

How an ancient genome duplication electrified modern fish

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At various chapters in the story of life on earth, certain characters arise that seem to open the door for explosions of new forms and species. From the flowers of angiosperms to the mammary glands of mammals, these so-called evolutionary innovations represent new features that change the playing field of evolution and carry with them a cascade of biodiversity. Where these novelties come from remains a major mystery for biologists, because innovations are so different from traits in related species that it is difficult to imagine how evolution can build such unique features from accumulated changes in DNA sequence. A study published by Arnegard et al. (1) illustrates how a combination of ancient gene duplication and good old-fashioned natural selection works together to produce a communication organ that led to the origin of hundreds of species of electric fish in two hemispheres (Fig. 1).

The myogenic electric organ has evolved repeatedly during the diversification of teleost fish (2). In some lineages, this organ exploits a sensory modality for communication entirely unique among vertebrates by producing electrical signals. Electric signals of these fish are analogous to acoustic songs of birds or insects, only sung in a different sensory realm. Two unrelated lineages of fish, the mormyroids of Africa and the gymnotiforms of South America, have evolved similar electric organs and use them to produce signals used in courtship and territoriality (3). Within each species, males and females produce recognizably different signals, and sexual selection seems to play a major role in the diversification of signal types (2).

The electric signal represents a private communication channel that does not compete for bandwidth with other signals in the environment and can be detected only by closely related species (and a few specialized predators). Environmental factors that degrade acoustic and visual signals have no effect on electric signals, thus allowing electric fish to exploit habitats with relatively few competitors for space and resources. As a result of this evolutionary innovation, both the mormyroid and gymnotiform lineages have radiated into hundreds of species that represent a significant proportion of the species found in their local waters (2).

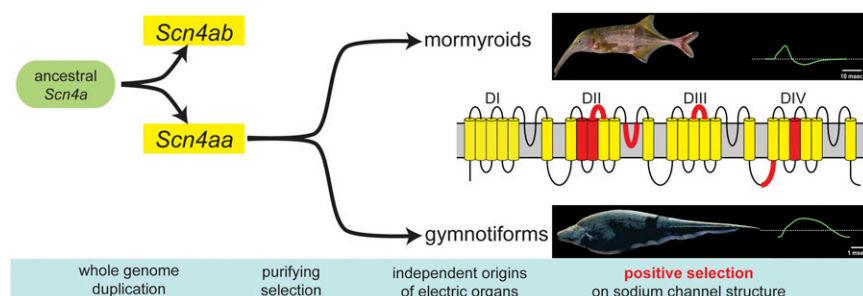


Fig. 1. A whole-genome duplication occurred early in the evolution of teleost fish, producing duplicate copies of the sodium channel gene *Scn4a* that was expressed in skeletal muscle. The two sister genes, *Scn4aa* and *Scn4ab*, continued to be expressed in skeletal muscle and experienced purifying selection that maintained protein structure. Roughly 100 Myr later, the *Scn4aa* gene was co-opted into novel electric organs in two independent lineages of fish, the mormyroids in Africa and the gymnotiforms in South America. Coincident with this change of expression, *Scn4aa* experienced a sudden 10-fold increase in the strength of selection, whereas the sister gene, *Scn4ab*, did not. Selection fixed amino acid substitutions in identical regions (shown in red) of the Na_v1.4a sodium channel in each fish lineage. Representative species and electric organ discharge are shown for mormyroids (*Campylomormyrus numenius*; photo by J.P. Sullivan) and gymnotiforms (*Sternopygus macrurus*; photo by P.K. Stoddard).

But how does evolution build such a novel structure and function? The electric organ is developmentally derived from skeletal muscle and requires the synchronized firing of multiple electrocytes to generate the electric signal (4). Pulses in current generate the discharge, and voltage-gated sodium channels unique to teleost fish are known to underlie the discharges from the electric organ (5). Sodium channels, in general, are encoded by a family of genes expressed in different tissues, the structure of which is highly conserved both across taxa and across tissues within taxa. Minor changes in channel structure are known to compromise their function as part of the machinery propagating action potentials in nerves and muscle, resulting in devastating effects on fitness.

The more specific question then is how can evolution take a gene so central to basic function as a sodium channel and co-opt it for a new function in a novel organ (and do so two times)? The answer starts with gene duplication. Roughly, 225–300 mya, during the early evolution of fish, teleosts experienced a whole-genome duplication (6). Some of the duplicate genes produced in that ancestor have been retained and presumably acquired new functions, whereas others were long ago lost as nonfunctional units. The entire family of sodium channel genes (*ScnA*), which numbered four at the time of genome duplication, was duplicated and

retained, generating the eight channel genes present in modern teleosts (7).

At this duplication, the gene-encoding skeletal muscle channel became two forms, *Scn4aa* and *Scn4ab*. Both forms are still expressed in skeletal muscle in most fish and retain the basic sequence structure essential for propagation of transmembrane current (7). Using newly developed phylogenies of gymnotiforms and mormyroids, Arnegard et al. (1) are able to pinpoint the evolutionary transition, ~100 Myr after the genome duplication, when one of these sodium channel forms, *Scn4aa*, was lost from skeletal muscle in two lineages. This event coincided with the origin of the electric organ and expression of *Scn4aa* in that tissue.

