The gar is a fish… is a bird… is a mammal?

David M Parichy

Teleosts have emerged as important model organisms, yet their ancestrally duplicated genomes sometimes complicate developmental genetic analyses and comparisons to humans. A new genome sequence of spotted gar, a fish related to teleosts but lacking a duplicated genome, now helps to bridge human and teleost biology.

Back in 1989, Chuck Kimmel published a review espousing the usefulness of zebrafish for research because of its advantages (transparency, fecundity and rapid development) and its similarities to other vertebrates: "The fish is a frog," "The fish is a chicken," "The fish is a mouse" (ref. 1). Since then, zebrafish and other teleosts have indeed been useful for developmental genetics and understanding human disease. Now, the shoal is joined by a new (and yet very old) fish—the gar (**Fig. 1**). On page 427 of this issue, John Postlethwait and colleagues present the spotted gar genome and show how it bridges human genetics, and development and evolution, to the tractable biology of teleost model species[2].

Genomes and bridges

With plenty of fish genomes sequenced[3], why did we need another? Fishes of the aquatic variety (we are all fishes phylogenetically) comprise half of vertebrate species, so adding a few more of their genomes seems not unreasonable. But why gar, a handsome fish and yet not one being studied intensively? The rationale came from something that is now known about zebrafish and other teleosts but was not known in 1989, namely that the ancestral teleost underwent a whole-genome duplication before giving rise to the ~27,000 teleost species of today[3,4] (**Fig. 1**, green bar). This duplication event provided evolutionary opportunities for the fish, as extra genes meant more raw material for selection[5,6]. It also has resulted in opportunities, as well as complications, for biologists.

Copies of genes can persist if they acquire novel functions or subdivide ancestral functions. Subfunctionalization can be useful for developmental geneticists, as less pleiotropy simplifies analyses and makes more traits 'visible' in forward genetic screens. Persisting copies also offer windows into the evolution of gene regulation[6] and have implications for human disease[7]. Yet, the most common outcome of gene or genome duplication is copy loss. Indeed, zebrafish has only ~1.3 times (not 2 times) as many genes as human, despite having an ancestrally duplicated genome[8]. Of course, as copies are lost, duplications of individual genes continue piecemeal, and copies that are retained can diverge at different rates. Adding further complexity, vertebrate ancestors underwent two rounds of genome duplication even before giving rise to teleosts[9] (**Fig. 1**, red bars).

With so many copies in play, it can be difficult to determine whether any particular genes are orthologous, that is, descended from a common ancestral sequence in a common ancestral species. Nevertheless, orthology assignments are critical for elucidating the evolution of gene functions and when using teleosts as models of

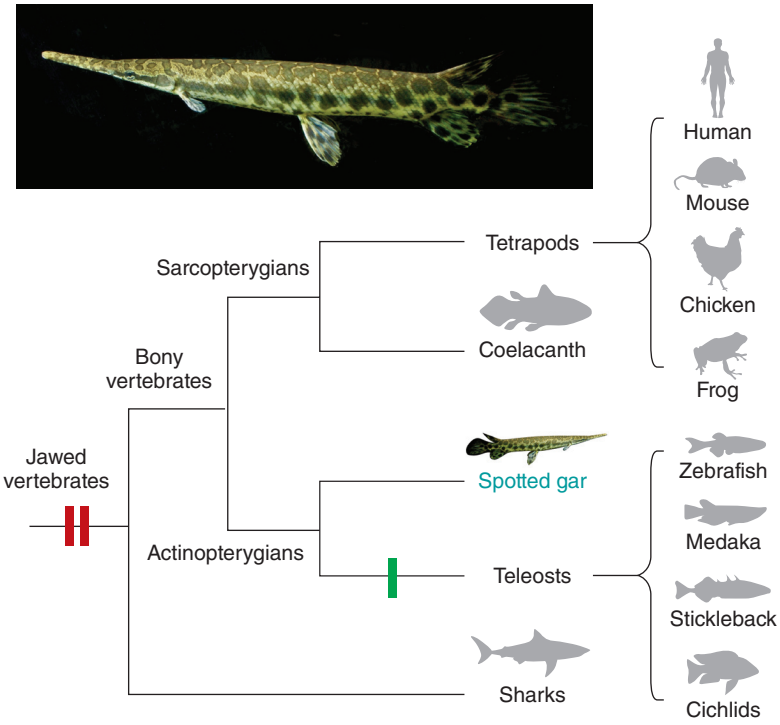

Figure 1 The convenient phylogenetic position and genome of spotted gar. The relationship of spotted gar to other vertebrate groups and model organisms is shown, with ancestral genome duplications indicated by colored bars along the lineages leading to all vertebrates and to teleosts. The photograph of spotted gar was taken by Solomon David.

