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Evolution and development: From the pet shop to the pelagic zone

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Flying fish and some of their relatives have evolved the ability to elegantly escape predators by gliding through air. A new study — involving a pet shop zebrafish mutant — offers glimpses into how fins might have been modified to enable this stunt.

> "There are these two young fish swimming along, and they happen to meet an older fish swimming the other way, who nods at them and says 'Morning, boys. How's the water?' And the two young fish swim on for a bit, and then eventually one of them looks over at the other and goes 'What the hell is water?'"
>
> David Foster Wallace — *This Is Water*.

Sure enough, there are fish for whom being out of the proverbial water is part of a lifestyle: there are mudskippers that hang out on tidal flats, or lungfish that can hole up in the ground to weather long droughts, but none escape their element more elegantly than flying fish. The 70 or so species of flying fish are found in the family Exocoetidae, part of the larger order of Beloniformes, which, among the halfbeaks, contains a few additional air-gliding fish[1]. Exocoetids break through the water surface propelled by their fast beating tail (Figure 1). With their ventrally enlarged hypoceral tail fin still in the water, they then taxi above the surface to pick up speed before they fully take to the air and glide on the airfoil afforded by their extended pectoral fins. The most accomplished flying fishes use four wings including enlarged pelvic fins to glide 50 or more meters before they have to taxi again[2]. With multiple rounds of flying and taxi, a fish can fly several hundred meters, outpacing large predators that haunt the waters underneath. Exocoetids are formidably adapted to this volant lifestyle: they have ultrafast muscles wagging their tails to propel them out of the water, a modified shoulder girdle and muscle system that lets them spread their 'wings', as well as an enlarged vestibular system to ensure balance in air and eyes that work well in both media; but their most obvious adaptation are their wing-like fins, whose evolutionary and developmental genetics are the focus of a new study by Jacob Daane, Matthew Harris and colleagues[3] in this issue of *Current Biology*.

With their wing-like fins, flying fish look like straight out of a Hieronymus Bosch painting and are a testament to the weird and wonderful awesomeness of nature and its — for want of a better word — creativity. But what makes flying fish so fascinating from an evolutionary perspective is that their aerial acrobatics — at least superficially — evoke an evolutionary transition — taking to the air — that has revolutionized the history of life on Earth. Only three vertebrate lineages — birds, bats and pterosaurs — have accomplished powered flight, and in each case conquest of airspace has led to spectacularly successful evolutionary radiations. Many more species, however, among them rodents, snakes or lizards, can glide, which is often invoked as a prelude to full flight. No wonder then that flying fish have captured the imagination of Charles Darwin, who mused that they "might have been modified into perfectly winged

Figure 1. Out of the water.
An exocoetid flying fish during taxiing.

animals", or latter day filmmakers who in '*The future is wild*' envisaged an evolutionary scenario, hundreds of millions of years away, in which fish evolve to conquer the bird-free skies to become 'flish'.

Since the beginning of the century, evolutionary biology has been dominated by the hunt for the genetic underpinnings of evolutionary — often adaptive — phenotypic change. Pinpointing evolutionary changes on the level of the genome is not just interesting in its own right; like all good science, it allows the formulation of new, more precise questions, such as: where do the mutations come from that selection acts upon or what makes certain genetic pathways more amenable to evolutionary modification? So far, the best understood examples in this space come from fairly minor phenotypic changes, often involving losses of a trait. And while trait loss is in itself an important feature of adaptive evolution, there is a sense that larger-scale evolutionary changes — often entailing the evolution of novel organs or the repurposing (co-option) of existing organs — have not been captured by this research program. Here is where flying fish appeal, because they embody both a major evolutionary change — taking to the air — and one that has a fairly discrete and understood phenotypic basis: modified fins.

Thanks to developmental genetics in zebrafish, many genes are known that control fin growth. Interestingly, rather than the same-old developmental signaling pathways, bioelectric components, such as ion channels or gap-junction proteins, have emerged as an exciting new module of developmental control in fins[4]. So far, how these proteins work and whether their biological role renders them particularly malleable as agents of evolutionary change is not entirely clear. Interestingly, the sexually selected fin ornaments of swordtails may have evolved via altered bioelectrical signaling[5].

Daane and colleagues[3] analyze two zebrafish mutants, one of which, *nr21*, has reduced fins. They identify the causative mutation as a gain-of-function mutation in a L-type amino acid transporter, *lat4a*, suggesting that this protein functions as a suppressor of fin growth. How it does that, the authors remain agnostic about. The second mutant is *long fin* (*lof*), an iconic zebrafish mutant that comes from a pet store. (These fish are great for novice Danioists, because their fins slow them down, making them easier to catch). Through an artisanal revertant screen, Daane and colleagues[3] identify the gene altered in *lof* as the potassium channel subunit *kcnh2a*. They also pinpoint the causative mutation as a small inversion that leads to the channel's overexpression; if you are so inclined, you may now nerd out about the fact that *lof* is a g-o-f.

As honorable developmental geneticists, Daane and colleagues[3] crossed the *kcnh2a* and *lat4a* mutants. The resultant trans-heterozygote is a hopeful monster if there ever was one — its fin configuration resembles that of flying fishes: while its unpaired dorsal and anal fins are normal-sized (here *lat4a* suppresses the fin overgrowth caused by *kcnh2a*), the pectoral and pelvic fins are elongated. The tail fin is uncannily reminiscent of the hypoceral caudal fin of flying fishes. But this semblance is only superficial, as the fins of flying fish have a number of additional adaptations, such as stiffened, v-shaped rays, that this mutant does not capture. Sadly, the authors do not report on whether these trans-heterozygotes can fly, but given the floppiness of their fins, these monsters might not be too hopeful.