Duplication of functional genes is thought to be an important route to evolutionary novelty, because one copy is free to accumulate mutations, whereas the other retains the original function (8). The puzzle of the electric fish story, however, is that the duplicate gene sat around for roughly 100 Myr without obvious function, expressed alongside another functional copy in the same tissue. The usual lifespan of a duplicate gene in animals is about 4 Myr (9). In this case, the

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building block of innovation hung around about 25 times longer than expected.

If the critical duplicate gene, *Scn4aa*, was indeed part of the crop of standing variation, only later adopted for a novel function, then the signature of selection on the DNA sequence should change distinctly after co-option. The dN/dS ratio measures the proportion of nucleotide substitutions that change amino acids [nonsynonymous (N)] to those with no effect on the protein product [synonymous (S)]. Exceptionally small values of the ratio indicate selection that limits changes in the sequence (i.e., stabilizing or purifying selection), whereas large values indicate many changes, which is expected under positive selection for new function or dramatically relaxed constraint. If *Scn4aa* was selected for a new function in the electric organ, the dN/dS should be large after co-option but very small before that. Moreover, a comparison of the strength of selection on the sister gene still expressed in skeletal muscle, *Scn4ab*, should show no increase in selection coincident with the origin of the electric organ. Arnegard et al. (1) find exactly this pattern. Before the radiation of the two electric fish lineages, both *Scn4aa* and *Scn4ab* experienced stabilizing selection as expected for an important gene under functional constraints. Immediately after the origin of each of the two electric fish clades, the *SCN4aa* gene showed a 10-fold increase in the strength of selection, but *Scn4ab* did not. This pattern is clear in each of the independent fish lineages.

The presence of an essentially neutral locus waiting to be co-opted for new function addresses another major issue in

the genetics of adaptation. Evolutionary geneticists often think of adaptation occurring through new mutations that arise and can then be favored and swept to fixation. This process is inherently slow,

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because the original mutations are, by definition, found at negligibly small frequency. However, alleles that are present as part of the standing crop of variation may allow adaptation to proceed much more quickly, because they are at higher frequencies to start (10). Co-option of the duplicate *Scn4aa* may follow such a scenario by taking advantage of existing variants in a sodium channel gene whose original function was accomplished by its sister *Scn4ab*.

The new electric organ sodium channel, *Scn4aa*, should show an additional signature if it was selected for modified function as a signal generator—convergent changes in similar regions of the channel in both the mormyroid and gymnotiform lineages. Arnegard et al. (1) use a sliding window analysis to show that selection was strongest in many of the same regions of the *SCN4aa* gene in both lineages of electric fish; roughly one-half of the targeted regions are identical between the lineages,

and most others are adjacent (Fig. 1). Meanwhile, a comparable analysis in closely related nonelectric fish reveals no positive selection at all in *SCN4aa*. As expected, the specific amino acid substitutions observed in both mormyroids and gymnotiforms included a number of sites that would be expected to affect the kinetics of channel activation, thereby changing the duration and frequency of electrical discharges. These positional targets of selection would have been unlikely to become fixed in a functional skeletal muscle sodium channel, because changes at many of these same positions are known to be associated with severely deleterious conditions in *Homo* and *Drosophila*.

The origins of the electric organ in mormyroids and gymnotids reinforce several emerging themes in our understanding of the origins of adaptation, most of which turn out to not be unique to those extraordinary traits that we recognize as evolutionary innovations. The importance of gene duplication and standing variation as materials that allow rapid and dramatic evolutionary changes is clear in electric fish. It is also evident that selection can generate remarkably parallel changes in functional genes at the level of specific amino acid positions and substitutions. There is undoubtedly much more to tell in this story of evolutionary innovation, including what drives the differences in gene expression that led *Scn4aa* to occur in electric organ (and not muscle) in the first place and what allowed a seemingly functionless duplicate gene to survive in the genome some 25 times longer than expected.

- Arnegard ME, Zwickl DJ, Lu Y, Zakon HH (2010) Old gene duplication facilitates origin and diversification of an innovative communication system—twice. *Proc Natl Acad Sci USA* 107:22172–22177.
- Arnegard ME, et al. (2010) Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *Am Nat* 176:335–356.
- Zakon HH, Lu Y, Zwickl DJ, Hillis DM (2006) Sodium channel genes and the evolution of diversity in communication signals of electric fishes: Convergent molecular evolution. *Proc Natl Acad Sci USA* 103:3675–3680.
- Bennett MVL (1971) Electric organs. *Fish Physiology*, eds Hoar WS, Randall DJ (Academic, New York), Vol 5, pp 347–491.
- Ferrari MB, McAnelly ML, Zakon HH (1995) Individual variation in and androgen-modulation of the sodium current in electric organ. *J Neurosci* 15:4023–4032.
- Hoegg S, Brinkmann H, Taylor JS, Meyer A (2004) Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. *J Mol Evol* 59:190–203.
- Novak AE, et al. (2006) Gene duplications and evolution of vertebrate voltage-gated sodium channels. *J Mol Evol* 63:208–221.
- Taylor JS, Raes J (2004) Duplication and divergence: The evolution of new genes and old ideas. *Annu Rev Genet* 38:615–643.
- Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. *Science* 290:1151–1155.
- Orr HA, Betancourt AJ (2001) Haldane's sieve and adaptation from the standing genetic variation. *Genetics* 157:875–884.