# Lost and found

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Can we ever hope to pin down the genetic changes that underlie the big steps in evolution? Possibly so, if a study of the variation in the pelvic fins of sticklebacks is anything to go by.

Darwin's lament[1] that "Our ignorance of the laws of variation is profound" has described one of the persistent problems in evolutionary biology for the past 145 years. How does genetic variation — the raw material of evolution — arise within populations, and how does it evolve to make species anatomically and behaviourally distinct? Ultimately, answers to these questions will be derived from understanding the actual genetic changes that control the evolution of anatomy or behaviour. These insights could explain a host of puzzles, from how rapid evolutionary change can occur in populations, to why morphological evolution often follows certain well-defined trends. On page 717 of this issue, Shapiro *et al.*[2] describe the genetic basis for one such trend: limb reduction in threespine stickleback fish.

Surprisingly, some of the most significant novelties in the history of life are associated not with the evolution of new structures but with the loss or reduction of primitive ones. In vertebrates, for example, the invasion of new ecological niches and the origin of new locomotor adaptations involve either the complete loss or partial reduction of appendages[3,4]. The complete loss of appendages has been involved in the evolution of new aquatic lifestyles in whales and burrowing niches in snakes, amphisbaenians (worm lizards) and caecilians (rubber eels). The reduction or loss of fingers and toes is associated with the evolution of jumping, flying and running in some amphibians, reptiles and mammals. In sticklebacks (Fig. 1), a large spine in the pelvic fin serves to limit predation in marine populations. But in some freshwater stickleback populations this pelvic spine is reduced or lost entirely — a trend that might be associated with lower concentrations of available calcium ions in freshwater lakes, or with the presence of large invertebrates that feed on sticklebacks by grasping the pelvic spines.

By comparing the genomes of sticklebacks with and without pelvic structures, Shapiro *et al.*[2] have identified the major gene responsible for the variation in pelvic size in these populations of threespine sticklebacks. By crossing a female from a population that has a complete pelvic skeleton with a male from a population that lacks pelvic spines, more than 375 progeny were produced after two generations. The offspring showed a range of conditions, from complete pelvic fins to reduced or wholly absent pelvic skeletons.

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Figure 1 **Sticklebacks under scrutiny. The threespine stickleback, *Gasterosteus aculeatus*, is shown in ventral view, with bony structures stained red. The right-most group of bones makes up the pelvic-fin structure under discussion here[2]. Inset, sticklebacks *au naturel*.**

By correlating how fin morphology segregates with each of 221 genetic markers in the offspring, Shapiro *et al.* showed that five regions of the genome are associated with pelvic reduction. One region accounted for a large amount of the pelvic reduction in freshwater populations; the other four explained smaller amounts of the reduction. Importantly, the Pitx1 gene maps precisely to the site that is associated with most of the variation. This is strikingly consistent with data from mice: mutants lacking Pitx1 function also have reduced hindlimbs, and do so asymmetrically, with the right limb more affected than the left[5–7]. Indeed, this same asymmetry is seen in the natural variation in pelvic fins within freshwater stickleback populations.

Pitx1 mutations in mice are often lethal, because they cause developmental abnormalities of the head, face and some glands. How, then, could alterations in this gene be involved in limb reduction in living populations of stickleback fish? The answer is that the regulation of Pitx1 — not the protein encoded by the gene — has changed. Thus, Shapiro *et al.* found that the sequence of the protein-coding region of the Pitx1 gene is identical between the different populations of sticklebacks. But the gene's expression pattern is altered markedly: the population with complete pelvic loss shows no Pitx1 expression in appendages but retains patterns of gene activity in other areas, such as the thymus, olfactory pits and caudal fins (Fig. 2, overleaf). This type of localized decrease in the activity of Pitx1 can result in pelvic-fin reduction without affecting other parts of the body.

Regulatory changes affect when and where a gene is active, not the actual product of the gene. So these types of changes are often involved in non-lethal and rapid morphological change, and are likely to be extraordinarily important components of evolutionary history[8,9]. Indeed, stratigraphic and geographic analyses suggest that limb loss in sticklebacks has evolved in fewer than 10,000 generations[2]. Extrapolating these results to other taxonomic groups leads to the conclusion that major morphological change can evolve rapidly through regulatory changes in a small number of genes. In addition, these changes can happen independently in different populations and species. Systematists have long known that some evolutionary changes occur more readily than others — different species often evolve similar traits independently. Shapiro *et al.* have shown that such patterns of parallel evolution might have the same genetic basis: similar genetic shifts seem to underlie pelvic reduction in stickleback populations in Canada and Iceland, more than 5,700 kilometres apart. Indeed, these results might speak to general principles

