most variable observed in a vertebrate appendage, offering a unique opportunity to explore the basis of hypervariation, which is generally assumed to reflect an absence of function. I argue that this variation reflects a lack of canalization as compared with other, more ancient lepidotrichs and suggest developmental context can affect the morphology of serial homologues.

1. Introduction

François Jacob [1] likened evolution to the action of a tinkerer, wherein design reflects the contingency of materials available and not simply adaptation for function. Indeed, the modular nature of organisms seems to facilitate tinkering [2]. Developmental modules are often redeployed in new contexts and for new functions, and this can result in large, discontinuous shifts in phenotype (i.e. saltation) when tissues or organs develop in locations where they were not previously [3,4]. Although such translocations are generally assumed to be maladaptive when observed within populations [5], there are numerous examples of lineages having evolved new and adaptive phenotypes in this manner [6–9]. Understanding how translocations affect patterns of morphological diversity requires documenting its instances, exploring its generative basis and resolving the apparent lack of congruence in adaptiveness at micro- and macroevolutionary scales [10].

Adipose fins, appendages that are positioned between the dorsal and caudal fins of many teleost fishes, primitively lack skeleton [11]. At least four lineages have independently evolved skeletonized adipose fins by the translocation of lepidotrichia, the ossified fin rays that support the distal portion of other osteichthyan fins, into this territory [11]. Here I describe the morphology and development of the adipose fin skeleton of the redtail catfish, *Phractocephalus hemioliopterus* (Bloch & Schneider 1801). The rayed adipose fin of *P. hemioliopterus* has long been noted [12–14], but this anatomy remains uncharacterized. The adipose fin skeleton of *P. hemioliopterus* exhibits diversity in its morphology and development that is unprecedented among vertebrate appendages and informs how morphological novelties can originate.

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2015.0415 or via http://rsbl.royalsocietypublishing.org.

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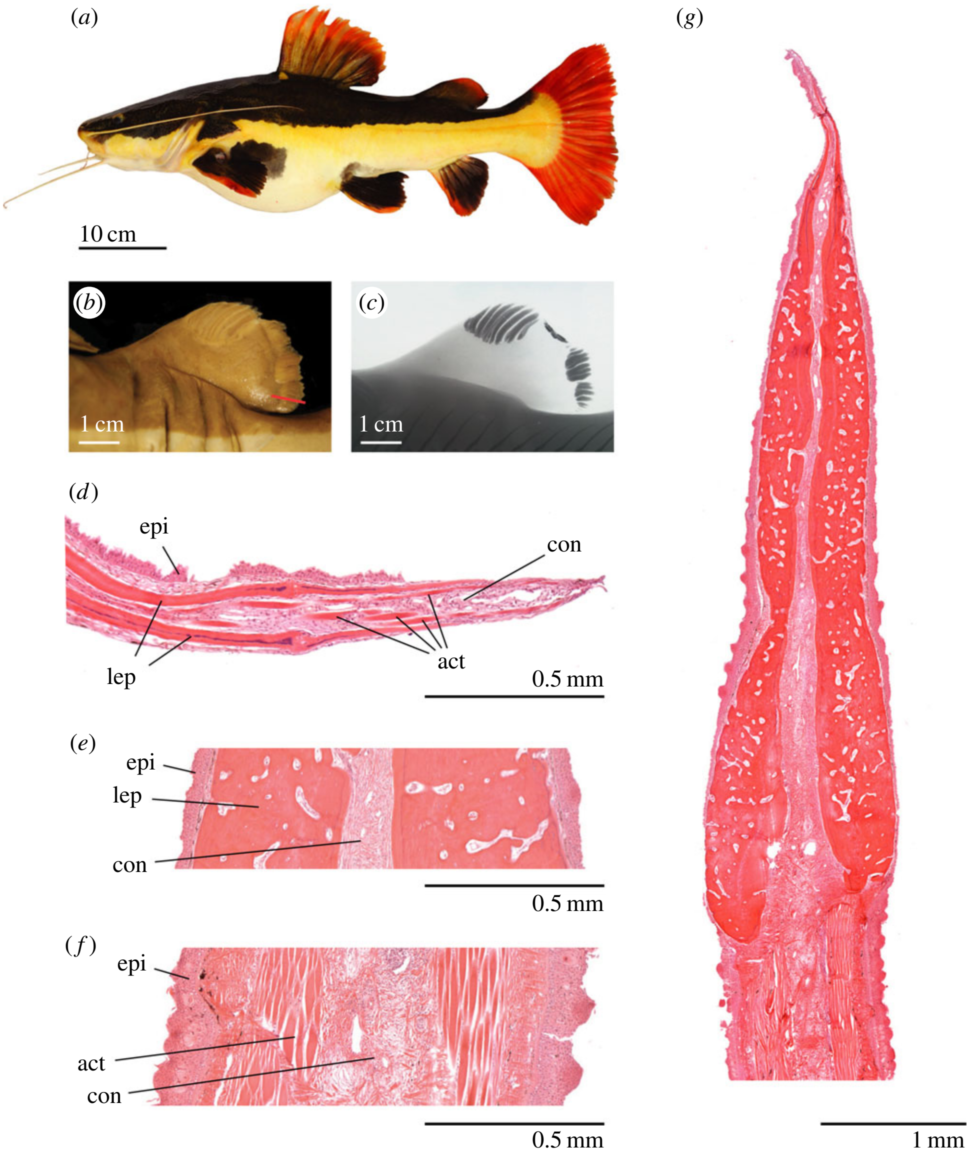

