# "Wrecks of Ancient Life": Genetic Variants Vetted by Natural Selection

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THE Genetics Society of America's George W. Beadle Award honors individuals who have made outstanding contributions to the community of genetics researchers and who exemplify the qualities of its namesake as a respected academic, administrator, and public servant. The 2015 recipient is John Postlethwait. He has made groundbreaking contributions in developing the zebrafish as a molecular genetic model and in understanding the evolution of new gene functions in vertebrates. He built the first zebrafish genetic map and showed that its genome, along with that of distantly related teleost fish, had been duplicated. Postlethwait played an integral role in the zebrafish genome-sequencing project and elucidated the genomic organization of several fish species. Postlethwait is also honored for his active involvement with the zebrafish community, advocacy for zebrafish as a model system, and commitment to driving the field forward.

Genetics blossomed as a science spurred by wise selections of compliant organisms. One hundred years ago, the first paper in the first issue of *GENETICS* used genetic maps and chromosome anomalies in *Drosophila melanogaster* to support the chromosomal theory of inheritance (Bridges 1916). Fruit flies, along with mice, mold, and maize (*e.g.*, Beadle and Ephrussi 1937; Beadle 1939; Beadle and Tatum 1941) have been joined by newcomers like nematodes, zebrafish, and *Arabidopsis*. Only recently, however, have biologists had access to the vast array of Darwin's "endless forms most beautiful and most wonderful" (Darwin 1859, p. 483) for in-depth genetic investigations of development, physiology, and evolution. Rapid genome sequencing, transcriptomics of small populations of differentiating cells, powerful bioinformatics, and broadly applicable genome-editing methods can now convert nearly any species inhabiting Darwin's "tangled bank" (Darwin 1959, p. 482) into a "model organism."

> I am only surprised that more wrecks of ancient life have not been preserved... (Darwin 1959, p. 136)

To Darwin, "wrecks of ancient life" were species that lost features associated with ancestral forms, like cave crabs that retain the eyestalk while missing the eye that crowns the stalk of terrestrial crabs (Darwin 1859). We called these rare species "evolutionary mutant models" because they offer important genetic variants that can shed light on the mechanisms of development and physiology in the wild (Albertson *et al.* 2009). Phenotypes exhibited by these "wrecks of ancient life" would be disease states in related species, but in particular environments are instead functional. Understanding the genetic basis for the wrecked phenotype provides insights into disease mechanisms, and learning how wrecked species cope with the altered phenotype can offer hints toward novel therapies.

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**—J.H.P.**

Darwin's prototypic living wrecks inhabit caves. Researchers have made remarkable progress investigating the Mexican blind tetra cavefish *Astyanax mexicanus* (Protas and Jeffery 2012; Stemmer *et al.* 2015) and have used the *Astyanax* genome sequence coupled with QTL analyses to identify candidate genes for cave phenotypes (McGaugh *et al.* 2014). In cave *Astyanax*, lens apoptosis induces eye degeneration. We investigated gene expression in the blind cyprinid cavefish *Sinocyclocheilus anophthalmus* and, in contrast to *Astyanax*, results suggested a lens-independent reduction in retinal cell proliferation and down-regulation of transcriptional factors that control retinal development and


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maintenance (Meng *et al.* 2013a,b). These results show that cavefish derived from different clades can use alternative genetic mechanisms to converge on a phenotype that in many ways mimics retinal degeneration in aging people. The full developmental genetic program that distinguishes retinal development in cave and surface fish is still being explored.

In 1871, Darwin realized that larval tunicates (urochordates) have morphologies similar to vertebrate embryos. He suggested that in "an extremely remote period" (p. 125) animals with these morphologies produced one lineage leading to vertebrates and another lineage "retrograding in development" (p. 125) to become today's tunicates (Darwin 1871). Tunicates are now recognized as forming the sister group to vertebrates (Delsuc *et al.* 2006). The urochordate *Oikopleura dioica* represents a curious case of a wrecked genome despite retaining the ancestral chordate body plan even in the adult. The *Oikopleura* genome has a reduced repertoire of developmental regulatory genes, drastically reorganized introns, and a highly altered gene order (Canestro and Postlethwait 2007; Canestro *et al.* 2010; Denoeud *et al.* 2010). In spite of its modified genome, we and others showed that the pattern of developmental gene expression in *Oikopleura* embryos suggests that supposed vertebrate innovations, such as placodes and the thyroid, originated in chordate ancestors (Bassham *et al.* 2008; Canestro *et al.* 2008). The recent development of methods to knockdown gene expression in *Oikopleura* now enables functional investigations of gene action in this morphologically beautiful chordate with a wrecked genome (Mikhaleva *et al.* 2015; Omotezako *et al.* 2015).

**Dr. Postlethwait's work began the molecular genetic era of zebrafish research and has helped to demystify the evolution of genes and genomes. He has also strengthened the zebrafish community through his generous data sharing, collaborative spirit, and help for dozens of labs in mutation and gene mapping.**
**—Alex Schier, Harvard University**