Mutant phenotypes resembling major evolutionary transitions, like a chicken mutant with saurian teeth[6] or the zebrafish with wing-like fins, are a tantalizing proposition. But this does by no means imply that changes in any of these genes underlay the evolution of flying fish fins in the Eocene ocean. It is unclear that such drastic phenotypic changes, were they to occur in the wild seas, could be anything but detrimental, especially as other systems, like the bones and muscle attachments of the shoulder girdle, on which their proper function is contingent, would not have had a chance to co-evolve. Instead of recapitulating actual evolutionary events, such mutants can, however, reveal how malleable a given developmental process is. A lot of the study of the developmental basis of evolutionary change still operates under this — highly successful — paradigm of developmental genetics, in which causative genes are identified through analysis of large-effect loss- and gain-of-function mutations with striking phenotypes; but especially when quantitative phenotypes are concerned, a more complex model is emerging where many genes exert small effects on a given trait[7]. And perhaps such subtler effects might provide more suitable raw material for selection to act upon.

Traditionally, the most successful case studies that have identified genes underlying evolutionary change have relied on association and mapping approaches that often involve crossing populations or species that diverge in the evolved trait of interest[8]. Given the deep evolutionary distances among exocoetids and their non-flying relatives, this is out of the question. Hence, Daane and colleagues[3] applied a so-called 'phylo-mapping' strategy that is aimed at uncovering differences in evolutionary dynamics across conserved regions of the genome, both coding and non-coding[9]. They sampled 35 species, comprising several exocoetids, some gliding halfbeaks, as well as non-gliding Beloniformes and outgroups, scanning their genomes at hundreds of thousands of sites. By comparing gliding and non-gliding fish, many hundreds of genomic loci emerge whose evolutionary rate is elevated in gliding species. How to make sense of this heap of differences? One way is to group these loci based on their ontology terms — functions ascribed due to homology to genes whose function is characterized in other animals. These terms are necessarily broad, and so the extracted information remains fairly coarse, too. Daane and colleagues[3] find — among others — elevated evolutionary dynamics in genes involved in limb and muscle development, as well as the vestibular system. The vagueness of the term 'involved' here betrays perhaps how thin the biological ice of assigning concrete functions — and testable hypotheses — based on gene ontology can be.

One unexpected find of Daane and colleagues[3] was a strong signal of an elevated evolutionary rate among genes affecting development of the *locus ceruleus*, a brainstem center mediating arousal and attention. This hints at a trait that had not been inferred from morphological studies alone and may make perfect sense for skittish flying fish. It also betrays the often-overlooked fact that almost nothing in morphological evolution makes sense, unless behavior is considered as well. The fancy fins make little sense if the animal does not in the first place have an inclination to sault out of the water. Flying fish may be one of the best systems to study such evolutionary transitions that originate in a particular behavior (and one that is not uncommon among fish).

In a next step, Daane and colleagues[3] focused their attention on genes, leaving conserved non-coding elements, like enhancers, aside. Given the particular phylogenetic pattern of flying in the Beloniformes, where all exocoetids and some non-excoetids are able to glide, several comparisons are possible: first, one can compare all flying *versus* non-flying lineages. These comparisons can be unbiased, looking for elevated evolutionary rates in flying lineages. This yields a set of 50 or so genes, most of which have not previously been linked to fin or appendage development, thus creating new raw material for hypothesis testing. A second approach is to look for shared amino acid positions in known fin development genes between flyers and non-flyers, which satisfyingly reveals that the newly found *lat4a* shows a common substitution in all gliders with larger pectoral fins.

As the best flyers, the exocoetids form a monophyletic group, so a lot of the evolution of such flying programs must have happened at the base of the lineage, leading up to their last common ancestor. When searching for signals in this part of the evolutionary tree, Daane and colleagues[3] again come up with several candidate genes, including potassium-channel subunits, connexins and, yet again, *lat4a*. All these leads are very suggestive of bioelectrical signaling machinery having played an important role in the evolution of elongated fins in flying fish, but they await functional testing *in vitro* or *in vivo*.

It is amazing that, through genomics powered by a phylogenetic framework and a robust understanding of developmental biology, erstwhile esoteric and elusive subjects like the evolution of flying fishes have come within reach. But to move beyond vague gene ontology terms and candidate gene lists, hard biological legwork lies ahead. More questions are just around the corner, perhaps most interesting among them the question of why flying fish have not evolved to become more "perfectly winged animals", given the seeming ease with which fin shape and size can be tinkered with. Why are there no flish? For the exocoetid ancestor in the Eocene, when skies were already filled with perfectly diversified birds, the answer is perhaps trivial — just watch flying fish escaping underwater predators only to get picked up by frigatebirds (https://youtu.be/bk7McNUjWgw). But there are fossil fish, from entirely different lineages, whose fins look suspiciously like those of flying fish[10]. These fossils date from the middle Triassic, a time well before even the first pterosaur took wing. The skies could have been all theirs! Of course, there are a myriad of ecological factors that may explain why this opportunity was missed; but maybe a deeper look at the physiological, developmental and genetic constraints that prevented fish from ruling the skies might be a worthwhile, if far-flung, endeavor. Hopefully, the excursion of evolutionary developmental biology into the world of flying fish does not remain a fleeting foray.

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