Figure 1. Histology of the adipose fin of *P. hemioliopterus*. (*a*) Photograph of *P. hemioliopterus* by Mark Sabaj Peréz (Specimen INPA43636). (*b,c*) Photograph and radiograph of the adipose fin of specimen FMNH 58032, which was serially sectioned. Red line in (*b*) indicates the position and orientation of histological sections shown in subsequent panels. (*d*) Actinotrichia are positioned at the distal margin of the fin, medial to lepidotrichia. (*e*) Lepidotrichia are medial to the epidermis and composed of paired ossified rods, which surround a core composed of connective tissue. (*f*) In non-skeletonized portions of the fin, parallel bands of actinotrichia sandwich the connective tissue core. (*g*) The left and right sides of the lepidotrichia can be asymmetrical in their length. Abbreviations: act, actinotrichia; lep, lepidotrichia; epi, epidermis; con, connective core.

2. Material and methods

To characterize adipose fin microanatomy, the adipose fin of specimen FMNH 58032 was decalcified by immersion in a 10% EDTA and 90% dH_2O solution at pH 7.4 for 3 days at 4°C, paraffin embedded, serially sectioned at 5 μm thickness, and stained with haematoxylin and eosin by the University of Chicago's Human Tissue Resource Center. Sections are transverse and parallel to lepidotrichia in the adipose fin.

To generate an ontogenetic series, specimens ($n = 53$) ranging in size from 6.1 to 90.0 cm standard length (SL) were X-rayed, size being regarded as a proxy for developmental stage. Adipose fins were X-rayed in lateral aspect, radiographs were digitized, and adipose fin area and the amount of skeleton in the fin were quantified using Adobe Photoshop 7.0C (Adobe System, Inc., San Jose, CA, USA) and Fiji [15] (electronic supplementary material, figure S1). A linear regression was used to test for correlation between the amount of skeleton in the adipose fin and SL. Analysis of covariance (ANCOVA) was used to test whether wild and aquarium individuals differed from one another in their patterns of skeletal growth with respect to SL. Statistical analyses were conducted using R [16].

3. Results

Lepidotrichia in the adipose fin of *P. hemioliopterus* are composed of bilaterally paired ossified rods (hemitrichia), which surround a connective tissue fin core and are adjacent to the basal surface of the epidermis (figure 1). At the distal margin of the fin, actinotrichia are medial to the hemitrichia (figure 1*d*). Lepidotrichia contain many intra-osseus lacunae and muscles do not attach to adipose fin lepidotrichia (figure 1*e,g*). Non-skeletonized regions of the fin are supported by actinotrichia (figure 1*f*).

In wild *P. hemioliopterus*, the amount of skeleton in the adipose fin is positively correlated with standard length (figure 2*a*). This is true when skeleton is measured as either an area ($F_{1,47} = 152.4$, $p < 0.0001$, $R^2 = 0.5589$) or as a fraction of the adipose fin area ($F_{1,47} = 19.94$, $p < 0.0001$, $R^2 = 0.2829$). Above 34 cm SL, all wild individuals were observed with lepidotrichia.

Lepidotrichia develop at the distal tip of the fin, along its trailing edge (figure 2*b*). Fin rays can begin differentiating at