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human biology. If trying to understand a gene involved in human disease using zebrafish, for example, one obviously hopes to knock out the 'right' locus. Gene trees can help, but analyses of shared gene order on chromosomes, or synteny, may be necessary. Braasch *et al.*[2] reasoned that orthology inferences would be aided were it possible to 'bridge' the vast phylogenetic distance between tetrapods and teleosts using a close relative of the latter that had not itself undergone the extra genome duplication. And this led them to gar.

Gars are a sister group of teleosts, and a genetic map of spotted gar (*Lepisosteus oculatus*) showed that its genome is unduplicated[10]. Now, the finished gar genome sequence confirms this species' usefulness and interesting biology.

Bridges and biology

Darwin considered gars and their relatives to be living fossils, and, indeed, Braasch *et al.*[2] find very low rates of protein evolution. Because the gar sequence is tied to a genetic map and faithfully indicates chromosome structure, it was also possible to uncover strikingly low rates of chromosome evolution, illustrated best by comparison with chicken: many entire chromosomes are conserved between the two species despite the phylogenetic chasm separating them.

Moreover, the usefulness of gar for sorting out orthologies is shown beautifully by analyses of secretory calcium-binding phosphoprotein (Scpp) genes, essential for vertebrate mineralized tissues. Some Scpp genes are needed for bone and dentin, whereas others promote mineralization of enamels[11]. Tetrapods and teleosts exhibit chromosomally linked Scpp genes (23 in human and 15 in zebrafish), yet these genes seemed to be virtually non-overlapping. Only two were clearly orthologous, and three critical for enamel were missing in teleosts, implying that they and bona fide enamel might be an innovation of the tetrapod-lungfish-coelacanth (sarcopterygian) lineage. In gar, however, lurk 35 Scpp genes. This allowed Braasch *et al.*[2] to trace clear lines of descent from a gar-like ancestor to sarcopterygians on one hand and teleosts on the other. Gar also has two enamel genes expressed in scales. So an enamel-like matrix likely evolved in earlier vertebrates, in association with scales, and was only later recruited to sarcopterygian teeth, while presumably being lost in teleosts. This conclusion was also reached by another group using these same publicly released genomic and transcriptomic data from gar, as well as independent paleontological evidence[12].

Finally, Braasch *et al.*[2] identify gar as a valuable model for the evolution of gene regulation. Candidate enhancers and repressors are often identified by genomic comparisons that highlight conserved noncoding elements (CNEs). But the species chosen are critical: too closely related and the genomes lack enough background variation for CNEs to stand out; too distantly related and the regulatory elements themselves are obscured. By comparing human to gar, and gar to zebrafish, it was possible to identify CNEs present in both human and zebrafish that were not evident in direct comparisons, including a conserved yet cryptic HoxD regulatory element in zebrafish able to drive limb expression in mouse. In total, Braasch *et al.*[2] found in zebrafish ~6,500 cryptic CNEs shared with human, of which ~1,000 have been associated with disease or other phenotypes in genome-wide association studies.

What's next for gar? The enhanced CNE detection afforded by the gar bridge will provide new opportunities for understanding gene regulatory variation and its evolutionary or pathological significance, whereas translational studies will benefit from improved orthology assignments. And, in the age of CRISPR/Cas9 mutagenesis and advanced transgenesis, functional studies are likely to be feasible in gar itself. Finally, with genome sequences now available for anchor species such as gar, shark[13], lamprey[14] and coelacanth[15], as well as tetrapods and teleosts, we may finally have a bridge to reconstruct the ur-vertebrate genome. It seems that the gar is a fish and also a whole lot more.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Tet proteins enhance the developmental hourglass

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A new study compares DNA methylation profiles in developing zebrafish, *Xenopus tropicalis* and mice and suggests roles for Tet proteins in demethylating conserved gene enhancers during the phylotypic period of early development. These findings provide an epigenetic underpinning for the 'hourglass' model.

Comparisons of vertebrate embryonic morphology highlight a striking trend: early-stage embryos of various species exhibit different morphologies, but these transiently converge just after neurulation—at the pharyngula stage—and then subsequently diverge. This phenomenon has been described to resemble an hourglass, with the bottleneck representing the developmental time point with the least phenotypic variability, which has been termed the mid-embryonic phylotypic period[1] (**Fig. 1**). During this period, the basic body plan is established, which is relatively well conserved among vertebrates. Until recently, this hourglass model has been based primarily on morphological comparisons and has lacked molecular evidence to explain this convergence. Now, a study by Ryan Lister and colleagues[2] reports that vertebrates achieve transcriptional competence for similar genes involved in body plan formation by changing the epigenetic state of their linked enhancers.

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