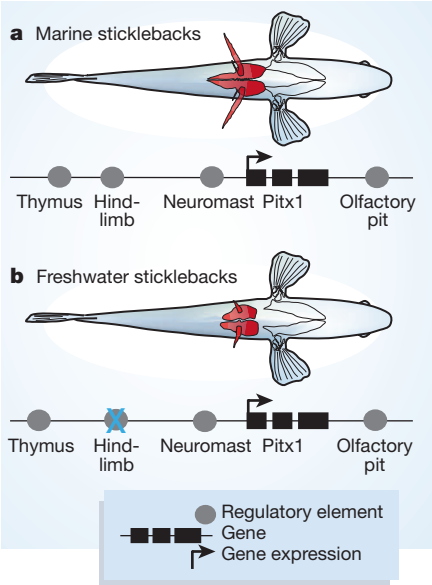


Figure 2 **Evolution in action: a genetic basis. Shapiro *et al.*[2] have found that variation in expression of the Pitx1 gene underlies variation in reduction of a large spine in the pelvic fin in threespine sticklebacks. The regulation of Pitx1 activity has been partitioned into discrete regulatory elements, each of which controls this gene in a particular tissue; changes in Pitx1 expression that result in pelvic-fin reduction can thus be uncoupled from the requirements of other parts of the body for Pitx1 activity.** a, **Shapiro *et al.* show that marine sticklebacks with intact Pitx1 regulatory elements develop robust pelvic spines.** b, **Mutations (X) that specifically affect hindlimb Pitx1 expression alter the pelvic-fin structures of freshwater species, whereas other Pitx1-dependent structures are unaffected. The neuromast is a mechanosensory organ. Figure adapted from ref. 2.**

of parallel evolution, because examples are now cropping up from diverse places. In bird plumage[10] and stickleback armour[11], the independent evolution of similar features in different populations appears to involve similar genetic modifications.

One of the central mysteries of evolutionary biology has been the relationship between microevolution and macroevolution[12]. How can an understanding of the evolutionary mechanisms that act in populations today explain the types of variation that distinguish higher taxonomic groups, such as genera, families or even phyla? Can an understanding of population-level processes explain major evolutionary events such as the Cambrian explosion — the period around 550 million years ago when complex animal life took off? Perhaps so. Shapiro *et al.*[2] might have discovered a smoking gun — a real example of a type of macroevolutionary change that is produced by genetic differences between populations. ■

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Astronomy

# The missing black-hole link

Nate McCrady

A class of black holes of intermediate mass is expected but has never been detected. The suggestion that these beasts might lurk behind powerful X-ray sources in nearby galaxies is now strengthened.

Black holes are known to exist in two mass regimes: those between two and ten times the mass of the Sun, known as stellar-mass black holes and formed from the collapse of the most massive stars; and those between a million and a billion times the mass of the Sun, the 'supermassive' black holes. Such behemoths, including the one at the centre of the Milky Way[1], are the engines powering quasars and active galactic nuclei. Between the two extremes are intermediate-mass black holes, although these have never been detected unambiguously. Observations from NASA's Chandra space observatory have stoked the debate over their existence. The images show extremely luminous, compact X-ray sources lurking in and around star clusters in nearby galaxies — sources that might be associated with intermediate-mass black holes.

Central to the discussion is the lack of a credible mechanism by which these objects could have formed. But dynamical simulations by Portegies Zwart *et al.*, reported on page 724 of this issue[2], show that stellar collisions in dense star clusters could 'run away' and lead to the formation of an intermediate-mass black hole at the cluster core. This process is particularly intriguing because the accretion of such black holes early in the galaxy-formation process is a crucial step in the formation of supermassive black holes at galaxy centres.

The nature of 'ultraluminous X-ray sources' (ULXs), identified in high-resolution Chandra images (Fig. 1), is a key question in the black-hole debate. Bright X-ray point sources in galaxies are generally either young remnants of supernovae, which fade over time, or the compact remnant of a massive star (a neutron star or a stellar-mass black hole). The latter shine as they accrete matter, typically from a companion star. The brightness of such an object generally cannot exceed the Eddington limit, which is the luminosity at which radiation pressure from escaping photons overcomes gravity and disperses the accreting material. ULXs are deemed 'ultraluminous' because the isotropic luminosities implied by their measured fluxes exceed the Eddington limit for the largest black holes formed from single massive stars. If ULXs are actually black holes accreting matter, then their masses must fall in the 100 to 1,000 solar-mass range — the missing intermediate-mass black holes.

Observations[3,4] indicate that ULXs tend to be located in or near star clusters in starburst galaxies, sites of very active star formation. This connection prompted the suggestion[5,6] that intermediate-mass black holes are formed in 'super star clusters' — young, dense clusters 10 to 100 times more massive than the ancient globular clusters they closely resemble. The largest stars formed in such clusters have masses 100 to 150 times that of the Sun, but they leave behind black holes of only 15 to 20 solar masses.

To form an intermediate-mass black hole, a mechanism other than star formation is required: Portegies Zwart *et al.*[2] propose runaway growth prompted by the collision of stars. In the field of a disk galaxy, outside clusters, collisions between stars are very unlikely, because their cross-sectional area is so small compared with the vast distances between them (galaxies are, in fact, far more common collision partners). Dense star clusters, however, are a different matter[7]. The most massive stars in a cluster tend to sink in towards the core, by transferring energy to lower-mass stars in a process known as mass segregation. Once the central density becomes high enough, collisions between high-mass stars can occur. But can these collisions create a 'runaway star' that builds sufficient mass to beget a 100–1,000-solar-mass black hole, before the high-mass stars explode as supernovae?

Portegies Zwart *et al.* have used many-body simulations to examine the behaviour of a pair of young super star clusters[8] in the nearby starburst galaxy M82 (Fig. 1). One cluster, MGG 11, coincides spatially with the brightest ULX discovered so far, the luminosity of which corresponds to an intermediate-mass black hole of 300–900 solar masses[9].