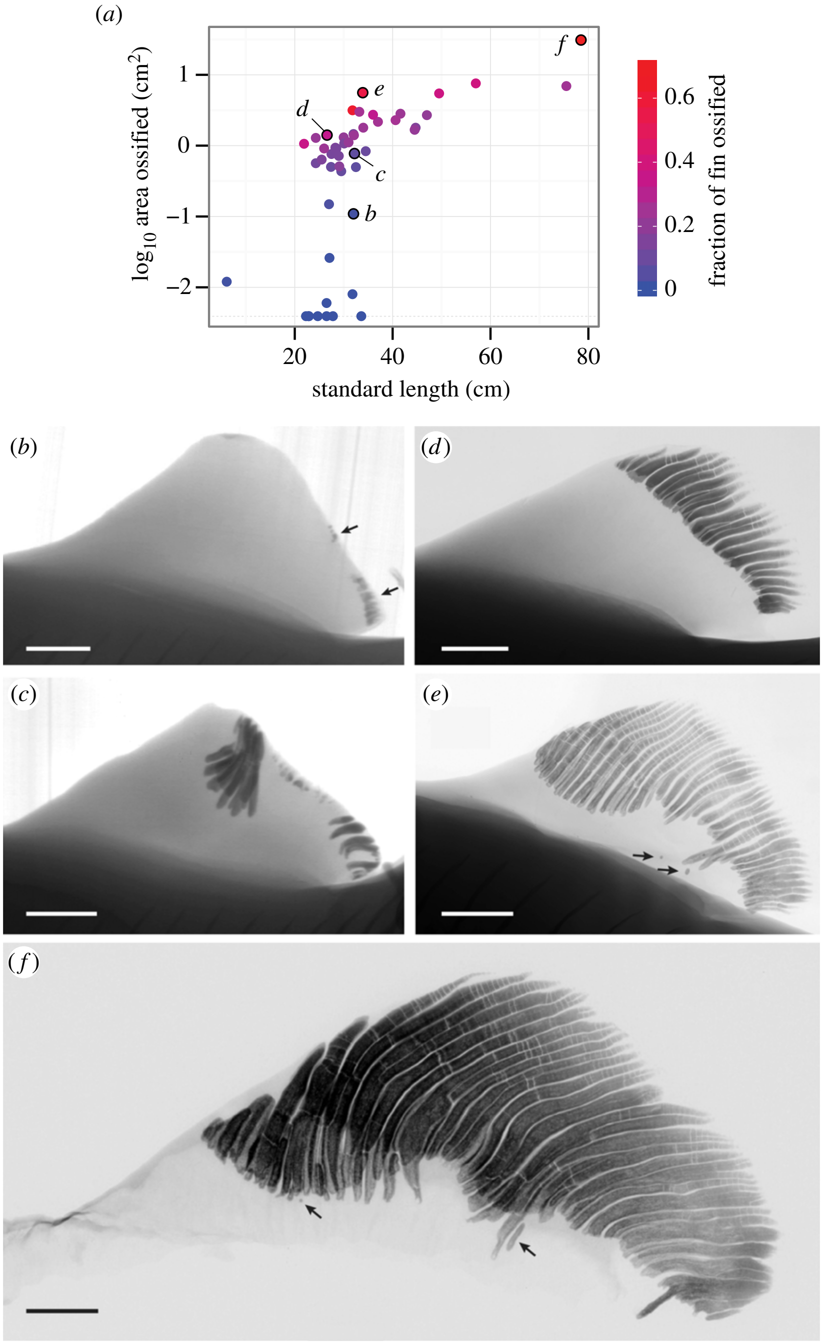

Figure 2. The development of lepidotrichia in the adipose fin of *P. hemioliopterus*. (*a*) In wild fishes, the amount of skeleton in adipose fins is significantly positively correlated with standard length. Specimens with no skeleton are assigned a *y*-axis value of -2.5. (*b–e*) Radiographs of representative individuals varying in their degree of ossification. Contrast has been adjusted to make skeleton more visible. Arrows in (*b*) indicate multiple distinct sites of ossification. Arrows in (*e*,*f*) indicate proximal asymmetrical ossified nodules. Specimens shown: (*b*) LACM 43295-52 #13; (*c*) LACM 43295-52 #5; (*d*) MCZ 6712; (*e*) MCZ 7615; (*f*) ANSP 179559. All scale bars = 1 cm.

multiple spatially discontinuous sites (figure 2*b*), and ossification does not always begin at the same position within the fin (e.g. specimens FMNH 71251 and LACM43295 #13). In several of the more heavily ossified specimens ($n = 9$), asymmetrical ossified nodules are observed proximally, and these do not extend to the fin's margin (figure 2*e*,*f*). Lepidotrichia are highly variable in their morphology, frequently wavy and of inconsistent width. Additionally, lepidotrichia do not exhibit consistent patterns of segmentation or branching, and this is true both between fins and when comparing adjacent rays. Adjacent fin rays can also be of markedly different lengths (figure 2*f*).

Aquarium-raised individuals have less skeleton than wild individuals. This difference is detected both when adipose fin skeleton is measured as an area (ANCOVA: $F_{1,49} = 36.218$, $p < 0.001$) or as a fraction of the adipose fin area (ANCOVA: $F_{1,49} = 21.325$, $p < 0.001$) (electronic supplementary material, figure S2). However, a limited sample of aquarium specimens precludes detailed description of their patterns of growth.