The voyage of the *Beagle* did not stray quite far enough South to encounter an amazing shipwreck of an animal, Antarctic icefish, which inhabit the icy (−1.9°) waters of the Southern Ocean. Icefish have acquired adaptations for survival in the cold, including antifreeze proteins (Devries 1971), a constitutive "heat shock" response (Place and Hofmann 2005), and modified membrane phospholipids (Logue *et al.* 2000). Icefish ancestors were bottom dwellers, possessing red blood and densely mineralized bones but lacking a swim bladder, which is the organ of neutral buoyancy in most fish (Eastman 1993). As the Southern Ocean cooled, fish species occupying the water column became extinct, providing unexploited habitats for ambush predators with neutral buoyancy (Eastman 1993). In the absence of a swim bladder, icefish ancestors accumulated mutations that cause lipid accrual, decreased skeletal ossification, and reduced bony scales (Friedrich and Hagen 1994; Near *et al.* 2009; Eastman *et al.* 2014). These phenotypes would be maladaptive in a person or even in most fish. Icefish are also unique among vertebrates in having lost functional hemoglobin genes and red blood cells, which generated an anemic phenotype compensated by the diffusion of oxygen through scale-less skin, decreased oxygen demand, increased heart size, high cardiac pumping volume, decreased blood viscosity, extensive vascularization, increased muscle cross-sectional area, and amplified mitochondrial density and lipid content in heart and oxidative skeletal muscle (Sidell and O'Brien 2006; Detrich and Amemiya 2010). For species with low bone density, we found that embryos maintain the youthful chondrogenic program and postpone or abandon the mature osteogenic program for most bones. This suggests that altered timing of skeletogenic gene expression may have been a significant adaptation in the radiation of Antarctic fish from the ocean bottom into the water column (Albertson *et al.* 2010). More work is required to identify the molecular genetics behind these heterochronic shifts. Recently developed tools now provide the means to study icefish and other wrecks of ancient life, just as mutations in model organisms like maize and *Drosophila* (*e.g.*, Postlethwait and Nelson 1964; Gelinas *et al.* 1969; Postlethwait and Schneiderman 1969, 1971; Postlethwait and Girton 1974) provide insights into the genetic mechanisms of development, organ function, and evolution.

*Natura non facit saltum* (Darwin 1859, p. 160)

Seven times in *On the Origin of Species*, Darwin invoked the concept that "nature does not make leaps. Over 50 years after Darwin's treatise was published, and now 100 years ago, an article published in the first year of the fledgling journal *GENETICS* discussed a situation in which nature *does* in fact make leaps—the origin of novel morphologies after a jump in genomic content by genome duplication (Tupper and Bartlett 1916). Genome duplication appears to have shaped vertebrate evolution in two rounds before the divergence of fish and mammalian lineages (Holland *et al.* 1994; Dehal and Boore 2005). It was previously known that gene families are often larger in teleosts than in mammals, but it was unclear if this condition arose due to excess preservation of tandem duplicates or to an additional genome duplication event, as suggested by S. Ohno (Ohno 1970). To resolve this question, we used genetic mapping to find the genomic locations of duplicated gene pairs in zebrafish. We found that gene pairs reside on duplicated zebrafish chromosomes, and these duplicated chromosome segments are shared with the pufferfish fugu, suggesting that distantly related teleosts share an ancestral genome duplication event (Postlethwait *et al.* 1994, 1998, 1999, 2002; Amores *et al.* 1998; Taylor *et al.* 2003; Jaillon *et al.* 2004). Gene expression patterns in zebrafish and other teleosts showed that gene duplicates from the teleost genome duplication (TGD) are often expressed in subsets of tissues or developmental times

shared by their mouse orthologs (*e.g.*, Ekker *et al.* 1992; Akimenko *et al.* 1994, 1995; Thisse *et al.* 1995; Risinger *et al.* 1998; Oates *et al.* 1999). These findings led to the idea that, after genome duplication, duplicated genes (called ohnologs when originating in a genome duplication) are redundant, so some of their ancestral functions—like expression domains, protein functional domains, or protein quantities—can reciprocally degenerate. But as long as the two ohnologs complement for essential functions, both can be retained in the genome, a process we called "subfunctionalization" (Force *et al.* 1999). Alternative outcomes include the loss of one of the copies or the origin of a new, positively selected function (which we called "neofunctionalization") (Ohno 1970; Force *et al.* 1999). Thus, although nature did make a leap—from diploid to tetraploid in perhaps a single clutch of fish ∼300 million years ago—gradual genetic changes afterward likely led to the origin of teleost morphological innovations (such as dorsal–ventral symmetrical tails that improve swimming and mobile upper jaw bones that facilitate prey capture). Perhaps these changes occurred because having twice as many mutation targets accelerated the genetic changes that led to teleost novelties. However, we still do not know whether or how genome duplication might increase the rate of speciation or increase the likelihood of evolutionary innovations.

> . . .seven genera of Ganoid fishes. . .these anomalous forms may be called living fossils. . . (Darwin 1859, p. 106)

Understanding the genetic mechanisms by which doubled genomes became modified in ancient teleosts requires the study of a surviving lineage that diverged from the teleost lineage before the TGD. Darwin's paradigmatic examples of what he called "living fossils" were fish that contain ganoid scales, such as spotted gar. The spotted gar represents a sister group of the teleosts, as we showed using genetic maps that capitalize on massively parallel DNA sequencing (Amores *et al.* 2011), novel software to analyze these sequences (Catchen *et al.* 2011a), and bioinformatic algorithms to perform comparative genomics (Catchen *et al.* 2011b). Analysis of the spotted gar genome showed that it links mammals to teleosts in ways that illuminate evolutionary mechanisms (Braasch *et al.* 2015). For example, conserved noncoding elements, many of which act as genetic regulatory elements, are often not detectable when directly comparing mammals to teleosts, but become evident when mammals are first compared to gar and then gar is compared to teleosts. Furthermore, these "cryptic" teleost elements can drive function in mammalian development in patterns similar to those of their mammalian orthologs (Gehrke *et al.* 2015). The gar genome promises to better link the duplicated genomes of teleost medical models to human biology.

> . . .so much variety and so little real novelty. . . (Darwin 1859, p. 185)

We are living in a remarkable time for genetics. For the first time, tools are available for mechanistic investigations of "so much variety" in the form and function of nonlaboratory species, allowing us to seek the origins of "real novelty."

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