4. Discussion

Skeletal rays in the adipose fin of *P. hemioliopterus* were previously diagnosed as lepidotrichia by their morphology—approximately parallel and sometimes branching ossified rods arranged in a series within a fin. Analysis of the microanatomy of these rays provides additional support for the hypothesis of their serial homology. As with other lepidotrichia, the lepidotrichia in the adipose fin of *P. hemioliopterus* are composed of bilaterally paired ossified elements adjacent to the basal surface of the epidermis, and actinotrichia are medial to the hemitrichia at the distal margin of the fin. Convergence could explain this morphological similarity, but a common developmental mechanism is likely. These hypotheses should be tested with molecular developmental data.

The development of lepidotrichia in the adipose fin of *P. hemioliopterus* is unique in its pattern and variation. Usually, lepidotrichia begin developing in larval fishes at the base of the fin by the membranous ossification of mesoderm [17,18]. Within a fin, lepidotrichia differentiate in an ordered, sequential pattern according to their position: ossification begins in one region and proceeds to adjacent rays, either uni- or bidirectionally depending on the site of initiation [19]. The site at which ossification begins is consistent within a species, but can vary between clades. By contrast, the skeleton of the adipose fin of *P. hemioliopterus* begins developing in adults. This appears to be unique among known fins, with rare exceptions in which lepidotrichia regenerate following their complete removal (e.g. [20]). Three other lineages that have independently evolved adipose fins with lepidotrichia (*Collossoma macropomum*, *Clarotes laticeps*, *Pygocentrus piraya*) have been proposed to develop this skeleton in adults [13,21,22]. Regrettably, their ontogenies remain uncharacterized. The adipose fin skeleton of *P. hemioliopterus* is also unique in that it begins differentiating at the distal tip of the fin, ossification can begin at multiple, non-contiguous sites within a fin, and the site of initiation is not consistent between individuals.

In *P. hemioliopterus*, the proportion of the adipose fin that contains skeleton increases as the fin grows. While several models might explain this pattern, bidirectional growth of lepidotrichia is likely. The branching of rays implies distalward growth, and variability in the proximal portion of individual lepidotrichs throughout the fin, specifically proximal asymmetrical ossifications in heavily ossified fins, implies proximal growth. This is surprising, as lepidotrichia are thought to only grow distally. In segmented lepidotrichia, growth is thought to proceed by the addition of ray segments, not by changes to fin ray segment length [23,24].

The skeleton of the adipose fin of *P. hemioliopterus* is among the most variable of any vertebrate appendage. No other fin skeleton described exhibits such a complete lack of pattern in the branching, segmentation and shape of rays, or regularly exhibits asymmetrical ossifications. Highly variable traits are generally interpreted to be non-functional and reflecting weak selective pressures [25]. Alternatively, highly variable morphologies could be adaptive but uncanalized. The observed differences between wild and aquarium-raised individuals implies plasticity, suggesting that the stimuli required for induction and maintenance of growth for this new fin skeleton is, at least in part, environmentally mediated. At present, adaptive hypotheses for this skeleton remain untested. However, the observation that many specimens (approx. 20%) had injured adipose fins (electronic supplementary material, table S1) and the fact that injuries were nearly always located at the posterior part of the fin suggests that evolution and development of skeleton in these fins might reflect a response to injury and potential fin biting by other fishes.

Tissues and organs that originate by translocation highlight the challenges of diagnosing structures as either iterative homologues or morphological novelties. Developmental context, in this case position, can dramatically alter the phenotype of an iterative homologue, including many key features of its shape, organization and growth patterns. Even the induction of the homologue can be switched from normal embryonic development to being plastically generated in adulthood. These data raise key questions about how translocation of body parts can lead to apparent morphological novelty.

Disclaimer. Opinions expressed in this paper are not those of the John Templeton Foundation.

Data accessibility. Histological sections have been deposited in the Fishes Collection at the Field Museum of Natural History (Chicago, IL). Radiograph data and information on X-ray methodology is available through Morphobank (http://www.morphobank.org, Project 7604).

Competing interests. I have no competing interests.

Funding. This project was supported by the National Science Foundation's Graduate Research Fellowship Program and by the John Templeton Foundation (Integrating Generic and Genetic Explanations of Biological Phenomena; ID 46919).

Acknowledgements. Thank you to the many curators and collections managers who aided in the collection of this data: K. Swagel, S. Mochel, C. McMahan, J. G. Lundberg, M. H. Sabaj, K. Luckenbill, D. Catania, J. Nielsen, C. A. Taylor, J. Armbruster, D. Nelson, A. Williston and K. Hartel. Thanks to L. Smith for imaging histological slides and to D. Krentzel, R. C. Albertson, B. Rubin, K. E. Criswell, R. K. Ho and M. I. Coates for feedback on the manuscript. Xingu specimens provided by iXingu Project, NSF DEB-1257813.

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